

Original Article

**Polyvinyl Chloride (PVC)-Glycerol with Chitosan Addition  
for Antibacterial Blood Bag Application**

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**ABSTRACT**

Blood bag is a medical device that stores and transports whole blood or blood components. The material that is often used for blood bag membranes is Polyvinyl Chloride (PVC), however the common problem that is bacterial contamination and that material have not antibacterial characteristic. Because of this matter, the aim of this research are a blood bag that has antibacterial function is needed and meet the ideal standard as bloodbag. Chitosan as a blood bag membrane material fabrication to get the antibacterial effect. Chitosan is chosen as a blood bag material fabrication to get the antibacterial effect. Chitosan has several specific biocompatibility properties, antibacterial, chelation, and biodegradability. This study used various Chitosan concentrations of 1.5%, 2%, 2.5%, and 3%, and Glycerol was added as a plasticizer. The composition of Chitosan: Glycerol is 1:1. Then, the mixture is added to the PVC solution in a ratio of 1:5 then poured into a petri dish. The results showed characterization that the biocomposite PVC-Glycerol with the addition of 3% concentration of chitosan was the best composition, the tensile strength test result of biocomposite is 21.20 MPa, the absence of membrane pores in the morphology of the blood bag, the hemolytic activity is 0.24%, and the inhibition zones of *E. coli* and *S. aureus*, respectively 11.66 mm and 12.66 mm in diameter. Based on the characterization results, the biocomposite PVC-Glycerol membrane with the addition of Chitosan has a very high potential as a candidate for blood bag membranes.

**Keywords:** Antibacterial, Blood bag, Membrane, Plasticizer, Poly Vinyl Chloride

**Highlights:** Bacterial contamination occurs during the process of taking and processing blood that is less aseptic because the issue material blood bag must have antibacterial. The PVC-glycerol-chitosan composites can be good candidates for ideal blood bag membranes because meet the standards of mechanical, physical, and biological tests.

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## INTRODUCTION

Currently, bacterial contamination of blood products is still a serious problem because it has a risk of fatal transfusion with the risk of bacterial sepsis.<sup>1</sup> Many cases of bacterial contamination still occur because of the Thrombocyte Concentrate (TC) storage suitable for bacterial growth. The source of bacterial contamination is obtained from the process of taking and processing blood that is less aseptic. Bacterial contamination is still a serious problem in the world with the risk of bacterial sepsis.<sup>2,3</sup> It also becomes an important problem in Indonesia due to the limitations of bacterial detection tools almost in every blood donation unit (UDD). The source of contamination bacteria can come from donor skin less aseptic, donor bacteremia, and processing of blood products.<sup>3,4</sup> Besides that, the storage conditions of Thrombocyte Concentrate at 20-24°C, processing at porous bag with agitation process, as well as the addition of preservatives in TC storage pouch can be an energy source for bacteria growth of contaminant bacteria getting better.<sup>5</sup> Cases of bacterial contamination have a risk of infection through higher blood transfusion than viral infection. Studies previously showed that 9.2% of 196 blood products are known to be contaminated with Gram bacteria positive and Gram-negative bacteria.<sup>6</sup> Results identification shows that there are staphylococci, *Bacillus sp.*, *Pseudomonas*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* in products stored in the blood. More than 50% of bacteria detected in the product TC blood are Gram-positive bacteria that can cause a transfusion reaction while Gram bacterial contamination negative is usually less however in the case of Gram bacterial contamination negative has a transfusion risk of up to one death.<sup>6,7</sup>

Blood is a very important part of the human body. The main function of blood in the body is to transport oxygen and substances

needed in the body. Blood deficiency in humans causes several diseases such as anemia, thalassemia, leukemia, sepsis, hemophilia, and kidney failure.<sup>8</sup> The main treatment of blood deficiency disease is the administration of blood transfusions by maintaining a hemoglobin level above 10 g/ml.<sup>9</sup> Blood transfusion is the infusion of blood components from one individual (donor) to another individual (recipient). Blood transfusion requirements must have a match between donor red blood cell antigens and plasma antibodies or recipient serum so that no hemolytic reactions occur.<sup>10</sup> The high need for blood transfusion is directly proportional to the amount of blood bag needed. The average need for blood in the hospital reaches 100 bags of blood per day with a blood size of 350 cc.

Under normal conditions, the amount of blood supply needed is 300 bags<sup>11</sup>, from the number of blood bags found defects in several bags of blood. Blood bag defects in the form of contamination of the blood bag by its ingredients, so that the blood in it becomes improper to transfuse. In addition, the Indonesian Red Cross (PMI) in several cities for several years destroyed around 1,000 blood bags that were not suitable for use. Besides its reactive nature and blood from the donor is contaminated with bacteria. Bacterial contamination was observed in 18 (9.2%) of the blood and blood components, of which 14 (77.8%) and 4 (22.2%) were Gram-positive and Gram-negative bacteria, respectively. The physical properties of blood bag material and bacteria are the main factors that cannot be used for blood vessels. The blood bag must be transparent, not damaged when bent on a small radius, flexible, heat resistant during the sterilization process, not easily damaged during the centrifuge, economical, and handling.<sup>12</sup> Many blood bags on the market today are made of Polyvinyl Chloride (PVC) with a plasticizer mixture. However, there is much evidence that blood

bags are exposed to or contaminated with bacteria, so there is a need for antibacterial blood bags.<sup>13</sup> The antibacterial property of blood bags aims to minimize the spread of bacteria in blood so the diseases caused by bacteria in the blood can be reduced. Antibacterial effects found in blood vessels are expected to be integrated with Polyvinyl Chloride (PVC)-glycerol biocomposite so that the original nature of the blood bag formed is maintained. With the presence of an antibacterial blood bag, blood damage due to bacterial contamination in the blood can be reduced.

One of the natural ingredients containing antibacterial is chitosan biopolymer. Chitosan is a chemical compound derived from chitin.<sup>14</sup> The addition of chitosan to Polyvinyl Chloride (PVC)-glycerol is intended to have antibacterial properties in the blood bag, in addition, chitosan biopolymers have properties that are bioactive, biocompatible, hemostatic, and can be biodegradable.<sup>13,15</sup> This study will modify the blood bag made from PVC-glycerol by adding chitosan to increase the antibacterial properties of blood bags. Research on PVC-glycerol-chitosan composites has been carried out by several previous researchers<sup>12</sup> who synthesized the composite with a ratio of chitosan-glycerol 1:1 and varied the concentration of chitosan between (0.5–2) wt/v%. The study results showed the presence of antibacterial properties on the membrane. In addition, the increase in chitosan concentration was also followed by an increase in tensile strength, but its value did not meet the standard tensile strength used as a bag of blood. The research of Omer et al<sup>13</sup> The PVC with the addition of clove oil showed antibacterial activities against four different bacterial strains (two-Gram positive: *Staphylococcus aureus* and *Bacillus cereus* & two-Gram negative: *Pseudomonas aeruginosa* and *Escherichia coli*).

The addition of chitosan is to provide antimicrobial and good biocompatibility.<sup>16</sup> Chitosan showed an intrinsic antibacterial

activity, impeding bacteria and fungi growth.

As an example, in *Staphylococcus aureus* cultures, chitosan stimulate structural changes membrane–the wall complex leading to the impairment of surface cell structures and bacterial death.<sup>17</sup> The standard tensile strength in a blood bag using PVC material is 14-26 MPa.<sup>18</sup> This research will focus on increasing tensile strength and antibacterial values by increasing the concentration of chitosan on PVC-glycerol-chitosan composites because it can increase the tensile strength of a blood bag. Thus the purpose of this study is to obtain blood bags that have physical, mechanical, and biological properties in accordance with the blood bag standard. Based on empirical research data<sup>6</sup> it is predicted that the greater the concentration of chitosan in PVC-glycerol-chitosan composites will increase the antibacterial properties. In addition, it is predicted that it will also increase tensile strength. Microscopic and macroscopic observations to determine the behavior alteration of the composite material. Microscopic observation of blood bag material was carried out through the Fourier Transformed Infra-Red (FTIR) and Scanning Electron Microscopy (SEM) tests. The macroscopic observation will be carried out through a tensile test, while the biological test will be carried out through an anti-bacterial test. In addition to these tests, a hemolysis test was also carried out to determine the interaction of blood with blood bag material, especially the response of red blood cells to the material.

The aims of this study are: 1) To explore the effect of chitosan addition on the PVC-glycerol on tensile strength, functional cluster, superficial surface morphology and pore size, anti-bacterial ability, and blood bag hemolysis percentage. 2) To know the optimal concentration of chitosan for PVC- chitosan-glycerol blood bag.

## MATERIAL AND METHODS

### Materials



Control variables in this study Polyvinyl Chloride (PVC) Concentration The independent variable of this study is the addition of Chitosan concentration 0 wt/v%, 1.5 wt/v%, 2 wt/v%, 2.5 wt/v% and 3 wt/v%, and the dependent variable is the characteristics of PVC - Biocomposite glycerol - chitosan. The materials used in this study were Polyvinyl Chloride (PVC), Chitosan (0%, 1.0%, 2.0%, 2.5%, 3.0%), Glycerol, Acetic Acid, Tetrahydrofuran (THF), Aquades. The tools used are a digital balance sheet, glass beaker, micropipette, petri dish, measuring cup, spatula, weigh paper, and magnetic stirrer. The tool used to carry out the characterization is Tool 8400 Shimadzu FTIR for the FTIR test, Imada HV-500 NII Autograph to determine tensile strength, water bath (Gemmyco YCW) for hemolysis test, petri dish for the antibacterial test, and using spectrophotometric UV-VIS device (Shimadzu UV-1800).

## Methods

### Blood Bag Synthesis Procedures

The blood bag is made by mixing 10% PVC dissolved tetrahydrofuran (THF) using a magnetic stirrer. Antibacterial blood bags made from chitosan dissolved in 1% acetic acid five variations of the solution were made with different concentrations of chitosan. Blood bag membrane with chitosan concentration of 0 wt/v%, 1.5 wt/v%, 2 wt/v%, 2.5 wt/v%, and 3 wt/v% and 10% PVC with a ratio of 2:10 and solution glycerol with chitosan comparison 1: 1. The mixing process uses a magnetic stirrer.

### Functional Group Test

This test is used to analyze functional groups of organic and inorganic compounds. The test was performed using a tool called Fourier Transform Infrared (FTIR) Shimadzu, 8400S.

The samples identified can be either solid samples or samples. The peak value of the light that didn't get absorbed by the detector is then processed using a computer using the Fourier transform method that could be calculated using the following formula:

$$F(\omega) = \int_{-\infty}^{\infty} f(t)e^{-j\omega t} dt \quad (1)$$

With  $f(t)$  as the original signal in the time domain, it will be transformed into  $F(\omega)$ , a function in the frequency domain by a continuous integral function. From this transformation, it will produce a graph representing the chemical bonds in the compounds contained in the sample.<sup>19</sup>

### Morphology Test

The Morphology Test was carried out using Zeiss's scanning electron microscope (SEM). Samples were cut transversely, sputtered with gold-palladium, then observed under SEM.

### Hemolysis Test

The hemolysis test was performed using human blood that had been given an anti-coagulant, *Ethylene Diamine Tetraacetic Acid Dipotassium Salt (EDTA)*. Blood-EDTA mixture taken 200 U<sub>l</sub> was diluted using 10 Ml of saline with a concentration of 0.9% then inserted into a micro tube, each tube containing 200 ul as a negative control. Blood-EDTA 200 ul was diluted with 10 ml of distilled water, after which 200ul was taken as a positive control. The sample was inserted into a microtube containing blood with saline and then incubated for two hours using a water bath at normal temperatur (37°C).

### Antibacterial Test

The antibacterial test aims to determine the ability of the PVC-Glycerol-Chitosan biocomposite membrane and mangrove extract to inhibit bacterial growth.

This test was carried out using two bacteria, namely *S.aureus* bacteria representing gram-positive bacteria and *E.coli* representing gram-negative bacteria. The strength of antibacterial inhibition was classified as weak showing an inhibition zone of  $<5\text{mm}$ , said to be moderate showing an inhibition zone of 5-10 mm, said to be strong showing an inhibition zone of 10-20 mm and said to be very strong when showing an inhibition zone of more than 20 mm<sup>12</sup>.

### Tensile Test

The tensile test is a destructive engineering and materials science test whereby controlled tension is applied to a sample until it fully fails. This is one of the most common mechanical testing techniques. It is used to determine how strong a material

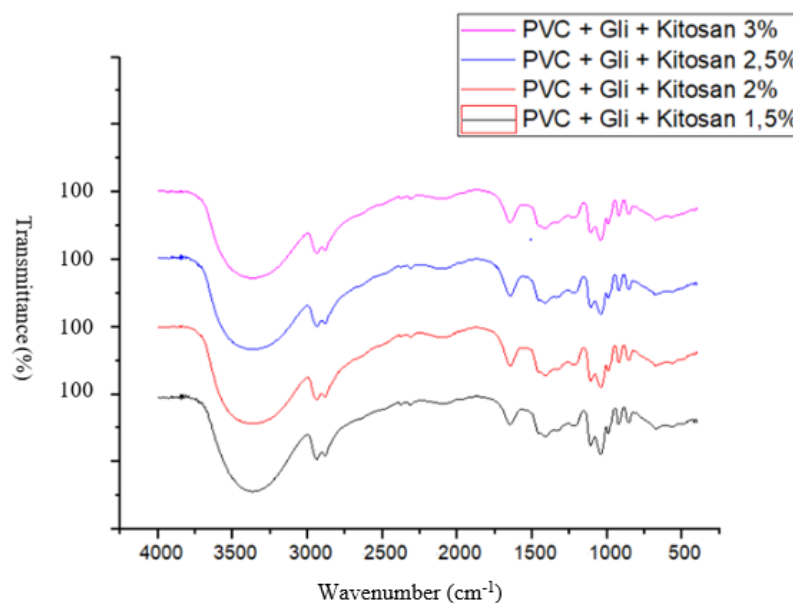
is and how much it can be stretched before it breaks.

The variable of the tensile test is carried out using a Shimadzu AGS-X tool using a tensile test frame of paper, with a gauge length of 10 mm. The sample is attached to the tool, then the frame is cut. Samples are drawn at a speed of 5 mm/minute (ASTM D 882–02).

## RESULTS AND DISCUSSION

### Fourier Transform Infrared (FTIR)

Characteristics of functional groups from PVC-glycerol biocomposite membrane samples with chitosan addition were analyzed using Fourier Transform Infrared (FTIR). The results of the FTIR spectrum of PVC-Glycerol biocomposite membrane samples with the addition of chitosan are shown in Figure 1.



**Figure 1.** Results of the FTIR Test

On the results of the FTIR characterization of PVC material, it is known that there is a C–H strain with a wave number of around 2970  $\text{cm}^{-1}$ . Typical absorption on PVC material appears at a wave number of 1425  $\text{cm}^{-1}$  which is the  $\text{CH}_2$  functional group. The C–H trans functional group is found at a wave number of around 960  $\text{cm}^{-1}$  and there is a C–Cl stretch with an absorption wave number of 682  $\text{cm}^{-1}$  on PVC material.<sup>13</sup> The FTIR spectrum of

glycerol material has a wave number of around 3390  $\text{cm}^{-1}$  indicating the presence of the –OH functional group. The absorption at wave number 2939  $\text{cm}^{-1}$  indicates the C–H functional group. the  $\text{CH}_2$  functional group is at the peak of wavenumber 1416  $\text{cm}^{-1}$  while the C–O functional group is also visible at the absorption wave number 1110–1043  $\text{cm}^{-1}$ .<sup>12</sup>

Test results In the FTIR spectrum of chitosan, there is an absorption at a wave



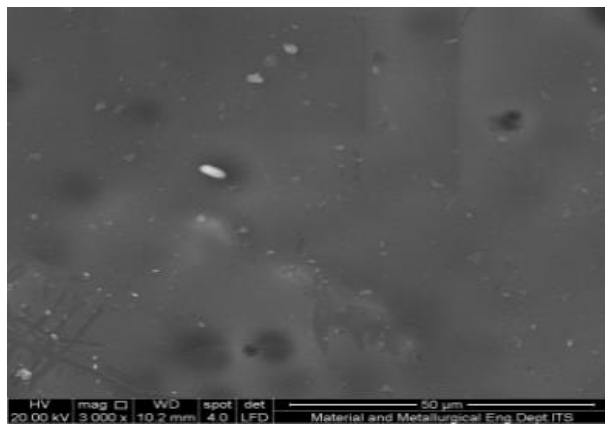
number of  $3433\text{ cm}^{-1}$ , indicating a stretch of the -OH functional group. Chitosan material has a typical absorption seen at the absorption wave number of  $1647\text{ cm}^{-1}$  indicating the N-H functional group of the amine ( $\text{NH}_2$ ) and the absorption wave number of around  $1151\text{ cm}^{-1}$  indicating the C-N functional group [20]. The FTIR test results for the PVC-Glycerol biocomposite membrane show the presence of an -OH group at a wave number of  $3381\text{ cm}^{-1}$ , a  $\text{CH}_2$  functional group at a wave number of  $1423\text{ cm}^{-1}$ , a C-H functional group at a wave number of  $2939\text{ cm}^{-1}$  and a C-O functional group at a wave number of  $2939\text{ cm}^{-1}$ . Wave number  $1111\text{-}1043\text{ cm}^{-1}$  contained in the glycerol material. The stretch function group C-Cl with a peak wave number of  $675\text{ cm}^{-1}$ , and the trans C-H functional group at a wave number of around  $960\text{ cm}^{-1}$  comes from PVC material. Membranes that have been tested FTIR showed absorption wave numbers that indicate the functional groups of PVC material, glycerol, and chitosan. In PVC material, it is known that there is a peak at the wave number of  $2973\text{ cm}^{-1}$  which indicates the C-H strain. Typical absorption on PVC material is found at the peak of the wave number of  $1413\text{ cm}^{-1}$  and  $675\text{ cm}^{-1}$  indicating the  $\text{CH}_2$  and C-Cl functional groups. The C-O group in the wave number with a range of  $1111\text{-}1043\text{ cm}^{-1}$  and the C-H functional group at the wave number  $2937\text{ cm}^{-1}$  are derived from glycerol material. The functional group with a peak at the wave number of  $3365\text{ cm}^{-1}$  indicates the -OH functional group. The amine functional group ( $\text{NH}_2$ ) which is owned by the chitosan material is shown by the absorption wave number of  $1647\text{ cm}^{-1}$ .<sup>20</sup>

The C-H trans functional group has a wave number of about  $993\text{ cm}^{-1}$  and there is a C-Cl stretch with an absorption wave number

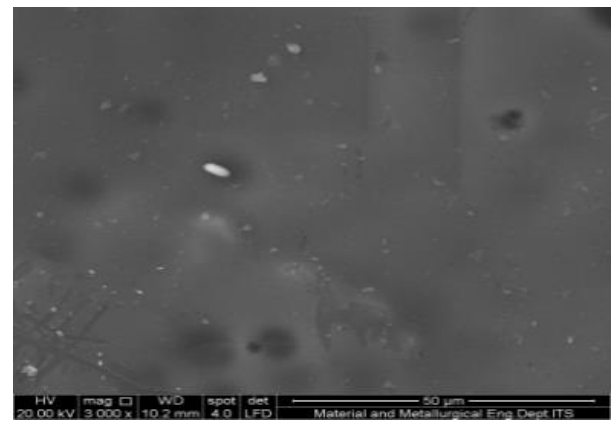
of  $675\text{ cm}^{-1}$  which comes from PVC material. In PVC-glycerol-chitosan biocomposite membranes, mixing between PVC material and chitosan material allows partial chain interactions or what is called dipole-dipole interaction between C-N bonds in chitosan and C-Cl bonds in PVC [20]. This kind of interaction may occur during the mixing process of the two solutions so that a mixture of PVC-chitosan has been obtained with several distributions between PVC and chitosan chains. The distribution of PVC and chitosan chains is influenced by the homogeneity of the solution, homogeneity or homogeneity is obtained in the process of mixing the two materials between the PVC solution and the chitosan solution that does not experience clumping and the mixing of the two solutions. The mixing of the two materials leads to the homogeneity of the solution.<sup>20</sup>

### Scanning Electron Microscopy (SEM)

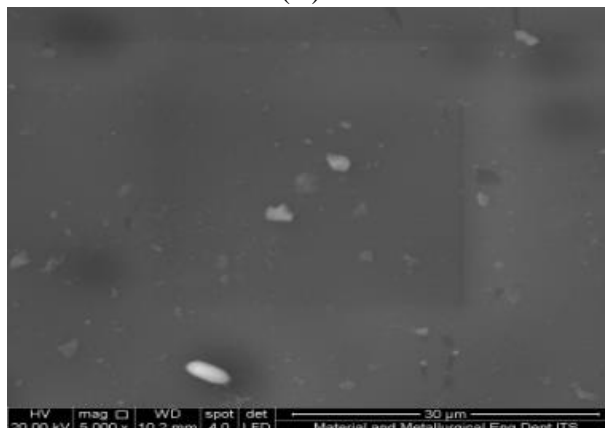
The observed concentration was 2.5% and 3% because the tensile test result met the standard. The sample of 2.5% PVC-Glycerol-Chitosan biocomposite membrane with a concentration of 2.5% shown in Figure 2 that have no pores but white spots or rough structure of the membrane caused by the bubbles in solution during printing process. The results of the Scanning Electron Microscopy (SEM) test of the PVC-Glycerol-Chitosan biocomposite membrane with a concentration of 3% shown in Figure 3 have a flat membrane structure and no pores indication. 3 % concentration PVC-Glycerol-Chitosan bio composite membrane has potential as a candidate for blood bag applications because it has no pore and has a smooth surface structure.



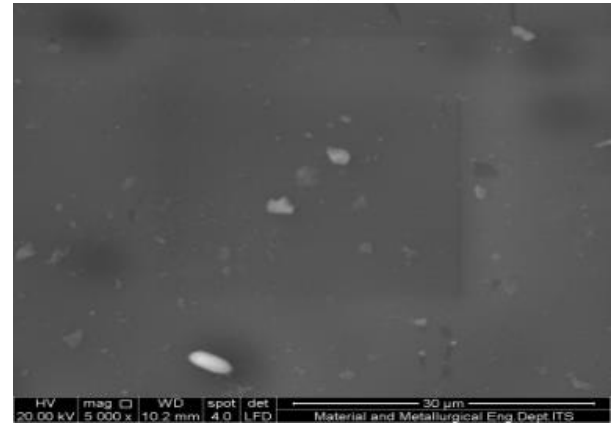
(A)



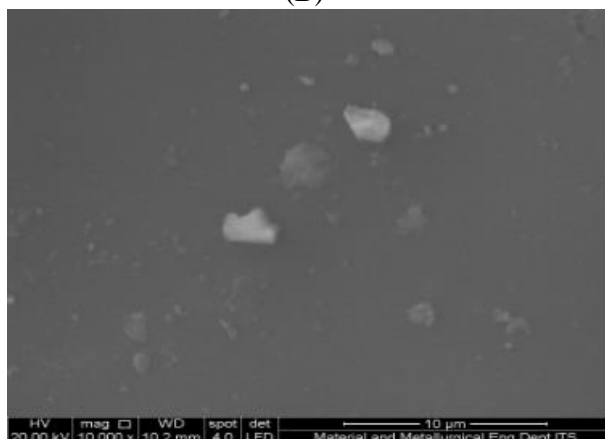
(A)



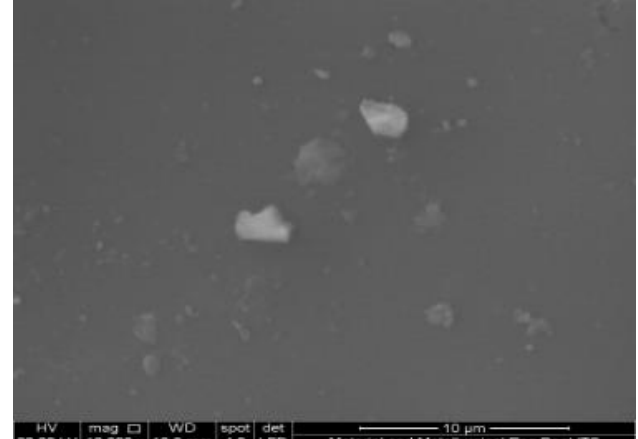
(B)



(B)



(C)



(C)

**Figure 2.** Result of Scanning Electron Microscopy (SEM) Test PVC-Glycerol-Chitosan biocomposite membrane with 2.5% concentration (A) 3,000X magnification, (B) 5,000X magnification, (C) 10,000X magnification.

**Figure 3.** Result of Scanning Electron Microscopy (SEM) Test PVC-Glycerol-Chitosan biocomposite membrane with 3% concentration (A) 3,000X magnification, (B) 5,000X magnification, (C) 10,000X magnification.

In the Scanning Electron Microscopy (SEM) test results, the PVC-Glycerol-Chitosan biocomposite membrane with a concentration of 3% has a flat membrane structure and does not show any pores. The PVC-Glycerol-Chitosan biocomposite membrane sample with a concentration of 2.5% showed no pores but there were white spots or rough structure on the membrane caused by the bubbles in solution during the molding process.

The absence of pores or no pores on the PVC-Glycerol-Chitosan biocomposite membrane is due to the addition of plasticizer material to the PVC solution. According to Xu et al<sup>21</sup>, the results of a membrane morphology test with a high plasticizer content equal to the amount of polymer solution or PVC can form pores because the plasticizer material prevents PVC polymer chains from forming a polymer matrix. One of the functions of the plasticizer is to act as a lubricant to allow the molecules in the plasticizer to be free from each other. Also to act as a partial solvent of polymer and can prevent pores in the membrane.<sup>21</sup> In accordance with this study, the ratio of the same or 1:1 between PVC and plasticizer resulted in the membrane becoming porous, so we used a ratio of 5:1 on the PVC and plasticizer solution, so as not to cause pores on the membrane. The increase in the ratio of 1:1 to 5:1 was due to the results in the ratio of 2:1, 3:1, and 4:1 in my research, which resulted in the membrane being porous and having a non-fine structure. Plasticizer in the application of blood bag membranes uses glycerol.

### Hemolysis Test

The hemolysis test (shown in Figure 4) was carried out to determine the hemocompatibility of the PVC-chitosan-mangrove membrane so that it knew the cause of the red blood cell lysis. Increasing the concentration of chitosan can reduce the hemolysis properties of a material or membrane, and increase hemocompatibility in

the membrane in accordance with the research conducted.<sup>22</sup> The hemolysis test results on PVC-glycerol-chitosan biocomposite membrane samples can interact with blood or not undergo hemolysis because the hemolysis percentage results are below 5%<sup>23</sup>, so PVC-Glycerol biocomposite membranes with chitosan addition are hemocompatible and allow biocomposite membranes PVC-Glycerol-Chitosan to be applied as a blood bag.

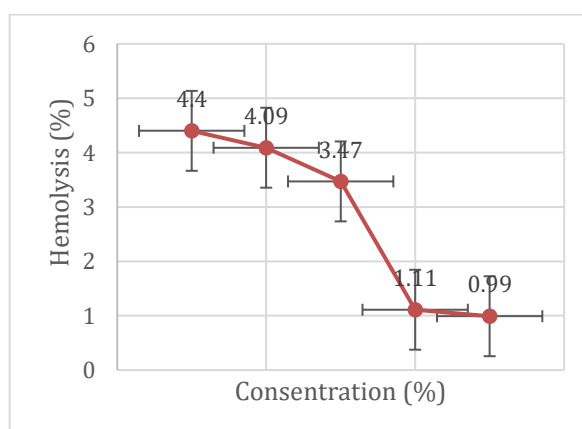


Figure 4. Results of Hemolysis Test

### Antibacterial Tests

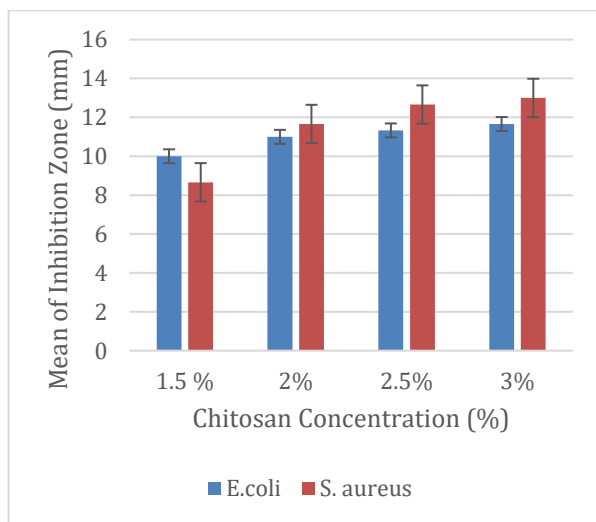
Antibacterial tests were conducted to determine the ability of PVC-Glycerol-Chitosan biocomposite membranes to inhibit bacterial growth. This test was carried out using two bacteria, namely *S. aureus* representing Gram-positive bacteria, and *E. coli* representing Gram-negative bacteria.

Analysis of the bacterial test was carried out by observing the inhibition zone. Measurement of the bacterial inhibition zone was carried out by measuring the diameter of the hole. The hollow formed is where bacterial growth is inhibited by the PVC-glycerol-chitosan biocomposite membrane, measuring the hollow using a caliper or ruler.

Antibacterial inhibitory power categorized as weak indicates a < 5mm inhibition zone, permitted to show a 5-10 mm inhibition zone, strongly supported showing a 10-20 mm inhibition zone, and proven to be



very strong if using an inhibition zone of more than 20 mm. Antibacterial tests were carried out three times for each type of bacteria with variations in the concentration of chitosan 1.5%, 2%, 2.5%, and 3%, and this test used 0.5 MacFarland. The test results of PVC-glycerol-chitosan biocomposite membrane bacteria have been stated in Figure 5. and Table 1.



**Figure 5.** Results of Inhibition/Clear Zone ( diameter) in Antibacterial Test

**Table 1.** Results of Inhibition/Clear Zone

Chitosan Concentration (%)	Mean of Inhibition Zone (mm)	
	<i>E.coli</i>	<i>S. aureus</i>
1.5%	10 ± 1,73	8.66 ± 0,55
2.0%	11 ± 1,73	11.66 ± 0,55
2.5%	11,33 ± 0,55	12.66 ± 0,55
3.0%	11.66 ± 0,55	13 ± 0,55

The bacterial test results of the PVC-glycerol-chitosan biocomposite membrane with a chitosan concentration of 1.5% had a bacterial inhibition zone diameter of 10 mm in *E.coli* bacteria and *S.aureus* bacteria had a diameter of 8.66 mm. Chitosan concentration of 2% showed an increase in the inhibition zone with a diameter of 11 mm in *E.coli* bacteria and an inhibition zone diameter of 11.66 mm in *S.aureus* bacteria. chitosan concentration of 2.5% had a diameter of 11.33

mm bacterial inhibition zone for *E.coli* bacteria and *S.aureus* bacteria had an inhibition zone diameter of 12.66 mm and at 3% concentration the diameter of inhibition zone for *E.coli* bacteria was 11.66 mm and *S.aureus* bacteria has an inhibition zone diameter of 12.66 mm, so the PVC-glycerol-chitosan biocomposite membrane bacterial test has strong antibacterial criteria but at a chitosan concentration of 1.5% in *S.aureus* bacteria it is categorized as weak because the diameter of the bacterial inhibition zone does not enter the strong category criteria.

The relationship of chitosan as an antibacterial depends on its affinity. The mechanism of very strong chitosan is with microbial DNA so that it can bind to DNA which then destroys mRNA and synthesis proteins. The antimicrobial affinity of chitosan in fighting bacteria or microorganisms depends on molecular weight and degree of deacetylation. Molecular weight and a greater degree of deacetylation show greater antimicrobial activity. Chitosan has a positively reactive, positively charged amine (-NH<sub>2</sub>) functional group, so it can bind to negatively charged bacterial wall cells.<sup>24</sup> The potential of chitosan as an antibacterial agent is based on the initial interaction between chitosan and bacteria involving electrostatics. Chitosan has a positively reactive, positively charged amine (-NH<sub>2</sub>) functional group, so it is able to bind to negatively charged bacterial wall cells. This bond occurs at the electronegative site on the surface of the bacterial cell wall. In addition, because -NH<sub>2</sub> also has a free electron pair, this group can attract Ca<sup>2+</sup> minerals found in bacterial cell walls with covalent bonding. Changes in the cell surface and loss of protective function in bacterial cells leads to a reduction in the number of bacterial cells. Gram negative bacteria with lipopolysaccharide in their outer layer have a negative pole which is very sensitive to chitosan. However, the antibacterial activity of chitosan varies and is influenced by many factors such as molecular weight, pH value, and water solubility.<sup>25</sup>

## Tensile Strength Test

The Tensile Strength test was carried out at the ULP Faculty of Physics, Malang. The sample was prepared by a size of 6cm x 1cm in a rectangular sample using a paper holder and the IMADA tensile test instrument with a load cell of 50N. The results of tensile strength characterization obtained values of PVC-glycerol-chitosan biocomposite membrane (as shown in Table 2).

**Table 2.** Tensile Strength Test Result

Chitosan Concentration %	Elongation Percentage (%)	Tensile Strength (MPa)
0	7,976 ± 2,535	9,046 ± 1,796
1,5	6,580 ± 2,085	11,480 ± 0,797
2	5,154 ± 1,682	14,055 ± 2,725
2,5	4,854 ± 1,448	18,979 ± 2,451
3	3,510 ± 3,653	21,202 ± 2,849

A Tensile Strength test was conducted to determine the tensile strength of PVC-glycerol biocomposite membranes by the addition of chitosan and coating of mangroves (*Aegiceras corniculata*). The results of the tensile strength characterization in tile strength can be seen in the greater concentration of chitosan, the greater the value of the tensile strength membrane. The increase in tensile strength is in line with the increase in the concentration of chitosan and this is related to the increase in hydrogen bonding formed in plastic films.<sup>26</sup> The PVC-Glycerol biocomposite membrane with the addition of chitosan, makes the formed bonds stronger and harder to break. The standard of tensile strength in a blood bag based on PVC material is 14-26 MPa<sup>18</sup>, it is in accordance with the standard of blood bag tensile strength with the concentration of chitosan 2 wt/v%, 2.5 wt/v% and 3 wt/v%.

## CONCLUSIONS

The addition of chitosan concentration can increase the physical and mechanical properties (tensile strength) of blood bags. The increase of chitosan concentration in the composite of PVC-Glycerol-Chitosan can meet the standards of tensile strength of the ideal blood bag membrane.

In the future the flexibility, and the condition under the thermogravimetric analysis to show weight loss in relation to alteration in the temperature of the membrane.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHOR CONTRIBUTION

Designed the study, synthesized, characterized, collected and analyzed the data : PW and AB. Manuscript preparation and writing : PW and TDPW. Manuscript correction : PW and S.

## REFERENCES

1. Booth C, Allard S. Blood transfusion. *Medicine*. 2017;45(4):244–50.
2. In FD. Bacterial Risk Control Strategies for Blood Collection Establishments and Transfusion Services to Enhance the Safety and Availability of Platelets for Transfusion: Draft Guidance for Industry.

- CBER, editor. Silver Spring: US Food and Drug Administration. 2016.
3. Schmidt M. Bacterial contamination of blood products. *ISBT Science Series*. 2013;8(1):177–80.
  4. Farzad BB, Farshad B, Zahra B, Nahid A, Mahsa KB. Bacterial contamination of platelet products in the Blood Transfusion Center of Isfahan, Iran. *GMS hygiene and infection control*. 2016;11.
  5. Ketter P, Arulanandam B, Cap AP. Platelets feeding Bacteria with lactate during room temperature storage: mitigated by refrigeration. *Blood*. 2017;130:2407.
  6. Horth RZ, Jones JM, Kim JJ, Lopansri BK, Ilstrup SJ, Fridey J, Kelley WE, Stramer SL, Nambiar A, Ramirez-Avila L, Nichols A. Fatal sepsis associated with bacterial contamination of platelets—Utah and California, August 2017. *Morbidity and Mortality Weekly Report*. 2018;67(25):718.
  7. Agzie M, Niguse S, Tsegay E, Kahsay G, Mahmud MA. Bacterial contaminants of stored blood and blood components ready for transfusion at blood banks in Mekelle, Northern Ethiopia. *BMC research notes*. 2019;12(1):1–6.
  8. Alder L, Tambe A. Acute anemia. *InStatPearls*. 2021. StatPearls Publishing.
  9. Goodnough, Lawrence Tim, and Stanley L. Schrier. Evaluation and management of anemia in the elderly. *American journal of hematology*. 2014;(1).88-96.
  10. Society AC. Getting a Blood Transfusion [Internet]. 2017. Available from: <https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/blood-transfusion-and-donation/how-blood-transfusions-are-done.html>
  11. WHO. Blood safety and availability [Internet]. 2022. Available from: <https://www.who.int/news-room/factsheets/detail/blood-safety-and-availability>
  12. Zahra, N. M., Siswanto and Prihartini, W. The Role of Chitosan on Polyvinyl Chloride (PVC)-Glycerol Biocomposites for Blood Bag Application. *Journal of Biomimetics, Biomaterials and Biomedical Engineering*. 2018;37:94–106.
  13. Omer AM, Tamer TM, Monem AE, Abd Elmoaty S, Abd El Fatah M, Saad GR. Development of PVC membranes with clove oil as plasticizer for blood bag applications. *J. Appl. Pharm. Sci*. 2016 Jul 28;6(7):085–93.
  14. Sudhakar YN, Selvakumar M, Bhat DK. Biopolymer electrolytes: fundamentals and applications in energy storage. Elsevier; 2018.
  15. Singh R, Shitiz K, Singh A. Chitin and chitosan: biopolymers for wound management. *International wound journal*. 2017;14(6):1276–89.
  16. Zhao D, Yu S, Sun B, Gao S, Guo S, Zhao K. Biomedical applications of chitosan and its derivative nanoparticles. *Polymers*. 2018;10(4):462.
  17. Vasconcelos DP, Fonseca AC, Costa M, Amaral IF, Barbosa MA, Águas AP, Barbosa JN. Macrophage polarization following chitosan implantation. *Biomaterials*. 2013;34(38):9952–9.
  18. Carr, Stephen. Material selection Analysis: Bag for Viable Blood Storage. Kittichai Sojiphan. KingMongkuts University of Technology North Bangkok. 2016.
  19. Munajad A, Subroto C. Fourier transform infrared (FTIR) spectroscopy analysis of transformer paper in mineral oil-paper composite insulation under accelerated thermal aging. *Energies*. 2018;11(2):364.
  20. Sobahi TR, Makki MS, Abdelaal MY. Carrier-mediated blends of Chitosan with polyvinyl chloride for different applications. *Journal of Saudi Chemical Society*. 2013;17(2):245–50.
  21. Jianying Xu, Lijuan Wang, Wei Shen, Rohani Paimin and Xungai Wang. The



- Influence of the Interior Structure of Aliquat 336/PVC Membranes to their Extraction Behavior, *Separation Science and Technology*. 2014; 39:15: 3527-3539.
22. Mohyeldin MS, Tamer TM, Abu Saied MA, Soliman EA, Madi NK, Ragab I, Fadel I. Click grafting of chitosan onto PVC surfaces for biomedical applications. *Advances in Polymer Technology*. 2018 Feb;37(1):38–49.
23. Hendershot MD, Melly F, Corfo BS. *ASTM Hemolysis*. NAMS, Washington.
24. Killay A. Kitosan sebagai anti bakteri pada bahan pangan yang aman dan tidak berbahaya. *Prosiding FMIPA Universitas Pattimura*. 2013:200–5.
25. Ardean, Cristina, et al. Factors influencing the antibacterial activity of chitosan and chitosan modified by functionalization. *International Journal of Molecular Sciences*. 2021; 22(14): 7449.
26. Selpiana S, Patricia P, Anggraeni CP. Pengaruh penambahan kitosan dan gliserol pada pembuatan bioplastik dari ampas tebu dan ampas tahu. *Jurnal Teknik Kimia*. 2016;22(1):18–26.