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Original Article

Development of Wound Dressing Hydrogel Based Combination of κ-Carrageenan and Chitosan

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Abstract

Wound dressing has an essential role in the wound-healing process because it can help accelerate wound healing and prevent infection. One of the wound dressings that needs to be developed is a hydrogel-based wound dressing because hydrogel can retain moisture and absorb accumulated fluid on the wound. Moreover, hydrogel can also be used as a matrix for therapeutic agent delivery. Therefore, this study aimed to develop a hydrogel film-based wound dressing using a combination of κ-carrageenan and chitosan. The optimum formula was determined based on the simplex lattice design. The parameters for optimization included swelling ratio, water vapor transmission rate (WVTR), and film degradation ratio. The optimum hydrogel film formula had a concentration of 1.5% κ-carrageenan and 0.5% chitosan. It exhibited a swelling ratio of 423.68 ± 60.52%, a WVTR of 831.54 ± 63.36 mg/cm^2.day, a pH of 5.2 ± 0.27, a tensile strength of 0.0681 ± 0.009 mPa, and a hydrogel film degradation of 66.64 ± 20.96% after three days. These results suggest that the optimum hydrogel film-based combination of κ-carrageenan and chitosan is a promising alternative wound dressing.

Keywords: κ-carrageenan, Chitosan, Hydrogel, Simplex lattice design, Wound dressing.

1. Introduction

Wounds are defined as disruptions or damage to the integrity and function of body tissues [1]. Frequent changing and reapplication of wound dressings can delay wound healing. High frequent changing of wound dressing can decrease the wound's temperature, affecting the cellular healing process and increasing the potential for harmful bacteria to enter the wound site [2]. Generally, wound dressings are used to protect wounds and provide an optimal environment to support wound healing. A wound dressing can accelerate wound healing if it can absorb exudate, retain moisture, and provide moisture to the wound area [3]. Absorption of exudate is an essential property of wound dressings. Excess exudate can contain

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tissue-destroying enzymes that can inhibit cell proliferation activity and degrade extracellular matrix and growth factors so that it can suppress the wound-healing process [4].

Biodegradable hydrogels have been extensively explored in the field of wound dressings because they do not need frequent replacement, thus minimizing pain [5-6]. Biodegradable hydrogels can be prepared from biodegradable polymers that can degrade into a loose complex structure, which the body's metabolism can eliminate. Among the natural polymers used in hydrogel formation are κ-carrageenan and chitosan [7-8].

κ-carrageenan can enhance the response of inflammatory cells in wounds and regulate tissue regeneration [9]. The administration of κ-carrageenan to mice has been demonstrated to accelerate wound healing compared to a control group that did not receive κ-carrageenan [9]. Additionally, κ-carrageenan has been shown to possess antibacterial properties, potentially serving as a new antibacterial agent against various pathogens by damaging bacterial cell walls and cytoplasmic membranes [10].

Chitosan exerts a positive influence on cell proliferation activity, which is significantly important in the context of wound healing. It is known to activate polymorphonuclear leukocytes and macrophages for phagocytosis, as well as induce the production of IL-1 (interleukin-1), TGF-β (transforming growth factor-β), and PDGF (platelet-derived growth factor) [11]. Furthermore, chitosan has been demonstrated to induce fibroblast proliferation, with the degree of deacetylation of chitosan being positively correlated with fibroblast activation [11]. Moreover, chitosan exhibits antibacterial activity against *Staphylococcus aureus* [12]. The antimicrobial activity of chitosan is attributed to the electrostatic interactions between the positively charged amino groups of chitosan and the negatively charged bacterial membrane [13].

In general, hydrogels are prepared by using κ-carrageenan with KCl as a cross-linker that will form a coil-to-helix structure, which makes the gel stiff, firm, and robust [12]. However, hydrogel-based chitosan is known to swell rapidly, with a capacity of nearly 1000% of its dry weight, due to the strong hydrogen bonds [14]. Therefore, a combination of κ-carrageenan and chitosan is needed to improve the performance of a hydrogel. Moreover, chitosan and κ-carrageenan can form hydrogels with stable ionic cross-links due to electrostatic interactions between the positively charged amine groups in chitosan and the negatively charged sulfate groups in κ-carrageenan [15].

This study aimed to determine the optimal concentration of κ-carrageenan and chitosan in forming hydrogel films. The optimum formula was determined based on the simplex lattice design using various concentrations of κ-carrageenan and chitosan. The optimized parameters considered were swelling ratio, water vapor transmission rate (WVTR), and film degradation. The optimized hydrogel film was expected to meet the specifications of a commercial wound dressing and could be considered an alternative wound dressing.

2. Materials and Methods

2.1. Materials

κ-carrageenan (pH 7.2 at 1.5% solution) was purchased from IndoFoodChem. Chitosan

(degree of deacetylation: 96.19%, MW: 168kD) was purchased from Chimultiguna. PEG 400 (MW: 375-450 g/mol; pH: 5.5-7.5; viscosity: 76-86 cSt at 20°C) was purchased from Alfa Kimia Jogja. Potassium chloride and sodium chloride were purchased from Xilong Scientific. Acetic acid was purchased from Merck.

2.2. Preparation of Hydrogel Film

The hydrogel film formula was optimized using the simplex lattice design (SLD) method, which involved obtaining various formula components, as shown in **Table 1**. Chitosan was dissolved in 0.125% acetic acid and homogenized by stirring at 200 rpm. Air bubbles in the chitosan solution were removed by using the water bath sonicator at 80°C for 30 min. Then, the κ-carrageenan was dissolved in distilled water at 80°C and homogenized by stirring at 200 rpm. KCl, a cross-linker that forms a coil-to-helix structure, was added to the κ-carrageenan solution while stirring at 80°C. Then, the chitosan solution was added to the κ-carrageenan solution and stirred for 5 min. In the last step, PEG 400, as a plasticizer, was added to the polymer mixture. 15 mL of the hydrogel solution was poured into a polystyrene petri dish with a diameter of 9 cm and a height of 3 mm.

The hydrogel solution was cooled to room temperature, followed by a cooling process at 4°C for 15 minutes.

After the formation of the hydrogel film, the sample was dried in an oven at 50°C until the film was almost dry. The hydrogel films were washed by dipping films in distilled water for 20 seconds. Then, the hydrogel film was dried by using an oven at 50°C until the surface of the hydrogel film was dry.

2.3. Characterization of Hydrogel Film

2.3.1. Physical Properties

The hydrogel films were observed organoleptically, including color, texture, and odor, and the thickness of the hydrogel was also measured using a digital thickness gauge (Mitutoyo).

2.3.2. pH Value

The pH of the hydrogel film was measured by dropping a universal pH indicator solution (LobaChemie) onto the hydrogel film.

Table 1. Components of hydrogel films

No	Components	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8
		Ratio (%)							
1	κ-Carrageenan	1.25	0.5	1.5	1	1.5	1	0.75	0.5
2	Chitosan	0.75	1.5	0.5	1	0.5	1	1.25	1.5
3	KCl*	0.93	0.37	1.11	0.74	1.11	0.74	0.56	0.37
4	PEG 400	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
5	Water	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100

Note: *every 1% of κ-carrageenan required KCl 0.2M

2.3.3. Swelling Ratio

The swelling ratio test was performed by placing a polyester nonwoven filter (5 µm pore size) moistened with the test medium in the center of a Petri dish (9 x 1.5 cm).

The polyester nonwoven filter was wetted by pouring 8 mL of simulated wound fluid (SWF) or distilled water as the test medium. The simulated wound fluid was made by dissolving 0.368 g of calcium chloride and 8.298 g of sodium chloride in 1 L of deionized water [14].

The hydrogel films were cut into approximately 1 cm x 1 cm pieces and weighed to determine their dry weight (W0). Then, the hydrogel film was placed on the polyester nonwoven filter and incubated in an incubator at 37°C. Samples were weighed after incubation at specified time intervals (W1). The percentage swelling ratio was calculated using equation (1) as follows:

$$\text{Swelling Ratio}(\%) = \frac{(W_1 - W_0)}{W_0} \times 100 \quad (1)$$

W_1: final weight of hydrogel at the time (mg)
W_0: dried weight of hydrogel (mg)

2.3.4 Water Vapor Transmission Rate

The hydrogel film was formed into a circle 2.8 cm in diameter and 7.5 cm high and placed over the mouth of a glass bottle containing 15 mL of distilled water at ¾ the distance from the hydrogel sample [15]. The bottles containing water were initially weighed before incubating (W_0). The film was adhered to the mouth of the bottle with PTFE adhesive. In the final step, the samples were incubated at 37ºC with 50% relative humidity for 24 hours. The WVTR value is calculated from equation (2) as follows:

$$\text{WVTR } (\text{mg/cm}^2.\text{day}) = \frac{(W_0 - W_1)}{\text{A x day}} \quad (2)$$

W_0: initial weight of bottle (mg)
W_1: weight of bottle after incubation (mg)
A: surface area of mouth glass (cm^2)

2.3.5 Film Degradation Evaluation

Hydrogel films were cut into approximately 1 cm x 1 cm pieces and individually weighed to determine the initial weight before swelling. The hydrogel film was placed on a polyester nonwoven filter, which had been moistened with the test medium (simulated wound fluid), in a Petri dish (as described in the swelling ratio test method) and incubated at 37°C. The weight of the film hydrogel was recorded at intervals of 1, 2, 3, 4, and 6 hours (time range adjusted), and the maximum hydrogel weight at swelling was marked. The percentage of film degradation was calculated using equation (3) as follows:

$$\text{Film degradation}(\%) = \frac{(W_0 - W_1)}{W_0} \times 100 \quad (3)$$

W_0: initial weight of film hydrogel (mg)
W_1: final weight of film hydrogel (mg)

2.3.6 Tensile Strength Evaluation

The hydrogel was cut into rectangular strips (2.5 cm × 5 cm). Tensile strength tests were performed using a universal testing machine (RTI-1225 A&D Company, Japan). The loading speed used in the tensile test was 10 mm/min with a load capacity of 2.5 kN. The value of tensile strength is also known as Young's modulus. The tensile strength test results are typically reported in units of N/mm^2 or MPa [16].

2.4 Statistical analysis

During the optimization process, the characteristic data of the hydrogel film were analyzed using the Simplex Lattice Design method. After the optimization process was completed, the resulting data were analyzed using One-Way ANOVA and Two-Way ANOVA with a confidence level of 95%, along with a one-sample t-test.

3. Results and Discussion

3.1. Preparation of Hydrogel Film

The physical characteristics of hydrogel films are shown in **Table 2**, and the images of their physical appearance are shown in **Figure 1**. All hydrogel films had a thickness ranging from 0.1 to 0.6 mm and were odorless. The color of the hydrogel film tended to be transparent, but it became more yellowish if it contained a relatively high concentration of κ-carrageenan. Moreover, the texture of the hydrogel film tended to be smooth, but it became rougher if the hydrogel film contained a relatively high concentration of κ-carrageenan. The rough texture of the hydrogel film was formed due to the presence of pores on its surface. The pores were formed because κ-carrageenan and KCl are well-known materials that can act as pore agents [17-18].

Table 2. Physical parameters of hydrogel films.

Run	κ-Carrageenan: Chitosan ratio (%)	Physical parameters of hydrogel film		
		Thickness	Appearance (Transparent)	Surface texture
1	(1.25: 0.75)	0.1 - 0.4	Yellowish Slightly milky	Slightly rough
2	(0.5: 1.5)	0.1 - 0.6	Slightly milky	Smooth slightly sticky
3	(1.5: 0.5)	0.1 - 0.5	Yellowish Slightly milky	Slightly rough
4	(1:1)	0.1 - 0.5	Slightly milky	Smooth slightly sticky
5	(1.5: 0.5)	0.1 - 0.5	Yellowish Slightly milky	Slightly rough
6	(1:1)	0.1 - 0.5	Slightly milky	Smooth slightly sticky
7	(0.75: 1.25)	0.1 - 0.5	Slightly milky	Smooth slightly sticky
8	(1.5: 0.5)	0.1 - 0.6	Slightly milky	Smooth slightly sticky

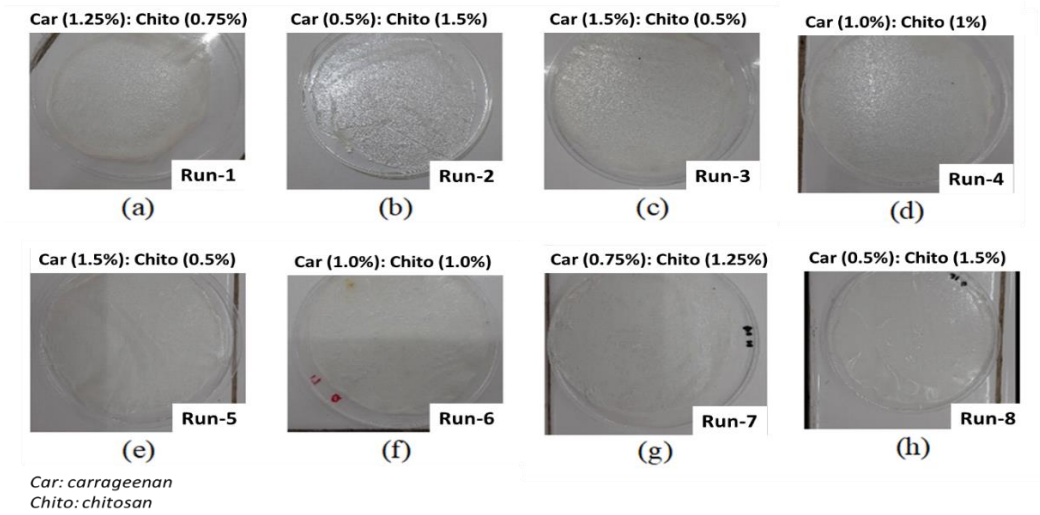

Figure 1. Appearance of hydrogel films, (a) Run-1, (b) Run-2, (c) Run-3, (d) Run-4, (e) Run-5, (f) Run-6, (g) Run-7, (h) Run-8.

3.2. pH Value of Hydrogel Film

The pH value of the hydrogel film for each Run can be seen in **Table 3**. The pH value obtained indicated that the pHs of hydrogel films were in the range of pH 5-5.5 and suitable for wounds because the pH values of chronic wounds are in the range of 5-8, and the pH of Class I pressure ulcers are in the pH range of 5.5-5.6 [19, 20]. The acidic wound environment can maximize oxygen delivery to meet body tissue repair needs through the Bohr effect [21]. The Bohr effect, which causes the pH of the wound to decrease, will decrease the affinity of hemoglobin for oxygen, thus increasing the delivery of oxygen to wound tissue [22].

Oxygen is an important component of fibroblast growth and collagen synthesis during wound healing. The proliferation and migration of fibroblast and keratinocyte cells will occur more rapidly in an acidic microenvironment [23]. Therefore, the pH value of the hydrogel films followed the targeted wound dressing product profile.

Table 3. pH values of hydrogel films (n=5).

Run	κ-Carrageenan: Chitosan ratio (%)	pH value
1	(1.25: 0.75)	5.2 ± 0.27
2	(0.5: 1.5)	5.3 ± 0.27
3	(1.5: 0.5)	5.1 ± 0.22
4	(1:1)	5.1 ± 0.22
5	(1.5: 0.5)	5.1 ± 0.22
6	(1:1)	5.2 ± 0.27
7	(0.75: 1.25)	5.2 ± 0.27
8	(1.5: 0.5)	5.1 ± 0.22

3.3. Swelling Ratio of Hydrogel Film

The swelling ratio is a phenomenon of increasing hydrogel weight by absorbing fluid [24]. Hydrogel films with high water content and swelling capacity are expected to be able to maintain a moist environment around the wound and simultaneously absorb exudate from the wound site [25]. In this study, hydrogel films were incubated in simulated wound fluid (SWF) media at pH 6.5 and distilled water. The equilibrium swelling ratio values for all runs are presented in **Table 4**.

Table 4. Swelling ratio of hydrogel films (n=3).

Run	κ-Carrageenan: Chitosan ratio (%)	Swelling Ratio (%)	
		Simulated Wound Fluid	Distilled water
1	(1.25: 0.75)	435.942 ± 31.27	700.01 ± 18.69
2	(0.5: 1.5)	581.012 ± 31.27	1006.14 ± 114.25
3	(1.5: 0.5)	297.334 ± 108.20	963.57 ± 190.04
4	(1:1)	532.335 ± 61.16	867.75 ± 34.88
5	(1.5: 0.5)	323.327 ± 72.99	1002.62 ± 88.16
6	(1:1)	498.641 ± 101.30	980.47 ± 81.47
7	(0.75: 1.25)	466.203 ± 24.60	798.62 ± 163.80
8	(1.5: 0.5)	524.72 ± 90.41	763.48 ± 115.23

Runs 3 and 5 exhibited the lowest swelling ratios among all runs due to their relatively high concentrations of κ-carrageenan. This phenomenon in the study resulted from the high degree of cross-linking between κ-carrageenan molecules, facilitated by the presence of KCl as a cross-linker. The high number of K^+ ions prevents electrostatic repulsion between the sulfonate groups in the κ-carrageenan structure, resulting in a high degree of cross-linking [26]. The high degree of cross-linking suppresses the hydrogel film's ability to absorb water, resulting in decreased swelling ability.

The swelling ratio of Run-2, 4, 6, 7, and 8 in SWF had insignificant differences (based on the two-way ANOVA test). This result showed that

κ-carrageenan only affected the suppression of the swelling ratio when the concentration of κ-carrageenan was dominantly high in the formula. Furthermore, the swelling ratio values of the hydrogel film in distilled water exceeded those in SWF. This phenomenon occurred because chitosan was neutral at pH 7, and the electrostatic interactions between κ-carrageenan and chitosan were low; thus, water can diffuse into the hydrogel network quickly [27].

3.4. Water Vapor Transmission Rate

The water vapor transmission rate is used to determine the ability of a wound dressing to control water loss at the wound surface [28]. The WVTR profile of all runs can be seen in **Figure 2 (a)**. The WVTR values of all samples ranged from 1433.96 ± 32.17 to 1659.47 ± 7.50 mg/cm^2.day. The result followed the requirement of the standard WVTR value of wound dressing products, which is 205-9360 mg/cm^2.day [29-30].

The WVTR values of all runs were not significantly different based on the results of the one-way ANOVA test. Therefore, there was no effect of κ-carrageenan and chitosan ratio on the WVTR value. The molecular structures of κ-carrageenan (with -OSO_3-, -OH groups) and chitosan (with -NH_2, -OH groups) contain functional groups that facilitate the absorption of moisture from the surrounding atmosphere. This hydration allows the hydrogel film to transfer water vapor from the wound to the external environment [31].

3.5. Degradation of Hydrogel Film

In this study, the degradation ratio of hydrogel film was evaluated for 6 hours, and the degradation profiles of hydrogel film can be seen in **Figure 2 (b)**. The hydrogel film with the highest concentration of κ-carrageenan exhibited the lowest percentage of film degradation (0%). On the other hand, increasing the concentration of chitosan in the hydrogel film had a high percentage of film degradation. This result occurred because the cross-linking between κ-carrageenan and KCl formed a coil-to-helix structure and maintained film integrity [11].

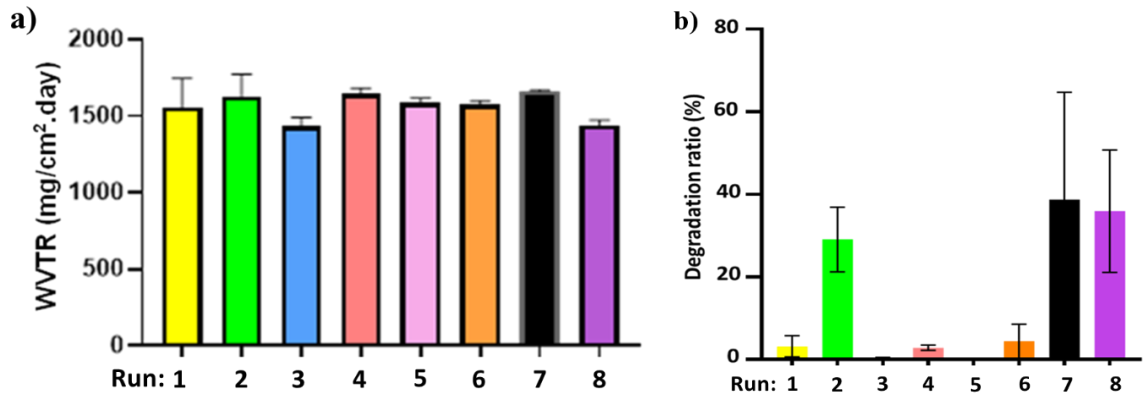

Figure 2. Hydrogel film profile of (a) water vapor transmission rate of hydrogel films, (b) Degradation ratio of hydrogel films.

3.6. Optimization of Hydrogel Film Formula-Based Simplex Lattice Design

The determination of the optimum hydrogel film formula was carried out using the simplex lattice design method. The parameters used to determine the optimum formula were swelling ratio, WVTR, and hydrogel film degradation values. The optimum formula was determined based on the target upper and lower limits of each parameter. The upper and lower limits of each parameter were based on the general characteristics of hydrogel-based wound dressings (**Table 5**).

Table 5. Criteria for determining the optimum formula of hydrogel film.

Parameter	Goal	Upper limit	Lower limit
Swelling Ratio (%)	in range	1000	100
WVTR (mg/cm^2.day)	in range	9360	205
Film degradation ratio (%)	minimize	25	0

All equations obtained for each parameter from the processed simplex lattice design method can be seen in **Table 6**. Regarding numerical optimization, the optimum formula was selected based on the highest desirability value, which is close to 1. Regarding the swelling ratio and WVTR equation, κ-carrageenan and chitosan gave a positive response, indicating that each component of κ-carrageenan and chitosan can increase the value of the swelling ratio and WVTR of hydrogel film. However, chitosan emerged as the most influential component on the swelling ratio and WVTR parameters, as it exhibited a higher positive value in the equation.

The effect of polymers on the film degradation ratio indicated that the addition of chitosan to the hydrogel film formula can increase the percentage of film degradation. However, a specific combination of κ-carrageenan and chitosan ratios can prevent film degradation. The highest desirability value was 0.996, which contained 1.5% κ-carrageenan and 0.5% chitosan, according to the desirability graph (**Figure 3**). Following the optimum formula of hydrogel film, the final hydrogel film was prepared and characterized before and after sterilization treatment. The characteristics of the optimum hydrogel film can be seen in **Table 7**.

Table 6. The simplex lattice design equation for each parameter of hydrogel film.

Parameter	Model	Equation (Y=)	p-value Model	Lack of Fit
Swelling Ratio	Linear	346.28A + 568.6B	0.0036	0.1023
WVTR	Quadratic	1498.43A + 1541.83B + 494.76AB	0.2340	0.8332
Film degradation ratio	Quartic	32.51B - 50.44AB - 51.38AB (A-B) + 198.98AB (A-B)^2	0.0046	-

Y = parameters, A = κ-carrageenan (g), B = chitosan (g)

Table 7. Characteristics of the optimal formula hydrogel film before and after sterilization treatment.

Parameter	Hydrogel film		Sig. (*2. tailed*)
	non-sterilized	sterilized	
Swelling Ratio (%)	427.02 ± 21.40	423.68 ± 60.52	0.9159
WVTR (mg.cm^{-2}.day)	993.23 ± 158.54	831.54 ± 63.36	0.1763
Film degradation ratio (%)	0	0	-
pH value	5.9 ± 0.22	5.2 ± 0.27	0.0022
Tensile Strength (MPa)	0.0348 ± 0.0071	0.0681 ± 0.009	0.0082

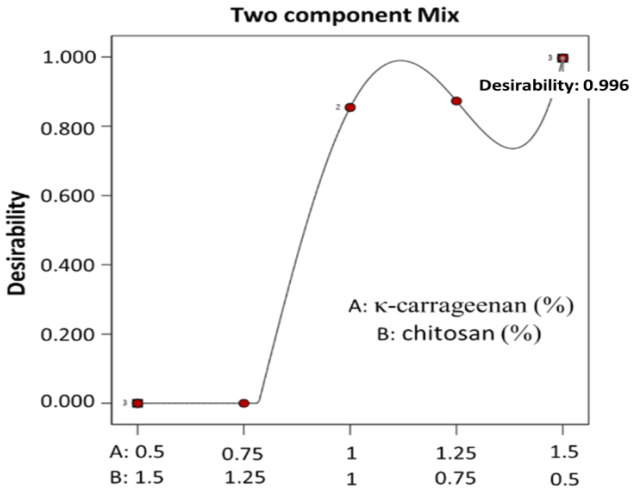

Figure 3. Desirability graph of hydrogel film formulation.

There were no significant differences between the swelling ratio, WVTR, and percentage of film degradation ratio between the hydrogel film before and after sterilization treatment. However, there were significant differences in pH and tensile strength values. The pH and tensile strength (Young's modulus) values of sterilized hydrogel films were higher than those of non-sterile hydrogel films. His phenomenon can be attributed to an increase in the degree of cross-linking density, which results from a reduction in the water content of the sterile hydrogel film [32].

Wound dressings must possess sufficient tensile strength to maintain their integrity when applied during wound care [33]. The optimal hydrogel film exhibited a tensile strength of 0.035 ± 0.007 MPa. This value indicates that the optimal hydrogel film is classified as elastic and follows the elastic modulus of dermal wounds (0.001–0.02 MPa) and human soft tissue (0.001–0.1 MPa) [34-35].

The sterilized hydrogel film was retested to determine the required time for the hydrogel film to be thoroughly degraded. The results showed that almost 100% of the hydrogel mass had been lost in 3 days (**Figure 4**). This result indicated that the replacement of hydrogel film as wound dressing will take place in 3 days. In general, hydrogel-based wound dressings have a replacement frequency of 1 to 3 days [36]. Therefore, our optimized hydrogel film can be an alternative wound dressing.

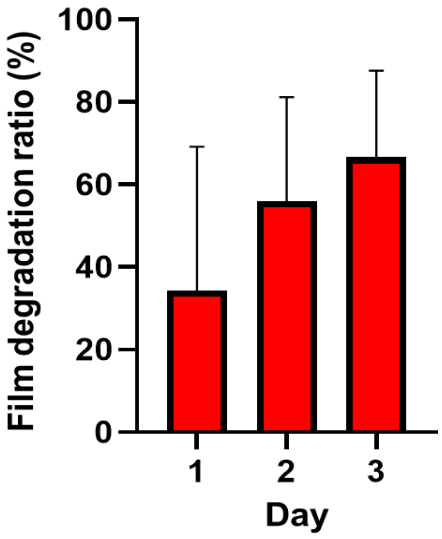

Figure 4. Degradation profile of sterilized hydrogel film.

4. Conclusion

We have succeeded in preparing a hydrogel film-based wound dressing with good properties as an alternative wound dressing. The optimum hydrogel film formula was obtained by combining 1.5% κ-carrageenan and 0.5% chitosan. The concentration ratio of κ-carrageenan and chitosan polymer influenced the value of the swelling ratio and film degradation and did not affect the WVTR value. Additionally, a high concentration of κ-carrageenan in the formula led to a significant decrease in both the swelling ratio and degradation ratio of the hydrogel film.

Acknowledgement

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Conflict of interest

The authors declare to have no conflict of interest.

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