Fabrication of silver-chitosan nanocomposite films and their antibacterial activity

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Abstract. The silver-chitosan nanocomposite film fabrication was carried out by the preparation of silver nanoparticles using chemical reduction methods assisted with microwave irradiation with chitosan as a reducing agent as well as a stabilizer. Silver nitrate is used as a precursor and NaOH as an accelerator. Silver-chitosan nanocomposite colloids were converted into films by casting techniques at the room temperature. Based on UV-Visible spectra, colloidal nanocomposites show absorption peak at the range of 409-412 nm. Based on TEM images, the silver nanoparticles are spherical shaped and distributed with a diameter of 6-10 nm. Based on FTIR spectra, interactions between silver nanoparticles and chitosan occur in the hydroxyl group (-OH) and amine group (-NH) of the chitosan polymer chain. Based on SEM images, the silver-chitosan nanocomposite films show a compact but rough surface morphology. The increase in tensile strength and decrease in elongation at break of the film was in line with the increase in the concentration of silver nitrate, while the value of the elasticity of the film fluctuates depending on the tensile strength and elongation at break. The increasing concentration of silver nitrate also causes an increase in the degree of swelling. silver-chitosan nanocomposite films showed better antibacterial activity against Escherichia coli than Staphylococcus aureus.

1. Introduction

Chitosan or β-(1,4)-2-amino-2-dioxy-D-glucose is a type of natural polysaccharide obtained from the chitin deacetylation process. Chitin is the second most biopolymer in nature after cellulose [4]. Chitosan has beneficial properties, including non-toxic, antibacterial, biodegradable, and biocompatible [1, 7]. In addition, chitosan has 3 types of functional groups, namely amine, primary and secondary hydroxyls. The existence of these three functional groups causes chitosan to have a high reactivity [11]. Chitosan has been widely used and applied in various fields, one of which is in the field of making environmentally friendly coating materials or films that have antibacterial activity.

To improve the mechanical properties and antibacterial activity of chitosan-based films, we need to combine them with other materials such as nano materials. Nano materials, such as silver nanoparticles, can be selected for dispersion into chitosan biopolymer structures to form silver-chitosan nanocomposites. Chitosan acts as a matrix or binding component while silver nanoparticles act as fillers of components [13].
Dispersion of silver nanoparticles which incidentally has antibacterial properties into chitosan biopolymers will produce a nanocomposite material with excellent antibacterial activity [12, 15]. In addition, the very broad surface of silver nanoparticles will interact with the polymer chains of chitosan, so the mobility of the chitosan polymer chains decreases. This interaction can improve the mechanical properties of the silver-chitosan nanocomposite produced [10].

Chitosan-silver nanocomposite films can be made by first preparing silver nanoparticles. The preparation process can be carried out by chemical reduction methods. Besides being easy and quite effective, the costs required are also relatively affordable. At least, there are two important components that must be involved in the synthesis process of silver nanoparticles using chemical reduction; they are 1) the reducing agent, which will reduce the Ag⁺ ions to Ag⁰ and 2) the stabilizing agent (stabilizer), which will provide stability to silver nanoparticles that are formed from oxidation, agglomeration and deposition processes [5, 19].

In the synthesis process of silver nanoparticles using a chemical reduction method, materials that are not environmentally friendly are often chosen as reducing agents. The strong reducing agents commonly used such as sodium borohydride and hydrazine may have a negative impact on the environment because they are toxic. Besides, conventional heating is also often used in the synthesis of silver nanoparticles. Besides still involving relatively high energy, the time required is also relatively long. The synthesis of silver nanoparticles can last for 12 hours using a temperature of 60°C [2].

Besides acting as a matrix, chitosan can also act as a stabilizer as well as a reducing agent in the synthesis of silver nanoparticles. This is based on the research by Bozanic [6] and Shah [15], who used chitosan as a stabilizer while Raghavendra [12], in their research used chitosan as a reducing agent. Raghavendra [12], in his research used microwave irradiation for 11 minutes with pauses for 45 seconds every one minute. Thus, the use of microwave irradiation assistance can provide time efficiency in the synthesis process of silver nanoparticles.

Based on the research conducted by Darroudi [9], the use of accelerators can also provide effectiveness and efficiency in the synthesis process of silver nanoparticles. The use of sodium hydroxide as an accelerator can affect the number and size of silver nanoparticles produced. Sodium hydroxide as an accelerator can reduce the size of silver nanoparticles and increase the amount along with the increase in the amount of sodium hydroxide used. The use of sodium hydroxide as an accelerator in the synthesis of silver nanoparticles has also been carried out by Susilowati [18] with the analysis of the results of the same synthesis.

Based on the explanation, this research on the film making from silver-chitosan nanocomposite materials was conducted. The making of silver-chitosan nanocomposite films was carried out by first preparing silver nanoparticles using the reduction method with the help of microwave irradiation. Sodium hydroxide was used as an accelerator. Chitosan was used as a reducing agent as well as a stabilizer. In addition, chitosan was also used as a biopolymer matrix in the synthesis of silver-chitosan nanocomposites. The colloidal silver-chitosan nanocomposite formed was printed into a film with the casting technique at the room temperature.

2. Experimental

2.1. Chemicals

The researcher purchased chitosan with molecular weight (MW) of 1071.579 kDa and degree of deacetylation (DD) of 70.356% from Surindo Cirebon Indonesia Biotech. Silver nitrate (AgNO₃), glacial acetic acid (CH₃COOH), and sodium hydroxide (NaOH) were purchased from Merck.

2.2. Preparation of silver-chitosan nanocomposite colloidal

Chitosan solution (1% w/v) in glacial acetic acid solution (1% v/v) was prepared first. The solution was stirred with a stirrer using a medium speed for 2 hours or until completely dissolved. Chitosan solution (1% w/v) that had been produced was then left alone for 24 hours. A total of 12.5 mL of chitosan solution (1% w/v) was poured into a beaker and stirred using a stirrer for 5 minutes. 0.25 mL
of AgNO₃ solution (0.012 g/mL) was added dropwise using a syringe while stirring with a stirrer for 5 minutes. After that, a 1.75 mL of NaOH (2 M) solution was added dropwise using a syringe while stirring with a stirrer for 5 minutes. The solution was then heated using a microwave with 100 watts of power for 4 minutes. After that, the gel formed was poured into a beaker containing 47.5 mL of chitosan solution (1% w/v), and then stirred using a stirrer until it dissolved to form colloidal silver-chitosan nanocomposites. The same procedure was performed to produce variations in the concentration of AgNO₃, which were then named A1 (0.5%), A2 (1.0%), A3 (1.5%), A4 (2.0%), A5 (2.5%), and A6 (3.0%). In this procedure, concentration of AgNO₃ was expressed in percentage or % (w/v, AgNO₃/Chitosan).

2.3. Preparation of nanocomposite films
The film was formed from colloidal silver-chitosan nanocomposites using casting techniques at the room temperature. Colloidal nanocomposites were poured into polyethylene molds (10 x 15 cm) and dried at the room temperature until the solvent completely evaporated (approximately 3 days). After drying, the film was neutralized using a solution of NaOH (1 M), and then rinsed using distilled water. After that, the film was dried again on a glass plate and then peeled off and stored in a desiccator. Characterization of film on SEM and FTIR was only carried out on sample films A3 and A6.

2.4. UV-visible spectroscopy analysis
To identify silver nanoparticles, absorption spectra of colloidal silver-chitosan nanocomposites were recorded at a wavelength range of 300 - 600 nm using the Ultraviolet-visible spectrophotometer Shimadzu UV3150. Before measurement, colloidal samples were diluted 10 times using distilled water.

2.5. Transmission Electron Microscopy (TEM) analysis
The shape and size distribution of silver nanoparticles in silver chitosan nanocomposite colloids were identified using TEM (JEM-1400) with a voltage of 80 KV.

2.6. Fourier Transform Infrared (FTIR) analysis
To identify the interactions between silver nanoparticles and chitosan in silver-chitosan nanocomposites, the transmittance spectra of nanocomposite films were recorded using FTIR (Shimadzu FT-IR 8201PC) in the wave range of 4000-300 cm⁻¹.

2.7. Scanning Electron Microscope (SEM) analysis
The surface morphology of the silver-chitosan nanocomposite film was observed by using SEM (Zeiss DSM 960) with a resolution of 10 µm, acceleration of 10KV voltage and magnification of 2500 times.

2.8. Tensile measurements
Tensile tests or mechanical properties tests on silver-chitosan nanocomposite films were carried out to determine the value of Tensile Strength, elongation at break, and elasticity (Modulus Young). Measurements were made using Universal Tensile Machine Type Z 0.5 with the stretching speed of 10 mm/min.

2.9. Swelling studies
Investigation of swelling properties or absorptive capacity of silver-chitosan nanocomposite films was determined in a Phosphate Buffer Saline/PBS solution (pH 7.4) at the room temperature. The film was cut first with a size of 2 x 2 cm, and then weighed (W₀). After that, it was soaked in PBS solution for 24 hours, and then dried and weighed. The procedure was repeated 3 times.
2.10. Antibacterial activity
The antibacterial activity test of silver-chitosan nanocomposite films against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria was carried out using the disk diffusion method. Before testing, the test bacteria, namely *S. Aureus* and *E. Coli*, were first incubated at 37 °C for 24 hours on sterile growth media. The sterile agar solution was poured into a sterilized petri dish (10 cm in diameter), and then left alone to condense. After that, the bacterial colony was placed on a petri dish containing agar evenly and incubated at 37 °C for 24 hours. Antibacterial activity testing was carried out by placing the silver-chitosan nanocomposite film discs on the surface of a filled petri dish so that the test bacteria were overgrown. After that, the incubation was carried out at 37 °C for 24 hours. The antibacterial effectiveness was assessed through measurement of inhibition zones (clear areas) that appear under and around the film discs on the agar media. The inhibition zones that appeared on the media are then named according to the variations in the concentration of AgNO$_3$ used, namely FA0 (chitosan), FA1 (0.5%), FA2 (1.0%), FA3 (1.5%), FA4 (2.0%), FA5 (2.5%), and FA6 (3.0%).

3. Results and Discussion
The silver-chitosan nanocomposite film was made by first preparing silver nanoparticles using the chemical reduction method. Then, the formed silver nanoparticles were dispersed into chitosan biopolymers to form colloidal silver-chitosan nanocomposites. The resulting colloidal color changes to yellow, brownish yellow, to dark brown/reddish, as shown in Figure 1. This indicates that silver nanoparticles have formed in colloidal silver-chitosan nanocomposites [3, 9]. The colloidal nanocomposites formed were then printed into films by casting techniques at the room temperature. The silver-chitosan nanocomposite film produced can be seen in Figure 2.

![Figure 1. Colloidal silver-chitosan nanocomposite with varying concentrations of AgNO$_3$.](image1)

![Figure 2. Silver-chitosan nanocomposite films with varying concentrations of AgNO$_3$.](image2)

3.1. UV-visible spectroscopy analysis
The formation of silver nanoparticles in colloidal silver-chitosan nanocomposites can be identified using UV-Vis spectroscopy. In addition, by using UV-Vis spectroscopy, the size of the formed silver nanoparticles can also be estimated. The UV-Vis spectra of each colloidal silver-chitosan nanocomposite sample can be seen in Figure 3.
Figure 3 shows that the maximum absorbance of each sample occurs in the range of wavelengths of 409-412 nm. This indicates that silver nanoparticles have been formed. These results are in accordance with the research of Raghavendra [12] and Shah [15], which state that the SPR band silver nanoparticles on UV-Vis spectra can provide characteristic peak absorbance spectra in the wavelength range of 400-450 nm.

Based on UV-Vis spectra (Figure 3), the increase in the concentration of AgNO$_3$ used is generally directly proportional to the increase in absorbance and decrease in the maximum wavelength even though the changes that occur are not so significant for maximum wavelength data. Increased absorbance shows that the concentration of silver nanoparticles formed also increases. The decreasing maximum wavelength indicates that the size of the formed silver nanoparticles also decreases. These results are in accordance with the research conducted by Susilowati [18]. In the sample A1, the sample with the smallest AgNO$_3$ concentration, the maximum wavelength shown is the smallest when compared to other samples. This is possible because the ability of chitosan to reduce the Ag$^+$ ion which is not too much is still at optimal conditions.

3.2. Transmission Electron Microscopy (TEM) analysis

The shape and size distribution of silver nanoparticles in silver-chitosan nanocomposite colloidal can be determined through TEM images shown in Figure 4 for A3 and A6 samples only to compare on shape and size in high and low content of silver nanoparticles in the colloidal.

Figure 4. TEM images for (a) sample A3 and (b) sample A6
Figure 5. Distribution of silver nanoparticle size for (a) sample A3 and (b) sample A6

Based on Figure 4, the resulting silver nanoparticles are spherical in shape. Based on Figure 5, silver nanoparticles are distributed in silver-chitosan nanocomposites with varying diameter sizes, which are in the range of 4-22 nm. Based on the frequency, the distribution of silver nanoparticles is dominated by particles with a diameter of 6-10 nm. When sample A3 and sample A6 were compared, it was seen that sample A6 had a higher concentration of silver nanoparticles than sample A3. This provides confirmation of the results of UV-Vis spectra analysis, which states that the increase in the concentration of AgNO₃ is directly proportional to the increase in the concentration of silver nanoparticles that are formed. The existence of larger particles is possible because of two things. First, there is overlapping or overlapping between particles [18]. Second, agglomeration of silver nanoparticles has taken place. This agglomerated tendency occurs because silver nanoparticles have a large specific surface area, so chemical bonds between particles form a strong electric dipole [5].

3.3. Fourier Transform Infra-Red (FTIR) analysis

The interactions that occur between silver nanoparticles and chitosan in silver-chitosan nanocomposites can be identified through the identification of functional groups using the FTIR spectrophotometer. FTIR spectra for some samples in this research can be seen in Figure 6.

Figure 6. FTIR spectra for samples FA0, FA3 and FA6

Sample FA0 is a sample of chitosan film without silver nanoparticle dispersion. Based on FTIR spectra, sample FA0 shows the absorption peak at the wave number 3448.72 cm⁻¹. This indicates that the sample FA0 has O-H and N-H amine stretching functional groups because the vibration band of the functional group is known to overlap at the absorption peak with a range of wave numbers 3500-
When the spectra of the three samples (Sample FA0, FA3 and FA6) were compared, it was seen that there was a shift and widening of the wave number in the peak region of the amine stretching of the O-H and N-H stretching vibrations. The wave number has shifted from 3448.72 cm\(^{-1}\) (sample FA0) to 3433.29 cm\(^{-1}\) (sample FA3) and 3425.58 cm\(^{-1}\) (sample FA6). Thus, the interaction between silver nanoparticles and chitosan occurs in the O-H (hydroxyl) and N-H (amine) functional groups. This is consistent with the results of the research by Raghavendra [12] and Shah [15]. They claim that the interaction between silver nanoparticles and chitosan occurs in hydroxyl and amino functional groups.

3.4. Scanning Electron Microscope (SEM) analysis
The surface morphology of the silver-chitosan nanocomposite film was observed using SEM. SEM images for some samples can be seen in Figure 7. Sample A0 is a pure chitosan sample without silver nanoparticle dispersion.

Based on SEM images from Figure 7, the increasing concentration of AgNO\(_3\) causes the film surface to become more uneven. This is possible because the concentration of silver nanoparticles that are formed has also increased, causing the polymer chain from chitosan to expand when interacting or bonding with silver nanoparticles. Based on SEM images, it also appears that in sample FA6, clumping occurs, which indicates that silver nanoparticles have experienced agglomeration. This is due to the high concentration and small size of the formed silver nanoparticles in sample FA6, as explained earlier in the UV-Vis spectroscopic analysis. The increasing concentration and small size of the formed silver nanoparticles will increase the tendency of silver nanoparticles to agglomerate, thus causing the surface of the film to become more uneven [15].

![Figure 7. SEM image of surface and cross sections of samples FA0, FA3 and FA6](image)

3.5. Tensile measurements
The tensile test or mechanical properties test of silver-chitosan nanocomposite films was carried out to determine the values of tensile strength, elongation at break, and elasticity (modulus young). The measurement results can be seen in Figure 8. Tensile strength values of silver-chitosan nanocomposite films showed downward trend with increasing AgNO\(_3\) concentrations. This is due to the silver nanoparticles cause inhibit the bond between the chitosan biopolymer chain in the film. High surface area of silver nanoparticles can interact and form bonds with the chitosan polymer chain, that
decreasing the bonding between the chitosan polymer chains. This causes the strengthening properties of silver-chitosan nanocomposite films to decrease [10, 16, 17].

![Graph](image)

**Figure 8.** Mechanic properties of silver-chitosan nanocomposite film (a) tensile strength, (b) elongation, and (c) elasticity

In sample FA1 (0.5% w/w), the tensile strength value shown is the highest than other samples. This may be due to the very small size of the silver nanoparticles, even being the smallest of all samples. With its small size, silver nanoparticles can form a strong bond with the chitosan biopolymer chain. In addition, the concentration of silver nanoparticles that are formed is still relatively small, so that the tendency of agglomerated silver nanoparticles becomes smaller. In the FA4 sample (2.0% w/w), the tensile strength value shows the lowest value among all samples. This may be due to the agglomeration or distribution of uneven silver nanoparticles in the chitosan biopolymer. The uneven distribution of silver nanoparticles makes the tensile strength of the film not homogeneous or unequal on each side. Increasing the concentration of AgNO3 tends to reduce the elongation at break of the film formed. This is caused by interactions that occur between silver nanoparticles and chitosan polymer chains. This interaction causes the mobility of the chitosan biopolymer chain to decrease, so that the strengthening properties of the silver-chitosan nanocomposite film decrease. In samples FA1 to FA5 (0.5% - 2.5% w/w), a reduction in the elongation at break of the silver-chitosan nanocomposite film occurred along with the increased concentration of AgNO3 used. In sample FA6 (3.0% w/w), the elongation at break experienced a greater increase than samples FA3 through FA5. This is possible because of the uneven distribution of nanoparticles in the chitosan biopolymer. Uneven distribution causes the film to have a non-homogeneous tensile strength value, so that when testing, the samples used are not uniform.
The elasticity (Modulus Young) value of the silver-chitosan nanocomposite film is upward trend with the increase in AgNO₃ concentration. The elasticity value is influenced by the value of the tensile strength and elongation at break of the film. The higher the tensile strength value is, the higher the elasticity value will be. The higher the elongation at break value is, the lower the elasticity value will be.

3.6. Swelling studies
Swelling test results can be seen in Figure 9. Based on the swelling test results, the increase in the concentration of AgNO₃ is directly proportional to the increase in the swelling degree of the silver-chitosan nanocomposite film.

![Swelling test results](image)

**Figure 9.** The degree of swelling of silver-chitosan nanocomposite films with varying concentrations of AgNO₃.

Because the increase in the concentration of AgNO₃ is directly proportional to the increase in the concentration of silver nanoparticles and the decrease in size, the degree of swelling has increased with increasing concentration and decreasing the size of silver nanoparticles. This is consistent with the research conducted by Shah [15], which states that the degree of swelling is influenced by the size of the nanoparticles, surface tension, and surface morphology of the nanocomposite film. The growth of silver nanoparticles can cause the chitosan matrix to expand and encourage the formation of an empty space/gap between the chitosan polymer matrices, so it can be easier for liquid to enter and bind to the silver-chitosan nanocomposite film. The contribution of amino and hydroxyl groups from chitosan is also known to cause an increase in the degree of swelling of silver-chitosan nanocomposite films [15].

3.7. Antibacterial activity
The antibacterial activity test of silver-chitosan nanocomposite films against Staphylococcus aureus and Escherichia coli was carried out using the disc diffusion method. Silver-chitosan nanocomposite film discs showed good antibacterial activity against the two bacteria, as evidenced by the appearance of inhibition zones (clear areas) around the film discs on the agar media that had been overgrown with bacteria.
Figure 10. Antibacterial activity of silver-chitosan nanocomposite films with varying concentrations of AgNO₃ against bacteria (a) *Escherichia coli* and (b) *Staphylococcus aureus*

The results of testing the antibacterial activity of silver-chitosan nanocomposite films can be seen in Figure 10. The wider clear areas that arise indicate that the inhibition of a compound against bacterial growth becomes stronger. Based on Table 1, the inhibition of silver-chitosan nanocomposite films against *Escherichia Coli* bacteria is stronger compared to the inhibition against *Staphylococcus Aureus* bacteria. In addition, the dispersion of silver nanoparticles into chitosan biopolymers is proven to have an influence on the activity of the resulting nanocomposite films.

Table 1. Inhibition zone diameter of chitosan silver-chitosan nanocomposite films against *Escherichia Coli* and *Staphylococcus Aureus* bacteria

| Sample | Inhibition Zone Diameter (mm) |
|--------|------------------------------|
|        | *Escherichia Coli* | *Staphylococcus Aureus* |
| FA0    | 0                      | 0                      |
| FA1    | 13.13                  | 11.11                  |
| FA2    | 13.75                  | 12.42                  |
| FA3    | 14.38                  | 13.07                  |
| FA4    | 14.38                  | 12.42                  |
| FA5    | 13.75                  | 12.42                  |
| FA6    | 15.00                  | 13.07                  |

The increase in the inhibition zone area for the two bacteria shows a trend that tends to increase along with the increase in the concentration of AgNO₃ used, which also means that the increase in the concentration of silver nanoparticles formed is also directly proportional to the increase in the area of the inhibition zone of the silver-chitosan nanocomposite film against the two bacteria. The antibacterial activity of this film nanocomposite solely comes from silver nanoparticles, because without silver nanoparticles (FA0), inhibition zone is zero for two bacteria.

4. Conclusion

Silver-chitosan nanocomposite films can be made using chemical reduction methods with microwave irradiation, where chitosan is used as a reducing agent as well as a stabilizer and NaOH as an accelerator in the preparation of silver nanoparticles. Based on TEM images, the resulting silver nanoparticles are spherical in shape and are distributed with different sizes in the 6-10 nm diameter range. Based on the analysis of functional groups using FTIR, interactions between silver nanoparticles and chitosan in silver-chitosan nanocomposite occur in the hydroxyl group (O-H) and
Amine group (N-H) of the chitosan polymer. Based on SEM images, the surface morphology of the silver-chitosan nanocomposite film showed an uneven surface in line with the increase in the concentration of AgNO₃. The decrease in the tensile strength and elongation at break occur along with an increase in the concentration of AgNO₃ used while the elasticity of the film fluctuates depending on the tensile strength and elongation at break. The increasing concentration of AgNO₃ used also causes an increase in the degree of swelling of silver-chitosan nanocomposite films. The silver-chitosan nanocomposite film showed high antibacterial activity against Escherichia coli than Staphylococcus aureus. The antibacterial activity of this film nanocomposite solely comes from silver nanoparticles.

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References
[1] Afonso C R, Hirano R S Gaspar A L, Chagas, E G, Carvalho R A, Silva F V, Leonardo G R, Lopes P S, Silva C F, and Yoshinda C M P 2019 Int. J. Biol. Macromol 132 1262-1273
[2] Ahmad M B, Lim J J L, Shameli K, Ibrahim N A, and Tay M Y 2011 Molecules 16 7237-7248
[3] Ahmad M B, Tay M Y, Sahmeli K, Hussein M Z, and Lim J J 2011 International Journal of Molecular Sciences 12 4872-4884
[4] Aranaz, I, Mengibar M, Harris R, Panos I, Miralles B, Acosta N, Galed G, and Heras A 2009 Current Chemical Biology 3 203-230
[5] Ariyanta H A 2014 Jurnal Media Kesehatan Masyarakat Indonesia 10 36-42
[6] Bozanic D K, Trandafilovic L V, Luyt A S and Djokovic V 2010 React. Funct. Polym 70 869-873
[7] Chang A K T, Frias R R, Alvarez, L V, Bigol U G, and Guzman J P M D 2019 Biocatalysis and Agricultural Biotechnology 17 189-195
[8] Dachriyanus 2004 Padang LPTIK Universitas Andalas
[9] Darroudi M, Ahmad M B, Abdullah A H, Ibrahim N A, and Shameli K 2010 International Journal of Molecular Sciences 11 3898-3905
[10] Hadiyawarman A R, Nuryadin B W, Abdullah M, dan Khairurrijal 2008 Jurnal Nanosains & Nanoteknologi 1 14-21
[11] Pratiwi R 2014 Oseana 39 35-43
[12] Raghavendra G M, Jung J, Kim D, and Seo J 2016 Int J Mol Sci 84 281-288
[13] Salari M, Khiabania M S, Mokarrama R R, Ghanbarzadeha B and Kafic H S 2018 Food Hydrocolloids 84 414-423
[14] Sastrohamidjojo H 2001 Yogyakarta Liberty Yogyakarta
[15] Shah A, Hussain I, and Murtaza G 2018 Int J Biol Macromol 116 520-529
[16] Sriyanti I 2009 Proceeding of The Third International Seminar on Science Education 1-6
[17] Sudibyo A, dan Hutajalu T F 2013 Jurnal Kimia dan Kemasan 35 6-19
[18] Susilowati E, Tiyon, Santosa S J, and Kartini I 2015 Indones J Chem 15 29 – 35
[19] Wahyudi T, Sugiyana D, and Helmy Q 2011 Arena Tekstil 26 55-60