

Synthesis of Hydrogel Film Based on Carrageenan Extracted from *Kappaphycus alvarezii*

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Abstract

Hydrogel based on kappa carrageenan extracted from *Kappaphycus alvarezii* was synthesized by film immersion in glutaraldehyde solution (GA 4% w/w) as crosslinker for 2 min and then followed by thermal curing at 110 °C for 25 min. The obtained crosslinked films were washed using ethanol to remove the unreacted crosslinker and finally dried to constant weight. The aim of this research was to investigate the effect of carrageenan recovery method on the prepared hydrogel properties. The method of carrageenan extraction strongly determined the swelling properties of crosslinked carrageenan. Hydrogel obtained from alkali treated carrageenan showed higher swelling ability compared to hydrogel from nonalkali treated carrageenan. Hydrogel from alkali treated carrageenan showed the ability of sensitive to pH media. Swelling degree of alkali treated carrageenan based hydrogels increased by increasing pH solution from about 5 g/g for neutral pH to 20 g/g for pH~13.

Keywords: hydrogel, kappa carrageenan, glutaraldehyde, swelling, *Kappaphycus alvarezii*

1. Introduction

Hydrogels are tridimensional networks of hydrophilic polymers which are able to swell in water. Hydrogel ability to swell in response to external stimuli as pH, ionic strength, temperature, electric fields depends on the nature of polymer chains and allows hydrogels useful in application such as controlled drug delivery, separation process or agricultural application (Hoffman, 2002; Gerlach et al., 2005; Peppas, 2004; Samchenko et al., 2011). Nowadays, preparation of hydrogels based on natural polymers especially polysaccharides have been explored extensively. Compared to synthetic polymer, the polysaccharides-based hydrogels exhibit several advantages, such as renewability, biodegradability and cheapness because the raw materials are locally abundant. The biocompatibility of polysaccharides is very interesting characteristic of material, mainly on biomedical applications. In this research, we converted polysaccharide extracted from seaweed into hydrogel.

Kappa carrageenans are linear polysaccharides sulfated galactan extracted from red seaweed (*Rhodophyta*), 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 (Campo et al., 2009). The presence of hydroxyl groups and sulfate groups in carrageenan structure cause the carrageenan 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 (Van de Velde et al., 2002), and potent as raw materials of hydrogels (Hoffman, 2002). Some gel applications need hydrogel properties which mainly can absorb and keep water without dissolution. For improving the stability in aqueous, the kappa carrageenan structures must be chemically crosslinked to produce hydrogel structure.

Preparation of hydrogel of kappa carrageenan graft polyacrylamide has been studied by Abd El-Mohdy and Abd El-Rehim (2009). Previous studies reported the preparation of kappa carrageenan base hydrogel by crosslinking with epichlorohydrine (Keppeler et al., 2009), CaCl_2 (Pascalau et al., 2012), and genipin (Meena et al., 2007). To our best knowledge, crosslinking carrageenan with glutaraldehyde into hydrogel film has not been reported. In this work, glutaraldehyde was chosen as the crosslinker. Glutaraldehyde is easily available and inexpensive. Its aqueous solution is known as an effective crosslinker for natural polymer, such as guar gum (Cunha et al., 2005),

alginate (Gerooge & Abraham, 2007), chitosan (Shang et al., 2008), and collagen (Verissimo et al., 2010). The previous studies stated that prepared hydrogels were suitable for applications in biomedical fields.

The gel properties of carrageenan are function of the recovery method from seaweed (Campo et al., 2009; Montolalu et al., 2006). Alkali treatment in carrageenan recovery from seaweed improved the gel strength of obtained carrageenan (Hilliou et al., 2006; Navarro et al., 2007). This research was focused on the evaluation of the effect of carrageenan produced with different extraction procedure of *Kappaphycus alvarezii* seaweed on the properties of prepared hydrogel. The hydrogels were prepared by using film immersion in glutaraldehyde solution and then followed by thermal curing method. The swelling properties of obtained hydrogel at different pH media was also studied for evaluating the suitability of hydrogel application.

2. Method

2.1 Materials

Seaweeds of *Kappaphycus alvarezii* were harvested from Makasar, South Sulawesi, Indonesia. The seaweeds were soaked in water for 2 h, and then washed using tap water several times to eliminate all impurities such as 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%) was used as alkali treatment before extraction process. Glutaraldehyde (wt 25% solution in water (Merck)) and all other chemicals were purchased and used without further purification.

2.2 Carrageenan Preparation

The procedure of carrageenan recovery from *Kappaphycus alvarezii* followed the previously reported method (Distantina et al., 2011) 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 °C for 30 min. After alkali treatment, the seaweed was washed with tap water and neutralized with HCl 0.1 N. A specified amount of distilled water was heated in a beaker as an extractor. After the temperature of the water reached 80 °C, the seaweed was then added into solvent, and the time of extraction started to be counted. A constant ratio of seaweed weight to solvent volume (1:50 g/mL) was maintained by adding hot water. After 1 h extraction, the filtrate was separated from residue and immediately poured into 4.5 L of cold (5 °C) ethanol (wt 90%) which caused precipitation of polysaccharides. The precipitation was allowed for 30 min while a gentle stirring was done. The precipitated carrageenans were collected. The obtained carrageenans were called as alkali treated carrageenan (AT). The other procedure of carrageenan recovery without alkali treatment step produced nonalkali treated carrageenan (NAT).

2.3 Film Preparation

Carrageenan films both AT and NAT were prepared by dissolution of the precipitated carrageenan in distilled water. The mixtures were heated and stirred until homogeneous solutions were obtained. The solutions were poured into plastic plate and allowed to solidify and then dried at room temperature to constant weights. The obtained films were cut into 1.5 cm x 1.5 cm pieces and the weight of each piece film was about 0.03-0.04 gram.

2.4 Carrageenan Characterization

The resulting carrageenans both AT and NAT were analyzed to evaluate their sulfate content, gel strength, and intrinsic viscosity. Percent sulfate content was determined using the method of sulfate hydrolysis followed by precipitation sulfate as barium sulfate (Jeffery et al., 1989). Percent sulfate content was calculated based on weight of free sulfate sample. The gel strength was determined using method described by Falshaw et al. (1998) with minor modifications. The dried carrageenan was diluted by KCl 0.09M with heating to obtain a 1.5% (w/v) carrageenan solution. Intrinsic viscosity was determined experimentally from measurement of the viscosity of dilute concentration of carrageenan aqueous solution (0.0038-0.0329 g/dL) using an Oswald glass capillary viscometer (Brand Germany, no.1) at room temperature. Intrinsic viscosity was evaluated by extrapolation of reduced viscosity to the value at zero carrageenan concentration.

2.5 Film Crosslinking

We prepared control (noncrosslinked) and crosslinked films. GA 4 wt% as the crosslinker was prepared by diluting GA 25 wt% with distilled water. For preparing the crosslinked film, the carrageenan films were immersed in crosslinker for 2 min. The surface of film were wiped with filter cloth and then cured at 110 °C in oven for 25 min. The crosslinked film was soaked in water with stirring for 1 min and then in ethanol for 4 h to remove unreacted GA. The wet hydrogels were dried at room temperature to a constant weight.

2.6 Hydrogel Characterization

Molecular groups were identified using FTIR spectrometer (Shimadzu IR Prestige-21). Both noncrosslinked and crosslinked film were powdered. Infrared spectra were obtained by using KBr pellet method with 10 scans and 16 cm^{-1} resolution. Assignment of IR spectra of obtained hydrogels was based on spectroscopy data summarized by Pereira et al. (2009).

For determining the value of swelling ability, a piece of hydrogel film was weighted and then placed in distilled water of 10 mL. The swelling degree was evaluated by measuring the weight before soaking (M_d) and the weight after soaking (M_w) 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 Equation (1). Each experiment was done at least one duplicate run and the mean value was used to display the data.

$$SD = (M_w - M_d) / M_d \quad (1)$$

To study the effect of extraction method on the swelling degree, the swelling tests were conducted in water (pH~7), phosphate buffer (pH~7.4), and NaOH 0.1N (pH~13).

3. Results and Discussion

3.1 Carrageenan Crosslinking

Figure 1 shows the FTIR spectra of control (noncrosslinked) film and crosslinked film. Sample A and C were noncrosslinked carrageenan film extracted from alkali treatment (AT) and nonalkali treatment (NAT), respectively. Sample B and D were crosslinked carrageenan films from AT and NAT, respectively. The characteristic infrared peaks of samples are presented in Figure 1 and Table 1.

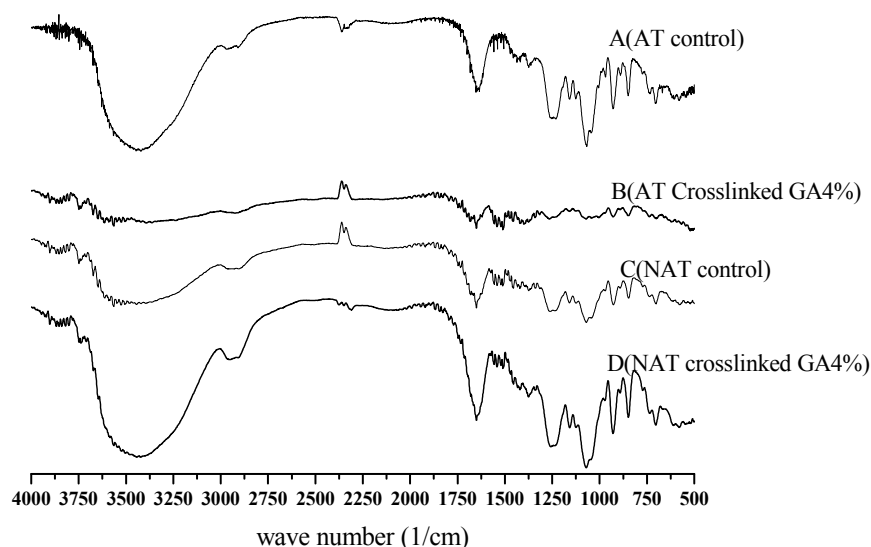


Figure 1. FTIR spectra of noncrosslinked (control) and crosslinked carrageenan film

Table 1. Characteristic IR peaks present in the sample

Functional groups	Peak (cm^{-1}) ^a	Peak (cm^{-1}) ^b			
		Sample A	Sample B	Sample C	Sample D
Galactose-4-sulfate	840-850	848.68	848.68	848.68	848.68
3,6 Anhydro-galactose	925-935	925.83	933.55	933.55	933.55
Ester sulfate	1210-1260	1257.59	1265.30	1273.02	1265.30
Hydroxyl	3200-3600	3448.72	3448.72	3417.86	3448.72

^a Literature values (Pereira et al., 2009).

^b Observed values.

In this research, infrared spectra of extracted carrageenan from *Kappaphycus alvarezii* for both AT and NAT show the features of kappa carrageenan. The changes of sample peak before and after crosslinking could not be seen clearly, as shown in Figure 1. Glutaraldehyde has been used as crosslinker for polymer containing hydroxyl groups. Some previous researchers reported that hydroxyls from polymer react with aldehyde forming hemiacetal structure (Chen et al., 2012; Kim et al., 1994; Lee et al., 2005; Mansur et al., 2008). The peak ascribed to acetal groups did not appear in observed FTIR spectra (Figure 1). This was probably caused by the low amount of acetal groups in crosslinked film.

Although it is difficult to exactly determine the conversion of crosslinked polymer, the physical characteristics of crosslinked polymer, especially swelling behavior, indirectly demonstrates the success of crosslinking reaction (Keppeler et al., 2009). The crosslinking is also expressed by the stability of the obtained hydrogel in aqueous solution as characterized by a significant decrease of swelling degree.

Figure 2 shows the values of swelling degree as function of swelling time in distilled water. The swelling degree expresses as gram of water uptake per gram of carrageenan film. The control films swelled and rapidly disintegrated in less than 30 min for both AT and NAT carrageenan. This facts show that carrageenan is a hydrophilic polymer. When it is contacted with water, it will swell and then gradually dissolve or disintegrate into the water. Therefore, when it is used carrageenan as the hydrogel, it needs to be modified to improve its solubility.

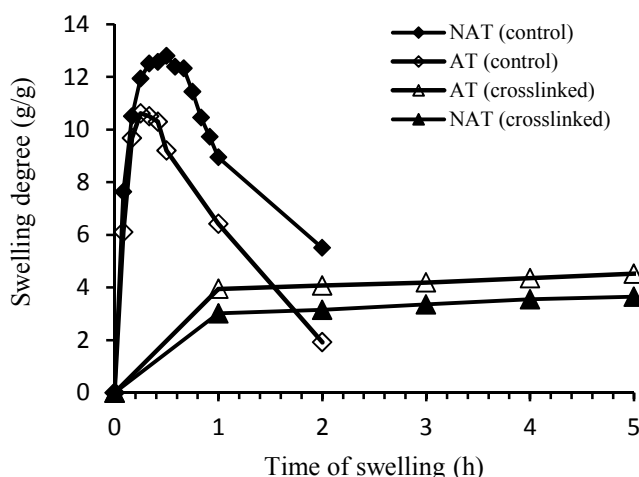


Figure 2. Comparison of control (noncrosslinked) and crosslinked film in distilled water

Comparing control and crosslinked films, the crosslinked hydrogels from AT or NAT were more stable at water (Figure 2). Crosslinked films exhibited hydrogel characteristics which did not dissolve easily in water and became water resistant indicating the more stable structures comparatively to the noncrosslinked film. The rigid structure of crosslinked film was formed by crosslinking. Crosslinking procedure by film immersion followed by high temperature curing can drive the reaction between hydroxyl groups of carrageenan and aldehydes of GA to form rigid crosslink structures. The decreasing of swelling degree of crosslinked film indicates the presence of crosslinked structure (Pascalau et al., 2012; Rasool et al., 2010). The crosslinked films showed lower swelling degree compared with the control, as observed from Figure 2.

Compared with commonly used hydrogel preparation procedure homogeneous system crosslinking in which the GA solution is directly added to the polymer solution, the film immersion and curing method used in this present research is simpler and more rapid. In addition, the amount of GA as crosslinker can be easily adjusted for controlling the hydrogel's swelling properties. Finally, the removal of unreacted GA is also easily conducted so that the hydrogel has far lower cytotoxicity.

3.2 Effect of Extraction Procedure on Swelling Degree

Crosslinked nonalkali treated carrageenan showed little ability to absorb water in buffer phosphate and NaOH 0.1M media, but crosslinked alkali treated carrageenan showed significantly higher swelling ability, as observed from Figures 3 and 4. The crosslinked film of NAT quickly reached the equilibrium swelling degree in less than 2 h of immersion.

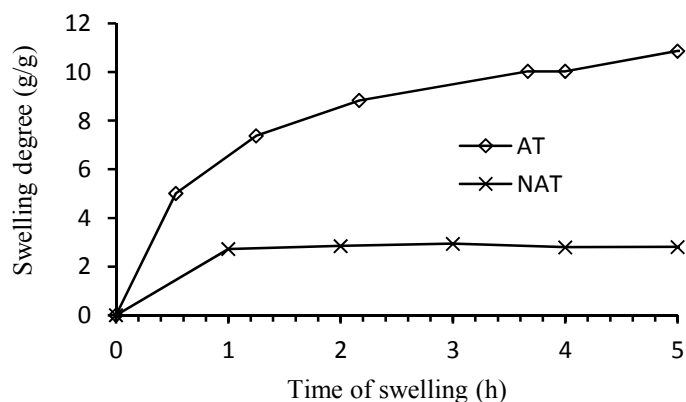


Figure 3. Swelling degree of crosslinked film in phosphate buffer

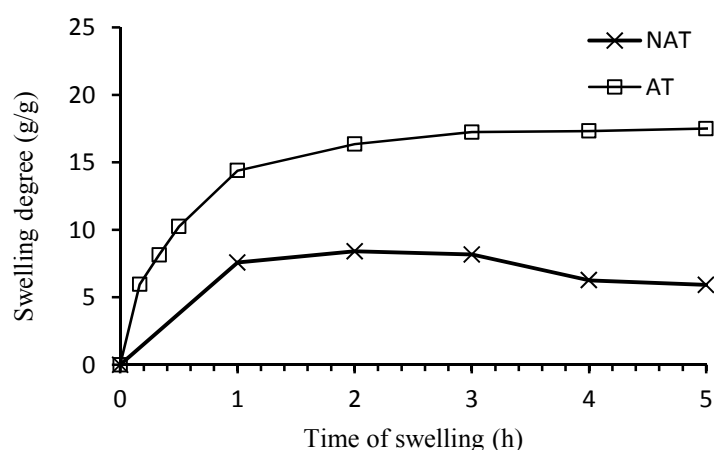


Figure 4. Swelling degree of crosslinked film in NaOH 0.1M

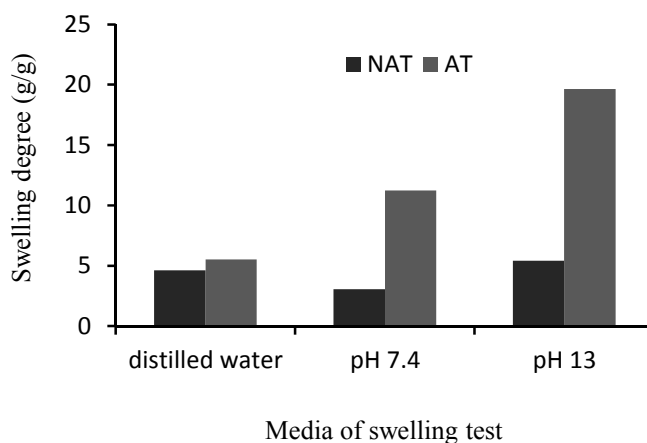


Figure 5. Swelling degree of crosslinked film in various media

Figure 5 presents results of swelling degree measurement after 24 h of immersion in distilled water (pH~7), phosphate buffer (pH~7.4) and in NaOH solution (pH~13). The effect of carrageenan recovery method on hydrogel swelling behavior is also presented in Figure 5. At distilled water, the crosslinked film of AT showed slightly higher value of swelling degree than that crosslinked film of NAT. When immersed at phosphate buffer and NaOH 0.1 M, the AT and NAT crosslinked film exhibited considerably different values of swelling degree. It is shown that the values of swelling degree of hydrogel prepared from AT were higher at all media tested as compared to the NAT. This is probably caused by the different structure between AT and NAT resulted during

carrageenan recovery process.

The different characteristics of NAT and AT carrageenan are displayed in Table 2. Alkali treatment in carrageenan extraction is an important and well-known reaction. It is used commercially to enhance gelation behavior (Campo et al., 2009). The reaction is shown in Figure 6. From Table 2, it can be seen that alkali treatment reduced the sulfate content from 19.5% for NAT into 16.7% for AT, indicating that carrageenan reaction occurred. Intrinsic viscosity value corresponds to the molecular weight. It was no significant difference of intrinsic viscosity between NAT and AT, expressing no polymer degradation occurred during alkali treatment. Alkali treatment using KOH 0.3N in this research caused slight improvement of gel strength.

Table 2. Characteristics of carrageenan extracted from seaweed

Carrageenan	Sulfate (%)	Gel strength (g/cm ²)	[μ] (dL/g)
NAT	19.52	191.09	51
AT	16.69	208.96	54

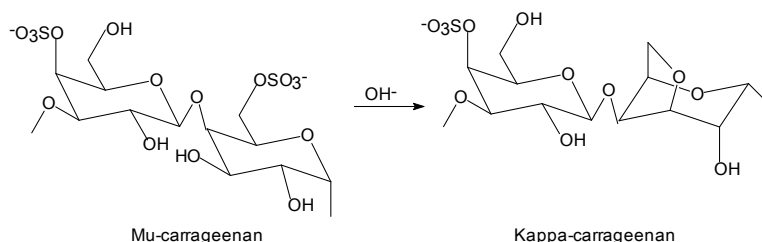


Figure 6. Kappa carrageenan reaction

Mu (μ) carrageenan is the biological precursor of kappa (κ) carrageenan. The seaweeds are usually extracted with alkali at elevated temperature to transform the biological precursor, μ carrageenan into kappa carrageenan (Ciancia et al., 1993; Van de Velde et al., 2002). The natural precursor of kappa carrageenan, μ carrageenan is non gelling carrageenan with galactose units in the 4C_1 conformation. The occurrence of 4C_1 conformation prevents the formations of helical strands and the gelation of the carrageenan. The 1C_4 conformation of the 3,6-anhydro-D-galactose units in kappa carrageenan allows a helical structure, which is essential for the gel forming properties (Falshaw et al., 1998; Van de Velde et al., 2002). Therefore, the carrageenan reaction produces gel forming structure, the 3,6-anhydro-D-galactose unit, chemical bonds that create a tridimensional hydrophilic structure. Alkali treatment in carrageenan recovery increases the amount of 3,6-anhydro-galactose unit of gel forming structure (Ciancia et al., 1993). The occurrence of this reaction can be also indicated by the reduction of sulfate content in carrageenan produced by alkali treatment.

Carrageenan of NAT also contained the 3,6-anhydro-D-galactose, as depicted by FTIR spectra (Figure 1). According to Van de Velde et al. (2002), naturally the 3,6-anhydro-D-galactose are formed enzymatically by a sulfohydrolase in the seaweed. Therefore, lower 3,6-anhydro-D-galactose unit in NAT probably causes the lower swelling degree compared to those of AT. The different swelling behavior of obtained hydrogel shows that alkali treatment in carrageenan recovery strongly determines the characteristic of hydrogel.

3.3 Effect of pH on Swelling Properties

Understanding the swelling behavior of hydrogels in the presence of ions is important from both practical and theoretical points of view. The high water content of the hydrogels and pH responsive properties provide them important characteristics that support personal hygienic (Hoffman, 2002), agricultural applications (Abd El-Mohdy & Abd El-Rehim, 2009), and tissue engineering (Lee et al., 2005). In this research, the crosslinked films were highly unstable in acidic pH. The swelling degree of crosslinked film at pH 1.2 could not be measured because the structure of hydrogel collapsed and disintegrated rapidly.

As shown in Figure 5, significant changes on swelling degree of crosslinked NAT film in different pH media was not observed. Maximum swelling degree of NAT hydrogels at all tested media was almost constant, about 5 g/g. The swelling degree of prepared hydrogels from AT in various pH solution was appreciably different compared to the swelling values in distilled water. The swelling degree at distilled water was lower than that at buffer phosphate solution. The swelling degree at pH~13 showed the highest value. Swelling degree of AT hydrogel

increased by increasing pH solution from about 5 g/g for neutral pH to 20 g/g for pH~13. Crosslinked films from NAT did not show the pH sensitive properties, but the crosslinked films from AT exhibited the pH responsive properties. Therefore, the obtained crosslinked hydrogels of AT film were chosen for studying the effect of pH on the swelling properties.

The presence of sulfate groups and hydroxyl groups in carrageenan structure make these hydrogels pH sensitive. Carrageenans contain at least one sulfate group per repeating unit, so the ionic concentrations inside are supposed to be high, which also means carrageenans are anionic polymers. All of the pH sensitive polymers contain acidic group, such as carboxylic and sulfonic acids, or basic group, such as ammonium salts, that either accept or release protons in response to changes in environmental pH (Gerlach et al., 2005; Rasool et al., 2010; Qiu & Park, 2001; Zhang et al., 2005).

When the system pH is higher than pKa of ionizable group, most of the group are dissociated, leading to the significant decreasing in hydrogen bonds. The value pKa of sulfonic acid is around 2.8. At pH media is higher than 2.8, the ionic groups $-\text{OSO}_3\text{H}$ are deprotonated resulting ionic groups $-\text{OSO}_3^-$ at hydrogel structure. The charges of hydrogel network change in aqueous media. Anionic polymers will be ionized at high pH, while cationic polymers will be ionized at low pH (Samchenko et al., 2011; Zhang et al., 2005). Due to the increase number of negatively charged, the electrostatic repulsion becomes dominant. These same negatively charged groups are repelled by each other. The negatively charged sulfate groups on different chains induce the electrostatic repulsion, as a result the distance between the chains increase. The space of network becomes larger, so that the network becomes more permeable to large molecules and much water can penetrate into the network, leading to the higher swelling degree. Comparing to distilled water (pH~7) and NaOH 0.1 M (pH~13) as media of swelling, the higher pH tend to increase the number of dissociated sulfate facilitating more amount of water diffusion into network to swell the hydrogel.

4. Conclusion

Kappa carrageenan hydrogels have been successfully prepared by crosslinking with GA using film immersion and followed by thermal curing. The procedure of carrageenan recovery from seaweed significantly affects the properties of obtained hydrogel. Comparing to the films on nonalkali treated carrageenan, the film of alkali treated carrageenan exhibited higher swelling degree. The prepared hydrogel of alkali treated carrageenan showed responsive to the change of pH. The pH sensitive properties indicate that glutaraldehyde crosslinked kappa carrageenan film may be developed as new natural based polymer with pH responsive properties.

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