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Hydrogels Based on Carrageenan Extracted from *Kappaphycus alvarezii*

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***Abstract*—**Preparation of hydrogel based on carrageenan extracted from *Kappaphycus alvarezii* was conducted with film immersion in glutaraldehyde solution (GA 4%w/w) for 2min and then followed by thermal curing at 110°C for 25min. The method of carrageenan recovery strongly determines the 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 showed the ability of sensitive to pH media.

***Keywords*—**Hydrogel, carrageenan, swelling, alkali treated.

I. Introduction

HYDROGELS are tridimensional networks of hydrophilic polymers which are able to swell in water. Hydrogels 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. Nowadays, preparation of hydrogels based on natural polymers especially polysaccharides, have been explored extensively. Compared to synthetic polymer, the polysaccharides-based hydrogels exhibited several advantages. They are more renewable, more biodegradable and cheaper because the raw materials are locally abundant. The biocompatibility of polysaccharides is characteristic of a material of great interest, mainly on biomedical applications.

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 [1]. The presence of hydroxyls and sulfate groups in carrageenan structure cause the carageenans tend to be hydrophilic. For improving the stability in aqueous, the kappa carrageenan structures must be chemically crosslinked to produce hydrogel structure.

Previous studies reported the preparation of carrageenan base hydrogel by crosslinking with epichlorohydrine [2], $CaCl_2$ [3], and genipin [4]. In this work, glutaraldehyde (GA) was chosen as the crosslinker. Glutaraldehyde is easily available and inexpensive. Its aqueous solution is reactive effectively crosslink natural polymer, such as guargum [5], alginate [6], chitosan [7], and collagen [8]. The previous studies stated that prepared hydrogel were potential for biomedical applications.

The gel properties of carrageenan are function of the recovery method from seaweed [1, 9]. Alkali treatment in carrageenan recovery from seaweed improves the gel strength of obtained carrageenan [10, 11]. This present research investigated the effect of carrageenan produced with different extraction procedure of *Kappaphycus alvarezii* seaweed on prepared hydrogel using film immersion in crosslinker solution and then followed by thermal curing method. The swelling properties of obtained hydrogel at different pH media were also studied.

II. Materials and Method

A. 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 1cm 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% solution in water (Merck) and all other chemicals were purchased and used without further purification.

B. Carrageenan Preparation

The procedure of carrageenan recovery from *Kappaphycus alvarezii* followed the previously reported method [12] with minor modification. The clean seaweeds were treated using KOH solution before being extracted. Thirty gram of seaweeds was soaked in KOH 0.3N overnight and then heated at 60°C for 30min. After alkali treatment, the seaweeds were 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

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reached 80°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 1hr extraction, the filtrate was separated from residue and immediately poured into 3 volumes of cold (5°C) technical ethanol (wt 90%) which caused precipitation of polysaccharides. The precipitation was allowed for 30min with stirring gently. The precipitated carrageenans were collected and dried at room temperature to a constant weight. 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).

C. Carrageenan Characterization

The resulting carrageenan both AT and NAT were analyzed of their sulfate content. Percent sulfate content was determined using the method of sulfate hydrolysis followed by precipitation sulfate as barium sulfate [13]. Percent sulfate content was calculated based on weight of free sulfate sample.

The gel strength was determined using method described by Falshaw [14] with minor modifications. The dried carrageenan was dissolved 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 using an Oswald glass capillary viscometer (Brand Germany, no.1) at room temperature.

D. Film Preparation

Carrageenan films both AT and NAT were prepared by dissolution of the dry carrageenan in distilled water, separately. 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 1.5cm x 1.5cm and the weight of each piece film was about 0.03-0.04g.

E. Film Crosslinking

GA 4 wt% was prepared by diluting GA 25 wt% 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°C in oven for 25min. The crosslinked film was soaked in water with stirring for 1min and then in ethanol for 4 hr to remove unreacted GA. The wet hydrogels were dried at room temperature to a constant weight.

F. Film Characterization

For determining the value of swelling ability, a piece of hydrogel film was weighted and then placed in an aqueous medium of 10mL. 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 (1). Each experiment was done at least one duplicate run and the mean value was used to display the data.

$$SD = (Mw - Md) / Md \quad (1)$$

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).

III. Result and Discussion

A. Effect of Extraction Method on Swelling Degree

Fig. 1 shows the values of swelling degree as function of swelling time in distilled water. The control films swelled and rapidly disintegrated, less than 30min for both AT and NAT carrageenan. This facts expressed that carrageenan is a hydrophilic polymer. When it was contacted with water, it would swell and then gradually dissolved or disintegrated into the water (Fig. 1). Therefore, when we use carrageenan as the hydrogel, it needs to be modified to decrease its solubility. In this work, chemically modified of carrageenan was conducted by film immersion in GA solution and then followed by thermal curing.

The crosslinked hydrogels from AT or NAT were more stable at water (Fig. 1), indicating the hydrogel did not easily dissolve in water like the control film. The crosslinked film had lower swelling degree compared with the control. Crosslinking procedure by film immersion followed by high temperature curing could drive the reaction between hydroxyl groups of carrageenan and aldehydes of GA to form crosslink structures. The swelling degree expressed as gram of water uptake per gram of carrageenan film. The decreasing of swelling degree of crosslinked film indicated the presence of crosslinked structure.

Fig. 2 shows the results of swelling degree measured after 24h initially swelling in distilled water (pH~7), phosphate buffer (pH~7.4) and in NaOH solution (pH~13). Crosslinked nonalkali treated carrageenan showed little ability to absorb water in all pH media, but crosslinked alkali treated carrageenan showed significantly high swelling ability (Fig. 2).

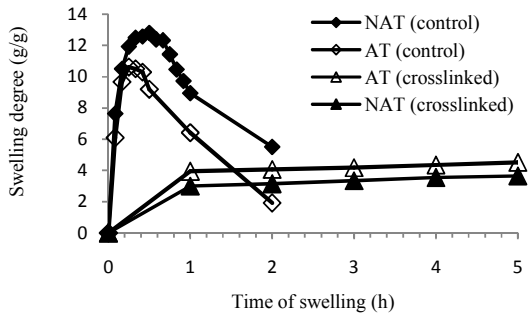

Fig. 1 Comparison of control (noncrosslinked) and crosslinked film in distilled water

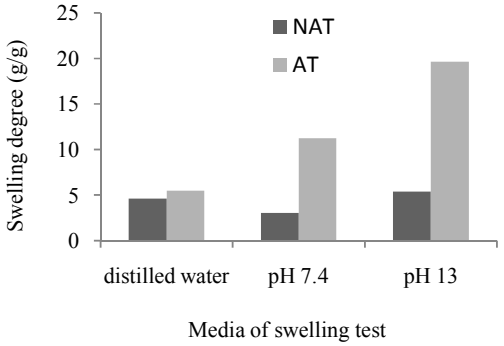

Fig. 2 Swelling degree of crosslinked film in various media

Generally, the values of swelling degree of hydrogel prepared from AT were higher than those from NAT at all tested media. This was probably caused by the different structure between AT and NAT resulted from different extraction condition. Some characteristics of NAT and AT carrageenans are displayed in Table I.

Alkali treatment in carrageenan recovery is an important reaction to enhance the gelation behavior [1]. The reaction is showed in Fig. 3. Mu (μ) carrageenan is the biological precursor of kappa (k) carrageenan. The seaweeds are usually extracted with alkali at elevated temperature to transform the biological precursor, μ carrageenan into kappa carrageenan [15], [16]. 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 formation 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 [15,17]. Therefore, the carrageenan reaction produces gel forming structure, the 3,6- anydro-D-galactose unit, chemical bonds that create a tridimensional hydrophilic structure. Alkali treatment in carrageenan recovery increased the amount of 3,6-anhydro-galactose unit of gel forming structure [16]. The occurrence of this reaction can be also indicated by the reduction of sulfate content in carrageenan produced by alkali treatment. From Table I, it can be seen that alkali treatment reduced the sulfate content from 19.5% became 16.7%, indicating that carrageenan reaction occurred. Intrinsic viscosity value corresponds to the molecular weight. There was no significant difference of intrinsic viscosity between NAT and AT, expressing no polymer degradation happened during alkali treated. Alkali treatment using KOH 0.3N in this research caused slightly improvement of gel strength.

Carrageenan of NAT also contained the 3,6-anhydro-D-galactose. According to Van de Velde et al. [15], naturally the 3,6-anhydro-D-galactose are formed enzymatically by a sulfohydrolase in the seaweed. Therefore, the lower 3,6-anhydro-D-galactose unit in NAT probably caused the lower swelling degree compared those of AT.

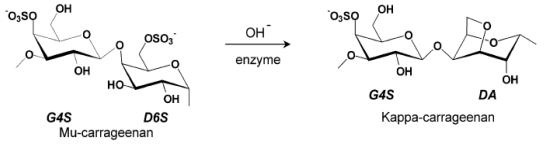

Fig. 3 Kappa carrageenan reaction [1]

TABLE I
CHARACTERISTIC 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

B. Effect of pH on Swelling Properties

From Fig. 2, it does not observe significant changes on swelling degree of crosslinked NAT film in different pH media. The swelling degree of prepared hydrogels from AT in various pH solution was appreciably different compared to the swelling degree values in distilled water. Crosslinked films from NAT do not show the pH sensitive properties, but the crosslinked films from AT exhibit the pH responsive.

Understanding the swelling behavior of hydrogels in the presence of ions is important view from both practical and theoretical points. 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 [18]. 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 sensitive to pH. The presence of sulfate and hydroxyl groups of carrageenan makes these hydrogels are pH sensitive.

Anionic polymers will be ionized at high pH, while cationic polymers will be ionized at low pH. The swelling degree at distilled water was higher than those at buffer phosphate solution. The swelling degree at pH~13 showed the highest value. When the system pH was higher than pKa of ionizable group, most of the group are dissociated, leading to the significant decreasing in hydrogen bonds. Due to the increase number of negatively charged, the electrostatic repulsion becomes dominant, which facilitated the diffusion of water molecules into network to swell the hydrogel.

At high pH, the ionic groups $–OSO_3H$ are deprotonated resulting ionic groups $–OSO_3^-$ at hydrogel structure. The charges of hydrogel network change in aqueous media. These same 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 become more permeable to large molecules and more water can penetrate into the network, leading to the higher swelling degree.

Compared with commonly hydrogel preparation procedure homogeneous system crosslinking, the film immersion and curing method used in this present research have several advantages. The preparation procedure is simple and rapid. More importantly, 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.

IV. Conclusion

Kappa carrageenan hydrogel membranes have been successfully prepared by crosslinking with GA. The procedure of carrageenan recovery from seaweed significantly affected the properties of obtained hidrogel. The prepared hydrogel showed responsive with the change of pH solution.

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