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Article

Preparation and Characterization of Glutaraldehyde-Crosslinked Kappa Carrageenan Hydrogel

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Abstract. Glutaraldehyde-crosslinked kappa carrageenan hydrogel was prepared using glutaraldehyde (GA) as the crosslinking agent. Kappa carrageenan film obtained from extraction of *Kappaphycus alvarezii* seaweed was immersed in GA solution (1-5 wt%) for 2 min and then cured at 110 °C for 25 min. The obtained crosslinked film was washed and soaked in the ethanol to remove the unreacted GA. The obtained film was air dried at room temperature to a constant weight. The infrared spectra, thermal analysis, and the value of swelling degree of obtained hydrogel showed that kappa carrageenan was able to be crosslinked using GA by film immersion and high temperature curing method without catalyst presence. GA concentration less than 0.027 g GA/g polymer was not able to crosslink hydroxyls group of carrageenan. The swelling degree in water media decreased up to 60% with increasing GA concentration from 3% to 5%. The kappa carrageenan hydrogel was found to be pH sensitive.

Keywords: Glutaraldehyde, crosslinked kappa carrageenan, hydrogel, swelling.

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1. Introduction

Hydrogels are three-dimensional hydrophilic polymer networks that can absorb and retain large quantities of water, saline or physiological solutions. Hydrogels have been widely used in agriculture, biomedical area, tissue engineering, biosensors, and sorbents for removal of heavy metals, and drug delivery. Recently, the hydrogels based on natural polymers, especially polysaccharides, have been investigated extensively due to more biodegradable, less toxic, more biocompatible, renewable, and cheaper because the raw materials are locally abundant than synthetic polymer hydrogels.

Kappa carrageenans are linear polysaccharides sulfated galactan extracted from red seaweed (Rhodopyta), such as *Kappaphycus alvarezii* (known as *Eucheuma cottonii* in industry) which is 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 sulfate groups in a certain amount and position [1]. The presence of hydroxyls and sulfate groups in carrageenan structure cause the carageenans tend to be hydrophilic. Kappa carrageenans 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 [2] and potential as raw materials of hydrogel [3]. The gelled carrageenan is still easily soluble in water. Some gel applications need hydrogel properties which can absorb and keep water without dissolution. For improving the stability of gel in aqueous, the kappa carrageenan structures must be modified to produce hydrogel structure. The aim of this work is proposed to enhance the stability of kappa carrageenan using chemical crosslinking technique.

Some crosslinking agents have been used to crosslink carrageenan, such as genipin [4, 5], vinyltriethoxysilane [6], $CaCl_2$ [7] and epichlorohydrin [8]. In this work, glutaraldehyde (GA) was chosen as the crosslinking agent. GA is easily available and inexpensive. Aldehydes are very reactive and have been used for crosslinking polymers. Many earlier authors have reported the use of glutaraldehyde as crosslinking agent for preparing hydrogels based on natural polymers, such as guargum [9], alginate-guargum [10], sodium alginate [11], chitosan-gelatin [12], collagen [13], and methylcellulose [14]. The obtained hydrogels were reported to be potential for biomedical application. To our knowledge, there is no study of preparation of hydrogel based on kappa carrageenan by chemical crosslinking using glutaraldehyde. In the present work, we develop method for preparing glutaraldehyde-crosslinked kappa carrageenan hydrogel by film immersion and curing method. The carrageenan extracted from *Kappaphycus alvarezii* seaweed was used as kappa carrageenan source. This work focused on study the effect of the glutaraldehyde concentration on preparation and properties of hydrogel. The obtained hydrogels were characterized using fourier transform infrared (FTIR), differential scanning calorimeter (DSC) and swelling measurement in various media. In addition, we present a preliminary result regarding the pH sensitive of obtained hydrogel.

2. Experimental

2.1. Materials

Seaweeds of *Kappaphycus alvarezii* were harvested from Makasar, South Sulawesi, Indonesia. The seaweeds were washed using tap water to eliminate all impurities such as the salt and sand. After washing, the seaweeds were cut into about 1 cm length, and finally sun dried to constant weight. The 'clean seaweed' sample was kept in a dry state until further processing was done. Technical grade of potassium hydroxide (purity 88%) were used as alkali treatment before extraction process. Glutaraldehyde 25 wt% solutions in water (Merck) and all other chemicals were purchased and used without further purification.

2.2. Carrageenan Preparation

The process of carrageenan recovery from *Kappaphycus alvarezii* followed the previously reported method [15] with minor modification. The clean seaweed was treated using KOH solution before being extracted. Thirty gram of seaweed was soaked in KOH 0.3 N overnight and then heated at $60^{O}C$ for 30 min. After alkali treatment, the seaweed was washed with tap water and neutralized with HCl 0.1N. A certain amount of distilled water as the solvent was heated in a beaker as an extractor. After the temperature of solvent reached $80^{O}C$, the seaweeds were then added into solvent, and the time of extraction started to be counted. The constant ratio of seaweed weight to solvent volume (1/50; g/mL) was maintained by adding hot water.

After 1 hr extraction, the filtrate was separated from residue and immediately poured into 3 volumes of cold (5^{O}C) technical ethanol (90 wt%) which caused precipitation of polysaccharides. The precipitation was allowed for 30 minutes with stirring gently. The precipitated carrageenans were collected and dried at room temperature to a constant weight.

2.3. Film Preparation

Carrageenans film was prepared by dissolution of the dry carrageenan in distilled water. The mixture was heated and stirred until a homogeneous solution was obtained. The solution was poured into plastic plate and allowed to solidify and then dried at room temperature to constant weight. The obtained film was cut of 1.5 cm x 1.5 cm and the weight of each piece film was about 0.03-0.04 gram.

2.4. Film Crosslinking

GA with a certain concentration (1-5 wt%) was prepared by diluting GA 25 wt% (gram GA/ gram GA solution) with distilled water. The carrageenan film was immersed in glutaraldehyde-water mixture for 2 min. The surface of film was wiped with filter cloth and then cured at 110^{O}C in oven for 25 min. The crosslinked film was soaked in water with stirring for 1 min and then in ethanol for 4 hr to remove unreacted GA. The wet hydrogels were dried at room temperature to a constant weight.

The amount of GA absorbed (X_{GA}; gram GA/gram polymer) during immersion was evaluated by determining the value of swelling degree of film at GA solution with a certain concentration (C; gram GA / gram GA solution). Briefly, the weight of film before immersion (Wb) and after immersion (Wa) were determined, then the amount GA absorbed was calculated using Eq. (1).

$$X_{GA} = \left(\frac{Wa-Wb}{Wb}\right)C \qquad (1)$$

2.5. Film Characterization

Molecular groups were identified using FTIR spectrometer (Shimadzu IR Prestige-21). Both control (noncrosslinked) and crosslinked film resulted from GA 5% were powdered. Infrared spectra were obtained by using KBr pellet method with 10 scans and 16 cm^{-1} resolution. Assignments of IR spectra of obtained hydrogels were based on spectroscopy data summarized by Pereira et al. [16]. Peak baselines and height were determined in transmittance (T) mode and peak height were converted to absorbance (A) [17]. Absorbance was calculated as Eq. (2).

$$A = -\log(T) \qquad (2)$$

The thermal stability was evaluated by the temperature profile of noncrosslinked and crosslinked film produced from GA 5%. Thermal analysis was carried out in air atmosphere using DSC (Linseis PT1600 Type) at heating rate 10^{O}C/min. An empty alumunium pan was used as a reference. The weight of the samples (in dry film form) was 0.03-0.05 g.

For determining the value of swelling ability, a piece of dry hydrogel film was weighted and then placed in an aqueous medium of 10 mL. The swelling degree was determined in different aqueous media, namely distilled water (pH~7), phosphate buffer (pH~7.4), 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. All weight measurements were conducted on a pan balance (Ohaus) having an accuracy up to fourth decimal. Swelling degree (SD) was calculated as Eq. (3). Each experiment was done at least one duplicate run and the mean value was used to display the data.

$$SD = \frac{(Mw-Md)}{Md} \qquad (3)$$

3. Results and Discussion

3.1. FTIR Spectra

Crosslinking carrageenan film with GA was carried under certain GA concentration. Figure 1 and Table 1 show the FTIR spectra of control (noncrosslinked) and crosslinked film that obtained by crosslinking carrageenan with GA 5%. The study of carrageenan spectra 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), 925-935 cm^{-1} (C-O of 3,-6-anhydro-D-galactose), and 840-850 cm^{-1} (C-O-SO_3 of D-galactose-4-sulfate). In this work, the infrared spectra of extracted carrageenan from *Kappaphycus alvarezii* show the main features of kappa carrageenan as summarized by Pereira et al. [16]. The sulphate and hydroxyl groups (3200-3600 cm^{-1}) correspond to the hydrophilic ability of carrageenan. The presence of hydroxyl groups in carrageenan chains may cause the carrageenan is possible to be crosslinked with GA.

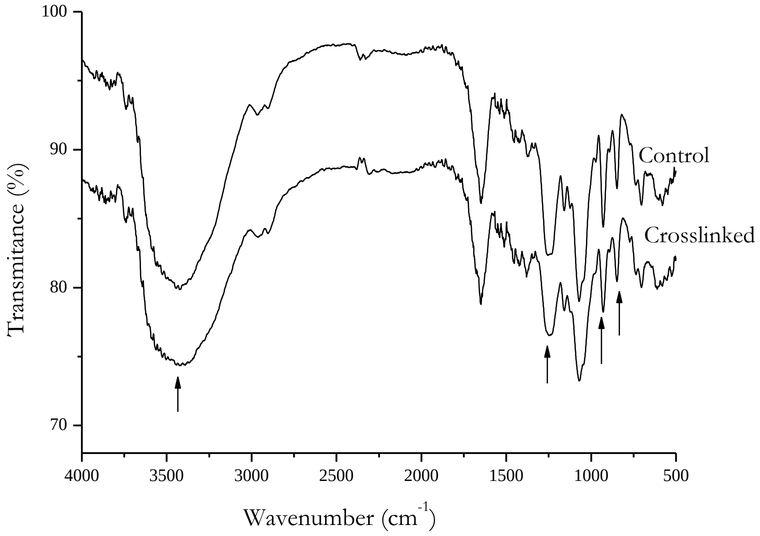

Fig. 1. FTIR spectra of carrageenan film before (control) and after crosslinking (crosslinked).

Table 1. Characteristic IR peak in control and crosslinked film.

Functional group	Control			Crosslinked (GA 5%)		
	Peak(cm^{-1})	A	A/A1	Peak(cm^{-1})	A	A/A1
D-galactose-4-sulfate	847	0.0607	1.0	848	0.0937	1.0
3,6-anhydro-D-galactose	928	0.0752	1.24	928	0.1062	1.13
Sulfate esters	1254	0.0845	1.39	1238	0.1164	1.24
Hydroxyl	3421	0.0979	1.61	3420	0.1286	1.37

A=*Absorbance*
A1=*Absorbance of D-galactose-4-sulfate*

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 [18]. Here, the chemical reaction between hydroxyl groups of carrageenan and GA as the crosslinking agent was confirmed by FTIR measurement. Figure 1 shows that there is no sharp difference peaks between control film (without crosslinking) and crosslinked film (with crosslinking).

The absorbance of peaks attributed to hydroxyls was normalized with respect to that of peak attributed to C-O-SO_3 peaks, which remained almost constant. The relative absorbance of the O-H groups was evaluated through the absorbance of ratio of O-H band to C-O-SO_3 band. This ratio was associated with relative amount of hydroxyls to D-galactose-4-sulfate. This absorbance ratio serves as semi quantitative index of chemical composition of film. We only observed a slight decrease of the relative absorption bands of hydroxyl group in crosslinked film (Table 1).

The reduction of this absorbance ratio, namely 1.61 for control film became 1.37 for crosslinked film, indicates that reaction glutaraldehyde and carrageenan has occurred. The hydroxyls had been consumed during crosslinking reaction. According to previous authors [19-21], in the crosslinking reaction, the aldehyde groups from glutaraldehyde reacted with hydroxyl group from polymer under acidic condition, and then formed acetal bridges. One aldehyde group links the polymer chains by reacting with the hydroxyl groups of polymer and produces a hemi-acetal structure [14, 21]. The peak of C-O-C groups (ascribed to acetal groups) did not appear in observed FTIR spectra (Fig. 1). This is probably caused by the low concentration of acetal groups in the film. Such low level of acetal groups could possibly be beyond the detection limit of spectroscopic analysis. The aldehyde of GA react with hydroxyl groups of polivinil alcohol and chondroitin sulfate, and the sulfate groups of chondroitin sulfate did not react with aldehyde groups [20]. In the present work, the reduction of hydroxyl absorbance of spectra IR indicated that hydrogels based on glutaraldehyde–crosslinked carrageenan were prepared successfully by film immersion and high temperature curing method without catalyst. The mechanism of crosslinking reaction was predicted as in Fig. 2.

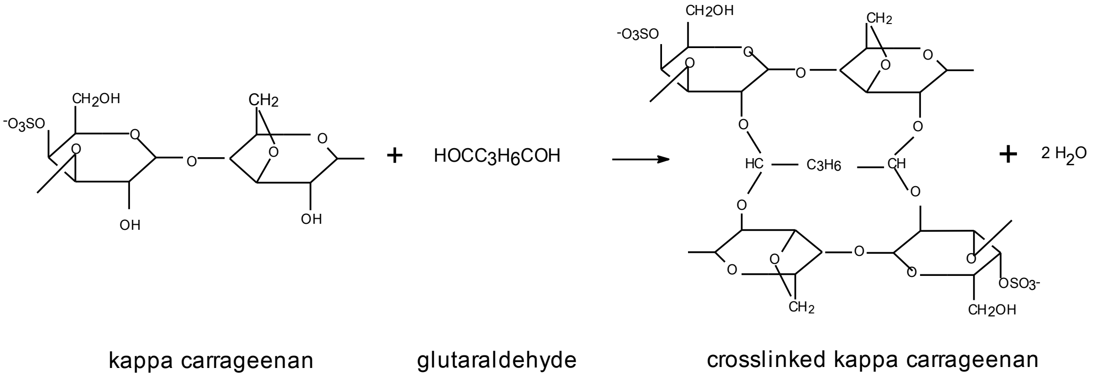

Fig. 2. Crosslinking reaction of carrageenan with GA.

3.2. Thermal Analysis

Figure 3 shows the thermal behavior of control and crosslinked carrageenan film (GA 5%) from DSC. The temperature values of endothermic and exothermic peaks of control and crosslinked are summarized in Table 2. The crosslinking treatment enhances the thermal stability as shown by the slight but appreciable shift of both endothermic and exothermic peak to higher temperatures. The calorimetric profile (Fig. 3) was composed of endothermic and exothermic peaks. The endothermic peak corresponds to early decomposition, water lost and the exothermic peak corresponds to the ongoing decomposition of the polymer [5, 9, 22]. The shift of all these parameters to higher temperature as shown in Table 2 indicates that the crosslinking treatment improves thermal stability of carrageenan film. The improvement of this thermal stability may due to the acetal bridges formed in the crosslinked film [14].

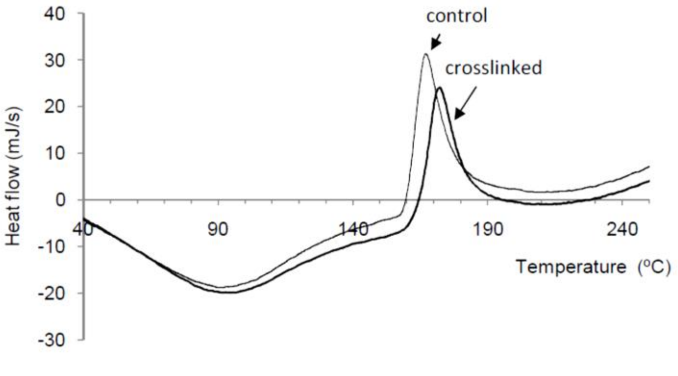

Fig. 3. DSC curve of control film and crosslinked carrageenan film.

Table 2. Comparison of thermal data of control and crosslinked from DSC curve.

Sample	**Endothermic peak temperature (^oC)**	**Exothermic peak temperature (^oC)**
Control	91	167
Crosslinked	96	172

3.3. Swelling Behavior

Beside IR spectra and DSC measurement, the crosslinked hydrogels were also tested with regard to their swelling in aqueous solution. Figures 4-7 show the comparison of that value of swelling degree of control film (noncrosslinked carrageenan) and obtained hydrogels with different GA concentration at various medium, measured at room temperature.

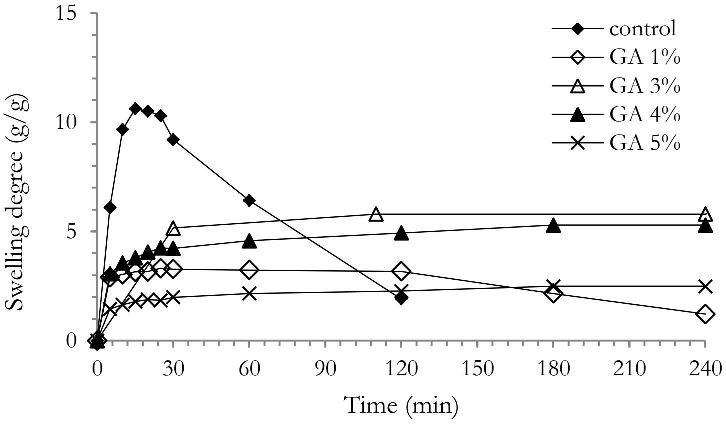

Fig. 4. Swelling degree of hydrogel with different GA concentration at distilled water.

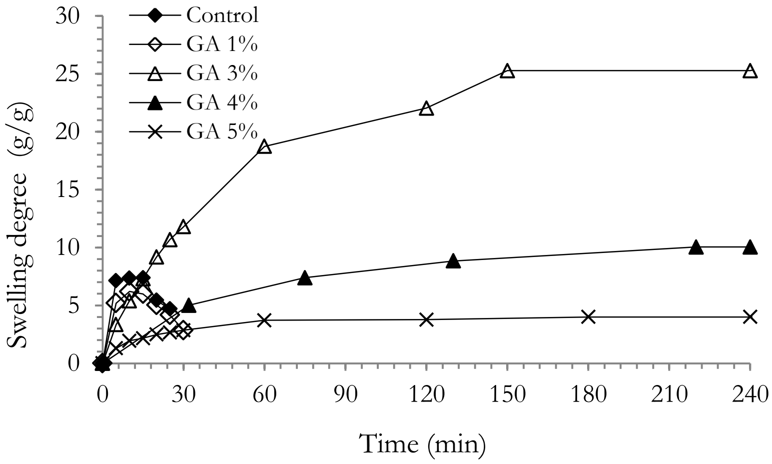

Fig. 5. Swelling degree of hydrogel with different GA concentration at phosphate buffer pH 7.4.

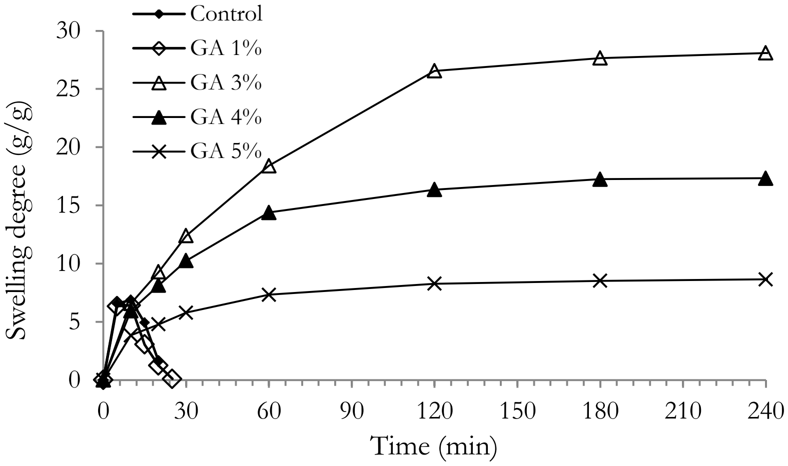

Fig. 6. Swelling degree of hydrogel with different GA concentration at NaOH 0.1N.

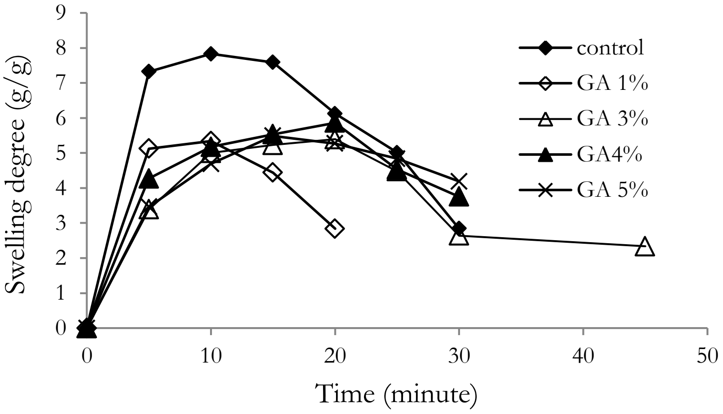

Fig. 7. Swelling degree of hydrogel with different GA concentration at HCl 0.1N.

The control film was still easy to dissolve in water. The control film attained the equilibrium state of swelling degree in water at about 30 min (Fig. 4), and after this time the film was not able to maintain the swollen state. Compared to the control film, crosslinked film (except GA 1%) was more stable in water and remained insoluble in water. The crosslinked carrageenan revealed improved properties in comparison to the control. These data exhibit that the aldehyde groups from glutaraldehyde were able to link the chains of carrageenan, as the result the crosslinked film may absorb water without dissolution and also the swelling degree decreased.

In this present work, the dry polymer film was immersed in GA solution for 2 min and then followed by curing at 110^{O}C. The mean value of GA solution absorbed in the immersion process for 2 min was observed about 2.7 g GA solution/g polymer. GA absorbed in the film from 1%, 3%, 4% and 5% of GA solution were 0.027, 0.081, 0.107, and 0.1343 g GA/g polymer, respectively. The products of reaction between carrageenan films with GA 1% still could not retain water compared with the higher GA concentration (Figs. 4-6). This indicates that GA concentration less than 0.027 g GA/g polymer was not able to crosslink hydroxyls group of carrageenan. The low GA concentration produced low yield of crosslinked structure. This means that low GA concentrations, under GA 1 wt%, are not recommended for the reaction with carrageenan.

The hydrogels obtained from GA 3-5% remained stable without noticeable changes in water, buffer and NaOH solution (Figs. 4-6). In the crosslinked structure, crosslinked hydrogels attain an equilibrium swelling degree in aqueous solutions that depend mainly on the crosslink density. In this work, the GA concentrations from 3% to 5% were used for studying the effect of GA concentration on the value of equilibrium swelling degree, evaluated after 24 hr since initially swelling measurement. In water media, the equilibrium swelling degrees were 7.34, 6.44, and 2.78 for GA 3%, 4%, and 5% respectively. In buffer and NaOH solution, the value of equilibrium swelling degree also decreased with the increasing GA concentration.

In general, there is a trend that with increasing of GA amount the degree of swelling decreased. The swelling degree, showed as grams of water absorbed per gram of carrageenan film, decreased up to 60% with increasing GA concentration from 3% to 5%, indicating increasingly crosslinked carrageenan structure. The decreasing of swelling degree indicates that there is a denser crosslinked structure. More crosslinked structure causes the smaller space in molecules, as the result the less water can be absorbed. It shows that crosslinked structure increases with the increasing of GA amount. Similar behavior was observed in hydrogel obtained from kappa carrageenan/acrylic acid crosslinked with vinyltriethoxysilane [6], alginate/kappa carrageenan with calcium ions [7], and kappa carrageenan crosslinked with epichlorohydrin at low concentration [8].

In our preliminary work, crosslinking reaction without high curing temperature step could not produce crosslinked structure, indicated by the swollen film was not stable in water and the swelling property was similar with the control film. The work of Kim et al. [19] showed that higher contact temperature between polyvinyl alcohol membrane and GA solution increased the consumption of GA and enhanced the rate of crosslinking reaction. It is obvious that reactivity of crosslinking carrageenan with GA can be controlled by high enough of GA amount (> 0.027 g GA/g polymer) and high curing temperature, probably due to the more available aldehyde group for crosslinking [23] and providing energy for endothermic crosslinking reaction. This means that degree of crosslinking of carrageenan film can be easily tailored by controlling the crosslinking condition.

Hydrogels may exhibit the swelling behavior dependent on the external environment. The ability of hydrogel to response the drastic changes can be investigated by their swelling behavior in different pH, temperature, ionic strength or electric stimuli [18]. Here, the obtained hydrogels were found to be pH sensitive as depicted Figs. 4-7. The swelling degree values in various pH solutions were much different, suggesting an ionic characteristic for this carrageenan gel.

Products of crosslinking reaction with GA 5% showed the value of equilibrium swelling degree in water, in buffer, and in NaOH solution were 2.78, 4.09, and 9.9, respectively. It is shown that the swelling degree in the phosphate buffer solution at pH~7.4 (containing salt compounds of NaH_2PO4 and Na_2HPO_4) were much higher than those in pure water pH~7. Hydrogels based on glutaraldehyde-crosslinked kappa carrageenan not only exhibit as pH-responsive but also salt-responsive hydrogel. The stability in different media further extends the potential of these crosslinked carrageenan films in biomedical application.

The hydrogels obtained from GA 3-5% remained stable and insoluble, without noticeable changes in all media, except in low pH solution. In acid solution (pH~1), the obtained hydrogel decomposed in a very short time, about 10 min after initially swelling to a certain value of swelling degree, around 5.5 (Fig. 7). The pH dependency can be understood by investigating the molecular structure of the polymer networks. The molecular structure of the networks significantly determines the stability of hydrogel in acid medium. The hydrogel contained acid labile acetal groups. The cleavages of acetal groups start to proceed as degradation of the swollen gel [24].

4. Conclusion

Kappa carrageenans extracted from *Kappaphycus alvarezii* were able to be crosslinked with glutaraldehyde by soaking the carrageenan films in GA solution (3-5 wt%) at room temperature and followed by curing at 110^{O}C for 25 min. The crosslinking treatment enhances the thermal stability of carrageenan. The crosslinking reaction with higher concentration of glutaraldehyde produced hydrogels with lower swelling degree. The kappa carrageenan hydrogels were found to be pH and salt sensitive. The obtained hydrogels were unstable at low pH in aqueous environments.

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