RESEARCH ARTICLE

Characteristics of Irradiated Hydrogel Formulated from Composite ι/κ-carrageenan Combined with Polyvinyl Alcohol

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Abstract

Eucheuma seaweed from tropical waters habitat mainly contains carrageenan, a type of hydrocolloid potential for hydrogel production. This study aims to determine the characteristics of hydrogel formulated from composite ι- and κ-carrageenan combined with polyvinyl alcohol (PVA) which, could be used further for wound dressing application. The concentrations of composite ι/κ-carrageenan used were 1.0%, 1.5%, 2.0%, 2.5% and 3.0% w/w, and the PVA concentration was 12.5% w/w. Composite ι/κ-carrageenan in a proportion of 6:4 by weight. The hydrogel was irradiated using ^{60}Co γ-rays with the irradiation dose of 25 and 2 kGy.h^{-1}. The concentration of composite ι- and κ-carrageenan influenced the gel fraction, water holding capacity, tensile strength, elongation, and surface morphology of the hydrogel. The optimum formula of irradiated composite hydrogel was obtained from 3.0% w/w of composite ι/κ-carrageenan. The formula had a gel fraction of 61.67%, water holding capacity of 1067.12%, tensile strength of 32.37 x 10^{-3} MPa, and elongation of 251.67%. Interestingly, the product with the optimum formula had a porous surface morphology and transparency, which are applicable for wound dressing purposes.

Keywords: *Eucheuma*, ι/κ-carrageenan, hydrogel, composite

Introduction

Eucheuma seaweed, as a source three types of carrageenan: iota (ι), kappa (κ), and lambda (λ) carrageenan can be used as raw material for various applications including hydrogels (Abad, Aranilla, Relleve, & Rosa, 2014). However only ι- and κ-carrageenan can be utilized for hydrogel production (Li, Ni, Shao, & Mao, 2014). Hydrogel synthesis involves heating and cooling carrageenan solution, freeze-thaw process, and polymerization of the solution under radiation (Hoffman, 2012).

The hydrogel can be applied in chemical, biomedical, or pharmaceutical products, including wound dressing. Hydrogel synthesis for wound dressing can be combined with other matrices such as polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), and hydroxyethyl starch. Composite of κ-carrageenan hydrogels with PVA has been reported by Kartika, Gardi, and Darma (2015) and Varshney (2007), with PVP by Abad et al. (2014) and Erizal (2008), and combination of ι- and κ-carrageenan with PVP by Utomo, Fransiska, and Darmawan (2016). In studying composite film produced characteristics from the mixture of carrageenan and polyvinyl alcohol, Meng et al. (2018) found that the composite film elongation was significantly increased when added bio-polyol. Moreover, in their study, potassium ion was proven to be more effective than calcium ion on promoting copolymer chains helix transition and thus higher tensile strength was obtained. Effect of potassium hydroxide on rheological and thermo-mechanical properties of semi-refined carrageenan (SRC) films has also been studied by Ganesan, Shanmugam, and Bhat (2018). Results showed that all the formulated films produced had high tensile strength ranging between 38.1 and 47.5 MPa. Balasubramanian, Kim, and Lee (2018) also studied the synergism between κ-carrageenan with xanthan gum and gellan gum in the processing of hydrogel film. The combination of the three-component produced hydrogel film with a good tensile strength of 19.1–31.0 MPa and elongation at break of 13–19% and tensile modulus of 1.6–2.4 GPa. The UV results indicated that the films were very transparent. A study of ι/κ carrageenan has been conducted previously by Utomo, Fransiska, and Darmawan (2016) suggested that combination between ι/κ-carrageenan in a proportion of 3:2 was the best formula. In addition, the synergism effect of carrageenan with PVA was proven to be better in terms of elongation capability (Meng et al., 2018).

This study aims to determine the characteristics of irradiated hydrogel formulated from composite ι/κ-carrageenan combined with PVA, which could be used further for wound dressing application. Parameters used to assess the quality of the hydrogel include gel fraction, water holding capacity, tensile strength, elongation, and surface morphology.

Material and Methods

The raw materials used in this study were dried *Eucheuma denticulatum*, and *Kappaphycus alvarezii* obtained from Nusa Penida Island, Bali, Indonesia in March 2015. Chemicals used were polyvinyl alcohol pa (Merck, Germany), potassium hydroxide (KOH), celite, potassium chloride (KCl), and isopropyl alcohol (IPA) (technical grade).

Extraction of ι and κ-carrageenan

Extraction of ι-carrageenan from *E. denticulatum* was conducted as follows: 2000 g of dry seaweed was soaked in water for 30 min, then washed and rinsed several times. The seaweed was pre-treated with 12 L of 8% KOH at 60-65 °C for 2 h to lyse the cell wall. After rinsing with water until neutral (pH 8), the pre-treated material was extracted using 40 L of tap water at 80-85 °C for 2 h. Celite (3% w/w) was added to the extract prior to the filtration process. The filtrate was subsequently mixed with 99% IPA with a ratio of 1:2 (v/v) and continued stirring for 1 h at room temperature until κ-carrageenan fiber was formed. The carrageenan fiber was sun-dried for two days.

A similar procedure was applied to *K. alvarezii* to obtain the filtrate of κ-carrageenan. The filtrate was cooled down to room temperature and precipitated with 3% KCl solution to produce κ-carrageenan gel. The gel was put in the filter cloth, pressed and then sun-dried for two days.

The ι and κ-carrageenan produced was firstly milled using a hammer mill and subsequently milled using ultra centrifugal mill/UCM (ZM 200 Retsch, Germany) with 0.08 mm sieve and 6000 rpm speed. To get the right particle size, then the ι and κ-carrageenan powder was filtered using Vibratory Sieve Shaker (VSS) (AS 200 Retsch, Germany) of 200 mesh size. Based on PSA results, the mean diameter of ι-carrageenan was 0.68 μm and κ-carrageenan was 0.64 μm.

Samples ι- and κ-carrageenan were analyzed using FTIR. To check the chemical bonds of κ-carrageenan compound, analysis was conducted using Fourier transform infrared spectrophotometer/FTIR (Spectrum One Series, Perkin Elmer). The samples were coated with potassium bromide (KBr) using a vacuum pump and then analyzed using FTIR at a wavenumber between 4000-450 cm^{-1}.

Preparation of Hydrogel PVA and Composite ι/κ-carrageenan

PVA solution (100 mL; 12,5% w/v) was autoclaved for 15 min and blended with 100 mL composite ι/κ-carrageenan in a proportion of 6:4 by weight based on the optimum proportion of i- and κ-carrageenan reported by Utomo, Fransiska, and Darmawan (2016). Various concentrations ie. 1.0%; 1.5%; 2.0%; 2.5%; and 3.0% of the mixture were prepared and heated at 80 °C for 15 min with continuous stirring until homogenous solution was formed (Dafader et al., 2015; Erizal, 2008; Varshney, 2007). The solution was poured into plastic containers (30 x 16.5 x 0.3 cm^3) and cooled down to 25 °C for about 1 h until hydrogel was formed. The hydrogel was then dehydrated using a freeze-thaw process. The freezing temperature was -5 °C conducted in a freezer for 18 h, and thawing was done for about 8 h. The process was conducted in four cycles of the freeze-thaw process (Kartika et al., 2015; Kenawy, Kamoun, El-Meligy, & Mohy, 2014). The hydrogel was irradiated with γ rays at the dosage of 25 kGy (dosage rate of 2 kGy/h) using ^{60}Co irradiator IRKA PATIR BATAN (Brant et al., 2012; Dafader et al., 2015; Varshney, 2007).

Characterization of Composite Hydrogel

Gel fraction and water holding capacity

Gel fraction and water holding capacity (WHC) tests were conducted using the literature (Dafader et al., 2015; Kenawy et al., 2014; Varshney, 2007). The hydrogel sample was cut into small pieces of 2 x 20.5 cm^3 dried in an oven at 60 °C for 24 h (Z). For the gel fraction test, the dry samples were packed in a 300 mesh stainless-steel screen. The screen containing the sample was then soaked in distilled water at a temperature of 80 °C in a water bath shaker set at 100 rpm for 24 h to remove dirt. Samples were then taken from the water bath and re-dried in an oven at 60 °C until a constant weight was achieved (X). For the WHC test, dry samples were soaked in distilled water for 24 h and then weighted (A). The sample was then dried in an oven at 60 °C for 24 h (B).

$$\text{Gel fraction} = \frac{\text{Weight of clean dry sample (X)}}{\text{Weight of original dry sample (Z)}} \text{ x } 100\%$$

$$\text{WHC} = \frac{(A - B)}{B} \text{ x } 100\%$$

Tensile Strength and Elongation Test

Tensile strength test of hydrogel was conducted using strograph-R1 Toyeseki (Erizal, 2008; Varshney, 2007; Wu, Bao, Yoshii, & Makuuchi, 2001). The sample with the dimension of 10 x 0.6 x 0.2 cm^3 was cut into a dumbbell shape to get a standard size and shape of the American Standard Testing Machine (ASTM) standard. The sample was tested using Instron-Tester R1 (Toyosaki, Japan) with a 30 mm/ min probe speed and sample length of 10 cm.

SEM and FTIR Analyses

The sample for SEM analysis was firstly freeze-dried for 2 x 24 h then coated with gold in a smart coater. The sample for FTIR analysis was similarly treated with the previous sample but coated with KBr powder in vacuum condition.

Statistical Analysis

The treatments (concentration of composite ι/κ-carrageenan were 1.0%; 1.5%; 2.0%; 2.5%; and 3.0% w/w) were carried out in triplicates. The data obtained were analyzed using ANOVA with Duncan's post hoc test on the SPSS software ver. 17.

Results and Discussion

The hydrogel prepared from composite ι/κ-carrageenan was transparent, shown in Figure 1. The physical characteristics analysis of hydrogel covering gel fraction and WHC using gravimetry, tensile strength and elongation are presented in Figures 2 and 3.

The tensile strength of the hydrogel ranged between 9.33 x 10^{-3} to 32.37 x 10^{-3} Mpa, and the elongation ranged between 113.33-251.67%. The highest tensile strength is achieved by hydrogel with the highest concentration of composite ι/κ-carrageenan. It could be due to the higher cross-linking between composite ι/κ-carrageenan with PVA. These two parameters increased with the increasing carrageenan concentration. When the concentration of carrageenan was 1% the tensile strength and elongation were 9.33 x 10^{-3} MPa and 113.33%, and respectively these parameters increased gradually until the concentration

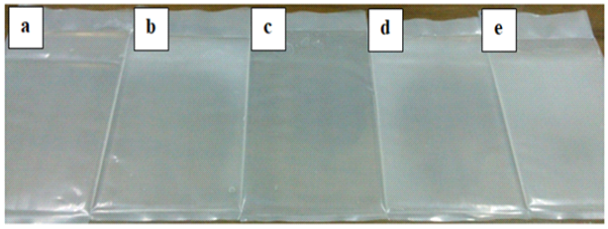

Figure 1. The irradiated hydrogel of PVA-composite ι/κ-carrageenan (a) 1%, (b) 1.5%, (c) 2%, (d) 2.5%, (e) 3% w/w loadings of ι/κ-carrageenan.

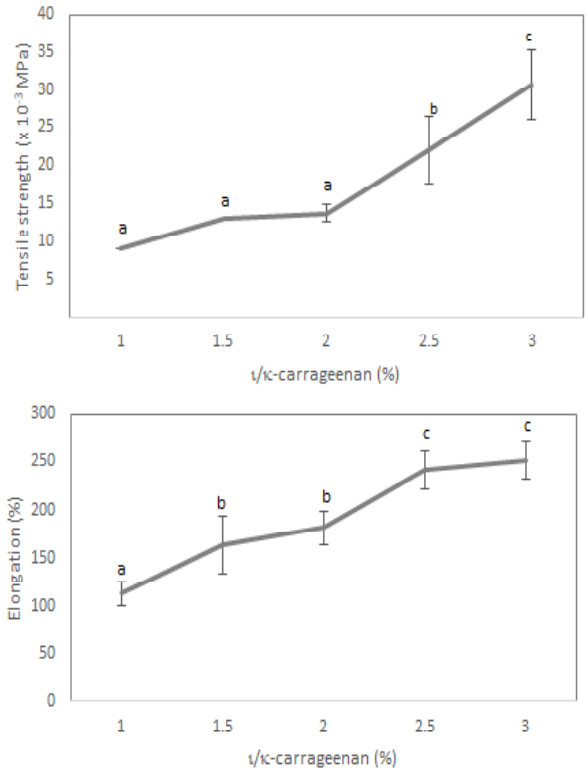

Figure 2. (a) tensile strength (b) elongation of hydrogel PVA-composite ι/κ-carrageenan.

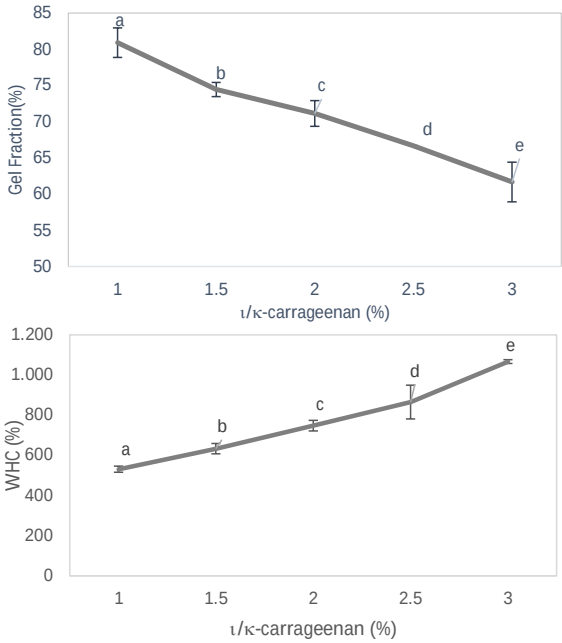

Figure 3. (a) Gel fraction, (b) Water holding capacity (WHC) of hydrogel PVA-composite ι/κ-carrageenan

of carrageenan was 3%. At this point, the tensile strength was 32.37 x 10^{-3} MPa and the elongation was 251.67 %. According to Varshney (2007), the mechanical properties of hydrogel can be represented by tensile strength and elongation, which are closely related to flexibility. According to Kenawy et al. (2014) polysaccharide addition can increase tensile strength since the cross-link increases. The tensile strength of PVA hydrogel added with polysaccharide (such as carrageenan) can directly increase tensile strength and elongation (Varshney, 2007). This indicates that there was a formation of three dimensional structure of the hydrogel. The addition of composite ι/κ-carrageenan from 1 to 3% of hydrogel was able to increase the tensile strength of the hydrogel. In contrast, elongation is closely related to the elasticity of hydrogel (Varshney, 2007). The highest elongation was achieved by hydrogel with the highest concentration of composite ι/κ-carrageenan of 3%. This condition might be caused by the higher proportion of cross link between composite ι/κ-carrageenan with PVA (Sabangan, 2014). A study conducted by Rosiak, Darmawan, and Zainuddin (2001) found that adding polysaccharide can increase elongation; however, too many polysaccharides will decrease hydrogel elongation (Kenawy et al., 2014). According to Sabangan (2008), hydrolyzed PVA would increase the elongation of hydrogel.

The tensile strength of Medifoam® N for comparison has tensile strength 0.033 kgf mm^{-2} equal to 0.33 MPa. The mean tensility of eight other commercially available polyurethane (PU) foam dressings ranged about 0.01-0.025 kgf mm^{-2} equal to 0.1-0.25 MPa. The greatest elongation properties among dressings tested were 1101 %. Medifoam® N also has suitable elongation property (412 %). The remaining eight products tested were ranged from around 180–377 % (Lee, Park, Kim, & Al, 2016). So far no certain quality standard for these parameters.

The gel fraction of the samples ranged between 61.67-80.91%. Gel fraction of the hydrogel PVA-composite ι/κ-carrageenan tended to decrease significantly with increasing carrageenan concentration (p<0.05). Fig. 4a shows that the gel fraction was 80.91% when the carrageenan concentration was 1% until finally, the gel fraction became 61.67% when the carrageenan concentration was 3%. Kokabi, Sirousazar and Hassan (2007) reported that the gel fraction of PVA-clay nanocomposite was 79.4-85.0%. Wikanta, Erizal, Tjahyono, and Sugiyono (2013) also reported that the gel fraction of PVA-chitosan hydrogel was 80-85%. This gel fraction is higher compared with our result. Carrageenan is a natural polysaccharide that is degraded on irradiation by breaking down the main chains (Hien, Phu, Duy, & Lan, 2012), does not form a cross-linking and is dissolved in water. The increase of concentration composite ι/κ-carrageenan up to 3% could cause degradation of composite ι/κ-carrageenan caused by γ-ray irradiation process. PVA probably had more profound effects than carrageenan, with the higher concentration of carrageenan indirectly lowering the concentration of PVA, hence lowered the gel fraction of the composite. Besides, some factors affect the hydrogel's gel fraction, such as polymer composition and irradiation dose (Erizal, 2012). The highest gel fraction is a hydrogel, with the lowest concentration of carrageenan. Hydrogel with composite ι/κ-carrageenan 3% had the lowest gel fraction, i.e., 61.67%. Yang, Liu, Chen, Yu, and Zhu (2008) reported that the hydrogels with a gel fraction of ~80% are suitable for applying wound dressings. It means that based on gel fraction, a hydrogel with 1% composite ι/κ-carrageenan was the best formula with a gel fraction of 80.91%. The degraded carrageenan molecules can prevent the cross-linking formation between PVA and carrageenan resulting in the decreased of gel fraction. The increased of ι/κ-carrageenan from 1 to 3% on the hydrogel can decrease the gel fraction produced.

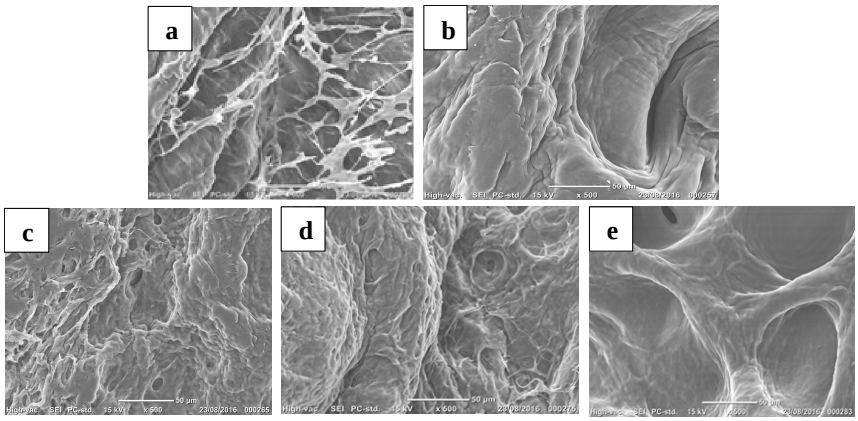

Figure 4. Micrograph of surface of hydrogel PVA-composite ι/κ-carrageenan at concentrations of (a) 1%, (b) 1.5%, (c) 2%, (d) 2.5%, (e) 3% w/w (500x magnification).

Fig. 4(b) shows that the WHC ranged from 531.17 to 1067.12%. The increased of WHC was caused by the increasing number of hydrophilic group in the hydrogel. Statistical analysis showed that increasing composite ι/κ-carrageenan concentration affected WHC significantly (p<0.05). Different from the gel fraction phenomena, the increasing concentration of composite ι/κ-carrageenan, increased the water holding capacity of the hydrogel. The water holding capacity started from 531.17% when the composite ι/κ-carrageenan was 1% and then continuously increased until 1067.12% when carrageenan concentration reached 3%. The characteristics of carrageenan were strong in retaining water; consequently, the higher concentration carrageenan applied in the composite, the higher the water holding capacity. WHC is a parameter showing the capacity of the hydrogel to hold water and swell. As stated by Carabeo (2005), a substance with WHC more than 10% of its initial weight is called hydrogel, and those higher than 95% of its initial weight is called superabsorbent hydrogel. When the hydrophilic group of hydrogel was ionized, the WHC increased due to the higher electrostatic force (Dafader et al., 2015). These phenomena showed the interaction between ι- and κ-carrageenan microparticle with PVA, water holding capacity increases proportionally with the increase of composite ι/κ-carrageenan concentration. Wikanta et al. (2013) reported that the WHC of PVA-chitosan was 1700-2036%. Soerens and Malik (2005) reported that the WHC of the hydrogel at a value of 20 g/g is ideal for absorbing an excess of wound exudates. Based on these results, the hydrogel with 3% carrageenan is the best formula because it have WHC value of 1067.12% (equal to 10.67 g/g)

WHC, tensile strength, and elongation increased with increasing carrageenan concentration. Gel fraction, WHC, tensile strength and elongation of the hydrogel were very much affected by the numbers of cross-links (Brant et al., 2012). According to Kenawy et al. (2014), hydrogel formulated with PVA can be synthesized using a freeze-thaw process. Hydrogel synthesized with PVA/PVP/PU through the freeze-thaw process followed γ-ray irradiation can improve the physicochemical characteristics (gel fraction, tensile strength and elongation) compared to those using γ-ray irradiation (Park & Nho, 2003). According to Yang et al. (2008), hydrogel from PVA/chitosan processed with γ-ray irradiation followed by freeze-thaw process had water holding capacity, mechanical characteristics, and thermal stability higher than those only treated with a freeze-thaw process followed with γ-ray irradiation. The irradiation using a dose of 25 kGy was based on a study conducted by Dafader (2015) that was also recommended for the sterilization process of health products (Varshney, 2007).

The effects of irradiation to hydrogel depend on compatibility degree, molecular interaction and type of homopolymer. Compatibility is affected by the molecular weight of the homopolymer. Among composite ι/κ-carrageenan and PVA, enable them to make hydrogen bonds, ionic bonds and electrostatic interaction. So that cross-linkings or degradation of composite ι/κ-carrageenan and PVA may occur on the hydrogel with a specific irradiation dose. According to Dafader (2015), when the hydrogel is synthesized from PVA and carrageenan and exposed to irradiation with a specific dose, the carrageenan will be degraded into low molecular weight compounds.

Surface Morphology of Hydrogel PVA Composite ι/κ-carrageenan

The surface morphology of the hydrogel was observed under the Scanning Electron Microscope (SEM) according to Nanaki (2015). Fig. 5 shows that the surface morphology of hydrogel PVA-composite ι/κ-carrageenan has porous surfaces of various sizes. The higher concentration of composite ι/κ-carrageenan, the neater and denser was the hydrogel matrix, while the porosity tends to be larger and smoother. It seems that this condition was due to the capability of the matrix to hold water. A similar study has been conducted by Yudianti et al. (2007) on the morphological surface of hydrogel from the seed coat of *Ocimum americanum* using SEM. The results showed that the higher the WHC value, the more porous the hydrogel surface. According to Varshney (2007), a hydrogel with a porous texture can absorb water and unlimited expansion until the hydrogel becomes fragile and broken down.

Spectrum FTIR micro particle of ι- and κ-carrageenan, PVA and hydrogel is presented in Fig. 6. Functional group characteristics of hydrogel wound dressing are listed in Table 1. Stretching at 806 cm^{-1} was due to the C–O–S bonding of 3,6-anhydro-D-galactose-2-sulphate of κ-carrageenan. Stretching at 850-853 cm^{-1} was indicative of C–O–S group of D-galactose-4-sulphate, while those at 923-927 cm^{-1} showed a group of C–O of 3,6-anhydrous-D-galactose. Glycosidic bond of α(1-4) hydrogel was visible at 1093-1097 cm^{-1}. Stretching at 1144 cm^{-1} on PVA showed the presence of C–O–H group. Absorptions at 1096 and 919 cm^{-1} on PVA were indicative of the C–O group and O–H. Absorptions at 1100 and 927 cm^{-1} were C–O of a secondary alcohol and O–H on PVA (Khan & Ranjha, 2014). Absorption 1260-1262 cm^{-1} on the hydrogel showed a sulfate ester group of O=S=O of ι and κ-carrageenan. It is concluded that there was no new chemical bonding between PVA and the composite of ι/κ carrageenan.

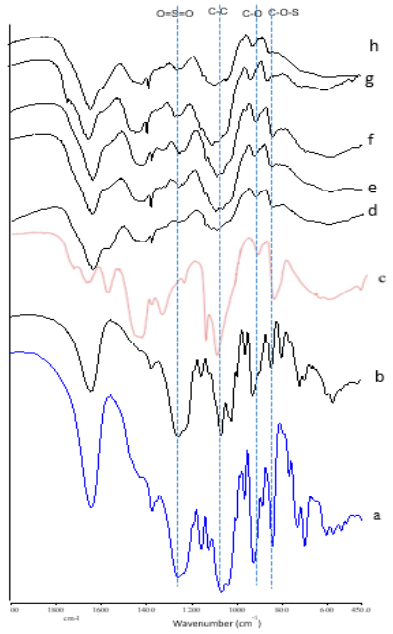

Figure 5. FTIR Spectra (a) κ-carrageenan, (b) ι-carrageenan, (c) PVA, hydrogel PVA-composite ι/κ-carrageenan at concentrations (d) 1%, (e) 1.5%, (f) 2% (g) 2.5% hydrogel, and (h) 3% hydrogel.

Table 1. Functional group characteristics of hydrogel wound dressing

Wavenumber (cm^{-1})				Functional group
ι-carrageenan	κ-carrageenan	PVA	Hydrogel	
806	-	-	-	C–O–S
850	846	-	853-850	C–O–S
933	928	-	923-927	C–O *stretching*
-	-	919	-	O–H *bending*
1028	1042	-	-	C–O–C *stretching*
-	-	1096	1096-1093	C–C *stretching*
-	-	1144	-	C–O–H *stretching*
1262	1262	-	1262-1260	O=S=O *stretching*

The missing of the absorption characterizes the changes of a functional group on hydrogel. In this case, absorption at 1144 cm^{-1} which represents C-O-H group and 919 cm^{-1} which represents O-H group were missing. Furthermore, an absorption at 806 cm^{-1} showed the presence of the C–O–S group of 3,6-anhydrous-D-galactose-2-sulphate of the κ-carrageenan microparticle.

Conclusion

The concentration of composite ι/κ-carrageenan affected the gel fraction, water holding capacity, tensile strength, elongation, and surface morphology of the hydrogel. There is no new chemical bonding between PVA and the composite ι/κ carrageenan. The optimum formula of irradiated composite hydrogel was obtained from 3.0% w/w of composite ι/κ-carrageenan, which had a gel fraction of 61.67%, water holding capacity 1067.12%, tensile strength 32.37 x 10^{-3} MPa, and elongation 251.67%. Interestingly, the product with the optimum formula had a porous surface morphology and transparency, which presumably applicable for wound dressing purposes.

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