EFFECT OF ELECTROLYTE ADDITION AND TYPE OF SOLVENTS USED ON THE QUALITY OF NANOFIBER SCAFFOLD

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ABSTRACT

Nanofiber-based scaffolding systems are now can mimic the natural extracellular matrix (ECM) of desired tissue to be regenerated and can replace organ transplantation in future. The objective of this study is to determine effect of electrolyte addition and type of solvents used on the quality of nanofibers scaffold. Besides that, the research will proceed to the fabrication of drug embedded nanofibers mat to detect the presence of the drug dissolved in nanofibers mat. In this study, needleless electrospinning technique is applied by using the advanced equipment called Elmarco Nanospider™ to fabricate nanofibers scaffold. Meanwhile, polyethylene oxide (PEO) and polycaprolactone (PCL) polymers were used in this studied. PEO solution was undergoes surface morphology studied by determine the effect of concentration and effect of additional electrolyte on the quality of nanofibers. While PCL polymer was used in study the effect of using different kind of solvents on the quality of nanofibers and also fabrication of drug embedded nanofibers. From the result, PEO 2 obtained a low diameter range of 100 nm to 125 mm and no bead formed compare to PEO 1. While for PEO 3 that contained electrolyte composition, it obtained a 75 nm to 100 nm diameter compare to PEO 2. For PCL that dissolve with DCM, nanofibers obtained were in range of 100nm to 300nm and smooth compare to PCL mixed with DMF and $CHCl_3$. However, since PEO cannot dissolve completely with curcumin and titanocene drug, PCL is used in this drug fabrication. PCL that mixed with curcmin obtained a higher existence with 18.79% in the nanofibers mat compare to drug titanocene with only 5.586%. In this study, SEM, Image J, FTIR and EDX were used in characterization study of nanofibers. As a conclusion, electrolyte addition can increase the conductivity and DCM solution with low boiling point can eject the solution more easily to the collector.

ABSTRAK

Objektif keseluruhan projek penyelidikan yang dicadangkan ini adalah untuk mengkaji kesan penambahan elektrolit dan jenis pelarut yang digunakan terhadap kualiti perancah nano serat. Selain itu, kajian ini akan diteruskan untuk fabrikasi nano serat yang berlapis-lapisan yang berhasrat untuk mengkaji pelepasan ubat dan sistem kawalan ubat dalam aplikasi kejuruteraan tisu badan. Ini adalah kerana sistem perancah berasaskan nano serat setakat ini boleh menterjemah tisu extracellular badan yang dikehendaki untuk dijana semula. Dalam kajian ini, kami mencadangkan untuk menggunakan teknik electrospinning needleless dengan menggunakan peralatan canggih yang dipanggil Elmarco Nanospider ™ untuk menghasilkan pelbagai lapisan perancah komposit yang mesra alam. Terdapat dua jenis polimer yang akan digunakan, iaitu polietilena oksida (PEO) dan polycaprolactone (PCL). Selepas itu, polimer akan digunakan untuk campuran dengan dadah curcumin untuk membentuk satu lapisan gandaan perancah berserabut komposit. PEO 2 memperoleh julat diameter rendah 100 nm hingga 125 mm dan tiada manik yang dibentuk berbanding PEO 1. Dalam kajian ini, PEO 2 memperoleh julat diameter serendah 100 nm hingga 125 mm dan tiada manik terbentuk berbanding PEO 1. Selain itu, PEO 3 yang mengandungi komposisi electrolit memperoleh diameter nano serat antara 75 nm hingga 100 nm. Bagi PCL yang larut dengan DCM, diameter nano serat adalah antara 100nm hingga 300nm dan lancar berbanding dengan PCL bercampur dengan DMF dan $CHCl_3$. Walau bagaimanapun, disebabkan PEO tidak boleh larut sepenuhnya dengan ubat curcumin dan titanocene, PCL telah digunakan. PCL yang bercampur dengan curcmin memperoleh kewujudan yang lebih tinggi iaitu 18.79% dalam nano serat berbanding dengan titanocene dadah yang hanya 5.586%. Dalam kajian ini, SEM, Image J, FTIR dan EDX digunakan dalam kajian pencirian nano serat. Kesimpulannya, penambahan elektrolit dapat meningkatkan kekonduksian dan DCM dengan titik mendidih yang rendah dapat mengeluarkan pelarutan polimer dengan lebih mudah.

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LIST OF ABBREVIATIONS

PCL	Polycaprolactone
PEO	Polyethylene oxide
PLA	Polylactic acid
SEM	**Scanning electron microscopy**
MSCs	Mesenchymal stem cells
THF	Tetrahydrofurane
DMF	Dimethylformamide
PEG	Poly (ethyleneglycol)
PEI	Poly(ethyleneimine)
LBL	Layer-by-layer
PLCL	Poly(l-lactide-*co*-ε-caploractone)
FTIR	Fourier Transform Infrared spectroscopy
HCOOH	Formic acid
CH3CO2H	Acetic acid
CH3CH2OH	Ethanol
$CHCl_3$	Chloroform
NaOH	Sodium Hydroxide
ECM	Extracellular matrix
TDC	Titanocene Dichloride
EDX	Energy Dispersive X-Ray Analysis

CHAPTER 1: INTRODUCTION

1.1 Background of study

Nowadays, the nanofibers scaffolds produced has play an important role in biomaterials division due to the higher demand of biomedical applications such as tissue engineering, wound healing, drug delivery and release control system. Human tissues are primarily composed of cells and extracellular matrix (ECM). ECM is the structural framework for tissues, also known as scaffolds, matrices, or constructs for cellular attachment, proliferation, and in growth ultimately leading to new tissue formation (Bae et al., 2013). The tissue engineering technologies paves a way for development of artificial scaffold materials. These artificial scaffold materials are substituted for organs and implanted in the needy patients for tissue restoration or regeneration. Nowadays, the tissue engineering have develop a porous structure scaffold required by mimicking the Natural ECM matrix of desired tissue to be regenerated. More recently, nanofiber-based scaffolding systems are being explored as scaffolds for tissue engineering (Hutmacher et al., 2000). In reality, what is the function of nanofibers in biomedical? The fabrication of nanofibrous scaffolds has provides the protein absorption and binding sites to cell receptor. Besides, it also provide greater surface area for cellular interaction which enables cell adhesion, spreading and proliferation in wound healing system and provide maximum volume fraction in fibers alignment for improve the strength of drug loaded nanofibrous mat in drug delivery system (Dias et al., 2013). In this research field, the synthetic biodegradable polymers which are polycaprolactone (PCL) and polyethylene oxide (PEO) have been used in drug loaded nanofiber mats. PCL has become widely and commercially use in biomedical because it is a type of synthetic polymers that can be degraded by microorganisms and lack of toxicity has made PCL suited to release control drug delivery system (John et al., 2008). Besides, PCL is a hydrophobic and semi-crystalline polymer; its crystallinity tends to decrease with increasing molecular weight and degrades very slowly in vivo and in vitro. PCL is resistant to chemical hydrolysis and behave achiral, which is a feature that limits the possibility of property modulation through the configurationally structure of polymer chains (Mark et al., 1990). While for PEO, it is the most suitable material for making nanofibers become stealthy, which is difficult to be detected by the immune system either through cell level or humeral reactions. This is due to

the highly hydrated and flexible hydrophilic PEO chains can form a steric barrier against the adsorption of proteins at the nanoparticle surface. Whenever, the proteins are no longer adsorbed (opsonisation), the phagocytosis of the nanoparticles is avoided and their lifetime is increased in the blood circulation (Aqil et al., 2008). Therefore, In order to produce the good quality nanofibers scaffold for making the r nanofibers mat for drug delivery system, we need to consider the process parameters such as flow rate, voltage, and distance. While for systematic parameters are polymer type, molecular weight, and solvent used. Besides, for solution parameters we need to consider the viscosity, concentration, conductivity, surface tension and also the charge of jet. Lastly for physical parameters is humidity, temperature and air velocity during the electrospun process of fibers (Katia et al., 2012).

1.2 Problem Statement

Currently, drug delivery system by tissue engineering is one of the vital and prosperous development research fields which involve medicine, biology and engineering technology in this new era. The objective of the development is to repair, replace, and maintain the function of tissue and organ of human body. The current challenge in utilizing tissue engineering is the design of the biocompatible support matrix called scaffolds, that can imitate the function and capacity to mimic the natural extracellular matrix component and collagen. A wide research has been carried out all around the world to produce the perfect scaffold materials. These researches potentially showed some positive results but they lack in few aspects like rate of degradation or the strength of the materials, due to this reason this scaffold materials are still not in wide use (Ishaug et al., 1997). Therefore, the selection of polymer material and solvent used is important to produce a good quality of nanofibers scaffolds that behave in the desired manner to generate tissues and organs of the desired shape and size. The selected material should provide better biodegradability; provide an excellent porosity with an appropriate pore distribution size distribution to allow diffusivity occur easily, biocompatible to promote cell adhesion, migration, proliferation, and differentiation of the cells (Wang et al., 2011).

In this present study, there are also varies parameters that will influence the nanofibers scaffold quality even though the electrospining process is simple. The Electrospinning process can be affected by few parameters, which are solution parameter, equipment parameter and ambient parameter, which makes this method very challenging to handle. The solution parameter includes conductivity, viscosity and also concentration of solution. Meanwhile, for equipment parameter that affect the Electrospinning process includes voltage, current and also distance between the wire electrode and also the collector while for ambient parameter, it includes temperature and humidity. Besides that, the nanofibers after electrospinning process often have beads as ‘‘by products’’. The formation of beads attached to fibers has been observed wisely. The beads formed might due to the instability of electric fields, polymer solutions and so on. Therefore, experiments with various conditions need to be taken out to get the good quality of nanofibers scaffolds. Preparation of the electrospinning solution plays critical role in embedding the drug for sustained release. The solution parameters need to be optimized and identifies the suitable method to embed the drug in the nanofiber matrix will be a challenging task for developing a Nano Drug Delivery System (NDDS) (Fong, et al., 1999).

1.3 Objective

1 To study the effect of electrolyte addition on the morphological quality and size of nanofibers scaffolds.
2. To study the effect of using different solvents with polymer solution on the morphological quality and size of nanofibers scaffolds.
3. To develop a drug embedded nanofiber scaffold.

1.4 Scope of Study

1. Preparation and characterization of various polymer solutions (PLA, PEO and PCL with and without electrolyte addition, using solvent such as ethanol and chloroform) that will be used to produce nanofiber scaffolds via viscometer and conductivity meter.
2. Development of a single layer nanofiber scaffolds via needleless electrospinning.
3. Morphological assessment of nanofiber scaffolds using SEM.

4. Measurement of fibre size distribution using a ImageJ and Origin software.
5. Development of a drug-embedded nanofiber scaffolds via needleless electrospinning.

1.5 Motivation

The main motivation of this present study is to form a nanofibers scaffold for the biomedical and healthcare purpose by using needleless electrospinning technique. Currently, various biodegradable polymers will be utilized to form a Nano fibrous scaffold. This scaffold will be applied in the drug delivery system. The main advantage of this method is reproducibility of the fibers at industrial level. The nanofibers scaffolds prepared in this work will undergoes various testing in order to have a better understanding on the properties such as mechanical, chemical and biological. Other than that, the produced fibers are inexpensive and has unique potential for improved properties like biochemical topographical and mechanical properties. The benefit of this study will be the better understanding of the process of electrospinning using needleless spinning technique and resulted scaffold in field of tissue engineering. Therefore, electrospun nanofibrous scaffolds have exhibited an importance role and higher potential in drug delivery system of tissue engineering field through both in vitro and vivo. This work can help to develop a detail knowledge on the cell-nanofiber interactions, biofunctionalization of drug encapsulation and application in regenerative medicine.

CHAPTER 2: LITERATURE REVIEW

2.1 Types of Nanofibers Scaffold Electrospinning Technique

Nanofibers is a one-dimensional naomaterials with a diameters of 1µm (1000nm), also known as ultrathin fibers. This intrinsic feature offers a drastically increased surface-to-volume ratio and high aspect ratio. Researchers from the engineering field used both the traditional and modern methods to produce a scaffold. The methods that are used for producing the scaffold are solvent casting and particulate leaching, phase separation, rapid prototyping, gas foaming, freeze drying and sponge replication electrospinning. All the above technique has its own advantages and disadvantages but this proposal we mainly focused on fabrication of scaffolds through needleless electrospinning process due to this reason the rest of technique were not explained which can be found elsewhere.

2.2 Electrospinning

Extrusion is one of the oldest technique used to produce the fibers in the last decade with advancements in researches a new techniques called electrospinning was developed recently to produce this fibers Electrospinning is one of the processing technique for the producing fibers. In this electrospinning fibers are produced through the electrical forces. In this technique the fibers are formed by self –organization through electrostatic force. This process consist of simple syringe, polymer solution, metal tip and aluminum foil placed opposite to the metal tip and high voltage is applied on the tip to form a self-assembled fibers which is collected in the aluminum foil (Wendorff et al., 2012). The inventor, William Gilbert first described the observation of liquid interacting with electrostatic force in 1600. He found that a spherical water drop on a dry surface was drawn up into a cone. The charges were induced to form on the drop surface with the help of electric fields, which produced the electrostatic forces opposing the surface tension of the liquid. In the early of 20^{th} century, Zeleny observe and evaluate the surface charge intensity by reading the pressure change and learn about the behaviour of fluid

droplets at the end of polymer jet. After 20years, Taylor in 1960s studied the shape of cone that the liquid droplets formed after eject from the polymer jet with the help of electric field and he observe that the conical interface between air and fluids was in stable form with an angle of 49.3^0. Therefore, the cone structure formation during electrospinning has since been named as "Taylor cone" (Wang et al., 2011).

2.3 Electrospinning Process and Principle

Electrospinning or electrostatic spinning is a simple technique which utilizes high electrostatic forces for fiber production. Electrospinning, consists of a container which carry the polymer solution. Besides, the metering syringe pump is used to control the flow rate of the polymer solution. In the electrospinning process, the polymer solution is delivered to the tip of the jet, and the needle of the syringe typically serves as an electrode to electrically charge the polymer solution and the counter-electrode is connected to the conductive collector screen. The polymer solution droplet will fall from the tip of nozzle under gravity if electric field is applied. However, voltage also plays an importance role in producing the electric fields. Under the strong electrostatic forces, charges will induce and accelerate to towards the metal collector. As the electric field is increased, the polymer liquid surface repels each other and shear stress will occur. The repulsive forces act in a direction opposite to the surface tension which result the form of conical shape (figure 1). The deformation of droplet will be formed into a cone shape (Taylor cone) when there have voltage difference. If the applied voltage is increased beyond this point a fiber jet will be ejected from the apex of the cone and be accelerated toward the grounded collector (Travis et al., 2008).

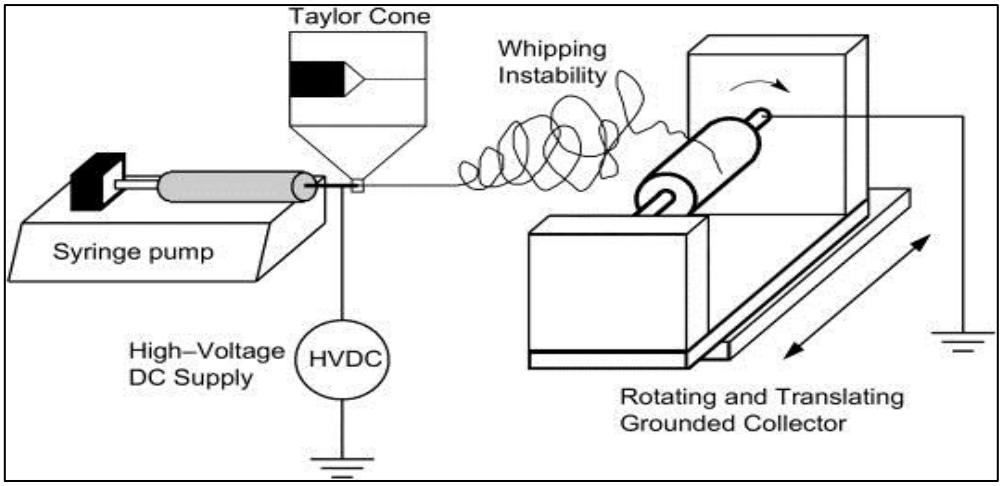


Figure 0.1: the schematic of typical electrospinning system (Wang et al., 2011)

2.4 Needleless Electrospinning

In this present of study, Elmarco Nanospider™ is the equipment that will be used to fabricate a drug embedded biodegradable composite scaffold, which will provide a path for commercial application. Elmarco Nanospider™, who using upward needleless electrospinning technique that have minimal dependence on the fluidic channel numbers to improve the fiber productivity. During the electrospinning process, a normal magnetic field will applied to the system, steady spikes were formed pertubing the interlayer interface. As voltage is applied to the polymer solution, thousands of jetting ejected upward to the opposite counter electrode wire.

The wire needleless electrospinning systems that will be used in this present study to produce nanofibers scaffolds contain a straight coil wire as fiber generator. As shown in Figure 2, when the voltage is applied, numerous polymer jets were generated from the wire surface and eject upward and will produce a finer and a narrower diameter distribution nanofiber scaffolds (Wang et al., 2011). In the upward Electrospinning process, the bubbles will be generated on the free surface of the polymer solution to initiate the Electrospinning process. It is totally different compare with the previous design, which a gas tube was inserted to the bottom of the solution tank to produce a high pressure gas. A flat aluminium plate was used as collector above the solution. Meanwhile, the production of nanofibers is depends on the gas pressure. Taylor cones were generated

from the bubbles as high voltage was applied to the solution. However, the nanofibers prepared by this method will form beads.

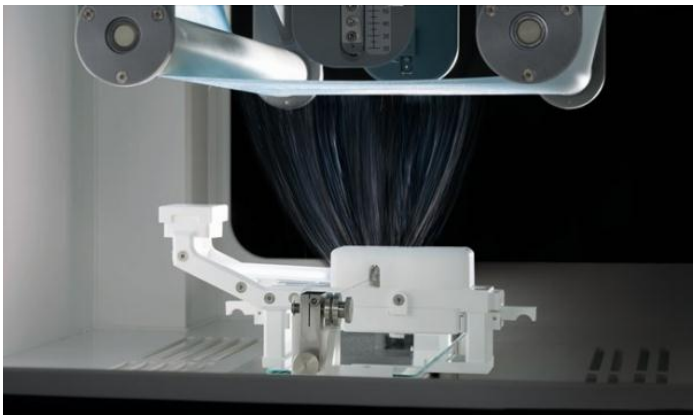


Figure 0.2: Needleless wire Electrospinning Process (Nanospider™) (Wang et al., 2011)

2.5 Polymers used in Electrospinning Process

Yoshimoto et al, 2003 suggested that electrospun PCL is a potential scaffold candidate for bone tissue engineering, they conducted an electrostatic fiber spinning to prepare a microporous, non-woven poly(e-caprolactone) (PCL) scaffolds. The Obtained electrospun PCL scaffolds was cultured in Mesenchymal stem cells (MSCs) derived from the bone marrow of neonatal rats. The cell-polymer constructs maintained the size and shape of the original scaffolds. Scanning electron microscopy (SEM), histological and examinations were performed. SEM showed that the surfaces of the cell-polymer constructs were covered at 4 weeks. In addition, mineralization and type I collagen were observed at 4 weeks (Yoshimoto et al.2003).

Other than that, Polyethylene Oxide (PEO) Polyethylene Oxide (PEO) is a polymer that is the addition of polyethylene oxide with water that usually designated by a number roughly corresponding to molecular weight. PEO has a high molecular weight than any other polymer which contain suitable viscosity condition for the production of good quality of nanofibers scaffolds, thus giving the supporting characteristics. In this research, PEO is used as polymer solution of the electrospun nanofibers because of it's

hydrophobic chain who allow the application if biomedical drug delivery system to occur easily in the human body extracellular matix system. In a research by Chen *et al*. (2011), the effect of PEO to the phase change of nanofibers can be manipulated by changing the composition of PEO in the nanofibers and the morphology of nanofibers will be changed according to the level composition of the core solution. A comprehensive review summarizing the effect of electrospinning parameters and potential applications of nanofibers in biomedical and biotechnology (Adnan et al., 2015).

2.6 Type of solvents used in Electrospinning Process

Table 2.1: Type of solvents used to improve the diameter in the quality of nanofibers scaffold

Polymer	**Solvent used ml**	**Concentration, wt%, g**	**Voltage, KV**	**Height (distance between jet and collector)**	**Flow rate**	**Humidity % and temperature, ^{0} C**	**Beads and diameter nm**	**Citation**	**Method electrospinning**
PCL	40 ml +chloroform 10 ml DMF	9 g	17KV	5 cm	2 ml/h	-	No beads formed Diameter: 910±400nm	(Ko Eun et al., 2015)	Single Needle electrospinning
PCL	40 ml chloroform + 10 ml ethanol	8 g	35 kV			23^{0} C, 45% H	Diameter: 701±227nm no beads formed	(Jakub et al., 2016)	Single Needle electrospinning
PCL	37.5 ml Formic acid+ 12.5 ml Acetic acid	7 g	11 kV	10 cm	0.4 ml/h	-	Diameter: between 65 nm and 250 nm -no beads formed	(Jhamak et al., 2015)	Single Needle electrospinning

Polymer	Solvent used ml	Concentration, wt%, g	Voltage, KV	Height (distance between jet and collector)	Flow rate	Humidity % and temperat-ure, ^{0} C	Beads and diameter	Citation	Method electrospinning
PCL	25ml tetrahydrofurane (THF)/*N*, 25ml *N*-dimethylfor-mamide (DMF)	7.5 wt%	12 KV	18 cm	1 ml/h	-	Diameter: between 250 to 700 nm -no beads formed	(Croisier et al., 2012)	Single Needle electrospinning
PCL	Chloroform:methanol (3:1)	-	-	-	-	-	Diameter: from 53.24±14.01 nm to 939.21 (±57.32) nm No beads formed	(Chen et al., 2007).	Single Needle electrospinning
PEO	Distilled water: ethanol (3:2)	3wt%	10.8 KV	10-15 cm	-	21^{0}C to 22^{0}C Relative Humidity: 28% and 56%.	Many beads formed	(Bharath et al., 2011)	Single Needle electrospinning
PEO	Distilled water: ethanol (3:2)	5wt%	10.8 KV	10-15 cm	-	22^{0}C Relative Humidity: 56%	Diameter: 450nm-850nm	(Bharath et al., 2011)	Single Needle electrospinning
PEO	Distilled water: ethanol	5wt%	10.8 KV	10-15 cm		22^{0}C Relative	Diameter: 271± 17nm	(Bharath et al., 2011)	Single Needle electrospinning

	(3:2)					Humidity: 40%			

From the above table, there is the type of solvents that used to mix with the polymer to produce nanofibers. For PCL polymer, the suitable solvents that mix well with PCL to produce a fine nanofibers are the pair of 40 ml chloroform+10 ml DMF with 9g of PCL pellets and also the pair of 37.5 ml Formic acid+ 12.5 ml Acetic acid with 7g PCL pellets.

2.7 Parameters affect Electrospinning Process

There are few challenges to produce a good quality of nanofibers mat with fewer bead formed and fine diameter. Therefore, there are few parameters need to be optimised for the formation of nanofibers scaffold which are solution parameters, equipment parameters, and also ambient parameters.

2.7.1 Solution Parameters

Solution parameters include polymer concentration, conductivity, surface tension and viscosity. The electrospinning process relies on the phenomenon of the uniaxial stretching of a charged jet. The difference of concentration of polymer solution will affect the charge of jet. For example, as the concentration of the polymer solution is low which cause the surface tension and electric field applied break the entangled polymer chain to become fragments before collecting to the opposite collector (Haider et al., 2013; Pillay et al., 2013). This fragments chain causes the formation of beads. Therefore, as the polymer concentration was increased, it will lead to a higher viscosity, which then also drive to increase the entanglement chain. As the increasing of entanglement chain, it will affect the surface tension which cause uniform beadless electrospun nanofibers. Besides, if the concentration is increased beyond the critical value, the polymer solution will faster dried up at the tip of the needle tip or electrode wire, which cause the tip of the metallic needle blocked, which ultimately results in defective or bead formation of nanofibers (Haider et al.,2013).

Moreover, conductivity also make a bigger influence in affecting the formation of taylor cone and helps in controlling the diameter of nanofibers. As the conductivity of the solution is low, the surface of the droplet on the wire will hardly to eject to upward to the counter electrode.

Increasing the conductivity of the polymer solution to a critical value will increase the charge of the polymer solution but it also will cause decrease in diameter of fibers (Sun et al., 2014). As the conductivity is beyond the critical value, it will hinder the formation of Taylor cone during Electrospinning process. The Taylor cone formation during Electrospinning process is depend on the Coulomb force which induced by strong electrostatic force created by the electrode wire. However, an ideal polymer solution will not have enough electric charges to eject the solution to the opposite electrode and it will not be sufficient to form a better Taylor cone to eject the polymer. In contrast, a polymer solution who contain conductivity ions will have sufficient charges to induce the electrostatic force to eject the polymer solution to form Taylor cone. The conductivity of the solution can be controlled by adjust the addition of salt such as NaCl. By adding the salt, it can increase the number of ions in the polymer solution, which increase the electrostatic force generated by the applied electric field. According to Zong et al. investigate regarding the effect of adding the salt to the polymer solution, (KH_2PO_4, NaH_2PO_4, and NaCl in 1% W/V) on the diameter of poly(D,L-lactic acid) (PDLLA), They obtained a smooth, beadles and also small diameter of nanofibers after adding the salt to the polymer solution separately (Zong et al., 2002).

2.7.2 Equipment parameters

Generally, the higher voltage power produced from the electrode will cause a spherical droplet to deform into a Taylor cone and form ultrafine nanofibers at critical voltage (Laudenslager and Sigmund, 2012). As the applied voltage is beyond the critical value, it will cause the bead formation on the surface of nanofibers and also increase in the diameter of nanofibers . This is due to the decrease in the size of the Taylor cone and increase in speed of the polymer carriage. Moreover, the diameter of the nanofibers was also reported to increase with an increase in the applied voltage. This increase in the diameter is due to an increase in applied voltage (Baumgarten, 1971).

Besides that, the uniform beadless nanofibers also can be created by adjusting the speed of the polymer carriage to control the flow rate for the polymer solution. This critical value varies with the polymer system. As the speed of polymer carriage is increase beyond the critical value, it will lead to the formation of beads. Therefore, increasing the flow rate

beyond the critical value will not only lead to increase in the pore size and diameter of fibers but also leads to bead formation (due to incomplete drying of nanofibers jet during the eject of the polymer solution between the wire and the collector wire at the top) (Megelski et al., 2002). Since an increase and decrease in the flow rate will influence the diameter of the nanofibers, a minimum flow rate is preferred to maintain the thickness balance between the polymersolution and replacement of that solution with a new one during wire eject formation (Megelski et al., 2002). Besides that, the formation of bead at an elevated flow rate and ribbon-like defect are also due to non-evaporation solvent and also low stretching of the polymer solution between the eject wire and also the collector wire. The present of the unspun droplet on the wire is due to the gravitational force (Shamim et al., 2012). Meanwhile, the surface charge density is also one of the factors that affect the morphology of the nanofiber. According to Theron et al. revealed that the flow rate and electric current are directly related to each other. Various polymer such as PEO, polyacrylic acid (PAA), polyvinyl alcohol (PVA), polyurethane (PU), and polycaprolactone (PCL), were being used as studied the effects of flow rate and surface charge density. From the studied, a research is observed by using PEO, which increase the flow rate simultaneously will decrease the surface charge density and increase the electric current. As the surface charge density decreased, it will allow the merging of the electrospun nanofibers during the eject process from the wire to the opposite electrode collector (Reneker et al., 2002; Theron et al., 2004).

Furthermore, the distance between the wire electrode and collector also play an important role in determining the morphology of an electrospun nanofiber. The distance between the wire electrode and collector is varies with the polymer system. Since the nanofibers morphology depends on the deposition time, evaporation rate and also whipping, a critical distance need to be measured to produce a smooth nanofibers (Bhardwaj and Kundu, 2010). There are some research studied on the effect of distance between wire electrode and collector snd concluded that when the distance is kept small, a large diameter of nanofibers is formed whereas as the distance was increased, then diameter of nanofibers will decrease (Baumgarten, 1971; Matabola and Moutloali, 2013; Wang and Kumar, 2006).

2.7.3 Ambient Parameters

Other than solution parameters and equipment parameters, there is also ambient parameter who affects the morphology of the nanofibers (Huan et al., 2015; Pelipenko et al., 2013).

Humidity is one of the ambient parameters who controlling the solidification process of the charged jet to eject the polymer solution. Nevertheless, this humidity condition mostly is depend on the chemical nature of the polymer. According to Pelipenko et al., research studied on changing in nanofibers diameter with adjusting the humidity condition by using PVA, PEO and their blend solution PVA/hyaluronic acid (HA), PEO/(chitosan (CS)). The research obtained as the humidity is increase from 4% to 60%, the diameter of the nanofibers decreased from 667 nm to 161 nm (PVA) and 252 nm to 75 nm (PEO). However, further increase in humidity will cause bead formation and almost no electrospinning for the blend (Pelipenko et al., 2013). According to the report by Park and Lee (2010), increase the humidity will cause the diameter of nanofibers to decrease. Besides that, humidity also play a critical role in obtained a good porous nanofibers when binary polymer solution system is applied. By using PMMA and a binary solvent system (dichloromethane (DCM) : dimethylformamide (DMF)) in ratio of 8:2, it can produce a highly porous nanofibers. The creation of the pores is depend on the different evaporation rates of two solvent. Since the more volatile solvent (DCM) will start to evaporate faster compare to DFM, (while the polymer solution is eject upward towards the electrode collector), cooling effect is occurred as difference in rate of evaporation of two solvents, this phenomenon is similar to perspiration. Condensation occurred during the cooling effect and condenses the water vapour into water droplets. The water droplets settle on the fibers. As the water droplets is miscible with DMF, two polymer solutions mix well with each other and results in the formation of porous electrospun nanfibers (Bae et al., 2013).

Furthermore, temperature also acts as an ambient parameter that will affect the rate of evaporation of the polymer solvents and also decrease the viscosity of the solutions. With the changing of temperature, it will affect average diameter of the nanofibers. Therefore, as the

temperature increase, it will induce the evaporation rate of polymer solvent to increase, the viscosity of the solutions will decrease as well.

2.8 Drug diffusion study (Simple diffusion)

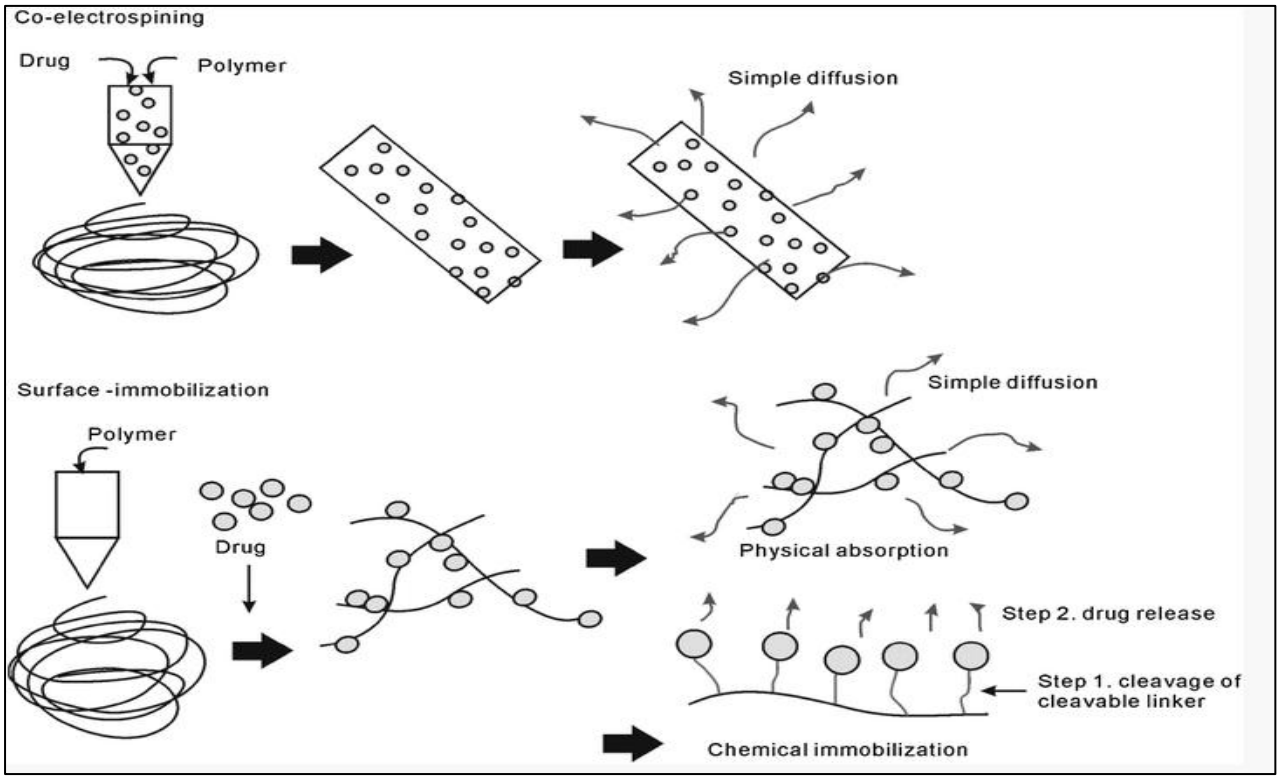


Figure 0.3: Schematic diagram of simple drug loading method within the nanofiber or on the surface of the nanofiber and its release kinetics. (Son et al., 2013)

From Figure 2.3, the simple diffusion of drug delivery system has separate into two methods. Drugs can be loaded to the nanofiber by the processing of co-electrospinning or surface immobilized.

2.8.1 Co-electrospinning of drug and polymer

The dissolve of the drugs and polymer in the same solvent and co-electrospin is the simplest method that embedded the drug with the nanofibers. The incorporation of drugs within

nanofibers that exhibit high drug-loading efficiency that can disperse well in fibers and sheet for human vitro and vivo. (Verreck et al. 2003). From the first report that describing the drug delivery system by Kenawy et al. (2002), poly(lactic acid) (PLA) were co-electrospun with tetracycline hydrochloride to observe the release controlled of tetracycline. The concentration of blending between the PLA and drug is one of the important criteria that need to be considered to improve the initial burst release. However, the experiment shows that over 50% of drug were released out within 5 days. Besides, the properties of drug have to be consider whether it is hydrophilic or hydrophobic characteristic. For instance, hydrophobic drugs such as paclitaxel have electrospun well with organic solvents (Xu et al. 2008, 2009), whereas for hydrophilic drugs such as peptides or protein will dissolve and mix well in aqueous phase (Gatti et al. 2013; Tang et al. 2012). However, the higher voltage during induce the nanofibers will cause damage and harmful to the drugs especially bioactive molecules (Yang et al. 2008). Moreover, the simple physical mixing drug-loading system also will exhibit an early stage burst in simple diffusion, which cause short release profile within several days.

2.8.2 Surface immobilization on the nanofiber

The surface immobilization of drugs method can avoid drug denaturation that caused by the high voltage during generation of the co-electrospun nanofibers. Besides, it is also able to control the amount of drugs loaded on the nanofibers by controlling the drug feeding ratio. The main forces to retain the drugs on nanofibers include electrostatic interactions, hydrophobic interactions, hydrogen bonding, and also van der waals interactions. Heparin, a negatively-charged polysaccharide that contain strong binding affinity for growth factor, is often used in immobilizing growth factors on the surface of nanofiber meshes. (Yoshida et al. 2006). Hydrophilic polymer such as poly(ethyleneglycol)(PEG) and poly(ethyleneimine)(PEI), were co-electrospun with PCL to maintain the mechanical strength of the nanofibers mesh and provide functional groups to the mesh. Furthermore, the physically trapping of the drug on the surface of nanofibers mesh can be improved by the Chemical conjugation methods which amount of incorporated drug can be controlled finely, exhibits slow drug release kinetics with reduced the initial burst release (Cho et al. 2010).

2.9 Drug diffusion study (Sheath nanofiber)

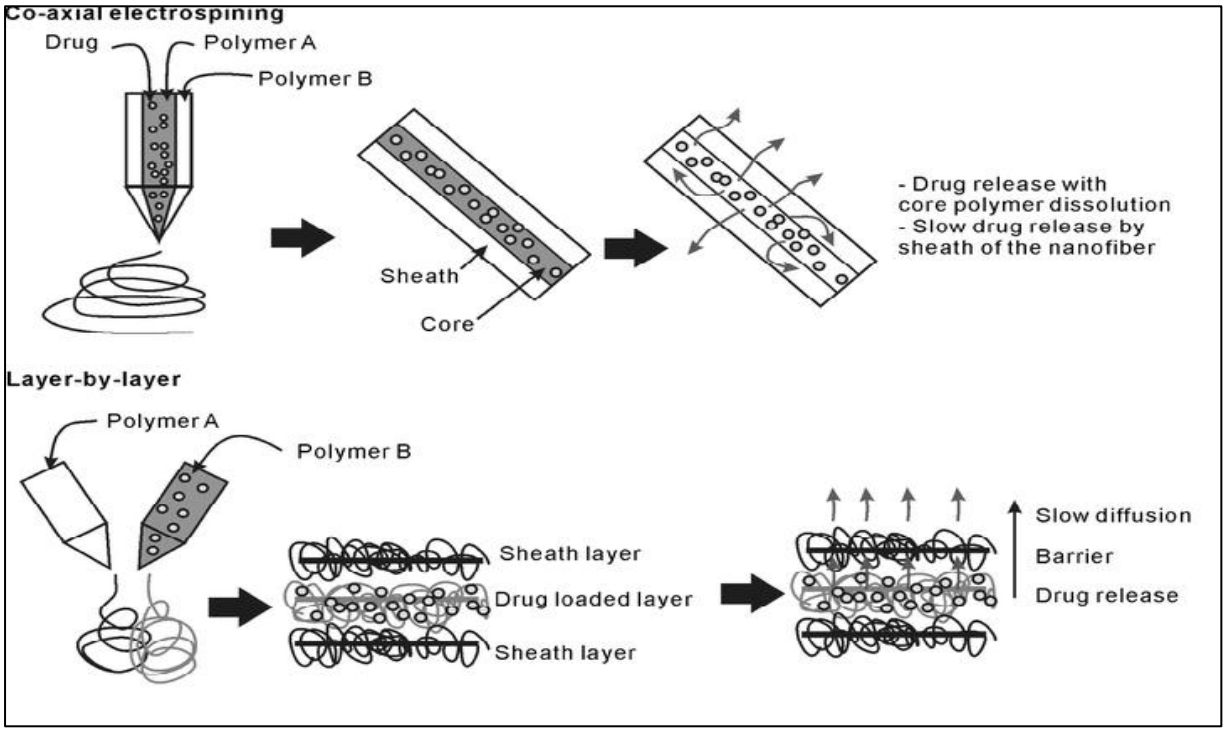


Figure 0.4: Schematic diagram of drug loaded nanofiber with sheath layer and its drug release kinetics (Son et al., 2013)

From Figure 2.4, the limitation of drug delivery system with simple diffusion method can be improved by adding the sheath layer on the solid fiber mesh. In this discuss topic, co-axial electrospinning technique has been developed by loading the drug within the core of nanofiber and nanofiber was also developed with subsequent electrospinning of two different solutions.

2.9.1 Co-axial nanofiber

Other than simple diffusion drug delivery system, there is a another method that will enhance a better diffusion which called co-axial electrospinning method. The co-axial is a horizontal arrangement of outer and inner needles that separate two different solutions. For co-axial electrospinning method, two immiscible polymer solutions that contain drugs are simultaneously electrospun in two separate capillaries (Wang et al. 2012; Yu et al. 2011). The inner solution in the inner capillaries most probably will load with desired drug. By using this method, the initial burst release time is decreased because of the slow diffusivity of drug after using the sheaths of nanofibers and the drug loading efficiency is very high compare to

co-electrospinning of drug with a single nozzle. PCL is one of the polymers that usually act as a barrier to simple diffusion of core-loaded drug due to its hydrophobic shell materials and slow degrading characteristics. After dissolution of hydrophilic portion in core, the swelling or dissolving of the core polymer resulting in the formation of pores in the shell and cause the drug to release (Liao et al. 2006). drug delivery systems are also possible using co-axial electrospinning. Besides that, drug delivery systems also can be developed by using co-axial electrospinning. In the previous study, drug release kinetics of the core/shell nanofiber can help in reduce the initial burst release of core-loaded drug by preventing the initial burst of shell diffusion, which was maximized up to 90% in 7 days (Choi and Yoo 2010).

2.9.2 Layer-by-Layer nanofibers

Nowadays, there are a lot of biomedical devices, nanoparticles, film and micelles apply the layer by layer nanofiber system. This is due to layer-by-layer (LBL) nanofibers reduce the initial burst with zero-order release kinetics. The system was applied to electrospun nanofibers by attaching 3 layers of nanofibers mat as shown in Figure 4 (Kidoaki et al. 2005). The concept is almost similar to co-axial nanofibers, which control the drug release by sheet barriers. nanofibers were introduced into the fabrication for three-dimensional tissue scaffolds and also drug delivery systems. The drug loading method was the co-electrospinning of a drug and polymer mixture. The electrospun nanofiber mesh can be fabricated by different solutions in certain sequential electrospinning. To determine the time-programmed dual release function of sequential nanofibers electrospun, poly(l-lactide-*co*-ε-caploractone) (PLCL) solutions that containing two different drugs were prepared. The first drug-loaded layer nanofibers mat was exposed to the air while the second drugs-loaded nanofiber sheet was sandwiched between the top and bottom barrier layers. As a result, it can be observed that the first loaded drug release earlier than the second loaded drug which is in between the layers.

2.10 Embedded Drug (Curcumin)

Curcumin (found in turmeric), is a yellow coloured phenolic pigment which can be extracted from rhizome of Curcuma longa Linn. It is a popular Indian spice that can be applied as herbal medicines for the treatment such as diabetic ulcers, anorexia, rheumatism and cough.

Besides, it have several therapeutic effects, such as anti-inflammatory, anti-oxidant, antibacterial, anti-carcinogenic and anti-infective. Curcumin also showed a significant effect in wound healing process by enhancing granulation tissue formation, collagen deposition, tissue regeneration, and wound contraction. This is due to the curcumin have the ability to reduce the body' natural response to cutaneous wounds such as inflammation.

Uncontrolled inflammatory may leads to tissue damage, which will cause inflammatory disorder such as rheumatoid arthritis (Joe et al., 2004). It is a crucial 2nd phase of the wound healing process which will goes through as the first step in optimum skin regeneration (Epstein et al., 1999). Curcumin was shown to inhibit the production of tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1), this main cytokines released from monocytes and macrophages that play important roles in the regulation of inflammatory responses. Besides, curcumin also have the ability to inhibit the activity of NF-(κ)B (nuclear factor kappa-light-chain-enhancer of activated B cells), a transcription factor that regulates many genes implicated in the initiation of inflammatory responses. NF-(κ)B is normally activated by various kinases (AKT, PI3K, IKK) and curcumin affects a variety of the pathways implicated in this activation . Therefore, uncontrolled and prolonged inflammatory will delay the wound healing stage and thereby slow down the healing process. With the help of curcumin to inhibit the inflammatory stage, the damaged skin will more readily to enter the healing stage such as proliferation and regeneration (Dania et al., 2014).

2.10.1 Titanocene Dichloride

Titanocene dichloride is an organometallic compound and is widely used in organometallic and organic synthesis. It also act as a catalyst and also a reagent. Between, it is a solid with bright red colour which is crystallized from toluene. Titanocene dichloride is a kind of inorganic antitumor agent which Köpf first found that titanocene dichloride have the function in inhibit the tumor activities. Titanocene dichloride is a drug that can be used in improve the efficacy of cancer chemotherapy and also can use to overcome the shortcomings such as instability and short half-life in the human body. The titanocene dichloride drug has a good control release system due to its in vitro antitumor activity against human lung tumor spca-1 cells which the control release can be gained for long time. The drug will mix with phosphate buffer solution (PBS) and were researched by UV–Vis spectrophotometer. The system was

also developed by electrospinning. Titanocene dichloride was also works well with the biodegradable poly(L-lactic acid) fibers. By using the XRD for the characteristic study, the results shows that the titanocene dichloride exists in amorphous form in the fibers. Therefore, the Titanocene dichloride also shows an obvious inhibition effect against lung tumor cells. Therefore, the titanocene dichloride can be used in the implantable anticancer drug in clinical applications in the future. Among the inorganic anticancer drugs, there are very less report discuss about the developing systems of other inorganic anticancer drugs. Between, there have been some reports on describing about the developing efficient systems of cisplatin. Compared with cisplatin, titanocene dichloride has better antitumor activity compare to cisplatin in antitumor activity against colonic adenoma, which contain higher antitumor activity against many tumor cells.

2.11 Scanning Electron Microscope (SEM)

According to the research of (Vassilev et al., 2005), SEM is an important equipment use in measure the chemical and physical characterization of a specimen. SEM will generate signals by using by focused electron beam to scan the surface of a sample. The three most common modes of operation in SEM analysis are backscattered electron imaging (BSE), secondary electron imaging (SEI), and EDS.

The technique and principle for the SEM to operate is, the electrode probe of SEM will scan through the surface specimen by using the beam deflecting coils within the electron-optical column. The scanning generator of display and photographic-recording cathode ray tubes is connected to the scanning coil. By reducing the current to reach the column coils when leaving the same current in cathode-ray-tube coils, magnification can be achieved. The cathode ray tube face that contains a raster about 10 cml can be used to scan on the specimen with a small raster of few microns. The magnification is given by the ratio of these two rasters and can be up to 100,000 times. Besides, the conventional optical microscope can up to 1000x. The wavelength of the imaging radiation has to decrease to obtain a better resolution. For measure a sample, the electrons in microscopy are usually accelerated to high energies between 2 and 1000keV, which the wavelength is about 0.027-0.0009 nm (Nixon, W. C, 1965).

Nowadays, SEM is one of the popular devices that use to measure and provide information on the sample surface topography, crystalline structure, chemical composition and also electrical behaviour of the top 1 μm specimen.

The advantages of SEM over optical microscopy are, it has a large depth of field that can focus the surface of the specimen regardless how rough of the surface. However, the surface of the sample has to be smooth to get a good quality of image. This is due to small depth of field need high magnification that very dependent on the surface of sample. Besides, it also has higher magnifications (up to 1,000,000x), with an ultimate resolution of 1 nm. The maximum useful magnification in an optical microscope is around 1000x. Besides that, various information such as crystal structure, chemical composition, electrical properties and surface morphology can be measured by switching different imaging techniques (Vernon-Parry, 2000).

2.12 Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectroscopy is an optical technique that has been used for characterizing a wide range of organic synthesis, polymer science, petrochemical engineering and mineral analysis. FTIR is much better than XRD because it can produce Compared to XRD, IR spectroscopy is rapid, and capable of because it can provide both chemical and structural information on a wide range of amorphous, semi crystalline, and crystalline materials. In particular, IR spectroscopy also providing discriminatory information on the different types of minerals present within the sample. The range of Infrared region is 12800 ~ 10 $cm^{-1}$and can be divided into near-infrared region (12800 ~ 4000 cm^{-1}), mid-infrared region (4000 ~ 200 cm^{-1}) and far-infrared region (50 ~ 1000 cm^{-1}). Meanwhile, the structures of molecules and also characteristic absorption of infrared radiation can be determined by using FTIR. Infrared radiation is passed through a sample in Fourier-transform infrared spectroscopy (FTIR). The sample will absorb the infrared radiation and some might be passed through the sample. The spectrum that showed out will represent the molecular absorption and transmission, creating a molecular fingerprint of the sample, which is no two unique molecular structures, produces the same infrared spectrum and wavelength. This is easier for the scientist to determine the unknown sample functional group, quality, consistency and amount of components in a mixture.

FTIR is widely used because it can produce an infrared spectrum of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the sample. In addition, the background spectrum must also be measured during analysis. This is a measurement with no sample in the beam. This can be used to compare the measurement with the sample in the beam to determine how much percentage has transmittance (Schmitt et al., 1998).

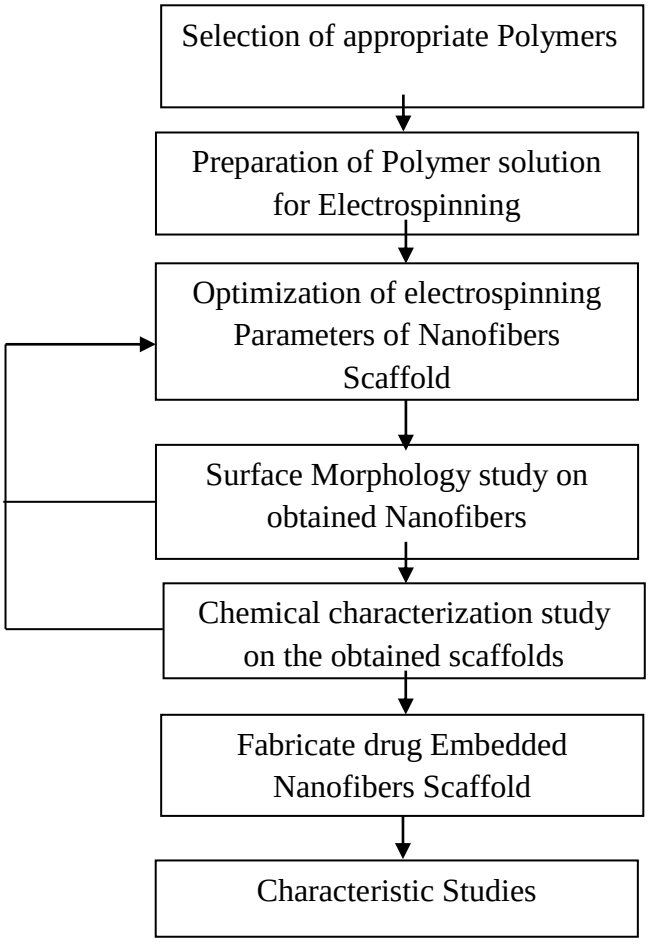
There are few advantage by using the FTIR. Analysis FTIR measure the sample in short period of time since it only require few second to run the sample and direct showed out the spectrum and wavelength peak. Besides, it also contain high sensitivity because the detector used is highly sensitive device.

CHAPTER 3: MATERIALS AND METHODS

3.1 Flow chart representation of Research methodology

3.1.1 Overview

The proposed research methodology is broken down into 3 stages. The first stages mainly deal with the preparation process of polymer and solvents. The polymers involve are Polyethylene oxide (PEO) and Polycaprolactone (PCL). Besides, the second stage deals with the selection of best processed nanofibers scaffold by using the NS Lab Nanospider system form Elmarco and learn to optimize the condition by studying the morphology of nanofibers. While for the 3rd stage, characteristic studies will be carried on by embedding the drug into the nanofibers to understand the existence of drug in fibers by using the Scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR), and Energy Dispersive X-Ray Analysis (EDX). Therefore, SEM, Image J and FTIR and Field Emission Scanning Electron Microscopy (FESEM) will be used in order to analysis the structural properties of nanofibers, fibers size distribution, and also chemical characteristics respectively.



3.2 Research Methodology

3.2.2 Stage 1

3.2.2.1 Materials and Methods

PEO (Mv-600,000), PCL(Mv-80,000), NaCl (Mv-58.44) and Titanocene dichloride (Mv-248.96) were obtained from Sigma-Aldrich and R&M chemicals. Dimethylformamide (Mv-73.09) and Chloroform (Mv-119.38) was purchased from Merck Chemicals. Deionised water was produced through MilliQ UV 8. Analytical grade Dichloromethane was purchased from Qurec(Asia) Sdn Bhd Chemicals.

3.2.2.2 Preparation of electrospun solutions

3 samples of PEO solutions were prepared by adding 7.167 $x10^{-5}$M for PEO 1 and 8.333$x10^{-5}$M for PEO 2 in each 0.1 L aqueous solution and also 8.333$x10^{-5}$M for PEO 3 with electrolyte additional in 0.1 L aqueous solution. Besides that, another 2 PCL samples, each with concentration 3.75 $x10^{-4}$M were mixed with single solvent system that was dichloromethane (DCM), and another PCL sample was mixed with 50 ml Dimethylformamide (DMF) and 50 ml chloroform ($CHCl_3$). respectively in order to prepare the polymer solution for electrospinning. These solutions were mixed homogeneously using a magnetic stirrer for 12 hr with constant temperature of 30^0C. Electrolyte addition was added in PEO 3 to improve the conductivity of the PEO polymer solution.

3.2.3 Stage 2

3.2.3.1 Electrospinning of nanofibers

Electrospinning technique was applied in fabricate the nanofibers scaffold by applying the NS Lab Nanospider system from Elmarco. This system consists of wire electrode based needleless electrospinning technique. The distance of the collector was set at 20 cm for all experiments. The collector is made of PP spun bound cloth fitted on the top of the wire electrode. The polymer ejected upward will collected on the collector at the top part. Besides that, the wire electrode is fitted with a polymer carriage reservoir which moves along the wire electrode in the bottom. The wire is fitted inside a groove which is passed through the polymer carriage. The groove with the size of 0.7 was used and the wire was adjusted to the middle of the groove. The prepared polymer solutions were loaded in the reservoir for

electrospinning. The reservoir carriage moved along the wire electrode at the speed of 100 mm/sec along with the applied voltage of 35kv for PEO solution while 45KV for PCL solution. The humidity is maintained around Rh 40% to 50% for PEO experiments. Fabrication of drug embedded nanofibers is studied by using PCL mix with DCM solutions that embedded with curcumin and Titanocene dichloride to study the present of the curcumin and titanocene dichloride particle that dissolve in the PCL nanofibers.

3.2.4 Stage 3

3.2.4.1 Measurement of solution Properties and Microscopy used

The samples in the experiments were carried out in temperature of 25^{o}C. Brookfield RST plus controlled stress rheometer fitted with a CCT 25 coaxial spindles were used to measure the viscosity of the polymer solutions. The system is controlled using Rheo 3000 software. The polymer solutions were loaded in to the MBT 25 coaxial cylinder. To find the reading of polymer viscosity, the constant shear rate method was applied to the polymer solution. The average viscosity was calculated using the Rheo 3000 software. 60 measuring points was taken for the period of 600 sec with 10sec for each point.

Multi-range conductivity meter (Hanna instruments HI-3388) fitted with HI-76301 probe was used in this experiment to measure the conductivity of polymer solution. Besides that, surface tension of polymer can be measured by using DIN certified Wilhelmy plate method in tensiometer. A Wilhelmy plate is a thin plate that made up of platinum is used to measure equilibrium surface or interfacial tension at an air–liquid or liquid–liquid interface. Since platinum material is chemically inert, it's been chosen as plate material to measure the viscosity of polymer. Wilhelmy plate will measure the force applied on it and operate at oriented perpendicular to the interface. This Wilhelmy plate can display low operator variance and it is widely used because of the higher accuracy determination of surface kinetics on a wide range of timescales. The experiment was conduct under the room temperature at 25^{o}C. 100 ml of polymer solution is loaded into the sample cup which is placed inside the sample holder and the height of the sample holder is raised till it reaches the plate and ensures the plate is not touching the sample initially.

For the SEM measurement, Hitachi table-top scanning electron microscope (TM3030plus) was applied to all of the fabricated nanofibers PEO and PCL samples for initial screening of

on the fiber diameter distribution range on the nanofibers quality. The The Hitachi TM3030Plus Tabletop SEM have been used because it has the highly sensitive low-vacuum secondary electron detector that able to measure the surface morphology of fine nanofibers. The nanofibers samples were imaged under the condition of 5 kv by using mix secondary electron (SE) and backscattered electron (BSE) option. Besides that, Hitachi TM3030Plus Tabletop SEM also offers both secondary electron images In low-vacuum mode and reflective electron images without any prior sample processing.

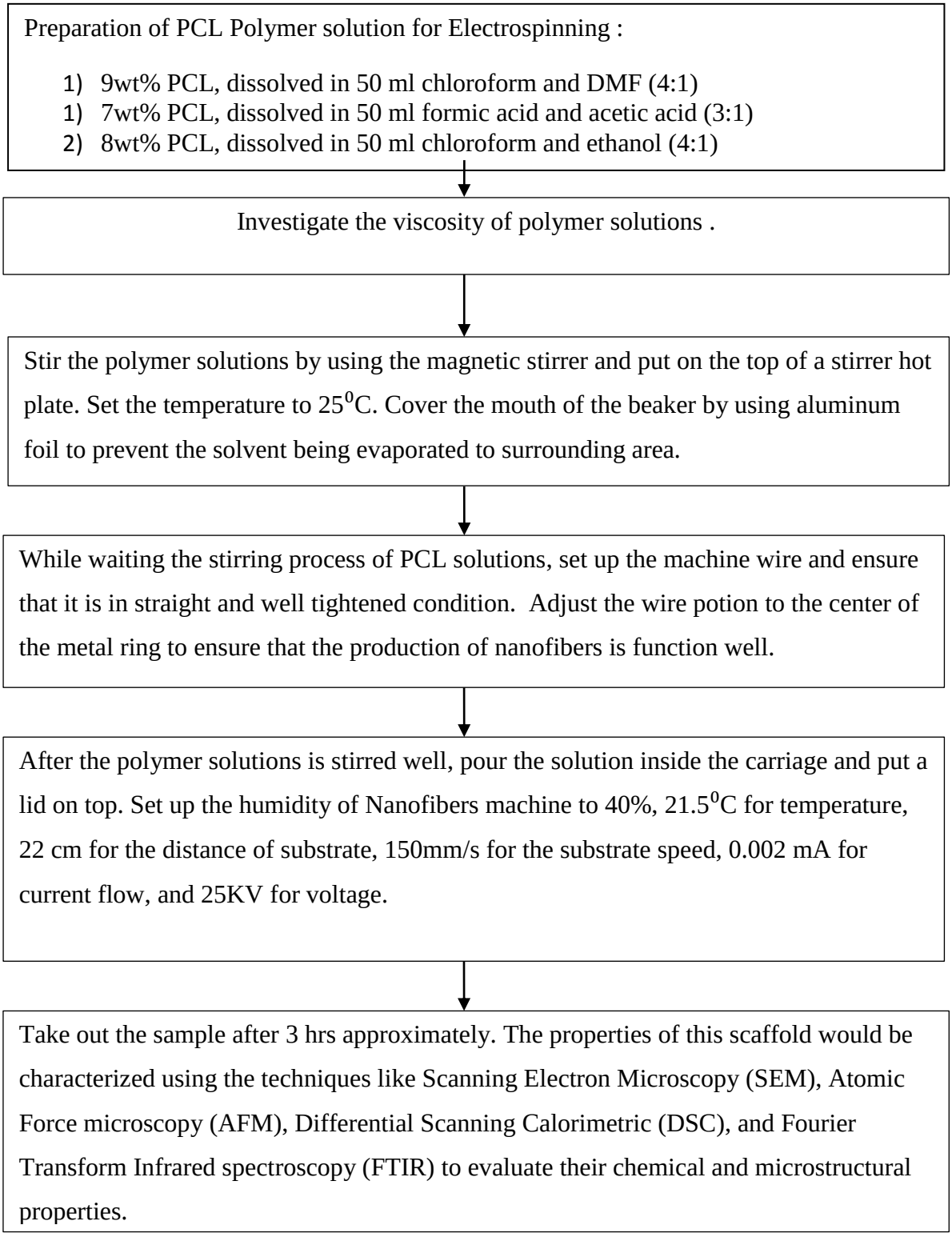
The sample with a good fiber quality (no bead or splash formation) was then imaged again using a field emission scanning electron microscopy (FESEM) (JEOL JSM-7800F). Imaging with FESEM is essential to ensure accurate measurement of fiber diameter at 3000x resolution. Image J software was used on all the SEM images to determine the fiber diameter distribution of PEO and PCL fiber. The known distance of image J software is depend on the drawing of scale setting selection line of a known length on the stage micrometer image. After setting the scale, unit of measurement is set to nanometer (nm). Graph of fiber diameter distribution is plotted after 100 measurements diameter data of each samples are calculated by using Image J software.

Fabrication of drug embedded nanofibers scaffold were carried out after the morphology of study of the diameter range of the nanofibers. Characterization studies were studied by using FTIR and EDX to detect the presence of drug that diffuses in the nanofibers.

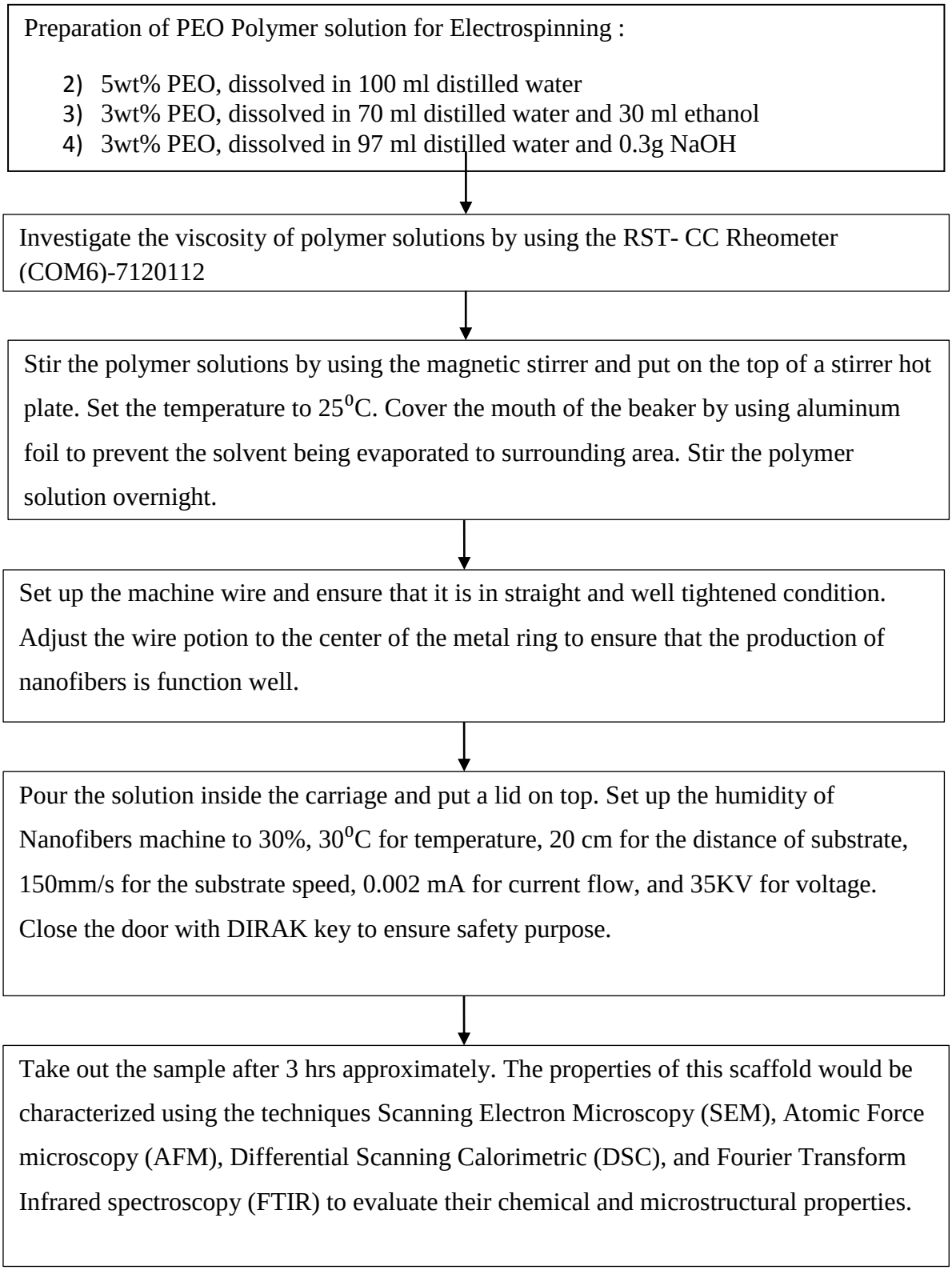
Figure 3.1: SEM model TM3030 plus

Figure 3.2: rheometer fitted with a CCT 25 coaxial spindles

3.3 Flow chart of Preparation of Poly-caprolactone (PCL) solution



3.4 Flow chart of Preparation of Polyethylene oxide (PEO)



3.5 List of Chemical

Table 2.2: Chemical used in the research field

No	Chemicals	Molecular Weight g/mol	Sources	CAS NO
1	Polycaprolactone (PCL)	80000	Sigma Aldrich	440744
2	Poly Ethylene Oxide (PEO)	600,000	Sigma Aldrich	25322-68-3
3	Curcumin Drug	368.38	Merck Millipore	458-37-7
4	Sodium Chloride (NaCl)	58.44	Merck	7647-14-5
5	Formic acid(HCOOH)	46.03	Merck	64-18-6
6	Acetic acid (CH_3CO_2H)	60.05	Merck	64-19-7
7	Ethanol (CH_3CH_2OH)	46.07	Merck	64-17-5
8	Chloroform ($CHCl_3$)	119.38	Merck	67-66-3
9	Dimethylformamide (DMF)	73.09	Merck	68-12-2
10	Sodium Hydroxide (NaOH)	40.0	Merck	1310-73-2

Chapter 4 RESULTS AND DISCUSSION

4.1 Overview

This section shows the preliminary results of the study of the quality and morphology of nanofibers produced in different solution parameters and additional of electrolyte addition and also the characteristic study of fabrication of drug embedded nanofibers mat. For the first stage, polymer PEO 1, PEO 2 and PEO 3 were used for study the optimising of solution parameter such as concentration such as conductivity, while PCL polymer were used in study the effect of using different solvent by adding PCL into DCM and another set was PCL adding with DMF and $CHCl_3$. The diameter distribution of nanofibers were measured by using software Image J from the SEM image to study and determine the quality and morphology of the nanofibers. Besides that, viscosity and surface tension also one of the important criteria that influenced the electrospinning of nanofibers. A comparison on the diameter of nanofibers distribution was studied extensively by many researchers such as (Bharath et al., 2011) and also fabrication of drug embedded nanofibers by using FTIR spectroscopy and EDX to determine the existence of drug in the nanofibers scaffold.

4.2 Morphology of Nanofibers (Fibers Diameter Distribution)

From Figure 4.1, Poly (ethylene oxide) (PEO, molecular weight: (600,000 g/mol, Aldrich) and ethanol (absolute, ACS reagent, ≥ 99.5%, Merck) were used as a biodegradable polymer in Electrospinning with different solvents condition and electrolyte addition, while PCL is used to mix with the DCM in different humidity condition.

4.2.1 Effect of Concentration (Viscosity) in Nanofibers Scaffold

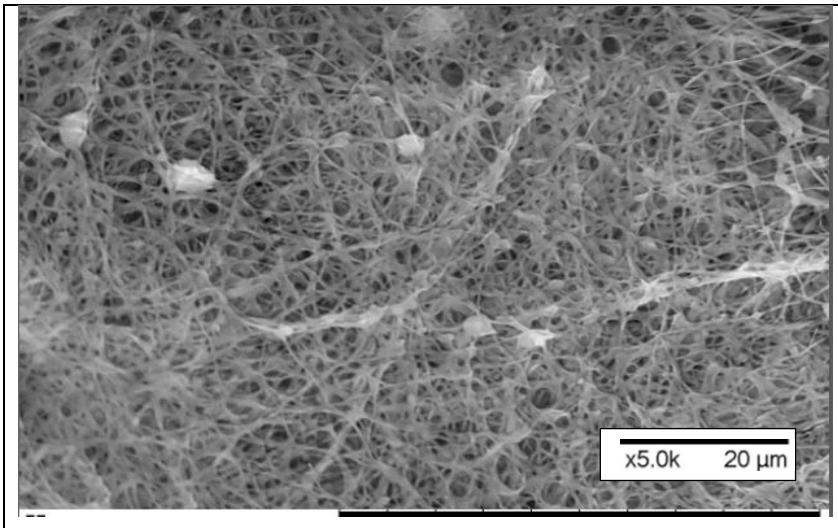


Figure 4.1: 7.167 x10^{-5}M PEO 1 with aqueous solution

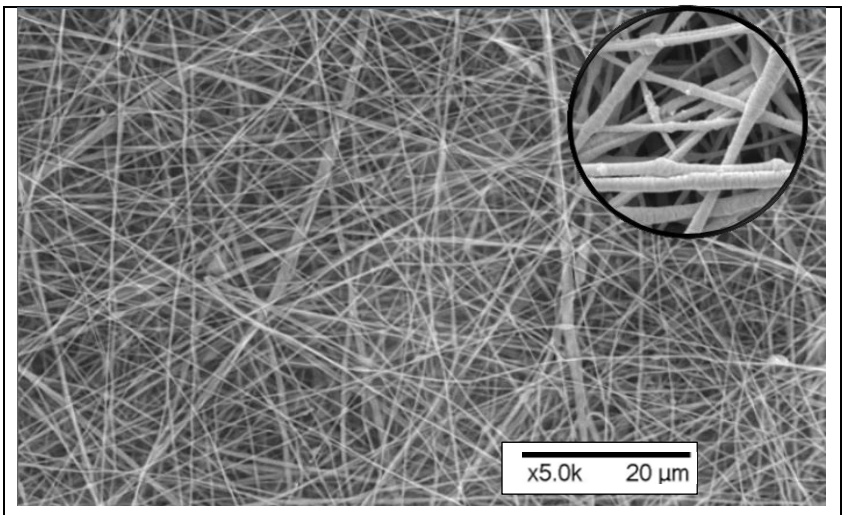


Figure 4.2: 8.333x10^{-5}M PEO 2 with aqueous solution

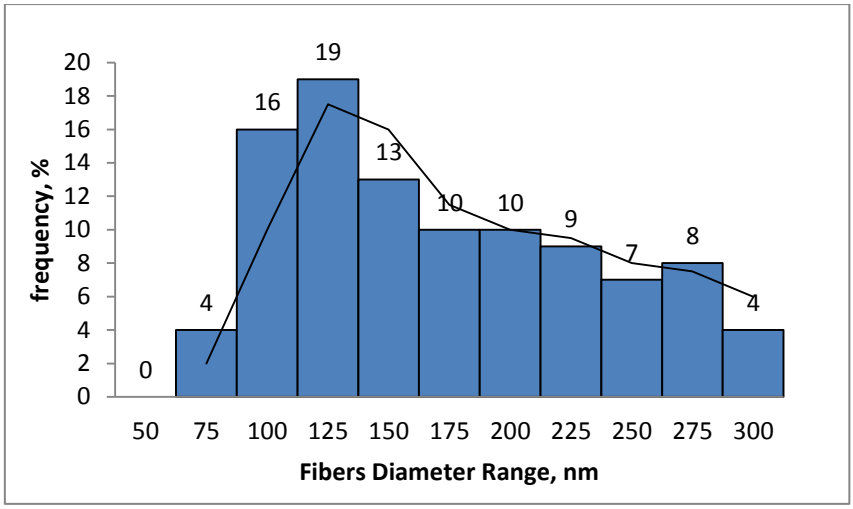


Figure 4.3: PEO 2 with aqueous solution

Figure 3.3: PEO 2 with aqueous solution

From PEO 1 and PEO 2, the solutions of PEO with different polymer concentrations were studied. Different concentration of PEO at 7.167 x10^{-5}M and 8.333x10^{-5}M of PEO were added approximately into the aqueous solution. The SEM image showed (Figure 3.1), many beads had formed in lower polymer concentrations, 7.167 x10^{-5}M (PEO 1) compared to PEO 2 (Figure 3.2) at 8.333x10^{-5}M. While for PEO 2, PEO polymer was greatly dissolved in the distilled water aqueous solution and many nanofibers with small diameter had formed. The viscosity of PEO 1 and PEO 2 were measured by using Brookfield RST plus controlled stress rheometer fitted with a CCT 25 coaxial spindles. The viscosity obtained for PEO 1 and PEO 2 were 79 cP and 417 cP respectively. From the Figure 4.3, PEO 2 has a lower fiber diameter distribution range between 100nm-125nm, while for PEO 1, The SEM image has investigate large amount of unwanted beads formed in the nanofibers. This was due to when the concentration of the polymer solution is low, the applied electric field and surface tension cause the entangled polymer chains to break into fragments before reaching the opposite upward electrode wire collector (Haider et al., 2013; Pillay et al., 2013). As fragments were formed, it will cause formation of beads or beaded nanofibers.PEO 1 had showed there were some beads formations in the fibers. Therefore, increasing the concentration will be decrease the beads formation. This was due to increase the concentration of polymer solution will cause the viscosity to increase as well, which also increase the polymer chain entanglement. These entanglements polymer chain overcome the surface tension and finally result in

uniform beadless nanofibers formed. However, if the concentration of the polymer solution is too high or beyond critical value, the high level of viscosity will hamper and block the flow of the solutions through the metal rings inside the polymer carriage and lastly leads to defective or beads formation. Besides that, as the viscosity of polymer solution change, the morphologies of the shape of beads will form a round droplet like shape (low viscosity) to a stretched droplet ellipse to smooth fibers (sufficient viscosity). Therefore, Increasing concentration of polymer solutions in certain suitable level will decrease the beads formation in nanofibers. Meanwhile in our research, 8.333×10^{-5}M of PEO is more suit to produce a good quality of nanofibers scaffolds compare to 7.167×10^{-5}M concentration of nanofibers scaffolds. The phenomenon of stretching of polymer solution on the surface of wire electrode is very important where the Electrospinning process relies on it. Concentration is one of the solution parameter that influence the stretching of polymer solutions on the electrode wire (Adnan et al, 2015).

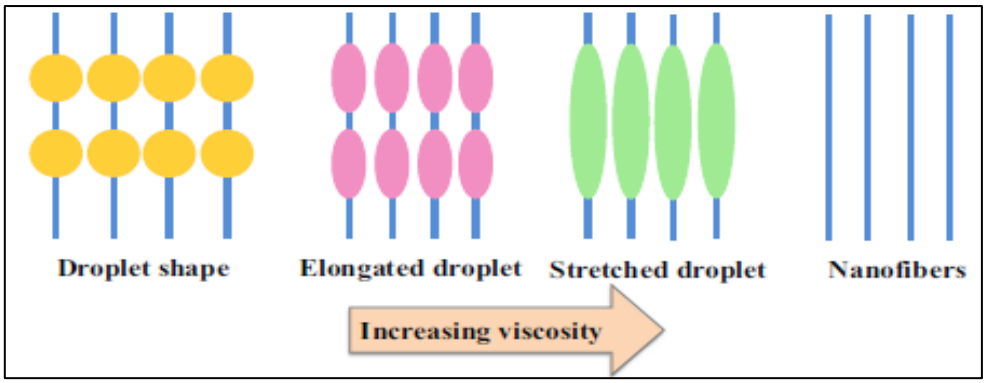


Figure 4.4: Viscosity effect on the elongation of polymer solution

4.2.2 Effect of Electrolyte Addition on Polymer solutions

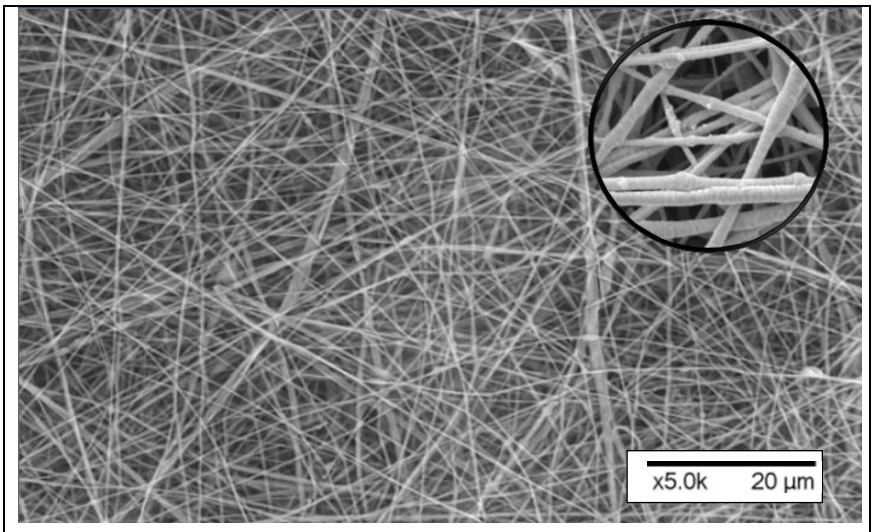


Figure 4.2: 8.333x10^{-5}M PEO 2 with 0.1L aqueous solution

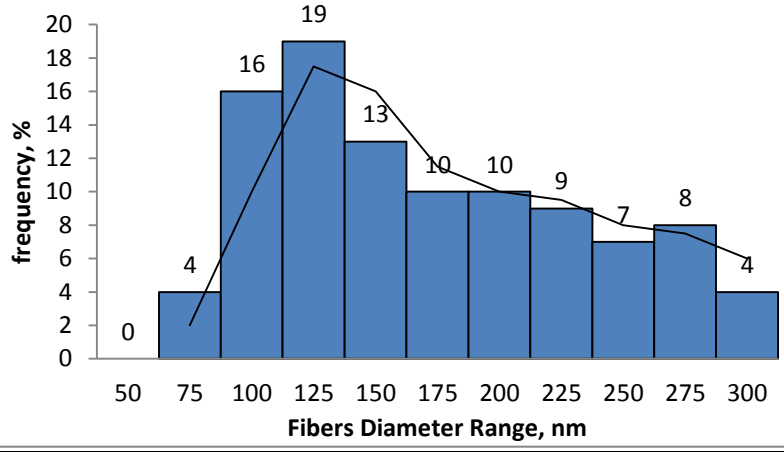


Figure 4.3: 8.333x10^{-5}M PEO 2 with aqueous solution

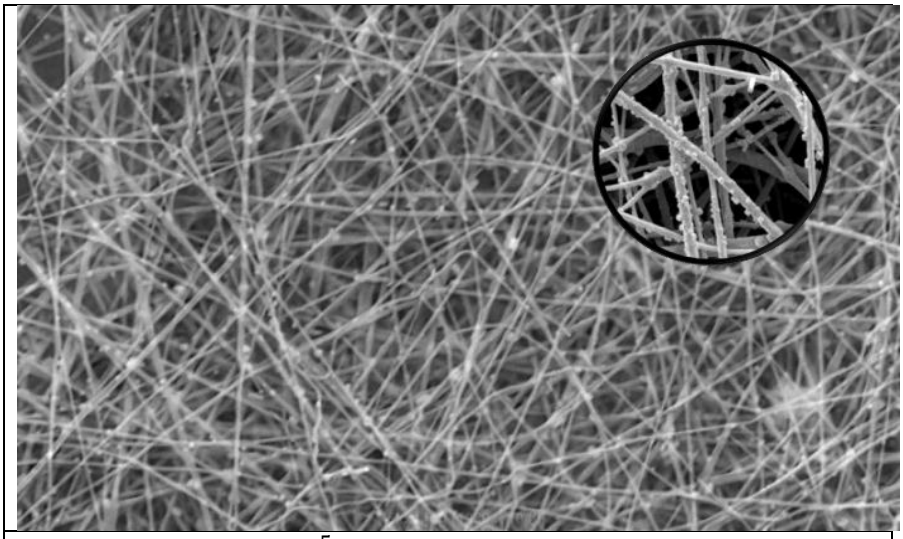


Figure 4.5: 8.333x10^{-5}M PEO 3 with 0.1L aqueous solution and 0.5g electrolyte addition

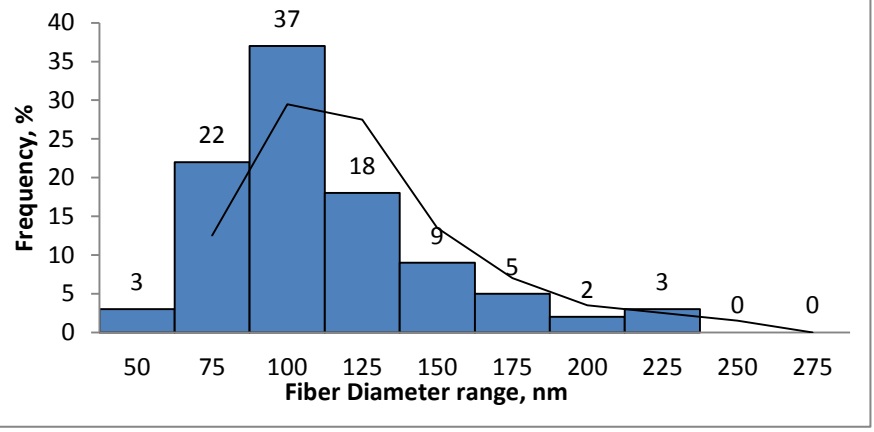


Figure 4.6: 8.333x10^{-5}M PEO 3 added with electrolyte addition in aqueous solution

Furthermore, electrolyte was added into aqueous solution and mixed with 8.333x10^{-5}M of PEO 3. In this study, PEO 2 and sample PEO 3 has the same concentration of polymer solutions, but sample 3 has the additional of salt. Electrolyte salt was added to increase the conductivity of the solution s and increase the movement of the mobile phase. Conductivity test has been applied to PEO 2 and PEO 3 system. The conductivity for PEO 2 was 0.06 mS/cm while PEO 3 solution contain 7.6 mS/cm conductivity. From the SEM image of PEO 2 and PEO 3 (Figure 4.2 and 4.5), there were salt attached at the surface of the nanofibers.

The diameter of the nanofibers in PEO 3 was lower than the diameter of fibers in PEO 2. The range of the nanofibers diameter distribution of PEO 3 (Figure 4.6) was about 75 nm to 100 nm, which was better than fibers in PEO 2 (Figure 4.3), around 100 nm to 125 nm respectively after the electrolyte addition was added. Solution conductivity is one of the solution parameters who play vital role in affects the Taylor cone formation and also the diameter of nanofibers. As the solution is low in conductivity, the bubbles is hard to be generated to ejecting to the opposite wire electrode collector, which will cause bead formation while as conductivity level is increases, it will increase and induce the charge on the surface of bubbles on the free surface of the electrode wire and form Taylor cone structure, the diameter of nanofibers decreased as well (Sun et al., 2014).

Furthermore, electrospinning process is dependent on the Coulomb force between the electrostatic force from electrode wire and also the surface charge from the polymer solutions. An ideal polymer solution without electrolyte addition will not have sufficient charges in solution to elongate the bubbles to eject upward to the opposite electrode wire collector. In contrast, conductive polymer solutions have sufficient charge to induce electrostatic force and initiate the electrospinning process. Therefore, the PEO 3 with electrolyte additional shows that the nanofibers produced not only smooth and beadless but were also small diameter compared to sample 2 (Zong et al., 2002).

4.2.3 Effect of different Solvents Used

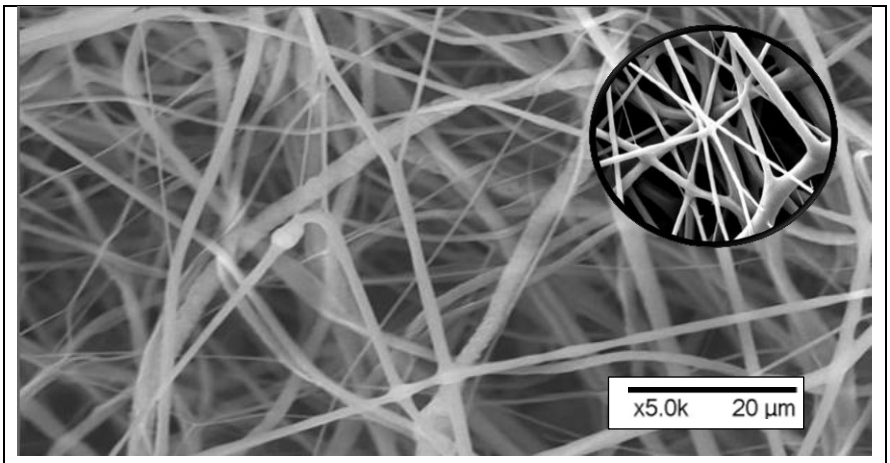


Figure 4.7: 3.75 x10^{-4}M PCL+ 0.1L DCM

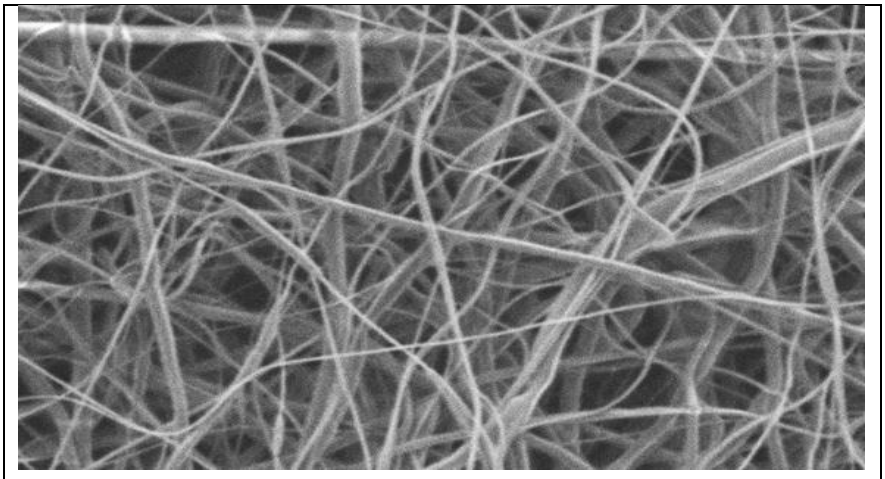


Figure 4.8: 1.125x10^{-3} M PCL + DMF: $CHCl_3$ (1/4)

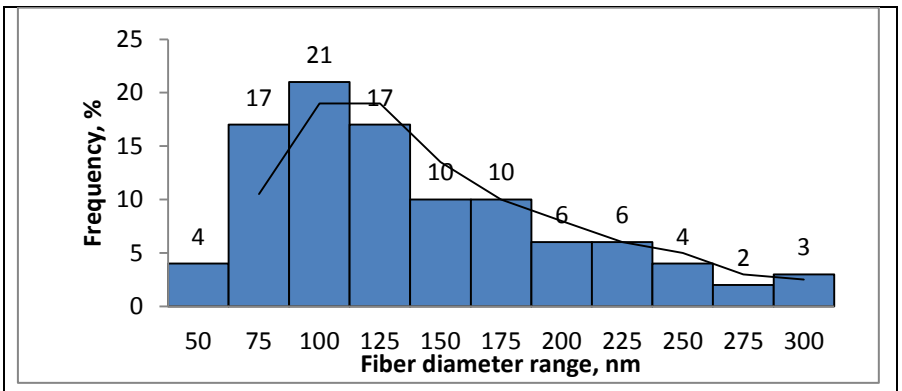


Figure 4.9: graph of 3.75x10^{-4} M PCL+ 0.1L DCM

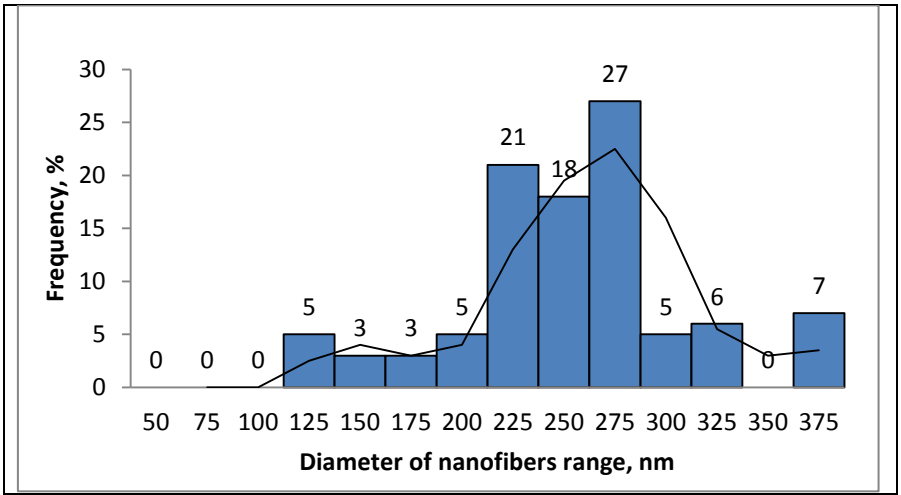


Figure 4.10: graph of $1.125x10^{-3}$ M PCL + DMF: $CHCl_3$ (1:4)

In this study, PCL was chosen in Electrospinning because of its biodegradable properties, and the most important is, curcumin drug and titanocene dichloride drug can dissolved well in the PCL polymer compare to PEO polymer due to its hydrophobic characteristic. Therefore, PCL is selected in the study. Surface morphology study of different solvents (DMF/$CHCl_3$ and DCM) was performed to investigate the formation of porous electrospun PCL fibers (Katsogiannis et al., 2015).

In the literature review, there were numerous reports on the production of PCL nanofibers by dissolving the PCL in different solvents. Meanwhile, PCL has dissolved in 2 different solvent (DCM and DMF/CHCl) to determine and observe the physical and mechanical properties of PCL nonwoven mats produced by electrospinning. From Figure 4.7, $3.75x10^{-4}$ M PCL was mixed with the DCM. As a single solvent, the electrospun fibers have a very regular and thin nanofibers diameter range between 50 nm to 125 nm (Figure 4.9), therefore, uniform and beadless electrospun nanofibers had formed in the Electrospinning process. While for the solvent system of DMF mixed with $CHCl_3$, Figure 4.10 has showed the fiber diameter distribution range, it was around 200 nm to 275 nm. While from the SEM image, there was no bead formed and uniform electrospun fibers were produced.

Meanwhile, for 2 difference boiling point solutions, DMF and $CHCl_3$ mixed together, the solvents can balance each other in term of volatility to fabricate nanofibers of appropriate

porous morphologies (Abdul et al., 2016). Compare to the literature review that PCL mixed in DMF:$CHCl_3$ in same concentration and condition, the fibers produced in this study was much more better than the literature review where the fibers diameter distribution range in this study was 200 nm to 275 nm compare to research of (Ko Eun et al., 2015), the fiber diameter was around 910 ± 400 nm, respectively.

However, the single solvent DCM mixed with the PCL (Figure 4.7 and Figure 4.9) has showed thin diameters and beadless uniform fiber compare to mixed solvent system. This was due to DCM has low boiling point and high volatility, which cause in low surface tension to eject the polymer easily upward to the collector (De Vrieze et al). Generally, the solvents with low boiling point and high volatility are preferred to mix with the polymers preferred because the polymer solutions is easier to evaporate during the eject and elongation of solutions upward to the opposite electrode wire. The humidty of this study was around 60% to 70% to prevent the dry up of the volatile solvents. When the humidity is lower, the solution will dry faster which cause the elongation of polymer solution decrease during the electrospinning process (Abdul et al., 2016).

Since the fibers diameter distribution in single solvent was much better than the mixed solvent system, PCL mixed with DCM was selected to be study further in the fabrication of drug embedded nanofibers system. The results of drug embedded fabrication nanofibers was observed and discussed in subtopic 4.4 below.

4.3 Drug embedded in nanofibers

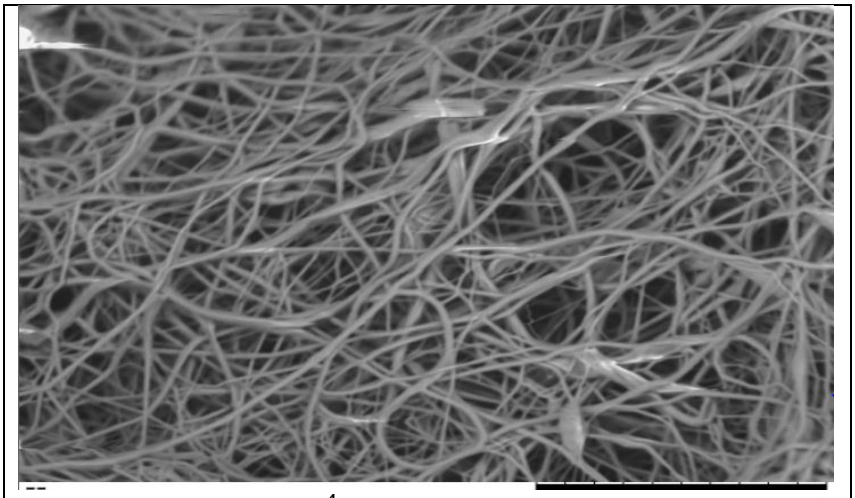


Figure 4.11: 3.75 x10^{-4}M PCL 1 + Curcumin drug embedded nanofibers

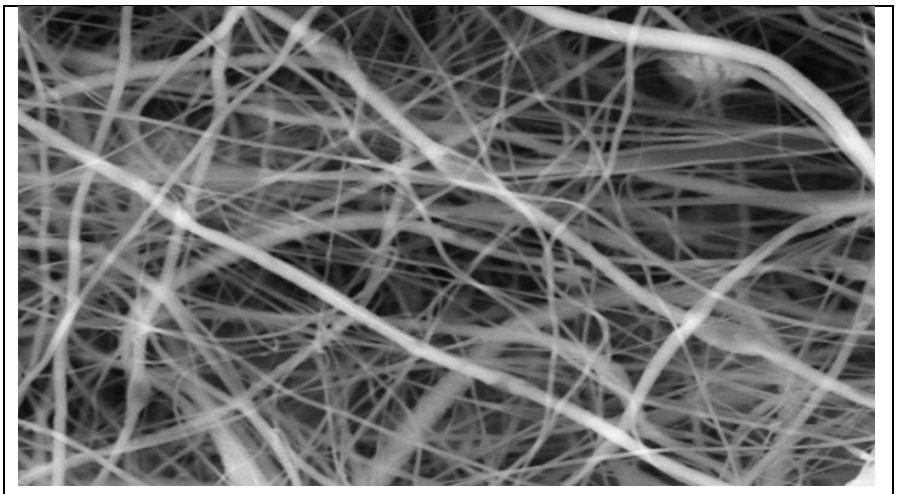


Figure 4.12: 3.75 x10^{-4}M PCL 2 mixed with Titanocene Dichloride drug embedded nanofibers

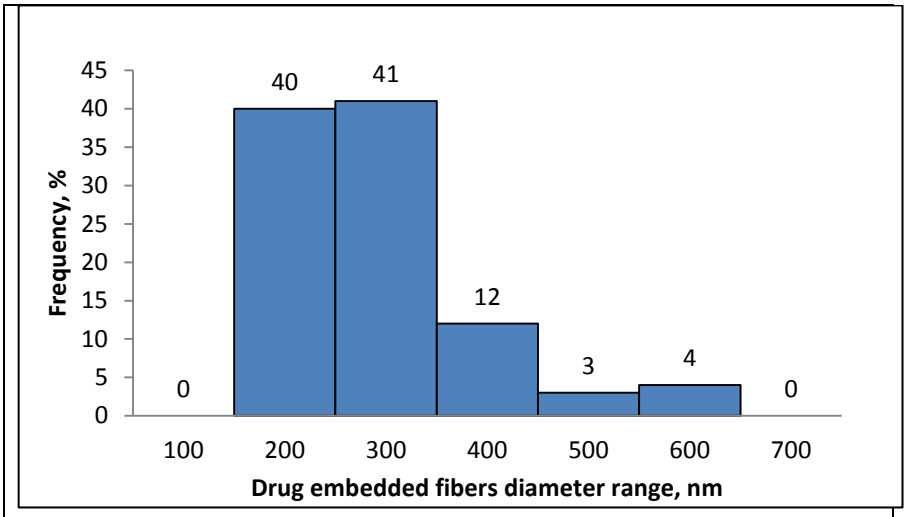


Figure 4.13: Fibers Diameter of Curcumin embedded Nanofibers

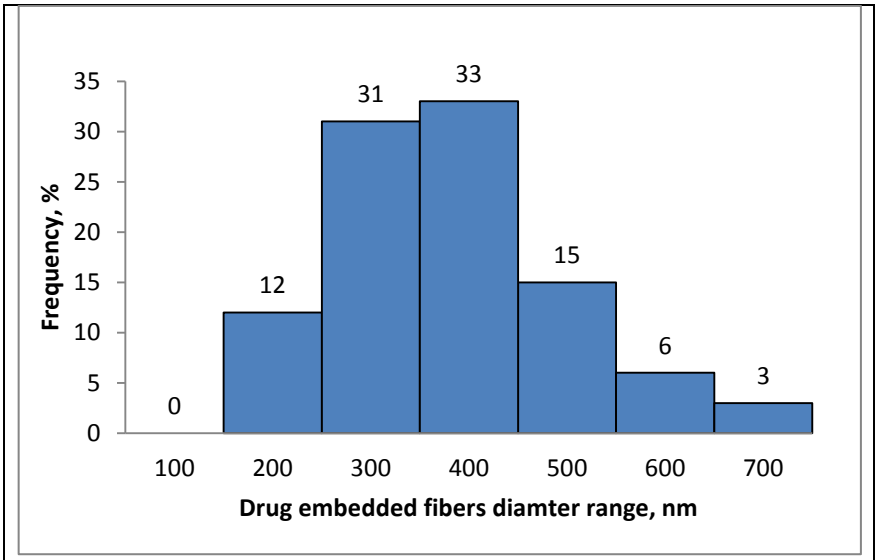


Figure 4.14: Fibers Diameter of Titanocene embedded Nanofibers

Figure 4.15: Surface of PCL mixed with DCM and curcumin

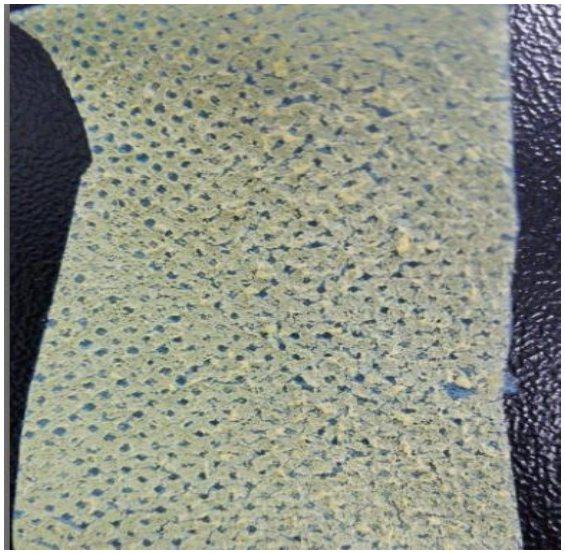


Figure 4.16: Surface of PEO mixed with ethanol and distilled water + titanocene dichoride

In Figure 4.11, 3.75 x10^{-4} PCL 1 has mixed with 0.1L DCM and curcumin while PCL 2 (Figure 4.12) has mixed with 0.1L of DCM with titanocene dichloride (TDC). From Figures 4.11 and 4.13, the PCL 1 fibers embedded with drug showed beadles and uniform fibers structure and the diameter obtained was within the range of 100 nm to 300 nm. Whereas for PCL 2, Figures 4.12 and 4.14 showed beadless fibers formed and the diameter range were around 200 nm to 400 nm. However, curcumin embedded with PCL 1 has obtained a lower average diameter range compare to drug TDC embedded with PCL 2. This may due to the possibilities of the clusters of the titanocene complex molecules on the surface of the microfibers that influence the quality of electrospun nanofibers (Napoli et al., 2013).

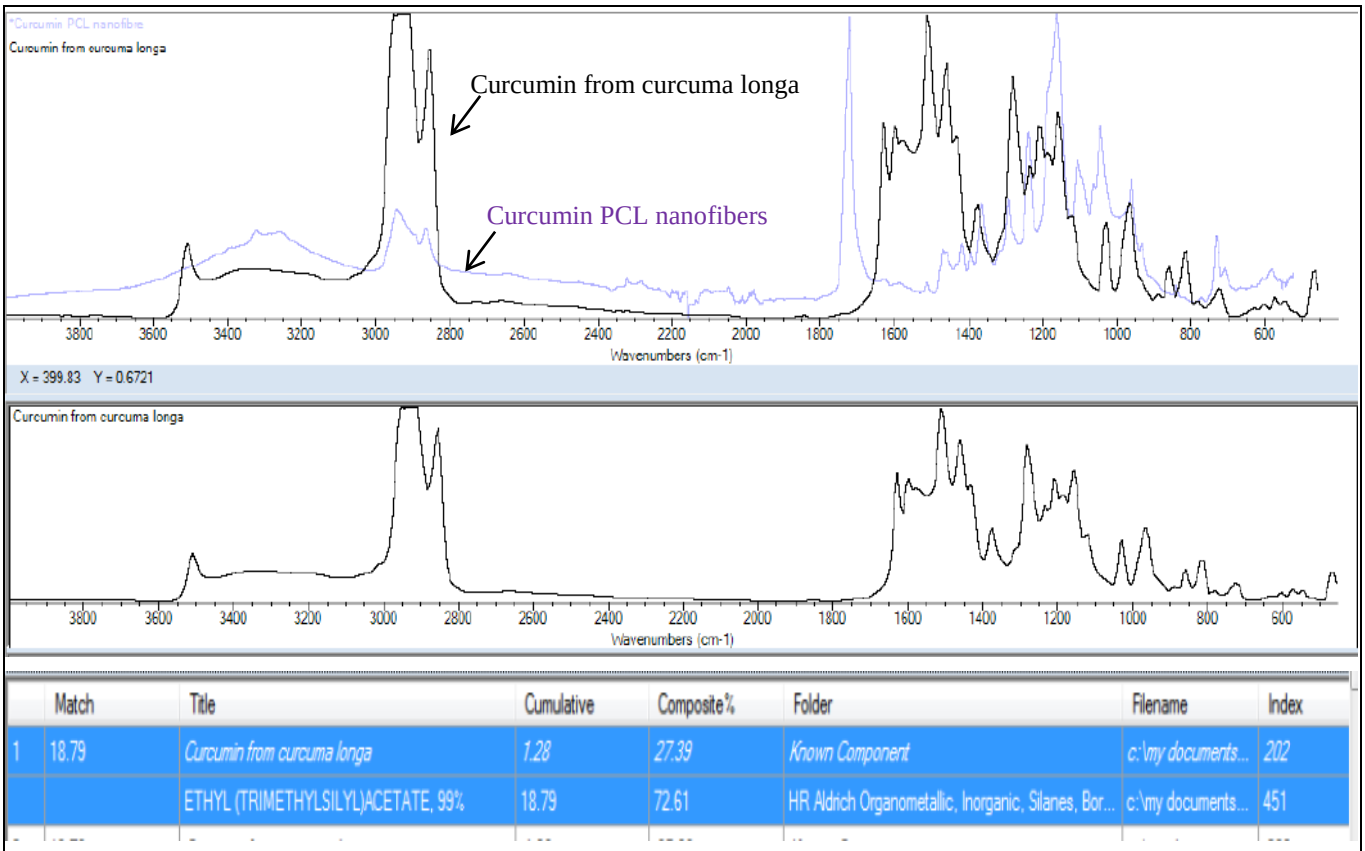
Nonpolar molecules that repel the water molecules are said to be **hydrophobic**; molecules forming ionic or a hydrogen bond with the water molecule are said to be hydrophilic.

Titanocene dichloride (TDC) has limited solubility in water, which is hydrophobic properties, that hard to obtain a hydrolytic stability. Normally after adding the titanocene dichloride (TDC), it will undergo dissolution after 5–10 min to give a clear yellow solution. However, the saturated yellow solution that frequently contains undissolved material, which cannot dissolve completely and mixed with the PEO (Aditya et al., 2015). This is due to PEO has a hydrophilic characteristic, which is not repel to water. Therefore, PEO was hard to mix completely with the drug titanocene dichloride (TDC). From Figure 4.15, the electrospun drug embedded nanofibers with PCL has a smooth surface which shows the PCL polymer

solution can dissolve completely with the curcumin drug. This is due to curcumin is a hydrophobic types of drug which can dissolve in organic solvents. For curcumin, it has low solubility in water, which easily degrades in alkaline pH conditions. (Anand, Kunnumakkara, Newman, & Aggarwal, 2007).

Figure 4.16 has showed the fabrication of the PEO mixed with the drug titanocene dichloride (TDC) that dissolved in the ethanol and distilled water solution. The nanofibers produced were spoiled and there were no suitable fibers formed on the collector (Mokdsi et al., 1998).

4.3.1 Existence of Drug Curcumin in Electrospun Nanofibers



	Match	Title	Cumulative	Composite%	Folder	Filename	Index
1	18.79	*Curcumin from curcuma longa*	*1.28*	*27.39*	*Known Component*	*c:\my documents...*	*202*
		ETHYL (TRIMETHYLSILYL)ACETATE, 99%	18.79	72.61	HR Aldrich Organometallic, Inorganic, Silanes, Bor...	c:\my documents...	451

Figure 4.17: Absorbance of drug curcumin in the nanofibers mat

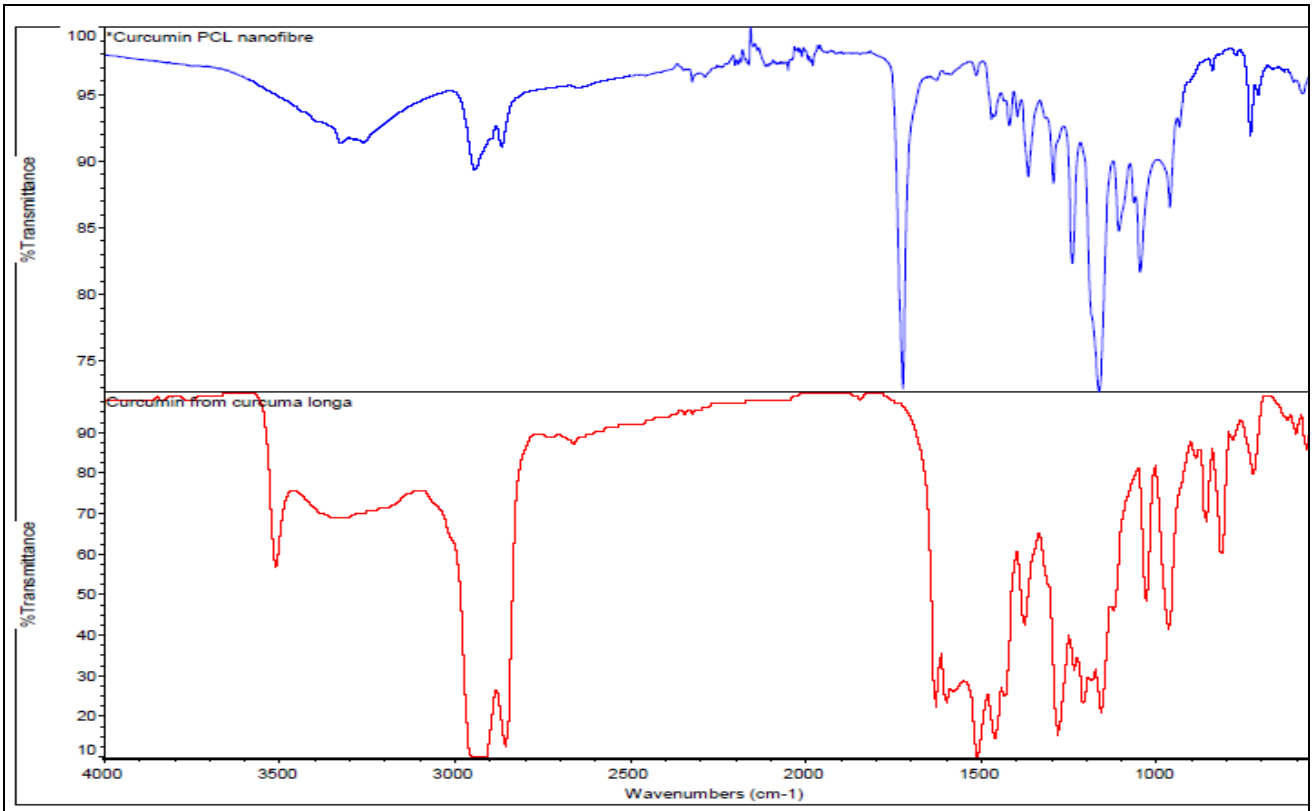
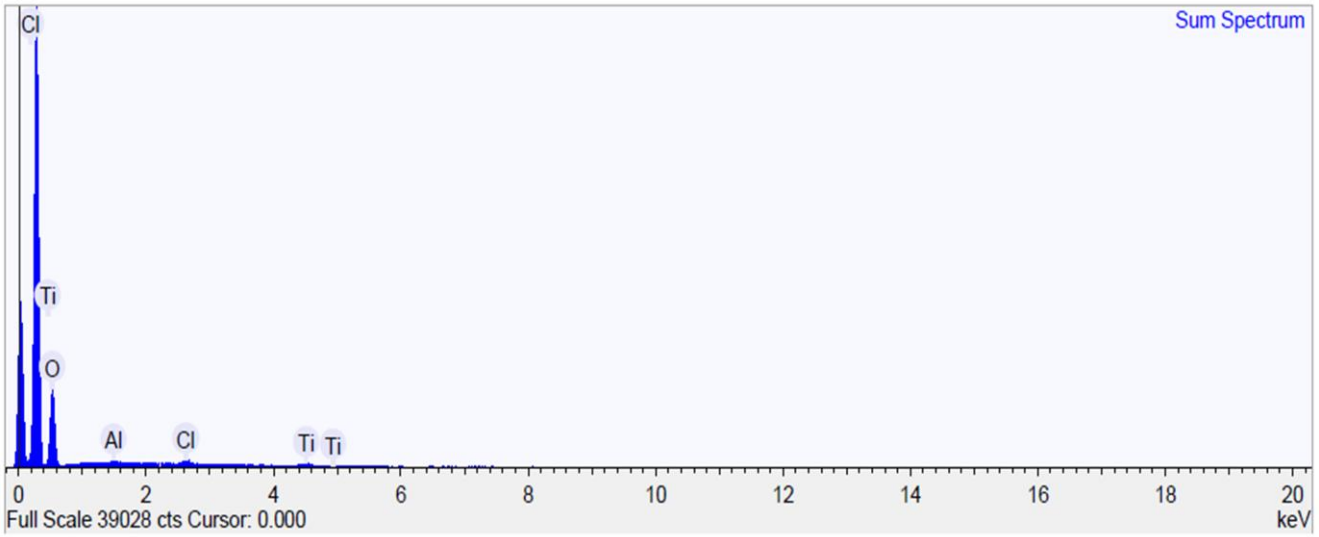


Figure 4.18: Transmittance percentage of drug curcumin in nanofibers mat

The FTIR spectrum of curcumin was shown in Figure 4.17 and 4.18. A broad peak at 3293 cm^{-1} and the sharp one at 3508 cm^{-1} indicate the presence of OH. The strong peak at1626 cm^{-1} has a predominantly mixed ν (C=C) and ν (C=O) character. Another strong band at 1601 cm^{-1} is attributed to the symmetric aromatic ring stretching vibrations ν ($C=C_{ring}$). The1508 cm^{-1} peak is assigned to the ν (C=O), while enol C-O peak was obtained at 1272 cm^{-1}, C-O-C peak at 1023 cm^{-1}, benzoate trans-C-H vibration at 959 cm^{-1} and cis CH vibration of aromatic ring at 713 cm^{-1}. Absorption at 1300–700 cm^{-1} corresponds to skeletal C=C vibrations and that at 1155 cm^{-1} corresponds to C=O=C vibrations. The sharp peak at 3508 cm^{-1} due to free hydroxyl (OH) group of curcumin. From the Figure 4.17, the drug curcumin has exist in a low intensity, which was 18.79. This indicate the existence of curcumin in the electrospun nanofibers scaffold (Krishna Mohan et al., 2012).

4.3.2 Existence of Drug Titanocene Dichloride (TDC) in Electrospun Nanofibers



Summary results

Element	Weight %	Weight % σ	Atomic %
Oxygen	88.294	0.353	94.798
Aluminum	1.534	0.165	0.976
Chlorine	4.586	0.196	2.222
Titanium	5.586	0.262	2.003

Figure 4.19: Composition of PCL embedded with titanocene dichloride measured by EDX

Energy dispersive X-ray spectroscopy, sometimes called energy dispersive X-ray analysis, is an analytical technique used for the elemental analysis or chemical characterization of a sample. To stimulate the emission of characteristic X-rays from a specimen, a high energy beam of charged particles such as electrons or protons, or beam of X-rays, is focused into the sample being studied. In Figure 4.19, the EXD analysis showed that the peak of Titanium was much higher respect to others element on the surface. This is an indication that the titanocene complex is located inside the fibers, probably with a very small fraction on the surface, which was 5.586% titanium weight being detected in the electrospun fibers (Napoli et al., 2013).

Chapter 5 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

As a conclusion, Nanofibers scaffold are now act as a structural mechanism in regenerate the human tissue by mimicking the natural extracellular matrix in human body. PCL and PEO polymer was used in this study because of its biodegradable properties. In the process of elctospinning, solution parameter such as concentration, type of different solvent used and the additional of electrolyte into the polymer solution have made a greater influence of the quality of nanofibers scaffold. After the fabrication of nanofibers, the morphology of the nanofibers has measured by using SEM and image J software. Whereas for the fabrication of drug embedded nanofibers scaffold, the characterization study of the product were measured by using FTIR, SEM, Image J and also EDX.

As for concentration, PEO 2 has obtained a lower average fiber distribution diameter range which was 100 nm to 125 nm compare to PEO 1. This was due to increase the concentration of polymer solution will cause the viscosity to increase as well, which also increase the polymer chain entanglement. It will result in uniform beadless nanofibers formed.

As for additional of electrolyte with polymer solution, PEO 3 who contain electrolyte addition has obtained a lower fiber diameter distribution range about 75 nm to 100 nm, that is much more better than PEO 2. As the polymer solution contain higher ionic molecules, it will eject the polymer solution to the opposite electrode wire and caused the diameter decrease as well.

While for using different solvent system, PCL that mixed with DCM has obtained a lower fiber diameter distribution range at 75 nm to 100 nm, which compare to PCL mixed with DMF and $CHCl_3$ at range 250 nm to 275 nm. PCL mixed with DCM was further been used in fabrication of drug embedded process. This was due to DCM has lower boiling point that easily to evaporate in humidity of 60-70% during electrospinning process.

Furthermore, for the fabrication of drug embedded nanofibers electrospinning process, PCL 1 (Curcumin embedded with PCL) has showed good fiber diameter distribution range about 100 nm to 300 nm compare to PCL 2 (titanocene dichloride embedded with PCL) which range around 200 nm to 400 nm. While for PEO, it was a hydrophilic polymer who cannot

dissolve completely with the drug. Therefore, the parameters need to be optimised to get a good quality of nanofibers scaffold.

5.2 Recommendation

In this study, humidity is the one of the important ambient parameters that need to be considered during Electrospinning process. As the humidity is not controlled well, fiber produced will undergo splashing and also beads formed. Besides that, Parafilm Sealing Film is needed in sealing the mouth of the beakers during the hot plate magnetic stirring process. This is due to the solvent mixed with the polymer contain low boiling point, which will evaporate to the surrounding. Other than that, low temperature for hot plate stirrer should be applied for the PCL polymer solution that mixed with solvents to prevet the solvents to evaporate at higher temperature. Furthermore, personal protect equipment (PPE) such as lab coat, lab shoes and goggles has to be used during the lab work. Lastly, the residual charge has to be discharged to prevent electric shock.

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