

Preparation of Hydrogel Based on Glutaraldehyde-Crosslinked Carrageenan

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Abstract. In this work, kappa carrageenan hydrogels were prepared by chemical crosslinking using glutaraldehyde (GA) as the crosslinking agent and hydrogen chloride (HCl) as the catalyst. The aim of this work was to study the effect of crosslinking process variables, namely the amount of GA and HCl, and time of reaction upon the swelling properties of the obtained hydrogels. Aqueous kappa carrageenan (3% w/v) was reacted with a certain amount of GA (25%) and HCl (0.1 N) at 40°C. After a certain time of reaction, the solution was cooled at room temperature producing gel form. The gel was soaked in the ethanol to precipitate the crosslinked polymer and to remove the unreacted residue. The obtained hydrogel was air dried at room temperature to constant weight. The FTIR spectra and the value of swelling degree of obtained hydrogel showed that carrageenan could be crosslinked using glutaraldehyde. There were no significant differences of the swelling degree of hydrogels synthesized by 5 to 120 minutes reaction time. The crosslinking with higher amount of glutaraldehyde and HCl may produce hydrogels with lower swelling degree. The kappa carrageenan hydrogel was found to be pH sensitive, indicating a high potential to be used in drug delivery polymer system.

Keywords: carrageenan, crosslinking, hydrogel, glutaraldehyde, swelling.

1. Introduction

Hydrogel is a hydrophilic polymer with three dimensional crosslinked structures and may absorb water and biological solutions without dissolution. Hydrogel had been widely applied as disposable diapers, agriculture media, controlled release matrices to deliver drug or cell, and matrices for generating the tissue and organs [1]. Compared to synthetic polymers, the natural polymers such as polysaccharides may produce better hydrogels due to more biodegradable, less toxic, more biocompatible, renewable, and cheaper because the raw materials are locally abundant. Kappa carrageenans are linear polysaccharides sulphated galactan extracted from red seaweed (Rhodopyta), such as *Kappaphycus alvarezii* (known as *Eucheuma cottonii* in industry) well cultivated in Indonesia. This natural polymers comprise of repeating units of (1,3)-D-galactopyranose and (1,4)-3,6- anhidro- α -D-galactopyranose with sulphate groups in the certain amount and position [2]. Kappa carrageenans are hydrophilic and have ability to form thermoreversible gel. Because of their gelling ability, carrageenans are widely used as agent for thickening and gelling in food and nonfood industries [3], and potent as raw materials of hydrogels [1]. Some gel applications need hydrogel properties which can absorb and keep water without dissolution. For enhancing the stability of gel in aqueous, the carrageenan structures need to be modified by chemically crosslinking. In this work, glutaraldehyde was chosen as the crosslinking agent. Aldehydes are very reactive and have been used for crosslinking polymer.

Many earlier authors have been reported the usage of glutaraldehyde as crosslinking agent for preparing hydrogels based on natural polymers, such as chitosan [4], guar gum [5], sodium alginate [6], collagen [7],

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alginate-guar gum [8]. The obtained hydrogels were reported to be potent for biomedical application. To our knowledge, there is no study of preparing of hydrogels based on kappa carrageenan by chemical crosslinking using glutaraldehyde as the crosslinking agent and hydrogen chloride as the reaction catalyst. In the present work, hydrogel were prepared by crosslinking them with glutaraldehyde in an aqueous medium. The aim of the present work was to study the effect of crosslinking condition, such as the amount of glutaraldehyde and HCl, and reaction time upon the swelling properties of obtained hydrogel. In addition, we present a preliminary result regarding the pH sensitive of obtained hydrogel.

2. Materials and Methods

2.1. Materials

Kappa carragenans (Sigma type I) were used as polymer source. The carrageenans consist of dominantly kappa carrageenan and less lamda carrageenan, and contain potassium 10.2%, calcium 2.7% and sodium 0.7%. Glutaraldehyde 25 wt% solution in water (Merck) was used as the crosslinking agent without further purification. Aqueous hydrogen chloride 0.1N was used as the catalyst.

2.2. Preparation of Hydrogel

Aqueous kappa carrageenan (3% w/v) was reacted with a certain amount of GA (25%) and HCl (0.1N) at ca. 40°C. The reaction condition and initial mixture composition of the crosslinking reaction were conducted as displayed in Table 1. The reaction was carried out by mixing the carrageenan solution and the mixture of GA and HCl. The homogenous state was kept by stirring the mixture using magnetic stirrer. In preparing HG2, the 10 mL sample was taken in a certain interval time of reaction. The sample was cooled at room temperature overnight producing gel form. In preparing HG1, HG3, HG4 and HG5, after certain time of reaction, the mixture was divided into two parts, ca. 10 mL per part and was cooled for overnight. The obtained gel was cut into four pieces and then soaked in ethanol overnight to precipitate the crosslinked polymer and to remove unreacted chemicals. The obtained hydrogels were air dried at room temperature to constant weight. The HGS was a hydrogel film formed without crosslinking reaction.

Table 1. Reaction condition and initial mixture composition for preparing hydrogel.

Sample code	Carrageenan solution	GA, mL	HCl, mL	Reaction time, minute
HG1	1.5 g/ 50mL	3	0	120
HG2	1.5 g/ 50 mL	6	0	5-120
HG3	0.6 g/ 20 mL	1.2	1	30
HG4	0.6 g/ 20 mL	1.2	1.84	30
HG5	0.6 g/20 mL	1.2	2.2	30

2.3. Characterization

Assignment of IR spectra of obtained hydrogels were based on spectroscopy data summarized by JECPA [9] and Coates [10]. Molecular groups were identified using FTIR spectrometer Shimadzu. For determining the value of swelling ability, a certain amount of dry hydrogel was weighted and then placed in an aqueous medium. The swelling degree was determined in different media, namely distilled water (pH~7), NaOH 0.1N (pH~13) and HCl 0.1N (pH~1). The swelling degree was evaluated by measuring the weight before soaking (Md) and the weight after soaking (Mw) in solution as function of soaking time at room temperature. Swelling degree (SD) was calculated as:

$$SD = (Mw - Md) / Md$$

3. Results and Discussion

3.1. IR Spectra

Fig. 1 shows the FTIR spectra of obtained hydrogel. The study of carrageenan by FTIR spectroscopy shows the presence of very strong absorption band in 1210-1260 cm⁻¹ region (due to the S=O of sulfate esters), 1010-1080 cm⁻¹ (ascribed to glycosidic linkage), 928-933 cm⁻¹ (3,-6-anhydro-D-galactose), and 840-850 cm⁻¹ (D-galactose-4-sulfate). The spectra of extracted carrageenan show the main features of kappa carrageenan. Fig. 1 shows that there is no sharp difference peaks of obtained hydrogel between without crosslinking (HGS) and with crosslinking (HG1, HG2, HG3, and HG4). We only observed a slight increase in the absorption bands at 1710 cm⁻¹ (HG2) and 1716 cm⁻¹ (HG4). These bands express the carbonyl group.

The presence of these new peaks may be due to the formation of new acetal groups. These new peak indicate that glutaraldehyde had been reacted with hydroxyl of carrageenan. According to previous authors [11-13], in crosslinking reaction, the aldehyde groups from glutaraldehyde reacted with hydroxyl group from polymer under acidic condition, and then formed acetal bridges. Mansur et al. [13] determined the amount of acetal bridges with the absorption bands of 1710 cm^{-1} in preparing hydrogels based on glutaraldehyde-crosslinked poly (vinil) alcohol. In the present work, spectra IR indicated that hydrogels based on glutaraldehyde-crosslinked carrageenan were prepared successfully. The mechanism of crosslinking reaction was predicted as in Fig. 2.

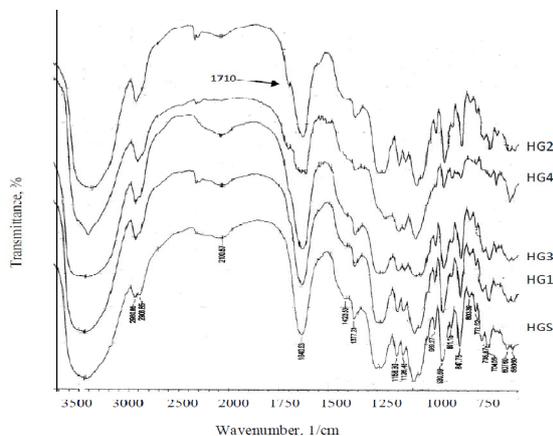
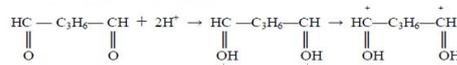


Fig. 1 FTIR spectra from samples.

a. Glutaraldehyde in acid medium:



b. Crosslinking reaction:

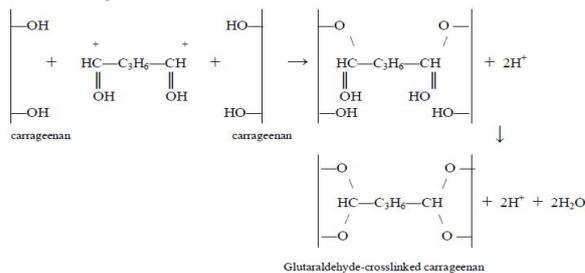


Fig. 2 Crosslinking reaction of carrageenan with GA

3.2. Influence of Reaction Time on Swelling Degree

Beside IR spectra measurement, the prepared hydrogels were also tested with regard to their swelling in aqueous solution. The influence of reaction time was investigated by preparing hydrogel without adding catalyst (HG2). The ability of dry hydrogel to absorb water indicates that hydrogels based on carrageenan were reversible gel, which the hydrogels could absorb water again after the wet hydrogels were dried.

Fig. 3 shows the effect of time reaction upon the value of swelling degree in distilled water medium (Fig. 3 (a)) and acidic medium (Fig. 3(b)) at room temperature (25°C). It is showed that length of reaction time from 5 minutes to 120 minutes did not give any significant differences of the swelling ability. These results indicate that crosslinking reaction between carrageenan and glutaraldehyde was spontaneous reaction, although without catalyst. In acidic medium, the hydrogel sampel was found to lose its swelling significantly after about 120 minutes, suggesting disintegration of the swollen gels.

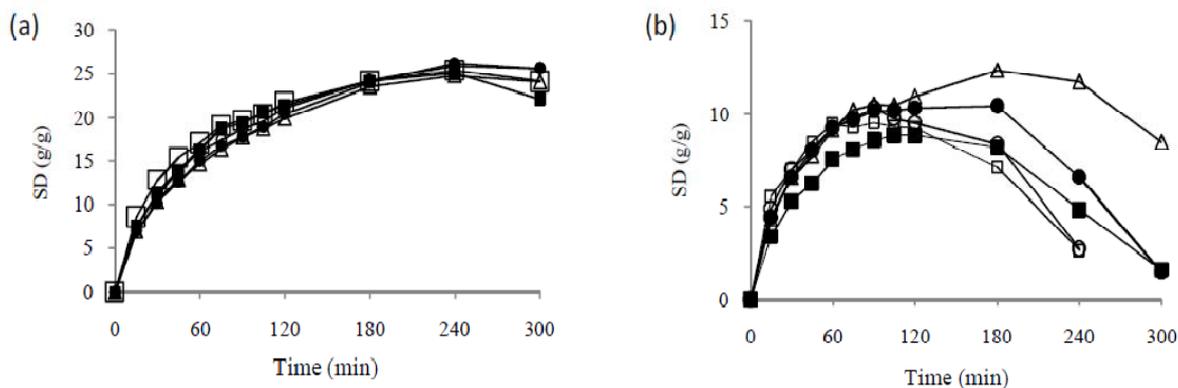


Fig. 3 Swelling degree of HG2 composition with different reaction time, namely , 5 min(□), 15 min (■), 30 min (○), 60 min (●), and 120 min (Δ), (a) at distilled water and (b) at HCl 0.1N.

3.3. Influence of Ratio of Carrageenan to GA Weight upon Swelling Degree

Chemical crosslinking is a direct reaction between linear polymer or branches and at least a bifunctional component, small molecular weight, and called as crosslinking agent or crosslinker. This component links the polymer chains with its functional groups [14]. In the present work, the aldehyde groups from glutaraldehyde able to link the carrageenan's chains, so that the swelling degree of crosslinked hydrogel decreased. Fig. 4 shows that value of swelling degree of obtained hydrogel without crosslinking (HGS) and

hydrogel with crosslinking (HG1 and HG2) in various medium. Compared to HGS, the HG1 and HG2 had lower value of swelling degree in all medium. The decreasing of swelling degree indicates that there was a denser crosslinked structure. More crosslinked structure causes the smaller space in molecules, as the result the water which can be absorbed was less. It shows that the crosslinked increases with the increasing of GA amount. The amount of GA may influence the pH of solution. In present work, the GA 25% exhibited acidic solution with pH about 3. The acidic condition may cause the aldehyde of GA was more reactive, producing hydrogel with more crosslinks number.

Figs. 3-5 show a comparison between the swelling degree of the hydrogels in distilled water, NaOH solution and HCl solution, measured at room temperature. Here, the obtained hydrogels were found to be pH sensitive as depicted Figs. 3-5. The swelling degrees values in various pH solution were much different, suggesting an ionic characteristic for this carrageenan gel. The swelling degrees were lower at strong acid and strong alkaline pH, relative to that at neutral pH. This properties shows that hydrogels based on glutaraldehyde-crosslinked carrageenan have high potential to be used as biomaterial.

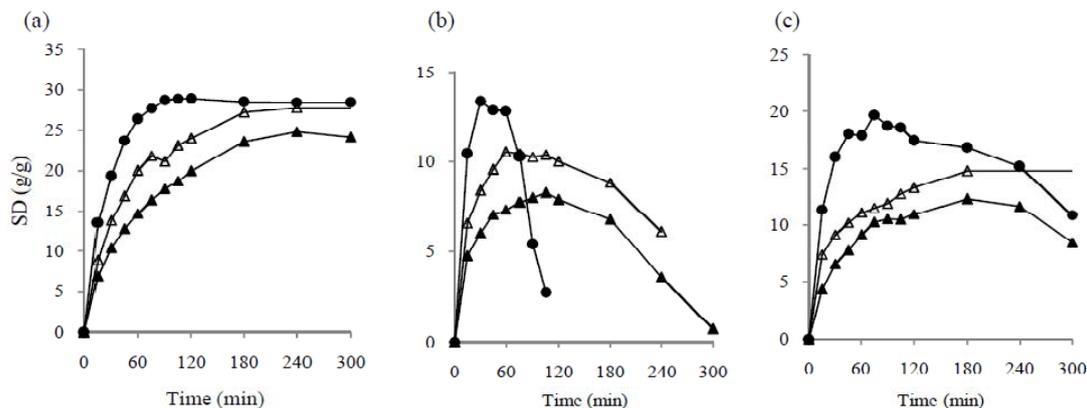


Fig. 4 Swelling degree of hydrogels with different amount of GA weight (HGS (●), HG1(Δ), HG2(▲)) (a) at distilled water, (b) at NaOH 0.1N and (c) at HCl 0.1N.

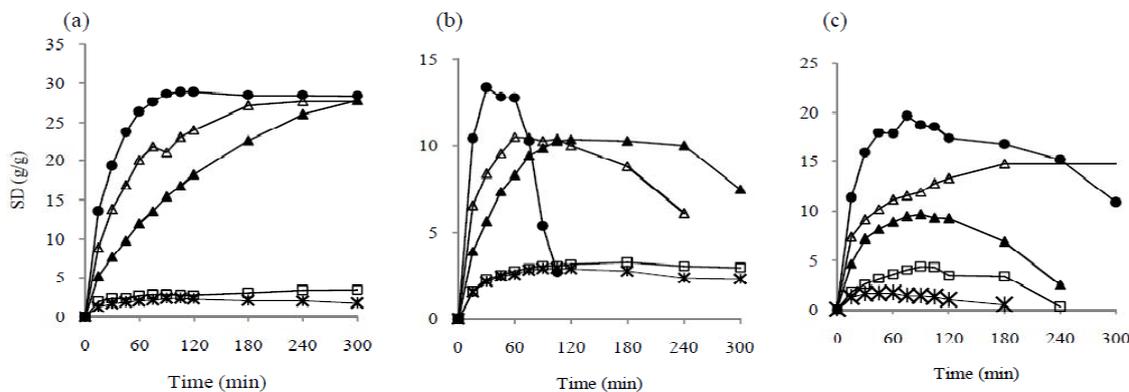


Fig. 5 Swelling degree of hydrogel with different HCl amount (HGS (●), HG1(Δ), HG3(▲), HG4 (□), HG5 (X)) (a) at distilled water, (b) at NaOH 0.1N and (c) at HCl 0.1N.

3.4. Influence of Amount of HCl upon Swelling Degree

The influence of HCl amount upon the swelling property was investigated using characteristic of HG1, HG3, HG4, and HG5. Fig. 4 shows the value of swelling degree with variation of HCl 0.1N volume as the catalyst. It is showed that with the more HCl volume, the swelling degree of obtained hydrogels decreased. Therefore, such findings have given strong evidence that the reaction of the carrageenan with GA has indeed occurred when this crosslinking agent is used and catalyzed by hydrochloric acid.

The amount of HCl affects the pH of initial mixture of crosslinking reaction which is the lower pH will be obtained with more HCl volume. The lower pH solution led the aldehyde groups of glutaraldehyde were more reactive. These aldehyde groups then reacted with the hydroxyl groups in carrageenan molecules, and as the results the number of crosslinks increased. The addition of HCl as the catalyst must consider the possibility of hydrolysis polysaccharides by this HCl. The aqueous carrageenans comprise of carbohydrate

structures that are easy to be hydrolyzed by acid catalyst. Here, the amount of HCl had not been caused the hydrolysis. This is indicated by the swelling degree decreased with the increasing of HCl volume.

4. Conclusion

Hydrogel properties are strongly influenced by the crosslinking reaction parameters, including the initial composition of the polymer mixture. The FTIR spectra of obtained hydrogel showed the new peak that indicated the formation of crosslinked structure. The crosslinking reduced the swelling ability of the hydrogel. There was no significant difference of the swelling properties between 5 to 120 minutes reaction. The increasing amount of glutaraldehyde and HCl will increase the crosslinked density, indicated based on the reduction of swelling ability of obtained hydrogel. The kappa carrageenan hydrogel was found to be pH sensitive, indicating a high potential to be used in drug delivery polymer system.

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6. References

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