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Optimization of Irgacure® 2959 Concentration as Photo-Initiator on Chitosan-Alginate Based Hydrogel for Colon Tissue Sealant

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Abstract. Tissue sealant is a material that is used as an adhesive to bond two tissue surfaces used during surgery. Chitosan and alginate are natural macromolecules used to manufacture hydrogels as tissue sealants because these two materials can strengthen bonds and the mechanical properties of tissue sealants. Irgacure® 2959, as a photo-initiator, can strengthen the cross-linking between chitosan-alginate to produce a tissue sealant with good mechanical strength. Therefore, this study aims to determine the effect of adding Irgacure® 2959 to chitosan-alginate hydrogel with different concentrations as a tissue sealant designed for the colon. The experimental design used a completely randomized design (CRD) with the addition of Irgacure® 2959 0; 1; 2; 3; 4%. The characteristics of the hydrogel as a tissue covering observed in this study include the degree of swelling, water resistance, simulated adhesion, degree of crystallinity, and ability to inhibit bacteria. The results of this study showed that the addition of 4% Irgacure® 2959 reduced the degree of swelling, increased the hydrogel's resistance to water, and increased adhesion. The interaction of chitosan and alginate with the addition of Irgacure® 2959 produces cross-links, as evidenced by a degree of crystallinity of 26.21%. Based on these results, the chitosan-alginate hydrogel with the addition of Irgacure® 2959 showed good potential in tissue sealant formulations, even though the inhibition of bacteria in all treatments showed low results, namely < 5 mm.

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

1. Introduction

Chitosan is a polymer with a polycationic linear chain consisting of one primary amine and two free hydroxyl groups (Bakshi et al., 2020). Chitosan is used in the biomedical field in the form of a hydrogel, which can be used as a tissue sealant to combine tissue surfaces during the operation process (Jarret et al., 2013). Hydrogels are hydrophilic and can absorb and release water. These properties make hydrogels usable as sealants because they have a moist environment, are flexible, have mechanical muscle strength, and are non-toxic, so they have the potential to be applied in the large intestine (Ehterami et al., 2019). Hydrophilic properties of hydrogels can influence cellular attachment between two surfaces (Dewi et al., 2020). Tissue sealants are more advantageous than sutures and

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clamps because the wound closure process becomes more accessible, faster, and less painful to minimize problems during the application and removal of sutures (Nayeb et al., 2011).

Chitosan is one of the biomacromolecules utilized. Chitosan has biocompatible, biodegradable properties, good tensile strength properties, is easily modified when applied to physiological pH, can act as an adhesive, is antibacterial, can help heal wounds quickly, and is non-toxic. As a result, it is suitable for use as a material in the production of hydrogels (Asadpour et al., 2020; Bagher et al., 2020; Bektas et al., 2020). Chitosan requires a crosslinker to increase its cross-linking strength so that the resulting hydrogel has strong adhesion and is not easily brittle. Alginate can play the role of a crosslinker because it has good biocompatibility, low toxicity, and can be modified easily via photocrosslinking (Lee & Mooney, 2012). According to Wang et al. (2017), who tried to make hydrogels from chitosan-alginate by physically cross-linking methods applied to olfactory nerve cells, which shows that the hydrogels were able to stimulate cell proliferation. Based on this study, chitosan-alginate can be used as a tissue sealant.

The hydrogel can use both materials in the form of chitosan-alginate to make hydrogels through the photocrosslinking method. Making hydrogels using the photocrosslinking method requires a photo-initiator. These compounds can form good mechanical bonds, maintain hydrogel properties in the long term, obtain uniform properties, and help form pores as a place for cell attachment (Qi et al., 2013). Irgacure® 2959 is the most commonly used photo-initiator because of its low cytotoxicity and possible immunogenicity, making it safe to make hydrogels as tissue sealants (Tomal et al., 2020). The added concentration of Irgacure® 2959 affects the cross-linking between polymers and the mechanical properties of the hydrogel as a tissue sealant. Therefore, this study aims to determine the effect of the addition of Irgacure® 2959 on the characteristics of the chitosan-alginate hydrogel. Its parts will be observed so that it can assess its potential as a tissue covering designed for the large intestine.

2. Methods

2.1. Hydrogel Preparation

The chitosan comes from Bio Chitosan Indonesia with a DD 98%, alginate from Leyoung Int, and Irgacure® 2959 from Jinan Huijinchuan Chemical Co., Ltd). The manufacture of the hydrogel begins with the manufacture of a 2% chitosan solution, which refers to the research of Baysal et al. (2013) with modifications. 2 grams of powdered chitosan were dissolved in a 0.5% (v/v) acetic acid solution. Stirred the solution using a hot plate stirrer (Arec Heating Magnetic Stirrer), at a speed of 400 rpm for 1 hour at a temperature of 40-45°C. Furthermore, making a 2% alginate solution refers to the research of Bagher et al. (2020) with modifications. 2 grams of powdered alginate were dissolved in 100 ml of distilled water. Stirred the alginate solution using a hot plate stirrer at a speed of 400 rpm for 1 hour at a temperature of 40-45°C. The selection of alginate chitosan concentration was carried out through a preliminary study by observing the viscosity of the solution visually. According to Sularsih (2013), when used as a wound healer in a chitosan solution thick enough to facilitate application, its mucoadhesive properties are expected to be better for wound healing and closure. The choice of 2% alginate solution is because, according to Chandramouli et al. (2004), the use of alginate concentrations of more than 2% is not possible to obtain homogeneous results due to an increase in solution viscosity and mass diffusivity, and higher alginate concentrations give heavier and thicker membrane results. Still, the porosity, strength, and elongation are decreasing.

2.2. Optimization of Chitosan-Alginate Solution Mixing Method and Concentration of Irgacure® 2959

Mixing chitosan and alginate solutions refers to the research of Baysal et al. (2013) with modifications. Chitosan solution and alginate solution were mixed in a 1:1 ratio. The selection of hydrogel from the mixing method is chosen based on its homogeneity. At this stage, optimization is carried out with five different methods, namely as follows:

a. 2% chitosan solution plus 2% alginate solution added dropwise at room temperature
b. 2% chitosan solution plus 2% alginate powder at 50°C
c. 2% chitosan solution at 40°C plus 2% alginate solution at room temperature directly
d. 2% chitosan solution plus 1% alginate solution directly at a temperature of 40-45°C
e. 2% chitosan solution plus 2% alginate solution directly at a temperature of 40-45°C

2.3. Method of Mixing Irgacure® 2959 into Chitosan-Alginate Solution

Optimization of the Irgacure® 2959 addition method was carried out in 2 ways: directly and added 30 minutes after the chitosan-alginate solution was mixed. Furthermore, optimization of the concentration of Irgacure® 2959 was carried out referring to the research by Qi et al. (2013) and study by Han et al. (2020), used concentrations of 0.5%, 0.75%, 1%, 1.25%, 2%, 3%, 4%, 5%, and 6%. The addition of Irgacure® 2959 was added in powder form to the chitosan-alginate solution. The choice of concentration used is based on the homogeneity of the hydrogel and its suitability for application as a tissue sealant.

After doing all the optimization, the next step is to make chitosan-alginate hydrogel with the addition of Irgacure® *2959*. The scheme for the illustration of the preparation of the composite can be seen in Figure 1.

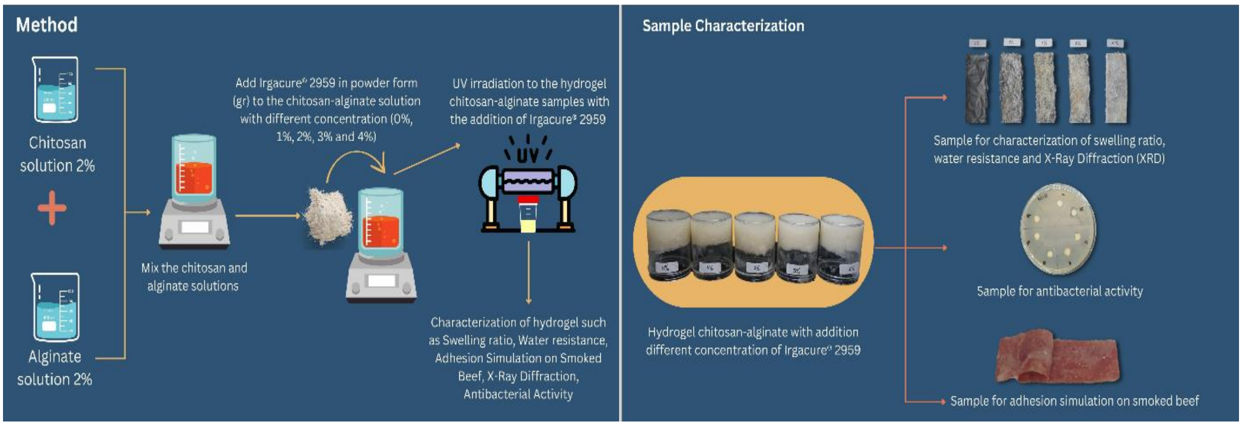

Figure 1 Illustration of the preparation of the composite

2.4. Analysis and Characterization of Hydrogels as Tissue Sealant

2.4.1. Swelling ratio

The swelling test refers to the research of Khan et al. (2020), which was modified. The swelling test was carried out using a 1x1 cm dried hydrogel sample that was immersed in 10 ml of phosphate buffer saline (PBS) solution at 37°C at 130 rpm for 24 hours on a water bath shaker. The water still on the surface of the hydrogel was removed using a tissue. The swelling ratio is calculated as the difference between the final weight after immersion and the initial weight, divided by the final weight of immersion, and multiplied by 100%.

$$Swelling\ (\%) = \frac{Wd - ws}{wd}\ x\ 100\% \quad (1)$$

Where Wd is the sample was weighed, and Ws is the initial weight

2.4.2. Water resistance

The water resistance test refers to the research of Sumarni et al. (2017). The sample used is a dry hydrogel measuring 1 x 1 cm. Then, put the sample into a beaker containing 10 ml of distilled water for 1 minute. After 1 minute, remove the sample and wipe away any adhering water with a tissue. Then repeat the process until you reach a constant weight. Then the percentage of water resistance is calculated as 100% minus the difference in the final weight with the initial weight divided by the initial weight multiplied by 100%.

$$Water\ Resistance\ (\%) = 100\% - \left(\frac{W - W0}{W0}\right) X\ 100\% \quad (2)$$

Where W0 is the initial weight of the sample, and W has finally weighed the sample

2.4.3. Adhesion simulation on smoked beef

This test was conducted using smoked beef media, which refers to the research of Ono et al. (2000). The adhesive simulation test is done by preparing two pieces of smoked beef measuring 2x5 cm with a thickness of 2mm. The created hydrogel is then applied to one part of the smoked beef and glued together. After that, the smoked beef is stored in the refrigerator for three days. The results are observed by opening the two smoked beef that have been glued together and then qualitatively observing whether the two smoked beef are glued together or not.

2.4.4. X-ray Diffraction (XRD)

The test was carried out by cutting the sample of 1.9x1.4 cm with a thickness of 1mm according to the size of the sample holder. Sample pieces are placed on the sample holder. The sample holder is then placed on the XRD tool for analysis. The analysis took six minutes using an X-ray diffraction device. The results will appear on the monitor screen with readings ranging from 5° to 40°. After obtaining the peak data, the percentage of sample crystallinity was calculated by dividing the area of the crystalline fraction by the area of the crystalline fraction plus the area of the amorphous fraction multiplied by 100. The formula for calculating the percent crystallinity is as follows:

$$\text{Crystallinity (\%)} = \frac{Area\ of\ crystalline\ fraction}{Area\ of\ crystalline\ fraction + area\ of\ amorphous\ fraction}\ x\ 100 \quad (3)$$

2.4.5. Antibacterial activity

The procedure for testing film samples was modified using the paper disc diffusion method on escherichia coli bacteria. Antibacterial testing using the disc method refers to the research of Mahdavinia et al. (2019) with modifications. Escherichia coli bacteria were grown on NB medium, and the density was measured through OD testing using a spectrophotometer. The bacterial suspension was taken in amounts as much as 0.1 ml and put into a petri dish that already contained a solid NA medium. The paper dish that will be used is immersed in the sample in each treatment for 5 minutes, then put into a petri dish. After that, the media filled with bacterial suspension is incubated for 24 hours at 37°C, then the inhibition zone formed is measured using a ruler. The diameter of the inhibition zone included was calculated as the difference from the diameter of the paper disk.

3. Results and Discussion

3.1. Optimization of The Method of Mixing Chitosan Solution and Alginate Solution

Mixing chitosan and alginate solutions was carried out to obtain a homogeneous solution. The mixing method's optimization results are given in Table 1**.** Based on the optimization results, the more plus signs indicate the thicker the solution obtained, and the more lumps, precipitates, fibers formed, and smoother the texture, so the method chosen for the next process is the one that has the fewest and most homogeneous fibers, namely the method with code E using a 2% chitosan solution plus a 2% alginate solution directly at a temperature of 40-45°C.

Table 1 Mixing Method of Chitosan and Alginate

Code	Method				Physical Observation of Solution			
	Chitosan (%)	Alginate (%)	Material condition	Temperature	Viscosity	Lumps	Fiber	Texture
A	2	2	S+S	27°C	++	+++	+++	+
B	2	2	S+P	50°C	+	+++	+	+
C	2	2	S+S	40°C 27°C	++	++	++	+
D	2	1	S+S	40°C-45°C	++	++	++	++
E	2	2	S+S	40°C-45°C	+++	+	+	+++

Information: +: low; ++: medium; +++: high; S: Solution; P: Powder

3.2. Optimization of Mixing Method and Concentration of Irgacure® 2959

Table 2 Irgacure® 2959 mixing method

Method	Physical Observation of Solution
Added directly	The solution is not mixed, the texture is rough, and there are clumps of fibre
Added after the chitosan-alginate solution was stirred for 30 minutes	The solution is well mixed; the texture is smooth and compact

The method of mixing Irgacure® 2959 into the chitosan-alginate hydrogel was carried out in two ways: directly added and added 30 minutes after the chitosan-alginate solution was stirred. The results of mixing are given in Table 2. Based on these results, the method of adding Irgacure® 2959 was chosen by adding it after the chitosan-alginate solution was stirred for 30 minutes. The choice was based on the fact that when Irgacure® 2959 was directly added together with chitosan-alginate, it could cause its bonding with alginate and chitosan to become less regular, resulting in an inhomogeneous solution, while the addition of Irgacure® 2959 30 minutes after the chitosan-alginate was mixed could provide space for the chitosan and alginate to be mixed beforehand, which made the bond between the chitosan-alginate and the Irgacure® 2959 more regular and more homogeneous.

3.3 Chitosan-Alginate Hydrogel Analysis and Characterization

3.3.1. The effect of variations in the concentration of Irgacure® 2959 on the swelling ratio

Based on the study's results, the addition of Irgacure® 2959 caused a lower degree of development. This follows the research of Qi et al. (2013) that the addition of Irgacure® 2959 at higher concentrations causes the degree of swelling to be lower due to an increase in cross-linking, which can cause the cross-linking to become stronger so that the structure will be tighter, and less water will enter. The percentage of development produced from chitosan-alginate hydrogel with the addition of Irgacure® 2959 as a photo-initiator is given in Figure 2.

Based on these results, this composite has potential as a tissue sealant because, Mukherjee et al. (2018), in its application as a wound healer, state that low swelling is needed to heal wounds because hydrogels with too high swelling can inhibit the rate of healing, and too much fluid absorption that occurs can cause dehydration in the wound, thereby slowing down the wound healing process. And hydrogels with good hydrophilicity can provide soft physical properties when applied to living tissue (Barleany et al., 2019).

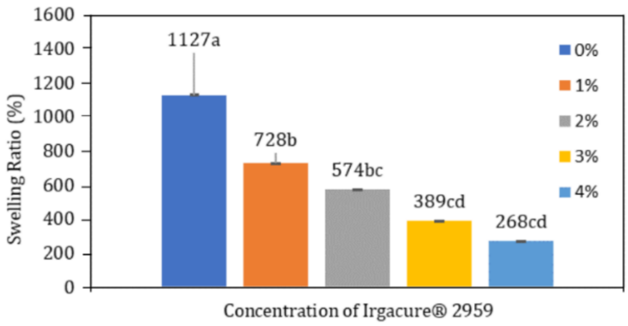

Figure 2 Swelling ratio of chitosan-alginate hydrogel with the addition of Irgacure® 2959 at different concentrations

3.3.2. The effect of variations in the concentration of Irgacure® 2959 on hydrogel resistance to water

The adhesive's resistance to water makes the tissue sealant not easily crushable or detached when attached to surfaces with a lot of water content, such as intestines. The addition of Irgacure® 2959 with a higher concentration causes its ability to bind water to increase. These results indicate that the addition of Irgacure® 2959 significantly affects the water resistance of the chitosan-alginate hydrogel as a tissue sealant. According to Rudyardjo (2014), when there is no addition of Irgacure® 2959 as a photo-initiator, the O-H group possessed by chitosan will bind less, so that its water resistance will decrease. On the other hand, with the addition of Irgacure® 2959, the O-H group will bind more and more, so its ability to hold water will increase. The average value of hydrogel resistance to water are given in Figure 3.

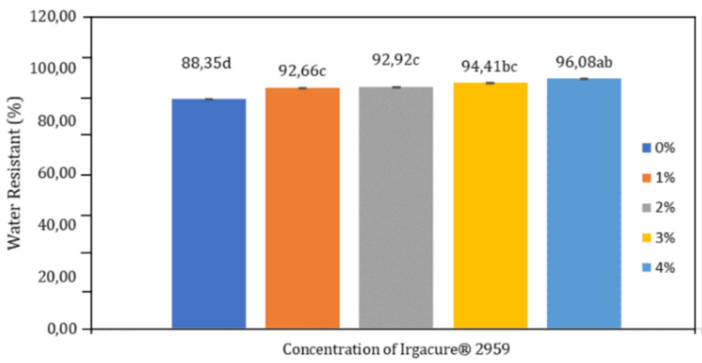

Figure 3 Percentage of hydrogel resistance to water

Based on the findings, this composite has the potential to be used as a tissue sealant due to its high-water resistance characteristics. This result is suitable as a tissue sealant. According to Briawan et al. (2011) the body contains 75% water and 25% solid matter, so a tissue sealant that has an elevated level of water resistance is needed so that when it is used as a tissue sealant in the intestine, it is not easily brittle but can still help the process of attachment and cell growth.

3.3.3. The effect of variations in the concentration of Irgacure® 2959 on the simulation results of adhesion to smoked beef

Adhesion simulation was carried out using smoked beef media because its structure is like tissue. Adhesion simulation was carried out to qualitatively determine the adhesive strength of chitosan-alginate hydrogel with the addition of Irgacure® 2959. The results of the simulation of adhesion can be seen in Table 3. The simulation results indicate that the higher addition of Irgacure® 2959 means that smoked beef can stick together. Based on Table 3, the more signs the smoked beef has, the stronger the bond and the more difficult it is to separate. In connection with these results, the addition of Irgacure® 2959 at a higher concentration can produce hydrogels with more potent adhesive abilities.

Table 3 Simulation results of adhesion with variations of Irgacure® 2959 with qualitative methods

Concentration of Irgacure® 2959	Adhesive Strength Level	Description
0%	+	Smoked beef can stick to each other, but when you take it off, it's still easy to separate
1%	++	Smoked beef can stick to each other, but when you take it off, it's still easy to separate
2%	+++	Smoked beef can stick to each other, but when you take it off, it's getting a little difficult to separate
3%	++++	Smoked beef can stick to each other, but when you take it off, it's hard to separate
4%	+++++	Smoked beef can stick to each other, but when you take it off, it's hard to separate

Information: +: less sticky; ++: slightly sticky; +++: a bit sticky; ++++: paste; +++++: very sticky

3.3.4. The effect of variations in the concentration of Irgacure® 2959 on the degree of crystallinity (XRD)

XRD is used to identify crystallites in materials using X-ray diffraction radiation to identify crystallite structures that appear as sharp or amorphous peaks (Hakim et al., 2019). The results of the XRD graph can be seen in Figure 4. Based on Figure 4, the graphic pattern of chitosan and alginate does not have a crystal peak, indicating that chitosan and alginate are amorphous materials. Furthermore, the chitosan-alginate graph pattern shows amorphous results with comprehensive graphs without clear crystal peaks. In contrast, the Irgacure® 2959 graph pattern shows the presence of crystallinity indicated by sharp peaks, and based on calculations, Irgacure® 2959 has a crystallinity percent of 63.39%. The addition of Irgacure® 2959 to chitosan-alginate showed a crystal peak seen at a sharp peak, indicating that the presence of Irgacure® 2959 caused cross-linking between chitosan and alginate. The percentage of crystallinity obtained after the calculation is 26.21%.

This value is lower than Irgacure® 2959 because of the cross-linking that occurs with chitosan-alginate. The percent crystallinity was obtained by calculating the area fraction of the crystalline material divided by the total area of the amorphous and crystalline materials. The results of mixing chitosan-alginate hydrogel with Irgacure® 2959 decreased crystallinity, indicating that there had been an interaction between chitosan-alginate and Irgacure® 2959, which was supported by the research of Cervera et al. (2004), which states that the decrease in intensity can indicate that there is an interaction that occurs between chitosan-alginate. Based on the XRD results, the addition of Irgacure® 2959 to the chitosan-alginate hydrogel causes crystallinity to occur and indicates that there has been a bond between chitosan-alginate and Irgacure® 2959.

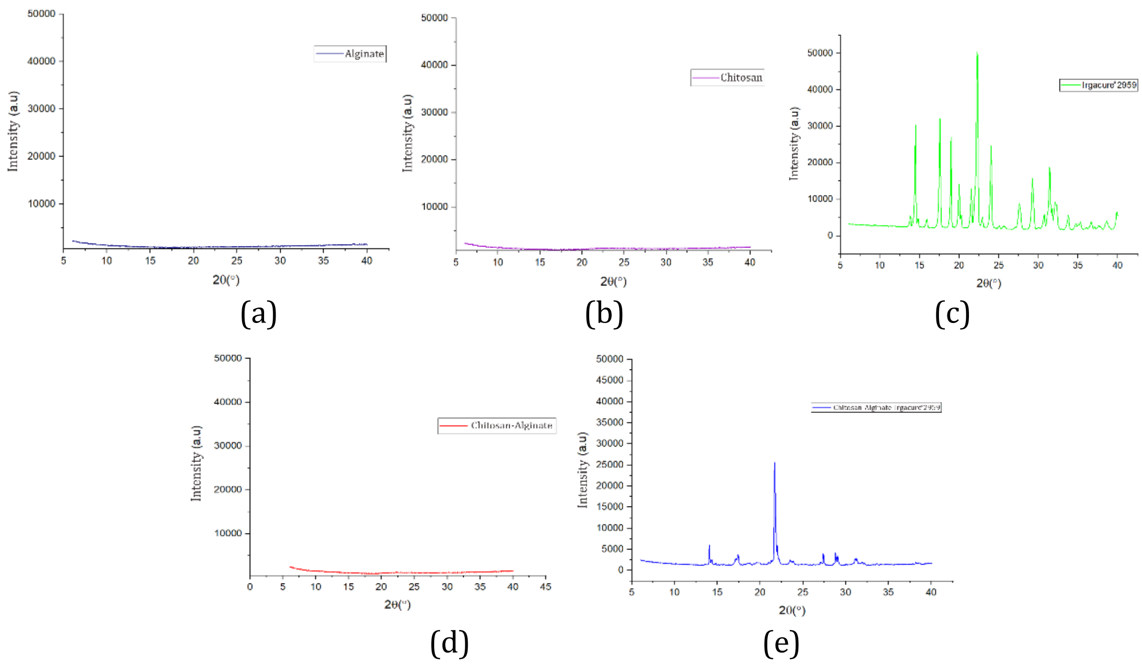

Figure 4 Graph of Crystallinity Degree (XRD) Pattern (a) Alginate; (b) Chitosan; (c) Irgacure® 2959; (d) Chitosan-Alginate; (e) Chitosan-Alginate-Irgacure® 2959

3.3.5. Antibacterial activity testing aims to determine the ability of the hydrogel to inhibit bacterial growth.

Characteristics of antibacterial activity were carried out only to find out whether the hydrogel still had antibacterial properties derived from chitosan after being mixed with alginate and Irgacure® 2959. According to Surjowardojo et al. (2015), the diameter of the inhibition zone formed at 5mm falls into the weak category. The results of the inhibition zone with the addition of Irgacure® 2959 treatment ranged from 0% to 4%, were included in the weak category because of < 5 mm. The mean value of the diameter of the bacterial inhibition zone on the chitosan-alginate hydrogel with the addition of Irgacure® 2959 is presented in Table 4.

Table 4 Average zone of inhibition formed on *Escherichia coli* bacteria

The concentration of Irgacure® 2959	Inhibitor Zone (mm)
Control negative	0^{b}
Control positive	4^{a}
0%	1.7^{ab}
1%	1.3^{ab}
2%	1.3^{ab}
3%	1.7^{ab}
4%	1.7^{ab}

^{abc}at each concentration, for different letters showed statistically significant differences with a 95% confidence level (p-value <0.05)

These results suggest that the addition of Irgacure® 2959 influences the inhibitory ability of bacteria in chitosan-alginate gel used as a tissue sealant. Still, between treatments, Irgacure® 2959 with different concentrations did not produce a significantly different effect. This happens because chitosan is bound to alginate and Irgacure® 2959, so there is a reduction in the positive charge on the chitosan group. Thus, the electrostatic interaction with the negative amount on the bacterial cell wall decreases (Pasaribu, 2020). The NH_3^+ glucosamine content in chitosan, which has a positive charge, will interact with the negative charge-forming proteins on the bacterial cell membrane, which can cause damage to the outer cell membrane and leakage of intracellular constituents of the bacteria so that the bacteria will die (Nurainy et al., 2008).

4. Conclusions

The additional concentration of Irgacure® 2959 to the chitosan-alginate hydrogel can be used as a tissue sealant. Increasing the concentration of Irgacure® 2959 results in a lower swelling percentage and increases the hydrogel's resistance to water, so the hydrogel is not easily brittle. Based on the characteristics, the best concentration is Irgacure® 2959 4%.

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