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Fabrication of Nylon-66 Membranes Coated with Violacein Pigment for Wound Dressing Application

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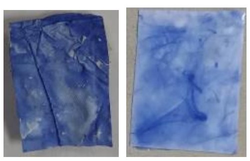
GRAPHICAL ABSTRACT



Images of (a) casted nylon-violacein membrane (b) electrospun nylon-violacein membrane

ABSTRACT

Wound dressing is used for effective wound healing and a suitable material must be used to cover wound. Nylon was chosen because it has high mechanical strength and has hydrophilic properties compared to other polymers. The incorporation of violacein pigment as natural antibacterial agent onto the nylon membranes will give added value to the wound dressing application. Two methods of fabrication were carried out which were electrospinning and casting technique. Based on the images obtained from Field Emission Scanning Electron Microscope (FESEM), the morphology of nylon membrane was made up of nanofibers which indicated the production of high surface area to volume ratio of electrospun membrane. Furthermore, the tensile strength of electrospun membrane, 8.0292 MPa is higher compared to casted membrane,3.284 MPa which shows better elasticity. By using Differential Scanning Analysis-Thermogravimetric Analysis (DSC-TGA), the decomposition temperature and the melting temperature of nylon membrane is high which are in the range of 477-485 ºC and 250-254ºC respectively, showing it has high thermal resistance compared to other polymers. From Attenuated Total Reflection-Fourier Transform Infrared Spectrospcopy (ATR-FTIR) results, there is a presence of C=C aromatic absorption indicating the presence of violacein pigment in the nylon membrane. Furthermore, the release rate of electrospun nylon-violacein membrane is higher than casted nylon-violacein membrane. For antibacterial test, the nylon-violacein membrane showed effective inhibition against Gram-positive bacteria but negative result towards Escherichia coli (Gram-negative). By comparing these two methods, we can conclude that electospinning technique is more suitable compared to casting technique in order to fabricate desired wound dressing. Also, the use of nylon materials as fabric and violacein pigment as natural antibacterial agent in wound dressing application are beneficial for effective wound healing.

Keywords: Nylon, electrospinning technique, violacein pigment, antibacterial activity

1. INTRODUCTION

Wound dressing is used for effective wound healing and a suitable material must be used to cover wound in order to prevent any infection. Previously, honey pastes, plant fibers, and animal fats were used as wound dressing materials. Recently, with natural biopolymer or synthetic biopolymer and fabrication techniques, a wound dressing material is expected to have extraordinary properties which enhance the healing process of a wound. The following properties are generally considered for all wound dressing materials which are able to maintain moist at the wound interface, absorb excess exudates without leakage to the surface of a dressing, provide thermal insulation and bacterial protections [1].

Nylons are synthetic polyamide polymers having good thermal and mechanical strength which make they suitable to be used in wound dressing application. The nylon polymers tend to be semi-crystalline and are generally very tough materials with good thermal and chemical resistance [2]. One of the most common polymeric solution used for wound dressing is nylon and the best technique for making wound dressing is electrospinning technique. This is because, the electrospinning of nylon nanofiber will form a high surface area to volume ratio, high porosity, and small pore size of nanofiber. Also, nylon is a hydrophilic polymer and thus, the nanofibrous dressings will be able to absorb wound exudates more efficiently than the typical film dressings [3]. In addition, the porous structure of a nanofiber is excellent for the respiration of cells which does not lead the wound to dry up. This indicates an appropriate control of a moist environment for the wound [1].

Some of the wound dressing have antibacterial agent functioning for killing bacteria or slow down the bacteria growth and help to heal wound quickly. *Chromobacterium violaceum* is a gram-negative bacterium that grows in soil and water. It produces violacein, a water insoluble purple pigment with antibacterial activity. The violacein pigment is a natural pigment with high biodegradability and low toxicity compared with synthetic pigment. In this study, violacein pigment extracted from *C.violaceum* will be used as antibacterial agent for wound dressing application [4].

2. EXPERIMENTAL

The experiment was divided into three main stages. The first stage was to fabricate the 14 wt.% of nylon solution using electrospinning technique and casting technique. The electrospinning of nylon was carried out under constant conditions of 20 kV voltage, 0.4 $mL.h^{-1}$ of flow rate, 15 cm tip-to-collector distance, in room temperature and 200 r/min of collector drum rotation speed. An aluminum foil on rotating drum with diameter of 8 cm was used as a substrate for collecting the obtained nanofibers. In casting technique, the nylon solution was fabricated using a dry-phase inversion method and wet phase inversion method. The homogenous clear solution was cast onto the glass plate using a casting knife and was dried at room temperature for 30 minutes. The thoroughly dried membrane was then immersed in deionized water and peeled from the glass plate. Figure 1(a) until Figure 1(b) shows the schematic diagram of electrospinning technique and casting technique. In order to choose the best technique for making wound dressing, the membranes were then characterized by using Attenuated Total Reflection-Fourier-Transform Infrared Spectroscopy (ATR-FTIR), Differential Scanning Calorimetry- Thermogravimetric Analysis (DSC-TGA), Field Emission Scanning Electron Microscope (FESEM) and tensile test.

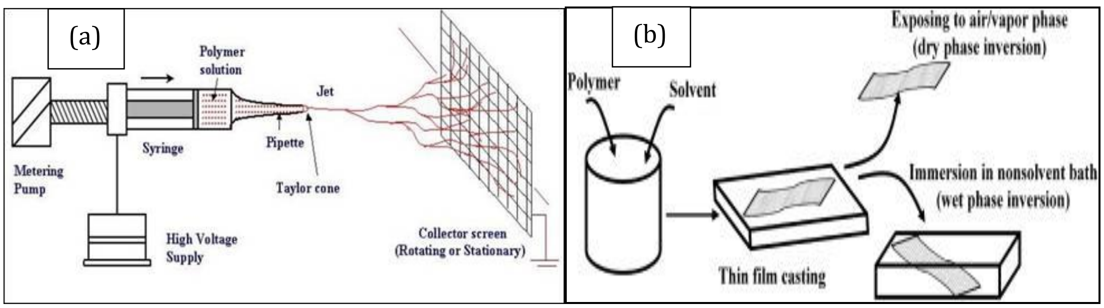


Figure 1 Schematic diagram of (a) electrospinning technique (b) Casting technique [3].

Next, the membranes produced were then coated with violacein pigment extracted from *C.violaceum* UTM5 using dip coating technique. After the fabrication of nylon, the nylon membranes were coated directly with violacein pigment. This technique required the membranes to be immersed in the violacein pigment with ethyl acetate as a solvent. The coated nylon membranes were then dried at room temperature and followed by testing the antibacterial activity and testing the release rate of nylon membranes coated with violacein pigment.

The evaluation of antibacterial activity of violacein pigment was carried out by disc diffusion method. In this method, Gram-positive bacteria (*S.aureus* ATCC 6538) and Gram-negative bacteria (*E.coli* ATCC 8793) were used against violacein pigment. The standard antibiotic solution used were vancomycin for Gram-negative bacteria and streptomycin for Gram-positive bacteria. The bacteria were grown for about 18-24 h and the bacteria were diluted until the optical density become 0.08-0.10. Then, 50 mL of the bacteria were streaked on Mueller-Hinton agar plates. The discs from the wound dressing were cut 6 mm and was added into 500 ppm of standard solution followed by placing onto Mueller-Hinton agar plates streaked with the bacteria. The agar plate was incubated for 1 day and the zone of inhibition was measured. The determination of release rate was obtained by measuring the absorbance of violacein pigment using UV-Visible spectrophotometry. The absorbance was then calculated using formula from Beer-Lambert Law which is $A = \varepsilon lc$. A is absorbance, ε is molar absorptivity, l is length of solution the light passes through in cm and c is the concentration of solution in mol dm^{-3}.

3. RESULTS AND DISCUSSION

3.1 Analysis IR spectrum

Based on the Table 1, the peak taken was only the significant peak for nylon. In all spectrum, the peak for C=O was belonging to amide group hence it clearly indicated the present of nylon. It also contains only one peak for N-H stretch as the nylon-6,6 contain secondary amine. For cast nylon-violacein membrane and electrospun nylon-violacein membrane, there is a presence of C=C aromatic absorption and C-H alkenes absorption indicated the aromatic ring in violacein pigment. Thus, this clearly shown the presence of violacein pigment when we incorporated violacein pigment onto the cast membrane and electrospun membrane.

Table 1 Analysis IR Spectrum of nylon membranes

Types of absorption	Frequency (cm^{1}) Casted membrane	Frequency (cm^{1}) Casted-violacein membrane	Frequency (cm^{1}) electrospun membrane	Frequency (cm^{1}) Electrospun-violacein membrane
C=O (amide)	1629.17	1630.45	1631.94	1633.52
N-H (stretch)	3297.80	3296.78	3298.01	3299.50
C-N (amine)	1199.48	1274.83	1199.10	1273.75
C-H (alkanes)	2950.00	2933.77	2932.15	2932.65
C=C (aromatic)		1534.78 and 1473.08		1535.52 and 1463.17
C-H (alkenes)		3079.68		3082.21

3.2 FESEM analysis

Based on the Figure 2, there is no significant differences between the casted nylon membrane and casted nylon-violacein membrane. The casted membrane has rough surface compared to electrospun membrane. Next, for electrospun nylon membrane and electrospun nylon-violacein membrane there was a formation of spindle-like shaped of nanofibers. The electrospinning of nylon produced nanosized fabric which contain nanosized pores with average diameter 127.4nm.

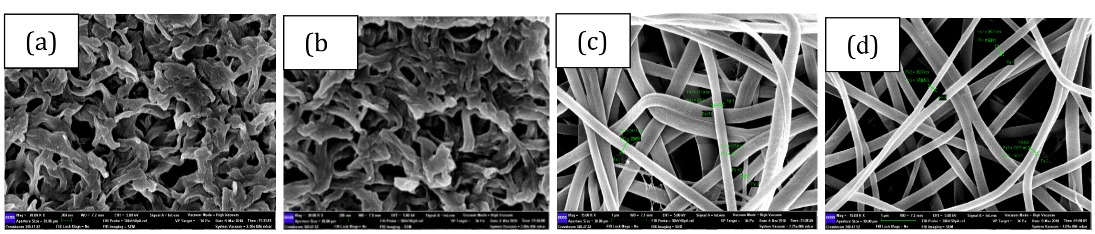


Figure 2 FESEM images for (a) casted nylon membrane, (b) casted nylon-violacein membrane, (c) electrospun nylon membrane, (d) electrospun nylon-violacein membrane.

3.3 Mechanical properties of Nylon membrane

From the mechanical test, the tensile strength, elongation break and Young's Modulus of elasticity were obtained. Tensile strength is the amount of force needed to pull the composites to the where it breaks. It can be defined as the maximum amount of tensile stress that can be subjected to before failure. From the graph below, the tensile strength were 3.284 MPa, 2.1538 MPa, 8.0292 MPa and 6.1517 MPa for casted nylon membrane, casted nylon-violacein membrane, electrospun nylon membrane and electrospun nylon-violacein membrane respectively. The tensile strength for electrospun nylon membrane is the highest followed by electrospun nylon-violacein membrane, casted nylon membrane and lastly was casted nylon-violacein membrane. This shows that the electrospun membrane has higher mechanical strength compared to casted membrane.

The elongation break is the ratio between changed length and initial length after breakage of the nylon membrane. It can be said as the capability of film to resist changes of shape without crack formation. From the graph below, the elongation break were 5.41, 4.47, 41.09 and 30.8 for casted nylon membrane, casted nylon-violacein membrane, electrospun nylon membrane and electrospun nylon-violacein membrane respectively. Supposedly the elongation break of electrospun membrane should be lower compared to cast membrane. This is because when the electrospun membrane has high tensile strength it should has low elongation break. The electrospun membrane supposedly can resist changes of shape thus the ratio between changed length and initial length after breakage should be lower. However the value of elongation break in electrospun membrane is high. This is might due to error during conducting the tensile test. The membrane were not gripped correctly on the machine and caused the membrane to slip away from the wedges grip.

The Young's Modulus measures the stiffness and elasticity of the composites formed. From the graph below, the Young's Modulus obtained were 0.6749 MPa, 0.8620 MPa, 0.3682 MPa and 0.2929 MPa for casted nylon membrane, casted nylon-violacein membrane, electrospun nylon membrane and electrospun nylon-violacein membrane respectively. Supposedly the Young's Modulus for electrospun membrane is higher compared to casted membrane. Electrospun membrane has higher tensile strength and the stiffness of membrane should be higher. However from the graph, the stiffness and elasticity obtained for electrospun nylon membrane is lower compared to casted membrane. This is might due to error during conducting the tensile strength.

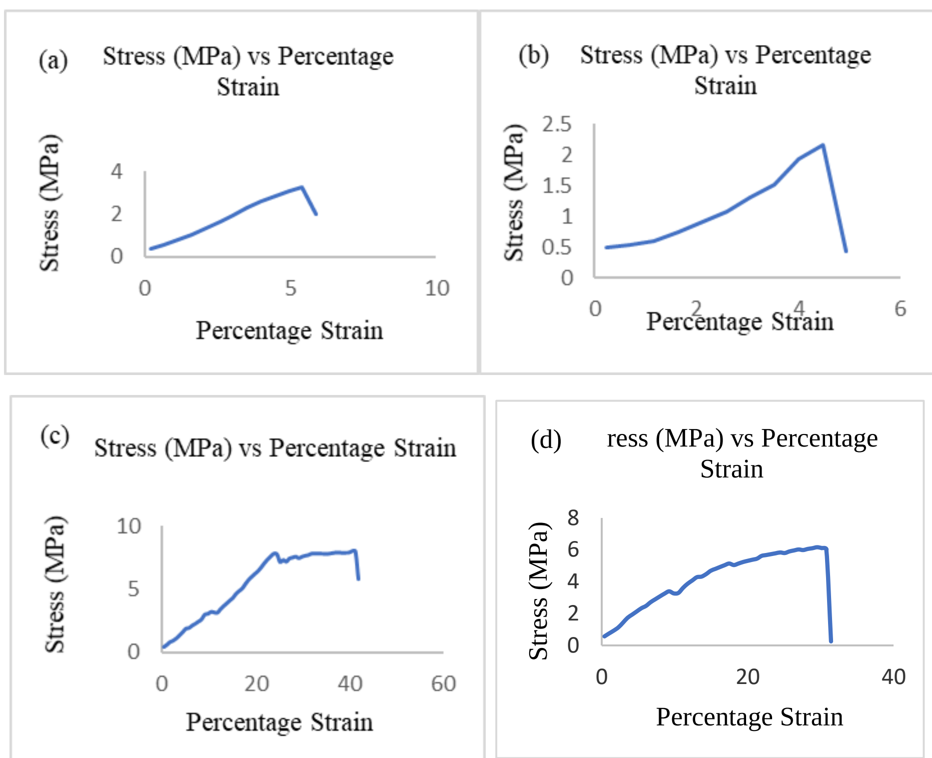


Figure 3 Stress vs Strain graph of (a) cast nylon membrane, (b) cast nylon-violacein membrane, (c) electrospun nylon membrane, (d) electrospun nylon-violacein membrane.

3.4 TGA analysis

TGA analysis was used to evaluate thermal degradation properties of nylon-66. These samples show rapid weight loss between 370-480 ^{0}C. TGA analysis was done in room temperature until reaches above its decomposition temperature which is 500 ^{0}C in the presence of nitrogen gas using 20 ^{0}C/min of heating rate. The nitrogen gas which is inert gas was used to avoid it from react with sample which can increase the mass of the samples.

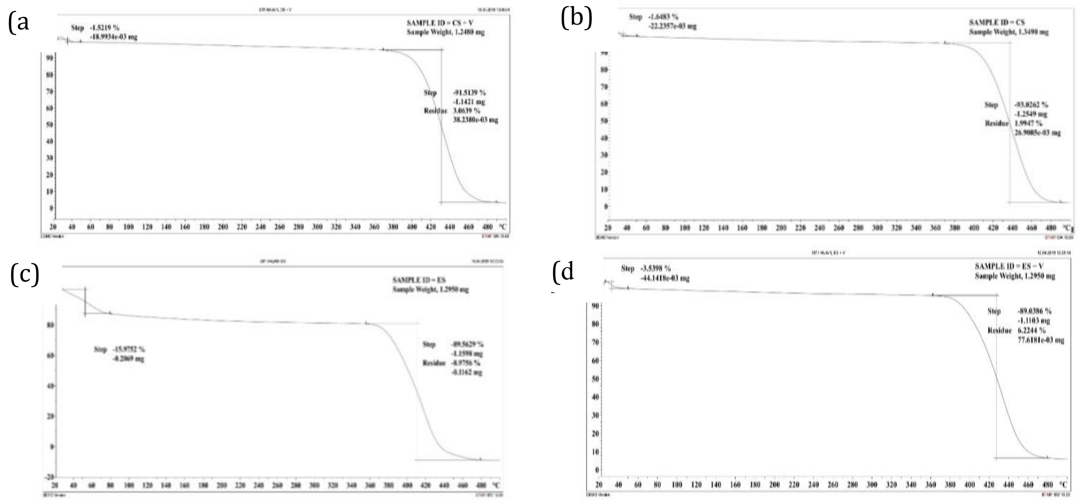


Figure 4 TGA Plot for (a) cast nylon membrane; (b) cast nylon-violacein membrane; (c) electrospun nylon membrane; (d) electrospun nylon-violacein membrane.

From Figure 4, in the step 1 of each sample, the weight of samples decreases because the moisture in each sample undergo evaporation. The water inside the sample was evaporated and it caused the weight loss. From the graph in Figure 4(a) until Figure 4(d), in the temperature range of 80-370 ^{o}C the graph remains constant for a wide range of temperature because the hydrophilic part of nylon structure help to retain moisture. Furthermore, nylon-6,6 also has hydrogen bonding which directly line up creating a strong, dense polymer structure [5]. Thus, it needs higher temperature to deform the structure of nylon. The initial decomposition temperature for both nylon membranes coated with violacein pigment is a little bit higher compared to pure nylon membranes because the violacein pigment contain aromatic ring. It needs more temperature to break the aromatic bond. All nylon membranes were completely decomposed in the temperature range of 477-485 ^{o}C.

3.5 DSC Analysis

Based on Figure 5, the melting temperature, glass transition temperature and crystallinity temperature were measured using DSC. Generally, the glass transition for pure nylon is 50-60 ^{o}C, the crystallinity temperature is 215-240 ^{o}C and the melting temperature which is nearest to crystallinity temperature is 255-265 ^{o}C [6].

The glass transition temperature is a temperature region where the polymer transitions from a hard, glassy material to a soft, rubbery material. From the DSC plotted, the glass transition obtained were 50 ^{o}C, 50 ^{o}C, 52 ^{o}C and 54 ^{o}C for casted nylon membrane, casted nylon-violacein membrane, electrospun nylon membrane and electrospun nylon-violacein membrane respectively. The glass transition temperature for electrospun membrane is higher than casted membrane. Next, the melting temperature is the temperature at which a given material changes from a solid to a liquid.

The crystallization of polymers is a process associated with partial alignment on their molecular chains. Polymers can crystallize upon cooling from the melt. The crystallinity temperature obtained were 240 ^{o}C, 242 ^{o}C, 244 ^{o}C and 248 ^{o}C for casted nylon membrane, casted nylon-violacein membrane, electrospun nylon membrane and electrospun nylon-violacein membrane respectively. There is no siginificant differences in crystallinity temperature but the highest crystallinity temperature obtained was electrospun nylon-violacein membrane compared to other membranes.

The melting temperature obtained were 250 ^{o}C, 252 ^{o}C, 253 ^{o}C and 254 ^{o}C for casted nylon membrane, casted nylon-violacein membrane, electrospun nylon membrane and electrospun nylon-violacein membrane respectively. The electrospun nylon-violacein membrane has the highest glass transition temperature, crystallinity temperature and melting temperature compared to other membranes [5]. This is because it has violacein pigment which has aromatic ring and it requires high temperature to break the conjugated double bond. The mechanical strength for electrospun membrane is also higher compared to casted membrane and thus it needs more heat to decompose the membrane.

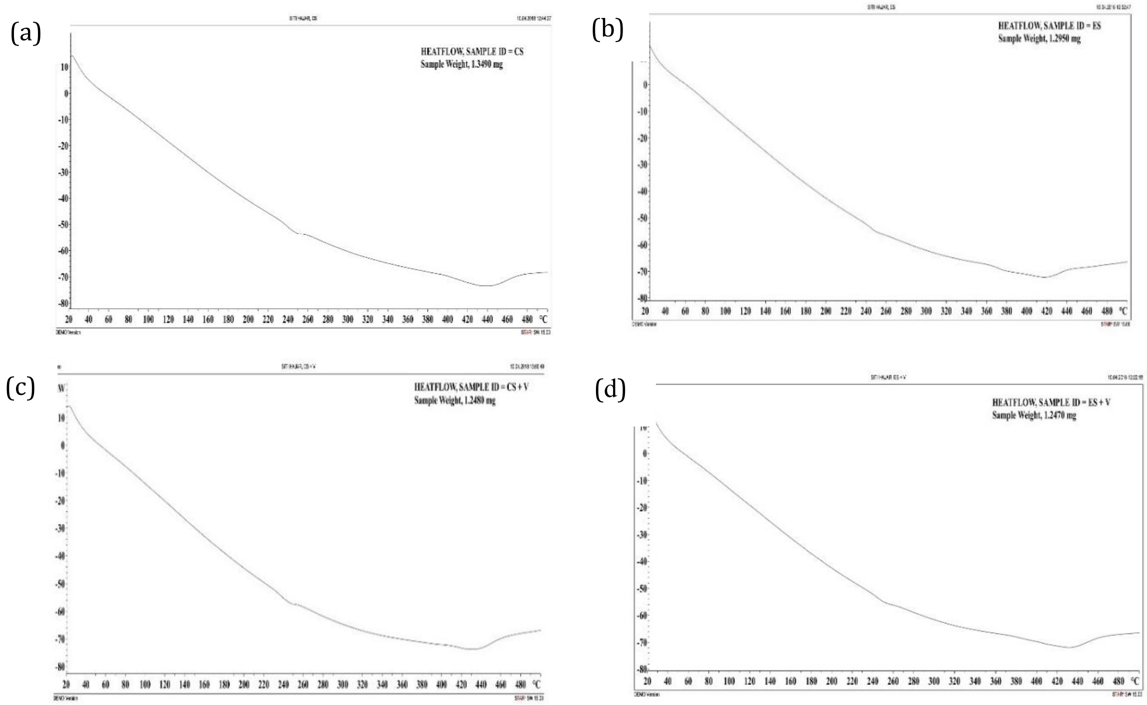


Figure 5 DSC Plot for for (a) casted nylon membrane, (b) casted nylon-violacein membrane, (c) electrospun nylon membrane, (d) electrospun nylon-violacein membrane.

3.6 Antibacterial Activity of Nylon Membrane Coated with Violacein Pigement

The evaluation of antibacterial activity was carried out using disc diffusion technique. The violacein pigment was active against Gram-positive bacteria (*Staphylococcus* ATCC 6538) [9]. The zone of inhibition was measured. The zone of inhibition measured for casted nylon-violacein membrane was 2 mm and for electrospun nylon-violacein membrane was 4 mm [8]. The zone of inhibition measured for electrospun nylon-violacein membrane is larger compared to cast nylon-violacein membrane. For Gram-negative bacteria (*Escherichia coli* ATCC 8793) there is no zone of inhibition which means the violacein pigment are not active against Gram-negative bacteria. Figure 6(a) until Figure 6(b) shows the zone of inhibition of nylon membranes coated with violacein pigment against *S.aureus* bacteria and *E.Coli* bacteria respectively.

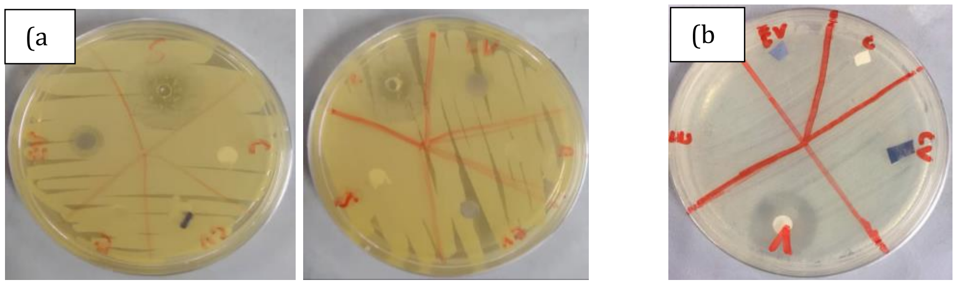


Figure 6 The zone of inhibition of nylon membranes coated with violacein pigment (a) against *S.aureus* bacteria (b) against *E.Coli* bacteria.

3.7 The Release Rate of Nylon Membrane Coated with Violacein Pigment

From the Figure 7, the release rate of violacein pigment is higher in electrospun nylon-violacein membrane compared to casted nylon-violacein membrane. For electrospun membrane, the concentration is constant after 10 hours while for cast membrane the concentration is constant after 8 hours. This indicated that the electrospun membrane can absorb and released out the violacein pigment higher compared to cast membrane. The higher the concentration the higher the higher the absorbance and the higher release rate of nylon membranes coated with violacein pigment.

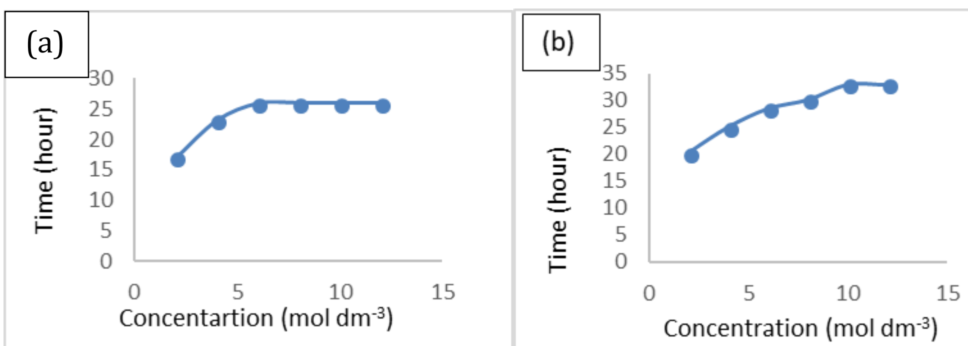


Figure 7 Graph time (hour) against concentration mol dm^{-3} of (a) casted nylon-violacein membrane (b) electrospun nylon-violacein membrane.

4. CONCLUSION

As the conclusion, the nylon membrane produced by electrospinning technique has nanofiber and has smooth surface membrane compared to membrane produced by casting technique. Moreover, electrospun membrane has high tensile strength indicated the high elasticity of the membrane and high mechanical strength of the membrane. It also has high thermal properties because the nylon membrane needs high temperature to be decomposed. The violacein pigment was incorporated into nylon membrane forming wound dressing application. The function of violacein pigment is to act as antibacterial agent. This is proven when there is a zone of inhibition of violacein pigment against Gram-positive bacteria but there is no zone of inhibition against Gram-negative bacteria. This is shows that the violacein pigment active against Gram-positive bacteria. Apart from that, the release rate of violacein pigment is higher for electrospun membrane compared to casted membrane making the technique more suitable to be applied in wound dressing application.

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