Synthesis and characterization of poly (lactic acid)/chitosan nanocomposites based on renewable resources as biobased-material

Suryani¹, H. Agusnar², B. Wirjosentono³, T. Rihayat⁴, Z. Salisah⁵
¹,²,³Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Sumatera Utara, Medan 20155, North Sumatera, Indonesia
⁴,⁵Departement of Chemical Engineering, Politeknik Negeri Lhokseumawe, Lhokseumawe 24301, Aceh, Indonesia

¹suryanisalim@yahoo.com
²teukurihayat@yahoo.com

Abstract. Biobased becomes one of the new breakthrough in the smart engineering, especially in biomedical applications, such as tissue engineering that serves as a supporting physical structure to trigger the growth of skin tissue. From various studies which had been done, it was known that the optimal Biobased healed wounds or injuries in a relatively short time. In this study, a Biobased natural polymer based e.g Poly(Lactic Acid) (PLA)/Chitosan Nanocomposites was made. PLA was synthesized from saba banana (Musa acuminata) as raw material using Ring-Opening Polymerization (ROP) method. PLA was mixed with Chitosan with Chitosan concentration variations of 1%, 3%, and 5% to form a nanocomposites. The analysis result showed that Chitosan concentration in PLA/Chitosan Nanocomposites sample affected the value of tensile strength. The highest value of tensile strength was obtained on a sample of 100 ml volume with a concentration of 3%, which was 120.396 MPa. The highest percentage of elongation was obtained in 100 ml volume sample with 5% concentration, which was 26.3686%. In the hydrophilicity test, the highest percentage of water absorption was obtained in a 200 ml volume sample with 5% concentration, which was 44.615%. The addition of Chitosan to the sample affected the functional group bonding, where there was a functional group of NH₂ at the wave number of 2923.92 cm⁻¹. The sample characteristics based on water absorption indicated that the sample was potentially to be used as Biobased construction material.

1. Introduction

Technological developments in various sectors of life have made it easily to deal with various problems, especially in the medical field that is closely related to the latest healing techniques. An example of the use of technology in the medical field is the technology of burns healing. Burns is an example of a physical injury that requires a relatively long time to heal. Burns tend to leave scars in the form of dark color caused by skin tissue damage. Therefore, various studies were conducted as an effort to approach for the field of tissue engineering in order to overcome the effects of a burn within relatively short [1]. One of technologies that are currently being developed in a variety of research is the manufacture of Bioscaffold which has now become one of the new breakthroughs in the field of skin tissue engineering. Bioscaffold serves as a supporting physical structure to trigger the growth of new skin cells. Bioscaffold based-material can be either synthetic or natural polymer material. One of natural polymers that can be used to manufacture a Bioscaffold is Poly(Lactic Acid) (PLA). PLA is a polymer which is most widely used in the biomedical field [2] because of its properties is good for biocompatibility, the degradation rate is relatively slow, and the melting temperature is high [3].
Centuries ago, biocompatible materials such as metals, ceramics, and polymers have long been used to perform surgical implantation. Metals and ceramics have a big impact in the medical field, especially in the tissue regeneration process. However, both materials have shortcomings because they have not biodegradable properties and their limited work ability. Polymer material is then developed as a replacement material before and received considerable attention because it is biodegradable and easy to process. It makes the polymer material has been widely used for tissue engineering applications [4]. Recently, research on the synthesis of polymers with macromolecular structure that clearly has been much done [5, 6]. Polymers with diverse structures, such as block, graft, star, gradient, hyper-branched, and comb, already used to stabilize colloidal, modification of crystal formation and transfer system of sophisticated drugs [7]. The use of polymers that are currently being researched by many scientists that is in the making Bioscaffold composed of natural polymers PLA.

PLA is a biodegradable thermoplastic in nature and derived from renewable plant sources, such as starch and sugar. Historically, the use of PLA is still limited to the fields of biomedicine, such as implantable devices, tissue scaffold, and the building blocks of stitches organs. This is because the price is relatively expensive. In addition, the availability of the PLA is in limited quantities and low molecular weight [7]. PLA with > 90% PLLA tend to form crystals, while the PLA with <90% PLLA tend to be amorphous. The melting point (Tm) and glass transition temperature (Tg) of PLA decreases with decreased levels of PLLA [8]. PLA was included degradable polymers produced by biotechnological processes, namely the synthesis of natural monomers such as lactic acid which is the result of fermentation of glucose. PLA became one polymer biodegradable which are widely used and are becoming the subject of research in recent decades. PLA can be produced through a variety of techniques and are commercially available in various qualities or grade. In addition, the relative PLA has good properties that can be used in various applications [2]. In addition, the PLA has drawbacks in nature, such as not resistant in environments with high temperature and has the possibility of contamination by microbes. This cause the PLA cannot be used in isolation and needed other supporting material with anti-bacterial properties to form a nanocomposite with PLA. One type of anti-bacterial material that can be used is Chitosan.

Chitosan is the main constituent of animal shells of crustaceans such as shrimp, mollusks such as snails and insects were obtained from the alkaline deacetylation of chitin. Chitin is the most widely available mucopolysaccharides naturally after cellulose in terms of the amount of production each year through the process of biosynthesis [10]. Structure of chitosan (chitin are acylated) is very similar to cellulose, but their utilization is still less than the cellulose, especially because it is not able to react chemically [11].

Utilization chitosan in the medical world lately provide significant benefits. Chitosan widely used in wound healing and prevent microbial activity for cationic nature. Chitosan also help the growth of granular tissue by activating and modulating the infected cells making Chitosan able to heal wounds. Chitosan acts as an important component in wound healing because it can bind to anionic red blood cells. Chitosan have anti-microbial properties with the incorporation of anions in the cell walls of bacteria and suppress the biosynthesis of the cell wall. This causes the bacterial metabolism is disrupted and bacteria was been disable [11]. Other than that, chitosan has bioadhesitvas properties, biodegradability and good biocompatibility [12, 13].

In this study, the PLA is produced through polymerization of lactic acid by the Ring-Opening Polymerization method. The lactic acid was produced from glucose fermentation process of raw materials starch. Starch was used in this study came from saba banana plant (Musa acuminata). The young who have a high starch content. Chitosan was used as supporting material in the manufacture of nanocomposites. PLA and chitosan then merged to form a nanocomposite with chitosan various concentrations of 1%, 3% and 5%. PLA/chitosan nanocomposites obtained is expected to be a constituent material Bioscaffold as evidenced through characterization, including analysis of the mechanical properties by tensile strength test and analysis of functional group was using a Fourier Transmittance Infrared (FT-IR).

2. Material and methods
2.1. Materials
This study used a saba banana (Musa acuminata) as a raw material to be taken its starch that was obtained from Lhokseumawe traditional markets, Aceh. Microbe Lactobacillus bulgaricus was used to ferment glucose from hydrolysis of saba banana starch results obtained from bacterial inoculation process. Bacterial inoculation is done at the Laboratory of Biotechnology and Food Technology, Department of Chemical Engineering, State Polytechnic of Lhokseumawe. Chitosan which is used as an anti-bacterial material, was obtained from the Laboratory of Chemistry, Department of Chemical Engineering, State Polytechnic of Lhokseumawe. In addition, chemicals such as glacial acetic acid, stannous octoate (Sn(Oct)₂), and hydrochloric acid (HCl), was obtained from Merck.

2.2. Synthesis of PLA
Saba bananas that had been cut with a thickness of ±3-5 mm is mixed with water at a ratio of bananas and water 1:3, blended for 10 minutes. Mashed banana is filtered and stored in a container, allowed to stand for 8 hours to form a precipitate. Liquids and solids are separated by decantation. The solids are dried in an oven at 40°C until dried, sifted and weighed to determine the amount of starch obtained. 25 grams of starch was hydrolyzed by adding 2 ml of HCl 5%, stirring with a constant speed of 350 rpm. The solution is heated until the temperature reaches 60°C and kept constant for 180 minutes to obtain a solution of glucose.

Glucose was tested using Benedict's reagent to prove their glucose content in the sample. Glucose was tested by titrated using reagents Benedict then heated in boiling water until the blue-green, yellow or red brick. Glucose is fermented for 72 hours with the help of microbes Lactobacillus bulgaricus to produce lactic acid. The lactic acid is then polymerized to form a PLA.

Polymerization of lactic acid was performed using methods Ring-Opening Polymerization (ROP). The process involves three distinct phases, namely polycondensation of lactic acid (prepolymerization), depolymerization thus forming a cyclic dimer (lactida) and ring-opening polymerization. Stannous octoate (Sn(Oct)₂) used as a catalyst.

2.3. Preparation of PLA/Chitosan Nanocomposites
Chitosan was dissolved in a solution of acetic acid (CH₃COOH). Once dissolved, the PLA and chitosan then mixed with chitosan various concentrations of 1%, 3% and 5% and variations in volume of 100 ml and 200 ml for each sample. The mixture between PLA and chitosan then was heated at 65°C and stirred with a constant so that a homogeneous mixture. Each nanocomposites then was inserted into the container mold. After completion of printing, nanocomposites then dried in an oven at 40°C for 3 days to dry. Once completely dry, nanocomposites removed from the mold. The samples were then characterized.

2.4. Mechanical properties
The mechanical properties of samples in tensile strength and elongation are tested using the tensile test equipment Universal Testing Machine (Model Exceed E43, Japan). Dimension of the sample was following the specimen dimensions on ASTM D 638 – 99.

2.5. FT-IR
FT-IR spectrum is measured in the wave numbers range of 500-4000 cm⁻¹ using a spectrophotometer Fourier Transform Infrared (Shimadzu IR Prestige-21 Serial No. A210048 02 519, Kyoto, Japan). Samples were adjusted to the size of 20 x 25 x 0.8 mm.

3. Result and discussion

3.1. Mechanical Properties of PLA/Chitosan Nanocomposites
Mechanical strength of Bioscaffold was an important factor that must be studied in depth to determine its application in the field of tissue engineering. The mechanical properties of appeal are very important because of the need for support during the process of culture and implantation in vitro. Additionally, the
The mechanical properties testing Bioscaffold are very important to know the homogeneity of a mixture of polymer material and to determine the mix of materials used in the manufacture Bioscaffold [14].

In this study, the mechanical characteristics of PLA/chitosan nanocomposites was analyzed by the tensile strength testing. Tensile strength testing data as a value of tensile strength values (MPa) and percent of elongation (%) are shown in Table 1.

Table 1. Tensile strength testing values of PLA/chitosan nanocomposites

| Chitosan (%) | Sample Volume (ml) | Tensile Strength (MPa) | Elongation (%) |
|--------------|--------------------|------------------------|---------------|
| 1            | 100                | 61.447                 | 6.7342        |
| 3            | 100                | 120.396                | 26.2282       |
| 5            | 100                | 61.961                 | 26.3686       |
| 1            | 200                | 26.7495                | 13.956        |
| 3            | 200                | 29.16                  | 20.4786       |
| 5            | 200                | 27.1376                | 20.5714       |

In the Table 1, we know that the increased concentration of chitosan has given an effect for the tensile strength values and percent of elongation of PLA/Chitosan Nanocomposites. This is explained through charts at Figure 1.

![Figure 1](image1.png)

Figure 1. Chitosan Concentration (%) vs tensile strength values (MPa) of PLA/chitosan nanocomposites samples

At a sample volume of 100 ml, the graphic shows that the highest tensile strength values were obtained at chitosan concentrations of 3%, which is equal to 120.396 MPa. This value increased to twice the value of tensile strength in chitosan concentration of 1%, which is equal to 61.447 MPa. However, at chitosan concentrations of 5%, the value of tensile strength experienced decreased to 61.961 MPa. This is because the chitosan concentration more than 3% causes the tensile strength decreased. In a sample volume of 200 ml, the graphic shows that the highest tensile strength values obtained at chitosan concentrations of 3% amounting to 29.16 MPa. This value is increased from the value of tensile strength in chitosan concentration of 1%, which is equal to 26.7495 MPa. However, at chitosan concentrations of 5%, the value of tensile strength decreased to 27.1376 MPa. As explained earlier, this is because the chitosan concentration more than 3% in the sample cause the tensile strength decreased so that from this analysis, it can be seen that the chitosan concentration of 3% is the best concentration to obtain maximal tensile strength values in the sample PLA/chitosan nanocomposites to a sample volume of 200 ml. In this analysis, it can be seen that the chitosan concentration of 3% is the best concentration to obtain the maximal tensile strength values.

Increasing of chitosan concentrations also affect the percent of elongation at PLA/Chitosan Nanocomposites sample. This is shown in Figure 2.
Figure 2. Chitosan Concentration (%) vs Elongation (%) of PLA/chitosan nanocomposites samples

At a sample volume of 100 ml, the graphic shows that the percent of elongation increased with increasing Chitosan concentrations. The highest elongation was obtained at a chitosan concentration of 5%, which is equal to 26.3686%. The lowest elongation was obtained at a chitosan concentration of 1%, which is equal to 6.7342%. A significant increase in percent of elongation occurs in the sample with a chitosan concentration between 1% and 3%, where the initial elongation of 6.7342% rose to 26.2282%. The increase in elongation is said to be significant due to the increase in % elongation reached more than three times than before. While the increase in percent of elongation between concentration chitosan 3% and 5% are not so significant. In this analysis, it can be seen that the chitosan concentration of 5% is the best concentration of the highest in the PLA/chitosan nanocomposites sample percent of elongation for a volume of 100 ml.

In a sample volume of 200 ml, the graphic shows that the percent of elongation increased with increasing Chitosan concentrations. The highest elongation was obtained at a chitosan concentration of 5%, which is equal to 20.5714%. The lowest elongation was obtained at a chitosan concentration of 1%, which is equal to 13.956%. A significant increase in percent of elongation occurs in the sample with a chitosan concentration between 1% and 3%, which amounted to 13.956% initial elongation increased to 20.4786%. The increase in elongation is said to be significant due to the increase in percent of elongation was greater than the increase in percent of elongation between chitosan concentration of 3% and 5%. In this analysis, it can be seen that the chitosan concentration of 5% is the best concentration of the highest in the PLA/chitosan nanocomposites sample percent of elongation to a volume of 200 ml.

3.2. FT-IR

This analysis was conducted to identify the functional groups contained in the PLA/chitosan nanocomposites sample. This analysis also aims to determine whether the addition of chitosan concentration affect the existing functional groups. Wavelength data obtained is displayed in Table 2.

| Sample  | Wavenumber (cm⁻¹) | Function Group | Wave Length (cm⁻¹) |
|---------|-------------------|----------------|--------------------|
| PLA     | 3503.71           | O – H          | 3550 – 3200        |
|         | 2897.72           | C – H          | 3000 – 2840        |
|         | 1759.12           | C = O          | 1760               |
|         | 1457.79           | CH₃            | 1450               |
| PLA/Ch  | 3437.63           | O – H          | 3550 – 3200        |
|         | 2923.92           | NH₂            | 3000 – 2800        |
|         | 2890.05           | C – H          | 3000 – 2840        |
Figure 3 was displayed analysis graphic of PLA samples functional groups. From the analysis, the results obtained are the PLA samples contained functional groups of OH, CH, C=O, CH$_3$ and CO. OH bond obtained has a wavelength of 3500-3650 cm$^{-1}$. Peak the wavelength area of 3503.71 cm$^{-1}$ shows the combined OH bond of acid and alcohol. In the wavelengths area of 2850-2970 cm$^{-1}$, there is a CH bond which is an alkane compound shown in wavelength of 2897.72 cm$^{-1}$. In the area wavelengths of 1690-1760 cm$^{-1}$, there is a C=O bond which is a carbonyl group demonstrated at a wavelength of 1759.12 cm$^{-1}$. While in the area of wavelengths 1340-1470 cm$^{-1}$, there are the CH$_3$ bonds shown at a wavelength of 1457.79 cm$^{-1}$, and the wavelength area of 1050-1300 cm$^{-1}$ CO bonds are shown at a wavelength of 1149.80 cm$^{-1}$.

Figure 3. FT-IR Spectrum of PLA

In the Figure 4, the graphic shows the functional group analysis of PLA/chitosan nanocomposites samples as a comparison. The graphic shows small change in shape after the PLA added Chitosan. OH groups on the PLA decreased wavelength FTIR spectra of samples Nanocomposites.

Figure 4. FT-IR Spectrum of PLA/chitosan nanocomposites for chitosan concentration of 3% and a volume of 100 ml.

4. Conclusion
Chitosan concentration in a sample of PLA/chitosan nanocomposites affects the value of tensile strength. The highest tensile strength value obtained at a sample volume of 100 ml with a chitosan concentration
of 3%, amounting to 120.396 MPa. The highest percent of elongation is obtained on a sample volume of 100 ml with a chitosan concentration of 5%, amounting to 26.3686%. The adding of chitosan in the sample affects the functional group bonding, where there is a new functional group NH₂ at wave number 2923.92 cm⁻¹.

Acknowledgments
The authors express their gratitude and thanks to the Higher Education Ministry for Financial Support through the Stranas Grant and Ditjen Penguatan Riset dan Pengembangan, Kemenristekdikti. The last but not least, gratefully acknowledgements for the super team from YPAR (Yayasan Puga Aceh Riset)/Research Puga Aceh Foundation.

References
[1] Guarino, V., Caputo, T., Altobelli, R., & Ambrosio, L. 2015. Degradation Properties and Metabolic Activity of Alginate and Chitosan Polyelectrolytes for Drug Delivery and Tissue Engineering Applications. AIMS Materials Science, 2(4), 497–502.
[2] Santoro, M., Shah, S.R., Walker, J.L., & Mikos, A.G. 2016. Poly(Lactic Acid) Nanofibrous Scaffolds for Tissue Engineering. Advanced Drug Delivery Reviews, 107, 206–212.
[3] Frydrych, M., Román, S., MacNeil, S., & Chen, B. 2015. Biomimetic Poly(Glycerol Sebacate)/Poly(L-Lactic Acid) Blend Scaffolds for Adipose Tissue Engineering. Acta Biomaterialia, 18, 40–49.
[4] Lopes, M.S., Jardini, A.L., & Filho, R.M. 2012. Poly (Lactic Acid) Production for Tissue Engineering Applications. Procedia Engineering, 42, 1402–1413.
[5] Rihayat, T., Suryani, S., & Zaimahwati, X. 2014. Preparation and properties and application of renewable source (palm oil polyol) based polyurethanes coatings and its thermal stability improvement by clay nanocomposites. Advanced Materials Research, 887-888, 566-569.
[6] Albertsson, A.C., Varma, I.K., Lochab, B., Finne-Wistrand, A., & Kumar, K. 2010. Design and Synthesis of Different Types of Poly (Lactic Acid). Poly (Lactic Acid): Synthesis, Structure, Properties, Processing, and Application, 43–55, New Jersey: John Wiley & Sons, Inc.
[7] Maharana, T., Pattanaik, S., Routaray, A., Nath, N., & Sutar, A.K. 2015. Synthesis and Characterization of Poly(Lactic Acid) Based Graft Copolymers. Reactive and Functional Polymer, 93, 47–67.
[8] Lim, L.T., Auras, R., & Rubino, M. 2008. Processing Technologies for Poly(Lactic Acid). Progress in Polymer Science, 33, 820–852.
[9] Auras, R., Harte, B., & Selke, S. 2004. An Overview of Polylactides as Packaging Materials. Macromolecular Bioscience, 4, 835–864.
[10] Ahmed, S. & Ikram, S. 2016. Chitosan Based Scaffold and Their Applications in Would Healing. Achievement in The Life Sciences, 10, 27–37.
[11] Saravanan, S., Leena, R.S., & Selvamurugan, N. 2016. Chitosan Based BioComposite Scaffolds for Bone Tissue Engineering. International Journal of Biological Macromolecules, 93, 1354–1365.
[12] Silva, S.S., Popa, E.G., Gomes, M.E., Cerqueira, M., Marques, A.P., Caridade, S.G., Teixeira, P., Sousa, C., Mano, J.F., & Reis, R.L. 2013. An Investigation of The Potential Application of Chitosan/Aloe-Based Membranes for Regenerative Medicine. Acta Biomaterialia, 9, 6790–6797.
[13] Zaimahwati, Agusnar, H., Rihayat, T., Reflianto, D., Gea, S. 2015. The manufacture of palm oil-based polyurethane nanocomposite with organic montmorillonite nanoparticle as paint coatings. International Journal of ChemTech Research, Volume 7, Issue 5, 2537-2544.
[14] Rihayat, T., Suryani, S., Zaimahwati, Z. (2014). Effects of heat treatment on the properties of polyurethane/clay nanocomposites paint. Applied Mechanics and Materials. 525, 97-100