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Stabilization of Kappa Carrageenan Film by Crosslinking: Comparison of Glutaraldehyde and Potassium Sulphate as the Crosslinker

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Abstract. The objective of this research was to crosslink kappa carrageenan films extracted from *Kappaphycus alvarezii* seaweed in order to form hydrogel structure that may be used as a controlled release delivery system. The effect of the crosslinker type namely glutaraldehyde and K_2SO_4 on the swelling degree in different media was studied. Crosslinking carrageenan with glutaraldehyde was conducted using film immersion in which carrageenan film was immersed in glutaraldehyde solution and then followed by thermal curing. Crosslinking carrageenan with K_2SO_4 was conducted using homogeneous reaction in which the K_2SO_4 solution was directly added to the carrageenan solution with heating. It is found that kappa carrageenan extracted from *Kappaphycus alvarezii* could be crosslinked using glutaraldehyde or K_2SO_4 indicated by the stability properties of obtained crosslinked films in distilled water, phosphate buffer, and NaOH solution. In distilled water (pH~7) and phosphate buffer (pH~7.4), the films crosslinked with K_2SO_4 exhibited higher swelling degree than that of glutaraldehyde. While in NaOH solution (pH~13) the films crosslinked with glutaraldehyde exhibited higher swelling degree than that K_2SO_4. Crosslinked kappa carrageenan showed swelling ability of pH sensitive.

Keywords: Carrageenan, crosslink, hydrogel, swelling degree, glutaraldehyde, K_2SO_4

1. Introduction

Our research related to the development of hydrogels based on natural polysaccharides, especially carrageenan extracted from *Kappaphycus alvarezii* seaweed from Indonesia. Hydrogels are tridimensional networks of hydrophilic polymers which are able to swell in water. 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 crosslinking technique. The study of swelling behaviors of hydrogels as a function of their ionic environment should allow for a better understanding of the potential of these crosslinked kappa carrageenan hydrogels. 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. .

2. Objectives

The effect of the crosslinker type, namely K_2SO_4 and glutaraldehyde, on the swelling degree of crosslinked carrageenan in different media was studied.

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3. Materials and Methods

3.1. 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.

3.2. Carrageenan Preparation

The procedure of carrageenan recovery from Kappaphycus alvarezii and carrageenan film preparation followed the previously reported method [1]. Alkali treatment was done in order to obtain gel forming structure in extracted carrageenan. The final films of carrageenan were kept in dry state until further processing was conducted.

3.3. Glutaraldehyde Crosslinked Film Preparation

We prepared glutaraldehyde crosslinked films using film immersion method and following by therma curing. 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. Glutaraldehyde (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 ^{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 h to remove unreacted GA. The wet hydrogels were dried at room temperature to a constant weight.

3.4. K_2SO_4 Crosslinked Film Preparation

We prepared K_2SO_4 crosslinked film by mixing carragenan solution and K_2SO_4 solution. The 2 gram carrageenans were completely dissolved in 30 mL distilled water by heating. The resulting carrageenan solution was mixed with 100 mL hot potassium sulphate aqueous 0.1 N in erlenmeyer glass and the reaction time started to be counted. The temperature was kept constant at 80^{O}C. After 30 min reaction time, the reaction was stopped by pouring the solution into 3 volumes of cold ethanol, so that the precipitated carrageenan could be collected. Crosslinked films 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.

3.5. Hydrogel Characterization

Percent sulphate content in crosslinked film was determined using the method of sulfate hydrolysis followed by precipitation sulphate as barium sulphate [2]. Percent sulphate content was calculated based on weight of free sulphate sample. 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 (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. Equilibrium 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 swelling behaviourof obtained hydrogels, the swelling tests were conducted in water (pH~7), phosphate buffer (pH~7.4), and NaOH 0.1N (pH~13).

4. Results and Discussion

Fig. 1 and 2 show the relationship between swelling degree value as a function of swelling time of glutaraldehyde crosslinked film and K_2SO_4 crosslinked film respectively. The swelling degree of control or non-crosslinked film in distilled water can be seen in Fig. 1. In this research, swelling degree was measured at room temperature and the distilled water, buffer phosphate and NaOH solution representatived the pH~7, pH~7.4 and pH~13 medium, respectively.

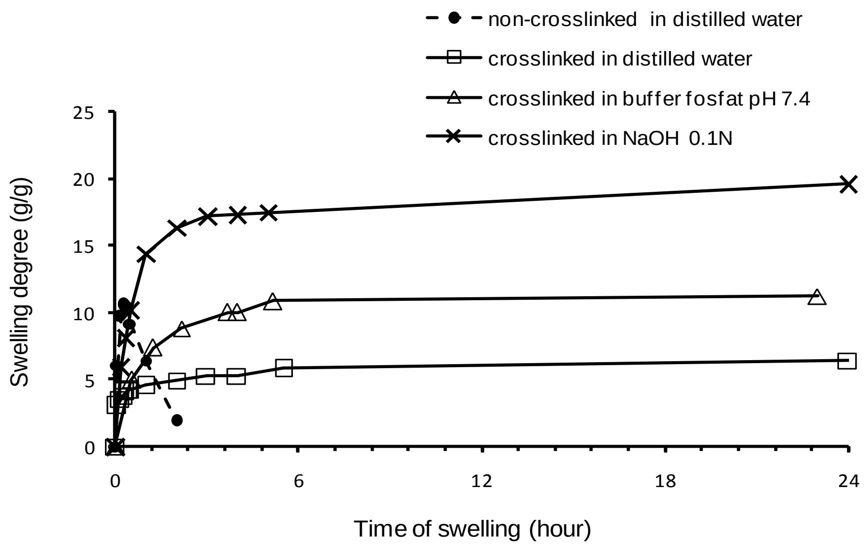

Fig. 1: Swelling degree of glutaraldehyde crosslinked hydrogel film in various medium

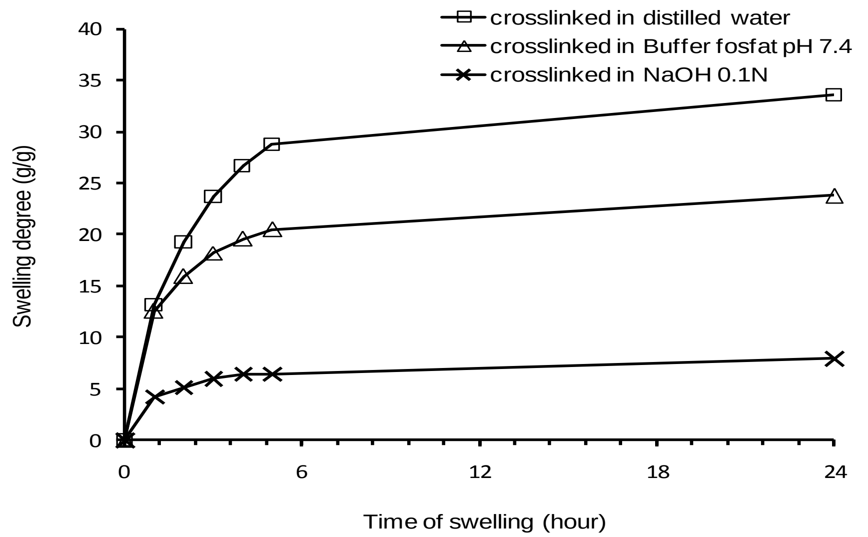

Fig. 2: Swelling degree of K_2SO_4 crosslinked hydrogel film in various medium

From Fig. 1, it can be seen that the control film was not able to maintain the swollen state after about 30 minutes initially swelling. The control film started to be unstable in water indicating it could not retain water. The swelling degree value of crosslinked film both obtained from glutaraldehyde and K_2SO_4 as the crosslinker exhibited improved properties in comparison to control film, as shown in Fig. 1 and 2. The crosslinked films were more stable in water and remained insoluble in water.

Crosslinking using glutaraldehyde involves reaction between hydroxyl groups from carrageenan and aldehyde groups from glutaraldehyde. The chemical reaction between hydroxyl groups from carrageenan and aldehyde groups from glutaraldehyde was confirmed by fourier transform infrared measurement [1]. Previous researchers also reported that hydroxyls from polymer react with aldehydes forming hemi acetal structure, such as polyvinyl alcohol [3], polyvinyl alcohol and chondroitin sulphate [4], and alginate-guar gum [5].

It was well known that potassium ions can bind strongly with sulphate groups forming gel state [6], [7]. Therefore, the networks of hydrogel structure in K_2SO_4 crosslinked film were composed of these interactions between K^+ ion and dissociated sulphate in carrageenan structure.

In water medium, the swelling degree of K_2SO_4 crosslinked film was higher than that of glutaraldehyde crosslinked film (Fig. 1 and 2). It is well known that swelling of hydrogel is primary driven by the electrostatic repulsion force of ionizable group in hydrogel structure [8]-[10]. Kappa carrageenans are linear polysaccharides sulphated galactan. This natural polymers comprise of repeating units of (1,3)-D-galactopyranose and (1,4)-3,6-anhydro-α-D-galactopyranose with sulphate groups in the certain amount and position [6]. Kappa carrageenan based hydrogels are composed of polymer chains containing charged groups, namely sulphate groups. Sulphate groups are ionizable groups that will be deprotonated at a certain pH. At pH medium is higher than the dissociation constant of sulphate (pKa~2.8), the ionic groups $–OSO_3H$ are deprotonated resulting ionic groups $–OSO_3^-$ at hydrogel structure. Therefore, the charges of hydrogel network change in aqueous medium. Due to the increase of negatively charged number, the electrostatic repulsion of sulphates becomes dominant. These same negatively charged groups are repelled by each other causing the space of networks become larger and then much water can penetrate into these networks.

Based on experimental, sulphate content in K_2SO_4 crosslinked film (36.33%) was higher than that in glutaraldehyde crosslinked film (16.69%). Crosslinking reaction using K_2SO_4 can increase sulphate groups in carrageenan structure. The higher number of sulphate groups in carrageenan structure caused polymer were more hydroplilic and increased the electrostatic repulsion. Therefore, in water medium, the swelling degree of K_2SO_4 crosslinked film was significantly higher than that of glutaraldehyde crosslinked film.

As shown in Fig.1 and 2, significant changes on swelling degree of crosslinked film in different pH medium were observed. These mean that the obtained hydrogels show the properties of pH sensitive. The swelling behavior was affected by the ionizable groups, name sulphate groups in carrageenan structure. It is well known that 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 [9]-[11].

Glutaraldehyde crosslinked film showed that the highest swelling degree was obtained at pH~13, but K_2SO_4 crosslinked film attained the highest swelling degree at pH~7, as depicted in Fig. 1 dan 2. This different trend indicates that different crosslinker results the different structure of crosslink. Crosslinking with glutaraldehyde produced chemical crosslink networks, namely acetal structure. In glutaraldehyde crosslinked film, the swelling was dominated by the presence of electrostatic repulsion of ionizable sulphate groups in alkali pH. Meanwhile, crosslinking with K_2SO_4 produced ionic crosslink networks formed by interaction between K^+ and sulphate groups. In alkali medium, the remained sulphates that did not interact with potassium ions will be neutralized by counter ions, such as Na^+. This neutralization reduced the electrostatic repulsion of sulphate groups causing the networks become more compact and swelling degree was lower compared with in water medium. However, because the sulphate content in K_2SO_4 crosslinked film was higher than that of in glutaraldehyde crosslinked film, it was predicted that the swelling of K_2SO_4 crosslinked film in pH~1 was still dominated by electrostatic repulsion.

In the next paper, we will report the chemical reaction between K_2SO_4 and carrageenan.

5. Conclusion

It is found that kappa carrageenan extracted from *Kappaphycus alvarezii* could be crosslinked using K_2SO_4 or glutaraldehyde indicated by the stability properties of crosslinked films in distilled water, phosphate buffer, and NaOH solution. In distilled water (pH~7) and phosphate buffer (pH~7.4), the films crosslinked with K_2SO_4 exhibited higher swelling degree than that of glutaraldehyde. While in NaOH solution (pH~13) the films crosslinked with glutaraldehyde exhibited higher swelling degree than that of K_2SO_4. Crosslinked kappa carrageenan showed swelling ability of pH sensitive.

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