

Investigating Fluid Parameters in Nanofiber Biomaterial Fabrication using Electrospinning

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Abstract

Fabricating nanofiber biomaterial using electrospinning is difficult due to its bioactive characteristics. Even though electrospinning is mentioned as the most well-established approach to produce nanofiber, it is undeniable that fluid factors involved in determining the product result. In this research, three influenced factors including viscosity, conductivity, and surface tension are investigated in the system of Biomaterial Composite that contains mixed Chitosan-Pectin material blended to the Polyvinyl Alcohol (PVA). Various concentrations were made up to create an assorted liquid profile to some extent influencing fluid characteristic which affecting fabrication result. This research also analyzed the interaction between group materials using Fourier Transform Infra-Red (FTIR). Moreover, bead and spray phenomena which are commonly occurred in the process of fabrication are also deliberated correlating with fluid parameters. This experiment revealed that the range of the ability of the composite solution that can be fabricated was from 90/10 to 60/40 with the average diameter size for each composition are 90/10 = 155,39 ± 43,68 nm, 80/20 = 99,03 ± 26,01 nm, 70/30 = 111,387 ± 50,06 nm, and 60/40 = 107,06 ± 47,36 nm. Regarding fluid characteristics, the discrepancy related to the effect of viscosity to nanofiber size has occurred due to the nonuniform shape and type that affected the average size of the nanofiber. Meanwhile, the conductivity parameter found as the main reason related to the limited ability of the electrospinning process. Furthermore, the surface tension parameters noted as a factor that influencing droplet and beads formation.

Keywords: electrospinning; nanofiber; viscosity; conductivity; surface tension

1. INTRODUCTION

Electrospinning is a method that can convert the solution into nanofiber by using electrostatic force by establishing potential differences between a tip of the needle as a positive pole and a collector as a negative pole. The electrospinning process is started by pushing the solution in a syringe until it creates a drop of solution hanging at the tip of a needle, called Tylor cone, which is caused by surface tension. Nanofiber that is produced by the electrospinning process is created from that Tylor cone which is being pulled and elongated by electrostatic force. The transformation process from solution into nanofiber occurs between base and collector caused by the evaporation process that is affecting the decreasing diameter relative to the elongated process [1].

The result of electrospinning fabrication influenced by exceptional parameters that are linked to each other caused the fabrication process is tricky. Those parameters can

be categorized into three groups which are: set up parameters, solution parameters, and environmental parameters. Set up parameters are the group of influence factors that correlated to the machine characteristic of electrospinning itself including voltage, distance between the tip of needle and collector, needle design, collector design, and flow rate setting. Meanwhile, solution parameters are the variable related to solution properties or fluid characteristics considered by viscosity, conductivity and surface tension. The third parameter which is the environmental factors are the parameters that are affected by temperature and humidity. The biggest challenge in understanding the electrospinning process is its fluid dynamic [1]. Fabrication in the electrospinning process is the transformation from the fluid solution from spinneret which has a diameter in millimeter to fiber with nanometer-sized which has four or five times smaller than spinneret diameter.

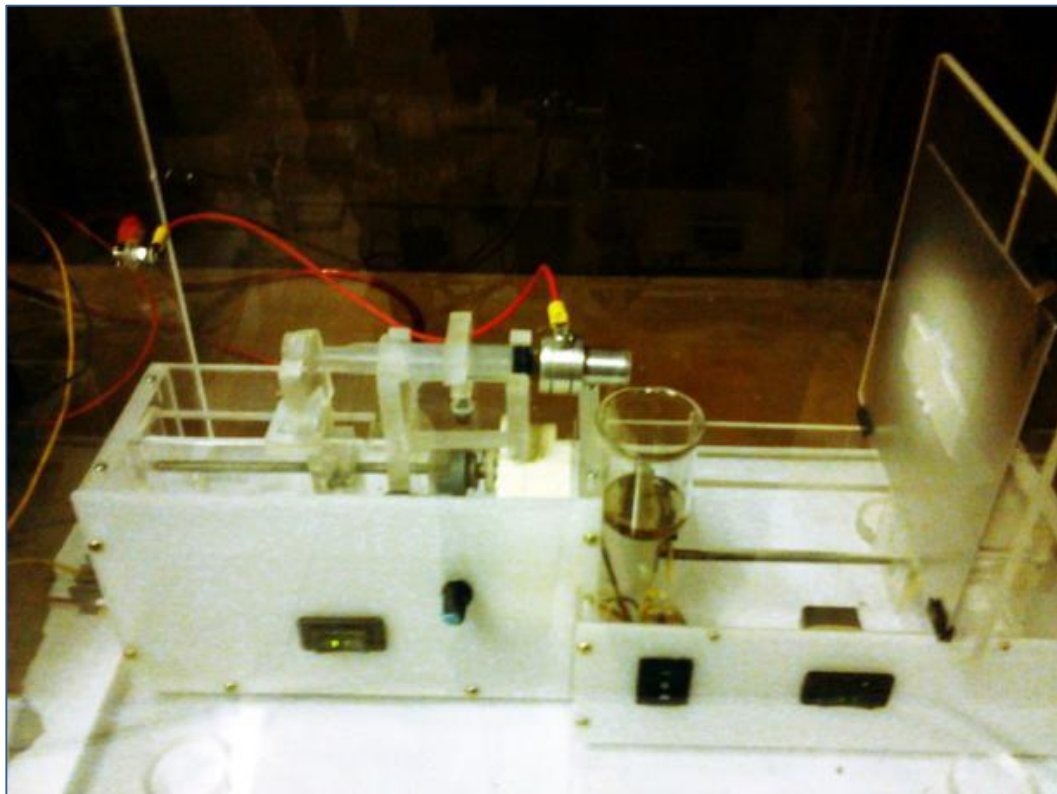


Figure 1. Electrospinning Process

This research imposed to investigate fluid parameters by varying composition PVA to chitosan-pectin that leads to different values of viscosity, conductivity, and surface tension. The other variables, set up and environment, are controlled to avoid disturbance while analyzing the system. Viscosity is defined as internal shear or fluid friction toward the layer where the fluid is flowing. It is friction between adjacent fluid layers when they move across one another or as the internal resistance of a fluid to flow as a measurement of the fluid shift. Viscosity also can be interpreted as the ability of a substance to flow in a particular media. Conductivity defined as how strong a solution can deliver electrons. It is used to measure the electrolyte solution. The greater the number of ions from a solution, the higher the conductivity value. Surface tension is the tension formed between fluid and air. It occurs because of the resultant differences in the attraction of molecules on the surface liquid [3].

Composite material based on PVA-Chitosan-Pectin is used in this experiment. PVA is a material that is often used as a polymer material for nanofiber fabrication using electrospinning. Various literature shows that PVA is a polymer that is often used as a guest polymer in various kinds of research to fabricate nanofiber with electrospinning [4][5][6][7]. This study used a guest polymer in the form of PVA to be able to fabricate

chitosan and pectin based on its ability to interact with them. Interaction of PVA with chitosan and pectin can occur through hydrogen bonds at the molecular level. This hydrogen bond is formed through the interaction of the amine and hydroxyl groups [7]. Chitosan is a natural product that is derived from polysaccharide chitin. Chitosan has the chemical name Poly D-glucosamine (beta (1-4) 2-amino-2deoxy-D-glucose). It has a chain that is shorter than the chitin chain. The solubility of chitosan in acidic solutions, as well as the viscosity of the solution, depends on the degree of deacetylation and the degree of degradation of the polymer [8]. Pectin is a polymer from D-galacturonic acid linked by 1.4 glycosidic bonds [9]. It is used as cross-linking for chitosan is a cationic polymer to form a polyelectrolyte complex. Chitosan experiencing crosslinking with pectin has been studied and shows an increase in hydrophilic nature, biocompatibility, and mechanical strength [10].

2. METHODS

The initial step in this experiment is making a specific ratio of the solution as a sample that has to be loaded in electrospinning. This step is conducted by making four types of solutions which are: Chitosan 3%, Pectin 3%, PVA 10%, and chitosan-pectin-PVA composite mixed solution. This research used local chitosan with a degree of deacetylation $\geq 90\%$ belong to Medical Grade produced by PT. Biotech Surindo. Meanwhile, pectin considered as high methoxyl with the degree of esterification >50 produced by Cargill. PVA that is used in this experiment is Gohsenol with a code of the product is C1210A57 produced by P.T. Brataco. And Acetic acid (Glacial) 100% Merck KgaA with Akuabides Sterile Water for Irrigation U.S.P. PT. Otsuka Indonesia is used to make solvent.

This research used PVA versus chitosan pectin ratio as a main modified variable to study influenced to fluid characteristics. The control group in this experiment are PVA, chitosan, pectin, and mixed chitosan-pectin which all is in the solution phase. Meanwhile, the experimental group is PVA/Chitosan-Pectin ratio that is varied 90/10, 80/20, 70/30, 60/40, and 50/50 respectively. The variation of PVA to chitosan-pectin is conducted from 90/10 until the fiber cannot be fabricated. This composition is used in volume (V/V).

The controlled variables in this study are based on optimum value form specific experiment, which are: Comparison of chitosan-pectin 1: 1 [10][11], 10% PVA concentration [4][6], 3% chitosan concentration in 1.5% acetic acid, Pectin concentration 3% (Adjustment ratio of 1: 1), Electrospinning set up parameters which include: a. Distance of needle tip and collector = 12 cm b. Voltage = 15kV (stable voltage) c. Needle diameter = 0.5 mm d. Flow rate = around 1.5 ml / hour.

Viscosity measurement was conducted by using the Ostwald viscosity meter by comparing two types of fluids which are samples and another liquid such as aquades. Based on this method the viscosity of a solution can be determined by the equation (1):

$$\eta_x = \eta_a \frac{t_x \rho_x}{t_a \rho_a} \quad (1)$$

Where:

η_a = aquades viscosity

η_x = sample viscosity

t_a = time flow of equates

t_x = time flow of sample

ρ_a = aquades density

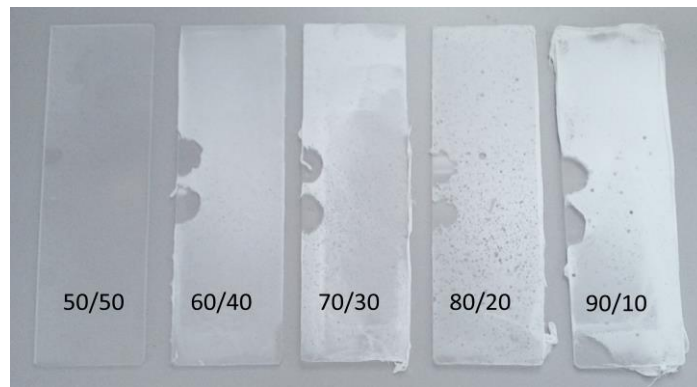
ρ_x = sample density

Conductivity values are measured by using Lovibond Conductivity Meter, LPPT UGM. Conductivity is the opposite of electrical resistance (R) and its unit is ohm⁻¹ (Ω^{-1}) or Siemen (S). The conductivity of a solution depends on the number of ions and the speed of the ions on the potential difference between the two electrodes. Factors that influence ion velocity include Ion weight and charge, hydration, solvent atmospheric orientation, ionic attraction, temperature, and viscosity.

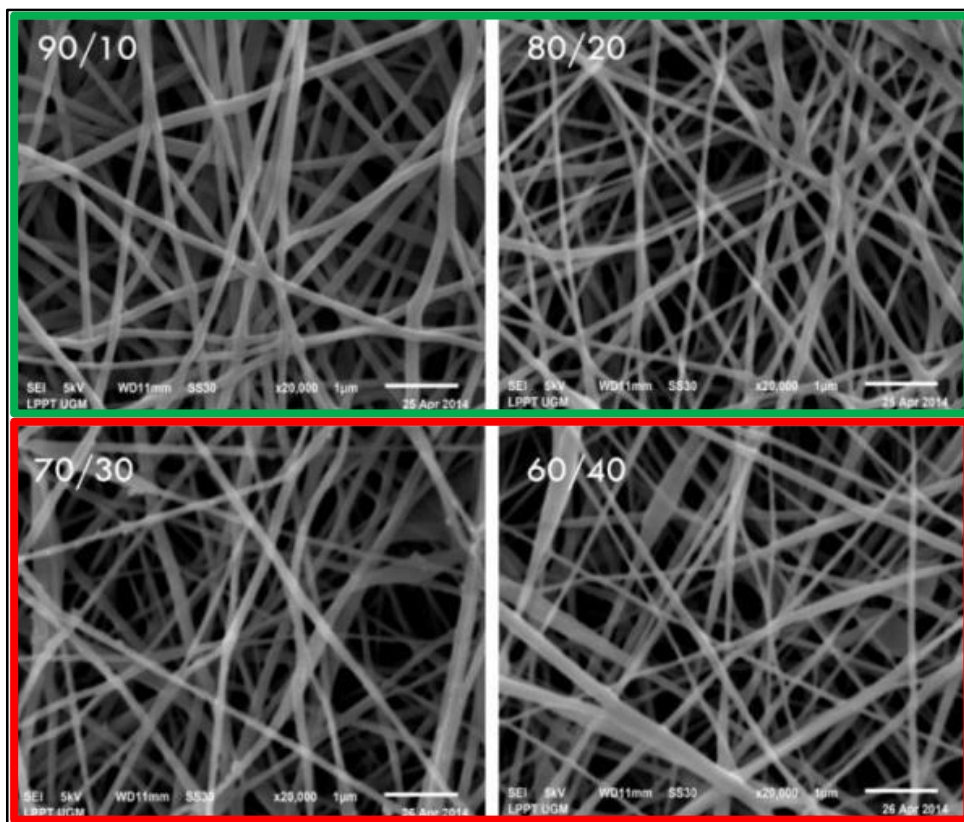
Du-Nouy ring method is one method that can be used to measure surface tension. The principle of this method is utilizing the force that is needed to release an iridium platinum ring which is proportional to the surface tension or interface tension of the liquid. Surface strength in this study was calculated using a Surface Tensiometer, Kruss, Karl Kolb, Heat and Mass Transfer Laboratory, Center for Engineering Studies in PAU UGM.

3. RESULT AND DISCUSSION

This study revealed that the range of the ability of the composite solution that can be fabricated was from 90/10 to 60/40. Figure 2a shows the fabrication results for each composition variation from 90/10 to 50/50 and it can be seen that the decreasing composition of PVA in composites, the ability of electrospinning to fabricate fiber is also reduced.



(a)



(b)

Figure 2. (a) The result of electrospinning from 90/10 until 50/50
(b) Results at magnification 10,000 times from 90/10 until 60/40

SEM is conducted to determine the size of the fiber fabrication results. Based on the size of the fiber, it can be seen that the fiber has reached the size of a nanometer. The results of SEM characterization for each composition can be seen in Figure 2b.

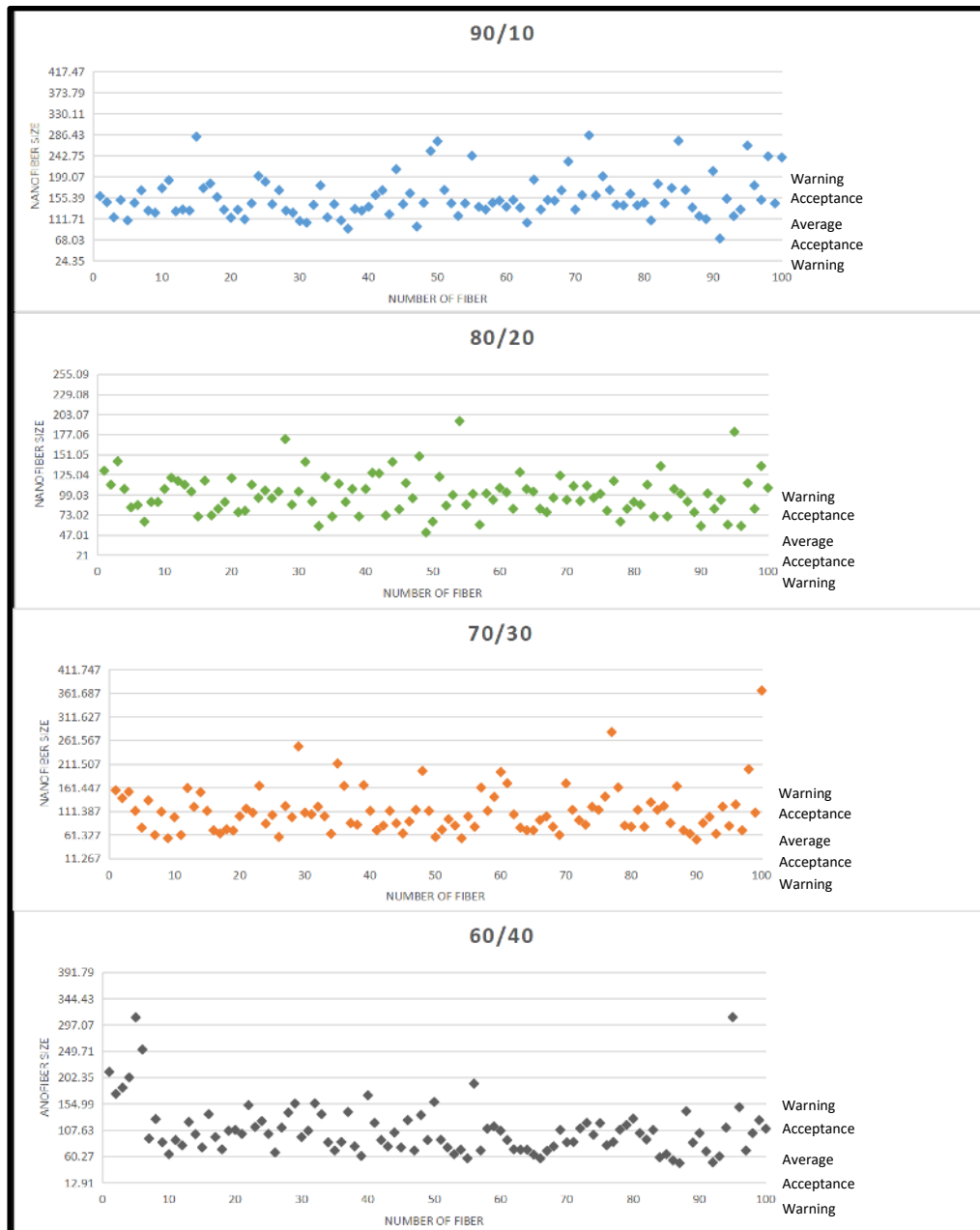


Figure 3. Fiber size diameter distribution ($n = 100$)

Based on the calculation, the average diameter size for each composition is 90/10 = $155,39 \pm 43,68$ nm, 80/20 = $99,03 \pm 26,01$ nm, 70/30 = $111,387 \pm 50,06$ nm, and 60/40 = $107,06 \pm 47,36$ nm. These results reveal that the fabrication results have reached nano size (below 500 nm) and a decrease in diameter is seen as chitosan-pectin composition increases. The results of the fiber size distribution for each composition can be seen in Figure 3.

Based on the result, the size of nanofiber decreases from $155,39 \pm 43,68$ nm (90/10 composition) to $99,03 \pm 26,01$ nm (80/20 composition). Then it goes up to $111,387 \pm 50,06$ nm (70/30 composition) before it decreased again to $107,06 \pm 47,36$ nm (60/40 composition). It is also found that the bead formation becomes higher from 90/10 to

60/40. The discussion related to this phenomenon will be present in chapter fluid parameters affecting fabrication result and bead phenomenon in nanofiber fabrication in this report journal. The distribution of nanofiber size from each composition can be seen in Figure 3.

In Figure 3 each composition was investigated by counting 100 fibers from a picture captured by SEM. The number of fiber counted by ImageJ software and they are inspected by Microsoft excel to find statistical data including average and standard deviation. To study how big the precision fiber from the fabricating process, we used a limit value that generated from average to standard deviation to find acceptance limit (average + standard deviation) and warning limit (average + two times the standard deviation) [12].

3.1. Fluid parameters affecting fabrication result

3.1.1. Viscosity effect and nanofiber size

The first parameter examined in this research is viscosity. The value of viscosity in this study tends to decrease from the composition of 90/10 to 60/40. This indicates that viscosity is an influential parameter in the ability of a solution to be performed electrospinning. The electrospinning process requires a large enough viscosity so that a fiber can be stretched extending towards the collector continuously to become a fiber.

This increasing value in size is due to the greater viscosity because solutions that have a large viscosity have strong cohesiveness [14]. Polymer molecules in solutions with large viscosity have bonded to links that are stronger than those with low viscosity solutions. These events cause greater resistance to the electrostatic force through the spinneret. The event resulted in the size of the fiber in high viscosity solutions having a larger size. The relationship between viscosity and diameter size can be seen in figure 5.

The results of the viscosity measurements in Figure 4 show that the greater the composition of the PVA in the solution will produce the greater the value of the viscosity from 90/10 to 80/20 composition but it has discrepancies in 70/20 and 60/40. The 90/20 to 80/20 results can be explained by the viscosity data that reported PVA has a relatively higher value of 55.77 N/m²s compared to the viscosity value of chitosan (18.85 N/m²s) and the viscosity of pectin (20.46 N/m²s). The reduced viscosity in the electrospinning process influences fabrication results. The higher the viscosity value of a solution, the larger the diameter of the fiber that will be produced from an electrospinning process. It also can be seen that the size of fibers that were captured in 70/30 and 60/40 have relatively heterogeneous shapes and types that affected the average size of the nanofiber.

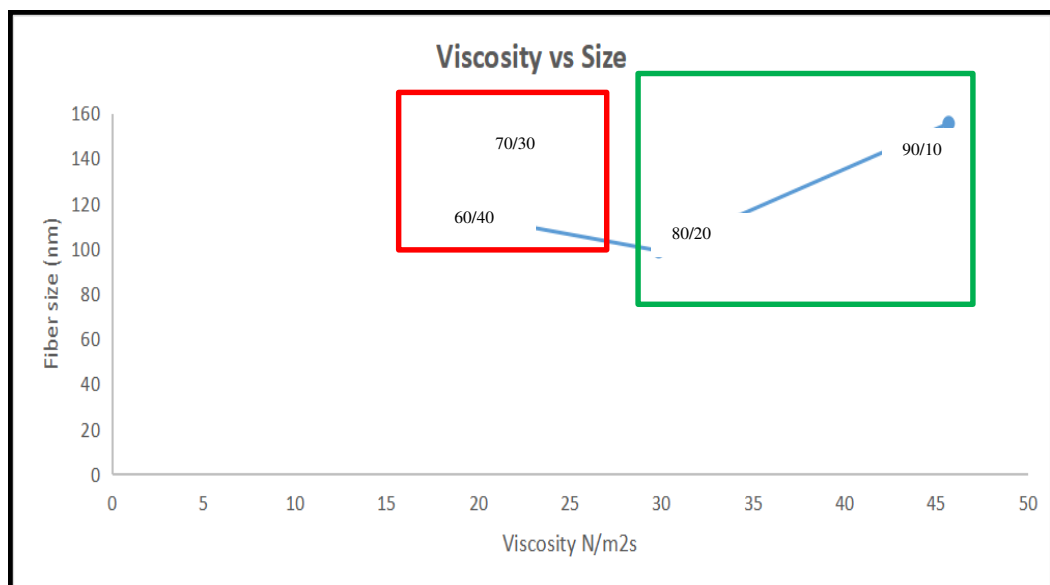


Figure 4. Correlation between viscosity and nanofiber size

Figure 5 can be seen that in the composition of PVA/chitosan-pectin 50/50 the viscosity value is calculated to be 29.66 N/m²S. This viscosity value is higher than the composition value of 60/40 which is equal to 20.25 N/m²S. This event was caused because the chitosan-pectin composition factor in the solution became dominant. The chitosan-pectin solution in this study has a higher viscosity than pure chitosan and pure pectin. This event is caused by the interaction of chitosan and pectin which are interactions that tend to form the gel phase [14]. The formation of the gel phase tends to cause the solution to become thick so that the time flowing in capillaries becomes large so that the viscosity value is high.

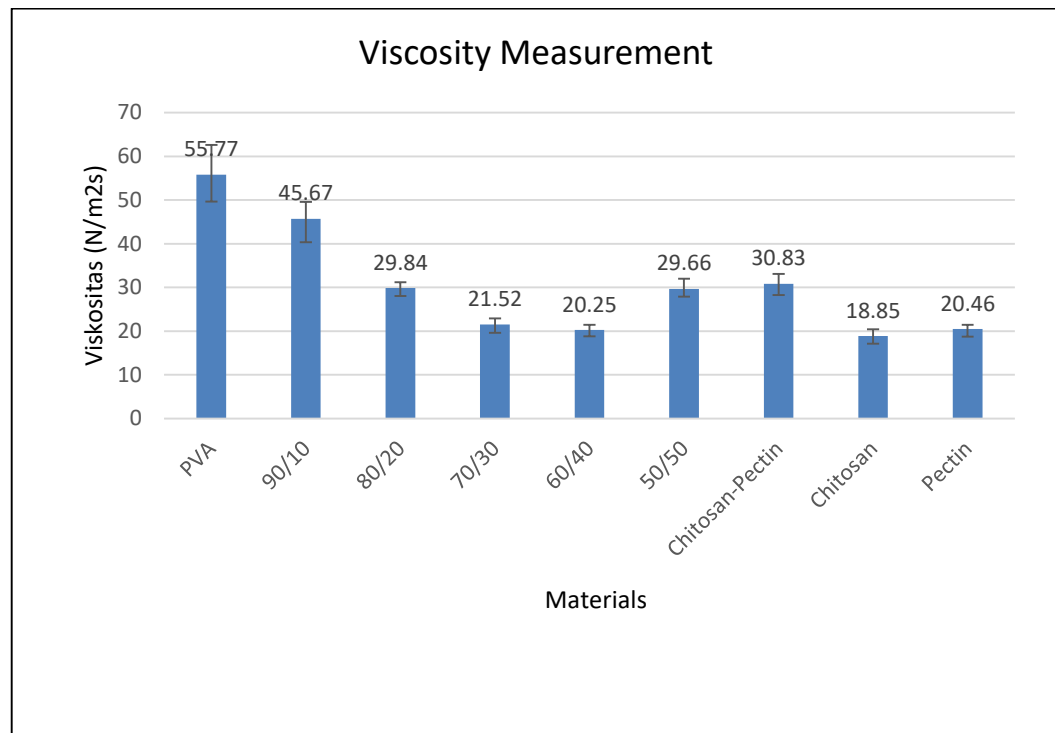


Figure 5. Viscosity measurement on various solutions

3.1.2. Conductivity affecting limits of fabrication process

This study found that the conductivity value increased along with the increase of chitosan-pectin composition. The conductivity parameter can be the main reason for the limited ability of the electrospinning process. It can be seen that in this study the strength of the attraction towards the collector raises the possibility of increasing the incidence of spray events in electrospinning. This research used chitosan and pectin which are ionic polymers. The addition of PVA in this composite solution also affects the conductivity value of the solution. Variation in composition in solution allows changes in conductivity which can be a parameter that affects the ability of electrospinning. The conductivity of the solution is affected by how many ions are present in the solution. The results of the measurement of the conductivity of the solution can be seen in Figure 6. Figure 6 shows that the composite conductivity rises with increasing chitosan-pectin composition in the solution. Based on the data it can be seen that the measurements of the conductivity values for each pure solution are: PVA of 0.60 mS/cm, chitosan at 7.45 mS/cm, and pectin at 0.72 mS/cm. The measurement of a chitosan-pectin solution obtained the conductivity value of 3.17 mS/cm. The large chitosan conductivity value will significantly affect the conductivity value of the composite solution.

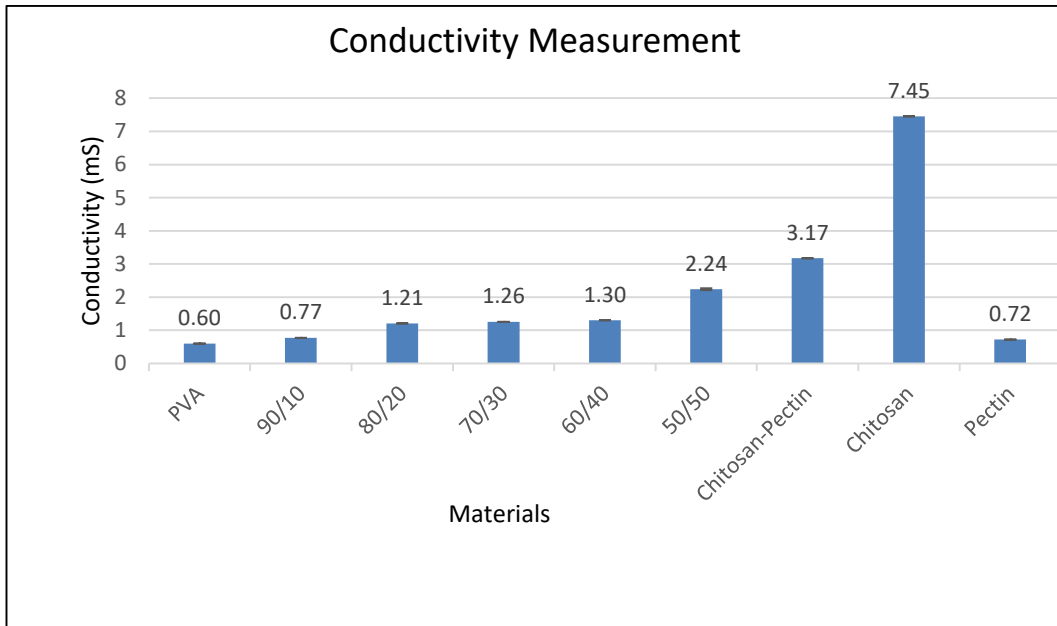


Figure 6. Conductivity Measurement on various solutions

The results of the measurement of conductivity parameters in this study show that the conductivity of the chitosan solution has a higher value compared to the composite solution. This high conductivity value is due to the positive ion charge in the chitosan molecular structure. The high conductivity value in this chitosan makes it difficult for pure chitosan to be fabricated by electrospinning [5]. The results of measurements on pectin showed relatively low conductivity values. This low conductivity value is due to the type of pectin used is high methoxyl (HM). HM pectin is a type of pectin whose esterification degree (DE) is more than 50%. The degree of esterification is the presentation of the carboxyl group of methyl esters in the polysaccharide chain of the pectin acid group [15]. High methoxyl pectin has a lower charge density than low methoxyl. This is because the COO group ratio compared to COOH is less. PVA itself has the lowest conductivity value because it does not have an ionic group.

Based on the results of the data in Figure 6, it can also be seen that the chitosan conductivity is much lower if pectin or PVA are added. This is because there is an interaction between chitosan with pectin and PVA. Chitosan bond with PVA can occur through hydrogen bonds at the molecular level [7]. While the bond between chitosan and pectin is formed through the interaction of the amine and hydroxyl groups [9]. Carboxyl group bonding to pectin and amine in chitosan is an interaction of complex polyelectrolyte formation [11]. Increasing the conductivity of the solution means increasing the elongation force caused by the electric voltage [14]. This study uses a constant voltage at a value of 15kV. When the conductivity value rises while the voltage is kept constant there is an increase in the tensile force in the electrospinning system. This event occurs because the conductivity causes the repulsive force on the surface of the beam to be even greater. If a solution's conductivity is zero, then the fiber cannot be formed by the electrospinning process.

Biomaterial group interaction influencing conductivity

FTIR analysis was carried out to find out information about the presence of chitosan, pectin, and PVA which affect the conductivity parameter. This method also can be used to seek information about their interaction regarding the bonding between polymers in a composite [13]. Based on the spectrometry graph in Figure 7 it can be seen that the composite FTIR spectrum is the combination of three material spectrum, namely chitosan, pectin, and PVA. This result proves that the chitosan-pectin-PVA nanofiber has been fabricated successfully by the electrospinning process.

An important spectrum that needs to be examined is the spectrum of the amine group that found in chitosan. Based on the identification table, the composite amine

(amide-II band) group is at a wavelength value of 1,573.91 or 1.597.06 cm⁻¹. Based on the spectrum graph, it can be seen that the spectrum for the amine group is sloping compared to the spectrum that appears in chitosan, which is at a value of 1,597.06 cm⁻¹. By seeing these results, it can be assumed that in this study an electrostatic interaction occurred between the positive charge of the amine group on C-2 from the pyranose ring chitosan and the negative charge on the carboxyl group on C-5 of the pyranose ring pectin [11]. This interaction results in the formation of a cross-linked polyelectrolyte complex (PEC). Another evidence that the formation of PEC appears is by the calculation of the conductivity parameters in Figure 6. The conductivity parameter calculation shows that the chitosan-pectin conductivity value is lower than the pure chitosan solution. This shows that the ions in the chitosan-pectin solution bind to each other due to their complementary charge.

The results of group identification for each material are presented in Table 1. It shows that in the composite material the presence of the three types of component materials can be identified by the presence of their characteristic wavelength. The interaction of carboxyl groups on pectin and amines in chitosan occurs in the region of 1.590,800 cm⁻¹ [10]. The table above shows the carboxyl group on pectin shown with a wavelength value of 1.751,36 cm⁻¹. Pectin's carboxyl group spectrum appeared composite material at a wavelength of 1.728,22 or 1.735,93 cm⁻¹. Based on the graph, it can also be seen that the spectrum of the pectin carboxyl group has become steeper.

Table 1. The identification wavelength of chitosan, pectin, PVA, and composite group

Wave Number (1/cm)						Vibration Type
PVA	Kitosan	Pektin	90/10	80/20	70/30	
3.448,72	3.441,01	3.387	3.348,42	3.356,14	3.332,99	O-H overlap N-H
2.931,8	2.887,79	2.939,52	2.939,52	2.939,52	2.939,52	C-H
-----	2.368,59	-----	2.368,59	2.368,59	2.368,59	C-N
2.337,72	2.337,72	2.337,72	2.337,72	2.337,72	2.345,44	CH-OH
-----	-----	1.751,76	1.728,22	1.735,93	1.735,93	COOH
1.635,34	-----	1.627,92	1.658,78	1.658,78	1.658,78	Amide II (C-O)
-----	1.597,06	-----	1.573,91	1.597,06	1.597,06	Amide II (N-H)
-----	-----	1.442,75	1.427,32	1.435,04	1.427,32	CH ₂
-----	1.381,03	-----	1.373,32	1.373,32	1.373,32	C-H from CH ₂ group
-----	-----	1.234,44	1.257,59	1.249,87	1.249,87	O-H
941,26	1.087,85	1.064,71	1.095,57	1.095,57	1.095,57	(C-O) (C-N) (C-C)

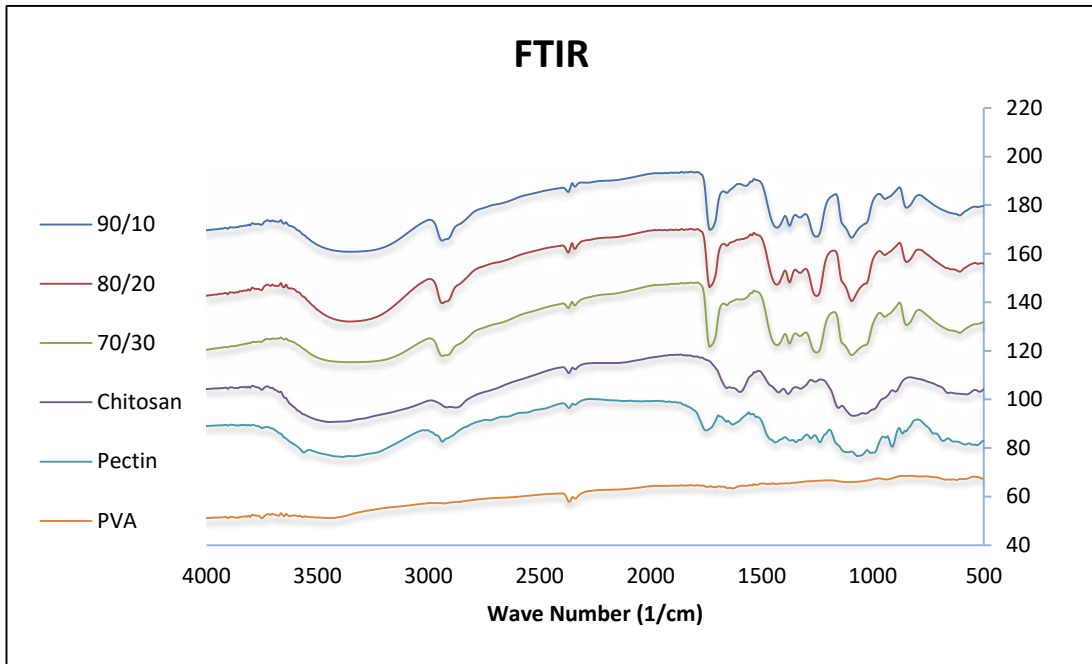


Figure 7. Comparison spectrum of FTIR on chitosan, pectin, PVA and composites

3.1.3. Surface tension effect influencing bead phenomenon

The third parameter examined in this study is surface tension. Surface tension is the parameter that plays a role because it is related to Tylor cone which is correlated to the ability of a solution whether or not a solution can be drawn by an electrostatic force caused by electric voltage. The fabrication process with electrospinning begins when the electrostatic force can overcome the surface tension. The greater the surface tension of a solution, the greater the voltage required. This study makes the electrical voltage a control variable that is equal to 15 kV. The 15kV value is the maximum stable voltage from the electrospinning device found on UGM LPPT. This research shows that the 50/50 solution can move from spinneret to collector. These results indicate that the electric voltage is strong enough to pull the solution to the collector. This study shows the surface tension factor is not a cause of the limited ability of composite compositions at 50/50.

The experiment result can be seen that the surface tension is increasingly correlated to a higher concentration on chitosan-pectin. Chitosan is a polymer that has polycationic properties. In this research chitosan dissolved in acetate solution that will cause it has many amine groups in its chain. Increased amine groups in chitosan will cause polycationic properties. This polycationic nature can increase the surface tension in the solution [15].

The formation of bead in electrospinning due to fiber in the fabrication process minimizes free energy in the system. If the attraction between the solvent and the polymer molecule (viscosity) dominates the attraction between the solvent particles (surface tension), the solvent molecule will hydrate the polymer. Smaller polymer molecules will adjust to the lowest free energy formation in the polymer. However, if the force between the solvent particles is dominant, the system will begin to adjust to the lowest energy formation in the solvent. This adjustment is done by reducing the surface area per mass unit so that it forms a ball [16].

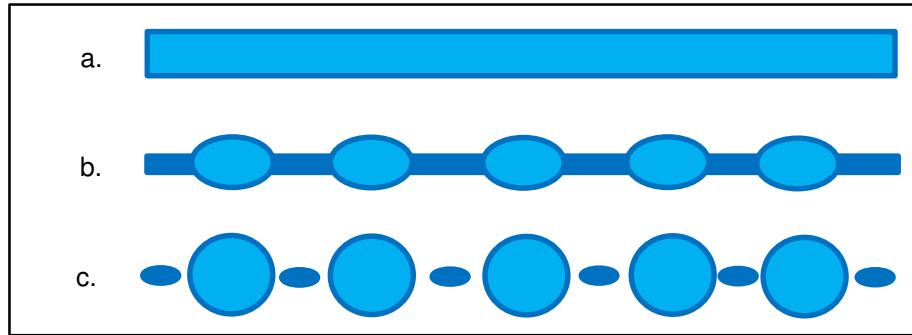


Figure 8. Illustration of (a) Fiber (b) Beads and (c) Droplet Formation in Electrospinning Process

This study also examined the effect of bead formation on changes in the composition of the solution. The process of bead formation in the electrospinning process is caused by surface tension [17]. Based on the surface tension parameters measurement, it is known that the greater the composition of chitosan-pectin in the composition will result in increased surface tension. The results of this experiment obtained data that the higher the surface tension, the bead formation will increase.

Viscosity and surface tension parameters are related to the cohesion strength of a solution [14]. The cohesion strength of a solution is caused by two types of interactions, namely: the interaction between solvent particles and solvent particles and the interaction between solvent particles and polymer molecules. Interactions between solvent particles affect surface tension, while interactions between solvents and polymer molecules affect viscosity. The cohesion strength of a solution can be seen from the viscosity value of the solution. A solution has a large viscosity value due to the chain linkages of a polymer so that they are interlocked with each other. A polymer chain that increases in length (increases in molecular weight) and an increased concentration makes greater attachment. The greater the viscosity of a solution in the electrospinning system, the electrostatic force needed to attract the solution will also be higher. However, the less viscosity of the solution the more prone the solution becomes spray. The spray phenomenon occurs because the polymer molecules are no longer related to one another due to an electrostatic force that is too strong.

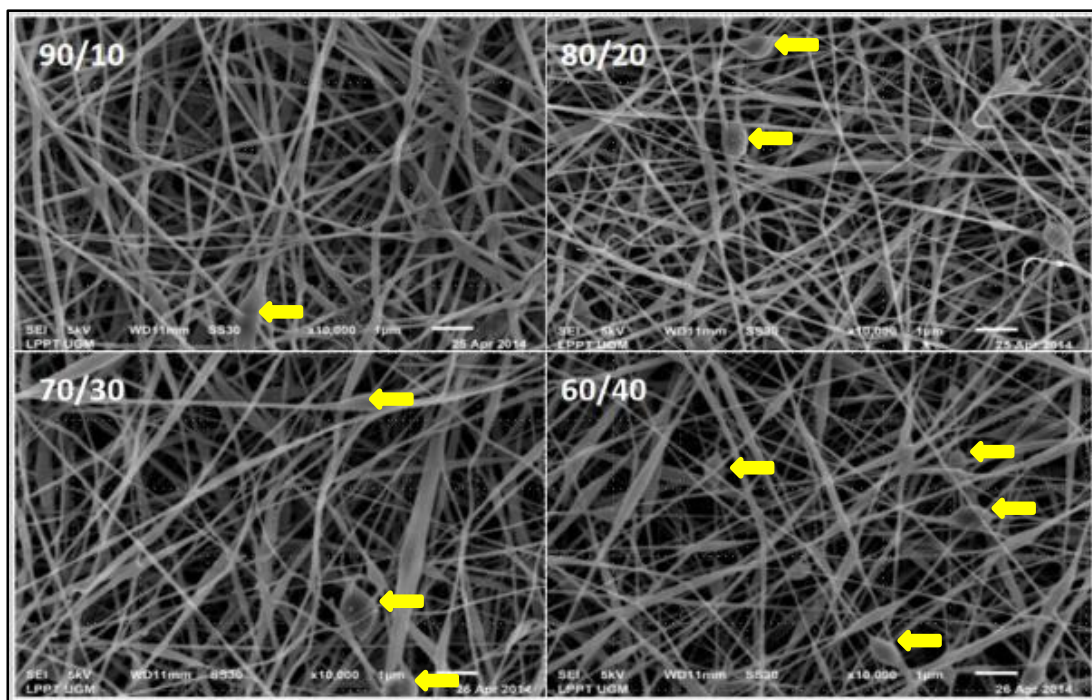


Figure 9. Beads Formation in various composition composite fabricated by Electrospinning Process

Viscosity is a parameter that is very influential in the process of fiber formation. The viscosity that is too low will result in the spray phenomenon. Conversely, if the viscosity is too high, it will be difficult for a solution to be pumped out of the needle tip on the syringe. The spray is a phenomenon where the solution cannot form continuous fibers and tends to form discrete drops of water. The greater the viscosity of the solution, the resulting fiber will be stable or uniform [18]. Besides, with greater viscosity, the bead, and branching of the resulting fiber decrease. However, it should be noted, that there is a maximum limit of the viscosity of the solution that can be carried out by the electrospinning process [17][18]. If the concentration of the solution is too large, it will cause the droplets to dry first at the tip of the spinneret before the jet is formed [18].

The assessment in this surface tension parameter shows that the value of the composite surface tension is influenced by the amount of chitosan-pectin in a solution. Measurement of surface tension in solution 90/10 has a PVA surface tension value of 44.20 mN/m. Subsequent composite measurements showed an increase in surface tension value along with an increase in the amount of chitosan-pectin. Measurements on the composition of 50/50 show that the surface tension rises to 51.10 mN/m. The higher the surface tension value, the more it will accelerate bead formation in the electrospinning process [15]. Based on

Figure 10, it can be seen that of the three types of composite composites, chitosan has the highest surface tension of 50.90 mN/m. This large chitosan surface tension value plays a role in increasing the surface tension value of the composite solution which increases with the addition of chitosan-pectin composition.

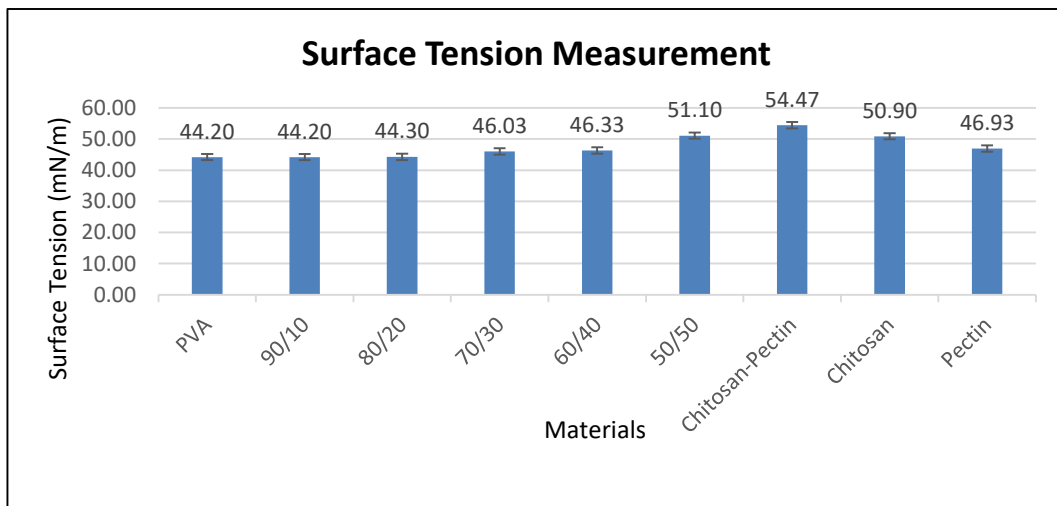


Figure 10. Surface tension measurement in various solutions

4. CONCLUSION

Fabricating nanofiber-based on biomaterial is tricky because of its bioactive characteristics. This experiment provides that chitosan which has a positive charge has the highest conductivity that influenced in electrospinning ability to produce nanofiber in this system. Viscosity which is also an important parameter that has to count into the investigation becomes a determining factor regarding nanofiber size but it is only until 80/20. Regarding discrepancy that shows in 70/30 and 60/40, it is because of the heterogeneity of shape and type of fibers that affect the average size of diameter affected by surface tension. Moreover, this parameter has noted as a strong effect to determine bead and droplet formation in the biomaterial electrospinning process.

This experiment suggests that biomaterial can be fabricated using electrospinning by manipulating or controlling the conductivity parameter. To further study in biomaterial fabrication using electrospinning, the negative charge that comes from bioactive material can be added to decrease conductivity value.

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