International Journal of Technology 13(8) 1715-1725 (2022)
Received August 2022 / Revised November 2022 / Accepted December 2022

International Journal of Technology

http://ijtech.eng.ui.ac.id

Optimization Concentration of Irgacure® 2959 as Photo-initiator on Chitosan-Kappa-Carrageenan Based Hydrogel for Tissue Sealant

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Abstract. Hydrogel is a three-dimensional network of hydrophilic polymers widely used in the biomedical field, one of which is a tissue sealant. The material that can be used in the manufacture of hydrogels is chitosan. The ability of chitosan in the formation of hydrogel is still limited by its swelling and adhesive properties, so it requires other materials such as crosslinkers, one of which is kappa-carrageenan which can stabilize and increase the viscosity of the hydrogel. The addition of Irgacure® 2959 as a photo-initiator to the hydrogel can produce free radicals that can bind to the active group of the polymer. This study aims to determine the effect of adding Irgacure® 2959 in the manufacture of hydrogel based on a chitosan-kappa-carrageenan solution formulated as a colon tissue adhesive. The treatments given were the addition of Irgacure® 2959 as much as 0%, 1%, 2%, 3%, and 4%. The characteristics of the hydrogel tested qualitatively (simulation adhesion test), degree of swelling, resistance to water, degree of crystallinity, and ability to inhibit bacterial growth. The addition of Irgacure® 2959 did not show significantly different results from the control that indicating it cannot increase the crosslinking between chitosan and kappa-carrageenan. This is predicted due to the presence of steric hindrance from kappa-carrageenan, causing very limited crosslinking due to the molecular size difference between chitosan and kappa-carrageenan. Further, it is necessary to optimize the ratio of the chitosan and kappa-carrageenan to get the balance ratio that supports the occurrence of cross-linking.

Keywords: Carrageenan; Chitosan; Hydrogel; Irgacure® 2959; Photo-initiator

1. Introduction

Hydrogels are three-dimensional networks of hydrophilic polymers with 90-99% moisture content that facilitate efficient mass and oxygen transfer and endogenous regeneration, drug delivery, and wound healing (Yegappan et al., 2018). One of the materials that can be used as raw material for making hydrogels is chitosan which has flexible, non-toxic, hydrophilic, anti-bacterial properties and provides wound healing effects, so it is widely used in biomedical and tissue engineering (Zhang et al., 2018). The properties of chitosan make it potential to be used as a raw material for making tissue sealant.

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doi: 10.14716/ijtech.v13i8.6166

Tissue sealant is a substance with the ability to bind two tissue substrates together. One of these tissue sealants can be applied to glue the tissue in the wound that is produced after the operation process on the colon tissue. Materials that are usually used as tissue sealants are macromolecules (Nayeb et al., 2011) which have biocompatible and biodegradable properties (Bao et al, 2020), one of which is chitosan (Asadpour et al., 2020). Chitosan was chosen as an alternative to reduce the shortcomings that arise from tissue sealants with previously used materials, namely cyanoacrylate, fibrin, and gelatin. Chitosan-based hydrogels are still limited by their swelling and adhesive properties and are weak in charge interactions without forming bonds between related chains (Phuong et al, 2019), thus requiring the addition of other materials as crosslinkers. Crosslinker material is added to form crosslinks between the polymers used which can affect the shape of the cavity in the hydrogel and affect the hydrogel's ability. The use of this crosslinker can help increase swelling ability and more stable bond strength (Ding et al., 2020). One of the materials that can be used as a crosslinker in chitosan hydrogel is carrageenan with good mechanical properties, biocompatible and non-toxic (Derkach et al., 2018) and can stabilize and form viscosity that it can be used as a gelling agent by forming a helical network with double molecule resulting from the sulfate group (Wang et al, 2018). There are various carrageenan types, including iota-, kappa-, and lambda-carrageenan (Darmayanti et al., 2016). In this study, we used kappa carrageenan as the basis for the hydrogel.

The use of chitosan and carrageenan in the manufacture of hydrogels is based on the properties of each polymer needed in medical applications. Chitosan-carrageenan film hydrogel has been made to be applied to fibroblast cells (Yu et al., 2018), showing that chitosan-carrageenan can heal cells because it is non-toxic, which causes cells to grow well. Chitosan-carrageenan-based hydrogel has the potential to produce a more flexible hydrogel so that it can be an alternative to tissue sealant that has previously been used but have brittle and less flexible properties. According to intestinal surgeons, 80% of cases of postoperative failure of the colon occur due to the use of adhesive tissue that is brittle and inflexible so that it is not compatible with peristalsis in the intestines so that chitosan-carrageenan hydrogel can be used as a tissue sealant, especially to be applied to the colon. Preparation hydrogels as tissue sealant by using crosslinking will produce better mechanical strength and hydrophilicity. The hydrogel produced by the crosslinking method produces a cross-linked structure that is not easily soluble in water, and the gel form can be maintained (Yu et al., 2018). The crosslinking method used in the manufacture of hydrogels, namely the photo-crosslinking method, refers to the research that used Irgacure® 2959 which has benzoyl and ketone active ingredients as photo-initiators in the manufacture of hydrogels (Qi et al., 2013). The addition of Irgacure® 2959 will change the crosslink strength, which can affect the results of the hydrogel characteristics test. Therefore, the chitosan-carrageenan-based hydrogel formulation was carried out with the optimization of Irgacure® 2959 as a photo-initiator with the aim of knowing the characteristics of the hydrogel as a colonic tissue adhesive.

2. Methods

The research was started by optimizing the concentration of chitosan and carrageenan solutions to find the concentrations to be used as the basis for making hydrogels which were selected based on the level of viscosity produced. Next, optimization of the solution mixing method was carried out to find the right method to use in the manufacture of hydrogels based on the homogeneity of the resulting hydrogels. The next step is to optimize the concentration of Irgacure® 2959 to determine the concentration range to be selected for further characterization of the hydrogel.

2.1. Optimization of chitosan and carrageenan solutions

Optimization of chitosan and carrageenan solutions needs to be done to determine the right concentration of chitosan and carrageenan for hydrogel manufacture. Optimization of the concentration of chitosan solution was carried out by dissolving chitosan powder (DD. 98 from Bio Chitosan Indonesia) with 0.5% acetic acid (Merck) at a concentration of 1%, 2%, and 3%. The optimization of the carrageenan solution was carried out by dissolving the carrageenan powder (CV Karagen Indonesia) using distilled water at a temperature of 50°C with a concentration of 1%, 2%, and 3%.

2.2. Optimization of the mixing method

Optimization of the mixing method was carried out by several methods, such as mixing the chitosan solution with the carrageenan solution directly, done by adding a solution of carrageenan (2%) into a solution of chitosan (2%) with a ratio of 1:1 then stirring using a magnetic stirrer. Mixing the chitosan and carrageenan solutions dropwise, done by adding a solution of carrageenan (2%) into a solution of chitosan (2%) dropwise while stirring continuously at a temperature of 60°C. Also, mixing the chitosan solution with carrageenan powder by using chitosan solution (2%) and carrageenan powder (2%, 3%, 4%, and 5%) was carried out by adding carrageenan powder into the chitosan solution slowly at a temperature of 50°C and stirring continuously. The observed results from the optimization of the mixing method were observed qualitatively, namely the homogeneity and viscosity of the hydrogel by tilting the hydrogel at an angle of approximately 45°C. The selected results based on hydrogel will be used to optimize the concentration of Irgacure® 2959.

2.3. Optimization concentration of Irgacure® 2959

The manufacture of hydrogels with the addition of Irgacure® 2959 refers to the earlier research (Qi et al., 2013) by dissolving chitosan (2%), which was stirred at 45-50°C for 1 hour. Then in a warm state, carrageenan powder (4%) was added little by a little while stirring for 15 minutes at 45-50°C. Irgacure® 2959 powder (Jinan Huijinchuan Chemical CO., Ltd) was added in the dark at a concentration of 1%, 2%, 3%, 4%, and 5% and continuously stirred at 40°C for 15 minutes. The hydrogel was irradiated with 8-watt UV light with a wavelength of 235nm for 15 minutes. The resulting hydrogel was observed for homogeneity, and then the concentration was chosen to be used in the chitosan-carrageenan hydrogel characteristics test. Based on the hydrogel produced, the concentration of Irgacure® 2959 was chosen to be used in the study with the following research design below (Table 1).

Table 1 Research design of chitosan-carrageenan hydrogel

Treatment	Chitosan (%) (solution)	Carrageenan (%) (powder)	Irgacure® 2959 (%)
A	2%	4%	0%
B	2%	4%	1%
C	2%	4%	2%
D	2%	4%	3%
E	2%	4%	4%

2.4. Chitosan-carrageenan hydrogel analysis and characterization

2.4.1. Swelling ratio

Swelling ratio testing was carried out using a hydrogel film that was cut to a size of 1cmx1cm and weighed (w_o). The hydrogel film was put into a phosphate buffer saline (PBS) solution and soaked for 24 hours in a water bath shaker at 37°C. After 24 h, the films were lifted, dried, and weighed (w). The swelling ratio is calculated by the formula:

$$Swelling\ ratio\ \% = \frac{w - w0}{w0} \times 100\% \quad (1)$$

Where w is hydrogel film weight after soaked (g) and wo is hydrogel film before soaked (g).

2.4.2. Water resistance

Water resistance testing is done by forming a hydrogel into a layer that is printed on silicone. The water resistance was tested by cutting the hydrogel film (1cmx1cm) and weighing it (W0), then putting it in aquadest at room temperature. The hydrogel is lifted, dried, and weighed (W) per one minute, then repeated until the weight is constant. Water resistance is calculated by the formula:

$$Water\ resistance\ \% = \frac{w - w0}{w0} \times 100\% \quad (2)$$

Where w is the hydrogel film's constant weight after soaked (g) and wo is dried hydrogel film (g).

2.4.3. Adhesion simulation on smoked beef

Simulation of adhesion was carried out by applying chitosan-carrageenan hydrogel between two pieces of smoked beef measuring 2x5 cm and then allowed to stick (Ono et al., 2000). Smoked beef is pulled on both sides to measure its adhesive qualitatively.

2.4.4. Degree of crystallinity (X-Ray Diffraction)

Testing the degree of crystallinity with X-Ray Diffraction (XRD) was carried out using a sample with a size of 1.9 x 1.4 cm with a thickness of 1 mm according to the size of the sample holder. The sample was placed in a holder and then analyzed with an X-ray diffraction tool for 6 minutes. The results will appear on the monitor screen. Furthermore, the degree of crystallinity is calculated using the formula:

$$\text{Crystallinity} = \frac{crystaline\ area\ fraction}{crystalline\ area\ fraction + amorf\ area\ fraction} \times 100 \quad (3)$$

2.4.5. Antibacterial activity

Antibacterial activity testing was carried out using the paper disk diffusion method using *Escherichia coli* bacteria. The bacteria to be used were first rejuvenated in a solution of Nutrient broth (NB) and incubated for 24 hours at 37°C. The bacteria that had grown on the NB were then taken to be poured and spread over the Nutrient Agar (NA). Furthermore, to test the antibacterial ability, paper discs with a size of 0.5 cm were immersed in a hydrogel sample aseptically, then placed on NA and incubated with an incubator (Isuzu Sesakusho Co., LTD) for 24 hours at 37°C. Antibacterial activity can be calculated by measuring the clear zone (inhibition zone) that appears around the paper disc.

3. Results and Discussion

3.1. Optimization of chitosan and carrageenan solutions

Based on the characteristics of the hydrogel, the concentration of the chitosan and carrageenan solution selected was 2%. This concentration was chosen because it has a viscosity that is neither too liquid nor too thick, so it can be suitable for its use as a tissue adhesive in wounds in the intestine. The gel used as a wound dressing should have a viscosity that is neither too liquid nor thick so that it can be easily applied (Sularsih, 2013). In addition, the selection of 2% chitosan concentration is expected to provide a good antibacterial effect (Damayanti et al., 2016); 2% chitosan has an optimal ability to form gels and has good antibacterial ability.

3.2. Optimization of the mixing method

The method chosen was based on the viscosity and homogeneity of the hydrogel. Hydrogel with a viscosity that is not too liquid and homogeneous is predicted to improve the mechanical characteristics of the hydrogel. Based on the resulting hydrogel, mixing chitosan and carrageenan in the form of a solution, either mixed directly or dropwise, will produce an inhomogeneous hydrogel characterized by the presence of lumps, and the resulting hydrogel is too liquid. The formation of lumps in the mixture was predicted because, in the form of a solution, the carrageenan sulfate group with a negative charge had bound to cationic water, thus triggering the formation of lumps when added to the chitosan solution. Therefore, optimization was carried out with another method, namely by adding carrageenan in powder form to the chitosan solution.

Optimization of the mixing method by adding carrageenan in powder using a solution concentration of 2% chitosan and 2% carrageenan produces a liquid solution so that optimization is carried out by increasing the concentration of carrageenan to 3%, 4%, and 5%. The addition of carrageenan in powder form to the chitosan solution can form a homogeneous hydrogel because it is predicted that the sulfate group of carrageenan can directly bind to the amine group of chitosan to form a crosslink. Based on the characteristics of the hydrogel produced, the use of a concentration of 4% carrageenan powder was chosen to be mixed in a 2% chitosan solution to add Irgacure® 2959 and test its characteristics.

3.3. Optimization concentration of Irgacure® 2959

The addition of Irgacure® 2959 was carried out to strengthen the crosslinking of the hydrogel (Qi et al., 2013) so that the characteristics of the hydrogel produced would be better. Also, the use of Irgacure® 2959 in hydrogels applied in the medical field is due to its good ability to tolerate fibroblasts. Then Irgacure® 2959 was also successfully used in an in vitro condrocytes encapsulation experiment. Photopolymerization reactions are becoming a system that is increasingly being used in medical applications as tissue engineering. it was stated that Irgacure® 2959 is a material that has a tolerance with a wide susceptibility to various cell types and chemical concentrations (Williams et al., 2005). So, the addition of Irgacure® 2959 is appropriate for the manufacture of the hydrogel as a tissue adhesive. The addition of Irgacure® 2959 to the hydrogel causes a change in the viscosity of the hydrogel. The more Irgacure® 2959 is used, would make the more hydrogel become thicker. Based on the characteristics of the viscosity and homogeneity of the hydrogel. The selected concentration of Irgacure® 2959 is 1%, 2%, 3%, and 4%. Hydrogels with good homogeneity or well-mixed are predicted to form more regular bonds in hydrogels so that they can strengthen bonds and produce better characteristic values. Irgacure® 2959 which is active and exposed to UV light will abstract protons and produce free radicals, namely benzoyl and ketyl to form intermolecular covalent bonds. These bonds will form a hydrogel network which is a growth mechanism for the photopolymerization chain (Pei et al., 2018). The hydrogel, with the addition of the selected Irgacure® 2959 concentration, was then tested for its characteristics, including swelling ratio, water resistance, degree of crystallinity (XRD), simulation of adhesion, and antibacterial ability.

3.4. Chitosan-carrageenan hydrogel analysis and characterization

3.4.1. Swelling ratio

The swelling ratio test is presented in Figure 1. The hydrogel forms a three-dimensional macromolecular network able to absorb the water beyond its own volume (Barleany et al., 2020). Based on the results of statistical analysis, the addition of Irgacure® 2959 in the manufacture of hydrogels does not have a significant effect on the swelling ratio value. An increase in the concentration of Irgacure® 2959 will cause the swelling ratio value to decrease due to an increase in intermolecular forces that strengthen crosslink (Qi et al., 2013). The higher the crosslinking that occurs, the denser the intermolecular walls and the lower the swelling ratio value (Cui et al., 2014). The higher the crosslinking, the denser the network and the smaller the pores formed so that the volume of solvent that can occupy the cavities and hydrogel can be used to swell less so that the swelling ratio value is small. However, in this study, the swelling ratio value did not decrease as the concentration of Irgacure® 2959 was added. This is because it is predicted that there will be a steric hindrance that occurs due to the size of the carrageenan molecule that is too large (Sedayu et al, 2019) so that it inhibits the formation of crosslinks in the hydrogel and does not provide a significant difference to the swelling ratio value.

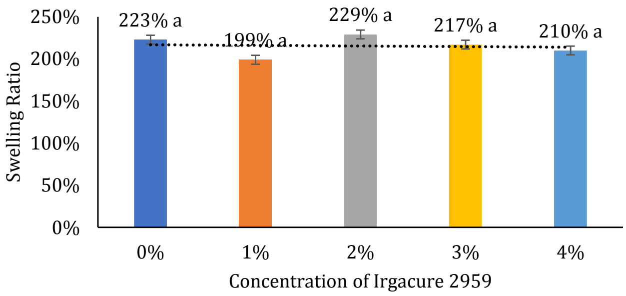

Figure 1 Swelling ratio test

<u>3.4.2. Water resistance</u>

The water resistance test is presented in Figure 2. Based on the results that have been statistically analyzed, the addition of Irgacure® 2959 to the manufacture of chitosan-carrageenan hydrogel did not give a significant difference to water resistance values. The water resistance of hydrogels is influenced by the bonds formed on the hydrogels. The addition of Irgacure® 2959 can cause the formation of crosslinks in the hydrogel (Qi et al., 2013). The crosslinks formed will make the intermolecular cavities smaller so that the volume of the cavities that can be occupied by the solvent will be smaller and cause the value of water resistance to increase. However, in this study, the increase of Irgacure® 2959 did not increase the water resistance value, which was predicted to occur due to the presence of steric hindrance in the hydrogel due to the large difference in the molecular size of the polymer used. The steric hindrance causes no increase in crosslinking that occurs in the hydrogel as the concentration of Irgacure® 2959 is added so that the water resistance value of the hydrogel produced is not significantly different.

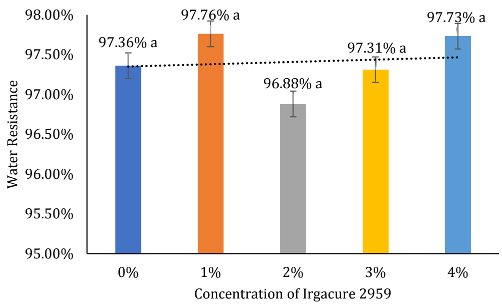

Figure 2 Water resistance test

<u>3.4.3. Adhesion simulation on smoked beef</u>

Hydrogel adhesion simulation was carried out by testing using smoked beef (Ono et al., 2000). The results showed that the increase of concentration of Irgacure® 2959 added to the manufacture of hydrogels did not give a significant difference when used to glue smoked beef. The hydrogel used to glue smoked beef produces a weak adhesion. The simulation shows that the resulting hydrogel has not shown the desired results because, qualitatively, the hydrogel is considered not strong enough to bind two tissues together. Qualitatively, the smoked beef that has been applied with hydrogel already feels attached to each other but is not too strong and is still easy to separate. This is in line with the results of the swelling and water resistance of the chitosan-carrageenan hydrogel, which showed no significant difference with the increase in the concentration of Irgacure® 2959 added.

3.4.4. Degree of crystallinity (X-Ray Diffraction)

Degree of crystallinity test of the hydrogel was carried out using X-Ray Diffraction (XRD), which aims to determine the crystalline phase formed and changes in the crystal structure that occur in the chitosan-carrageenan hydrogel. The test is carried out with X-Ray Diffraction and then connected to an interface on a computer to find out the results obtained (Kurniawan & Setiyorini, 2014). The results of the XRD test are in the form of crystallinity and amorphous value data that compose the test sample (Warsiki et al., 2020). Based on the results of the degree of crystallinity test, which showed changes in the crystal structure, it could be seen that the bonding occurred in the chitosan-carrageenan hydrogel. XRD test results can be seen in Figure 3.

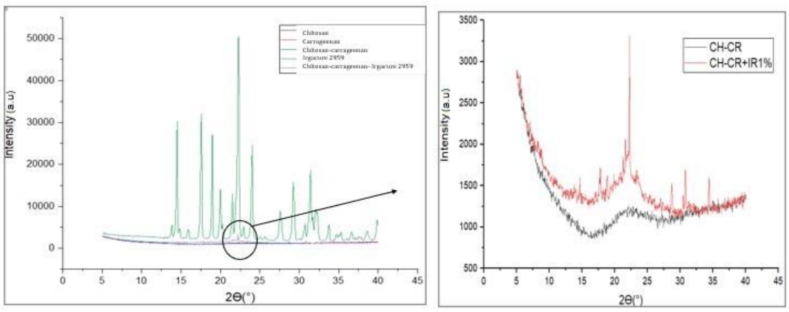

Figure 3 Hydrogels X-Ray Diffraction result

Based on the XRD results, chitosan does not have a diffraction peak or can be referred to as amorphous according to the earlier research (Nugroho et al., 2011), which shows that there is no diffraction peak in chitosan because it is predicted that the chitosan has dissolved well. The XRD results of carrageenan also show that there is no diffraction peak as in the study because the carrageenan polymer is non-crystalline material, so it does not produce a diffraction peak (Ulfah & Nugraha, 2014). The diffractogram of the chitosan-carrageenan hydrogel sample also showed no peak appearance because both materials were non-crystalline materials. XRD test results on Irgacure® 2959 showed a diffraction peak with a degree of crystallinity of 63.39%. Then the chitosan-carrageenan hydrogel with the addition of 1% Irgacure® 2959 was chosen because it has the lowest swelling and the highest water resistance value showing diffraction peaks with a degree of crystallinity of 25.84%. The appearance of diffraction peaks on XRD results of chitosan-carrageenan hydrogel with the addition of 1% Irgacure® 2959 indicates that there are crosslinks formed but in small amounts. These peaks can be formed, indicating the level of crystallinity due to changes in the bonds between polymers due to the addition of Irgacure® 2959. Based on the data, it shows a change in the bond that occurs so that the resulting test value changes even though it is not significantly different.

3.4.5. Antibacterial activity

Antibacterial test was carried out to determine the ability of chitosan-carrageenan hydrogel to inhibit bacterial growth. Wounds on the body are susceptible to infection by bacteria that can hinder the wound-healing process, so tissue adhesive is needed that can inhibit bacterial growth (Kurniawaty & Putranta, 2019). The ability to inhibit bacteria can be seen from the resulting clear zone, which is formed to show the effectiveness of chitosan in inhibiting bacterial growth (Amanda et al., 2020). The results of the ability chitosan-carrageenan hydrogel test with the addition of Irgacure® 2959 to inhibit bacterial growth can be seen in Figure 4. In the test, the negative control used nutrient broth solution, and the positive control used 2% chitosan solution.

Table 2 Inhibition diameter zone

Concentration of Hydrogel	Control -	Control +	0%	1%	2%	3%	4%
Clear Zone (cm)	0.00	1.33	1.33	1.17	1.00	1.00	0.83

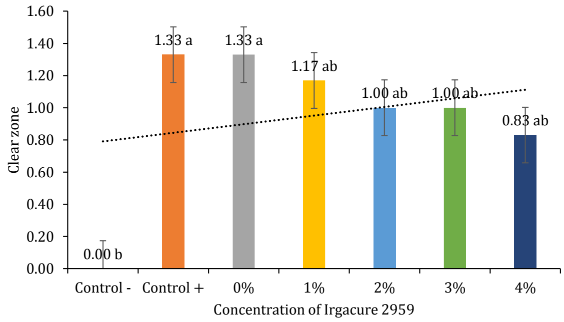

Figure 4 Antibacterial activity test

The clear zone formed in NA is included in the weak category in inhibiting bacterial growth (Surjowardojo et al., 2015). Based on the results of statistical analysis, the addition of Irgacure® 2959 to the hydrogel was not significantly different from the hydrogel without the addition of Irgacure® 2959. Although not significantly different, increasing the concentration of Irgacure® 2959 used is known to decrease the hydrogel's ability to inhibit bacteria. The ability to inhibit bacteria in hydrogels comes from the polycationic amine group of chitosan, which will interact with the bacterial cell wall so it can disrupt the metabolism of bacteria and inhibit their growth (Nurainy et al., 2008). The non-formation of these bonds causes the number of amine groups to remain unchanged so that the chitosan-carrageenan hydrogel has the same ability as the chitosan solution to inhibit bacterial growth. While the chitosan-carrageenan hydrogel with the addition of Irgacure® 2959 decreased the ability to inhibit bacteria due to the amine group in chitosan partially bound to Irgacure® 2959.

The absence of significant differences in the test results for each parameter is thought to be due to the presence of steric hindrance formed due to the large difference in molecular size between chitosan and carrageenan, making it difficult for interactions to occur (Chang et al., 2018). The use of a higher ratio of carrageenan resulted in more molecules of carrageenan sulfate groups, thus inhibiting the formation of bonds in the hydrogel, and resulting in less crosslinking, so that the hydrogel density distance did not change and resulted in the degree of swelling, which was not significantly different from the control. The steric hindrance in the hydrogel causes a cavity to form due to the bond does not change so the water resistance of the hydrogel and the ability of the cavity in the hydrogel to store the solvent to be used for the development of the hydrogel was not significantly different for each hydrogel with the addition of Irgacure® 2959.

The changes in test values in the test of the characteristics of the chitosan-carrageenan hydrogel with the addition of Irgacure® 2959 can occur due to changes in the bonds that occur in chitosan and Irgacure® 2959. This change in bonding is indicated by the XRD results of the chitosan-carrageenan hydrogel with the addition of Irgacure® 2959 as much as 1%, which changes by showing the presence of a distraction peak or the presence of crystalline in the hydrogel is different from the chitosan-carrageenan hydrogel. This change was supported by the antibacterial test results on the hydrogel, which showed a decrease in the value of the chitosan-carrageenan hydrogel with the addition of Irgacure® 2959. This decrease in antibacterial ability was due to the free amine group in chitosan, which played a role in attacking cell membranes in bacteria due to its binding to Irgacure® 2959. The amine groups that attack the protein on the bacterial cell membrane are the same, causing the antibacterial properties of the chitosan solution and the chitosan-carrageenan hydrogel to have the same value. The glueing ability which was assessed qualitatively from the chitosan-carrageenan hydrogel with the addition of Irgacure® 2959, showed the same results in each treatment, which means that the results of the quantitative test on the hydrogel showed no significant difference. It is necessary to optimize the chitosan-carrageenan ratio with a higher amount of chitosan or reduce the amount of carrageenan used so that the sulfate group molecules no longer inhibit the formation of bonds so that cross-links can occur and form stronger bonds.

Based on the research that has been done, it is known that the addition of Irgacure® 2959 to the manufacture of chitosan-carrageenan-based hydrogel with a ratio of 1:2 using the photo-crosslinking method has not shown good potential as a tissue adhesive based on the results of characteristic tests. It is necessary to optimize the ratio to eliminate steric barriers in the hydrogel so that it can produce cross-links that will improve the water resistance properties and degree of swelling of the hydrogel. The application of tissue adhesive hydrogel to be used in the intestine must have good water resistance because the location of the intestine is in an environment that is always wet to minimize damage and failure during the process of glueing the tissue. The degree of swelling is an important indicator to determine the success of the treatment procedure. Hydrogels are designed to help reconstruct damaged tissues, so the increased volume will contribute to hemostasis without disturbing surrounding tissues. Further, it is necessary to optimize the ratio of the chitosan and kappa-carrageenan to get the balance ratio that supports the occurrence of cross-linking so that it can help determine which hydrogel is good to use as a tissue adhesive, especially to be applied to the colon.

4. Conclusions

The Irgacure® 2959 concentration that added to the chitosan-carrageenan-based hydrogel did not significantly affect the values for swelling ratio, water resistance, simulation of adhesion, and antibacterial activity. This is predicted because there is steric hindrance due to differences in the size of the chitosan and carrageenan molecules, thus inhibiting the formation of crosslinks in the hydrogel. Further research is needed to obtain hydrogel results that can be used as tissue sealants.

Acknowledgments

This paper was funded by the Lecturer Research Grant of Agriculture Faculty 2021, Universitas Gadjah Mada so this research that became part of the first author's thesis could be carried out properly.

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