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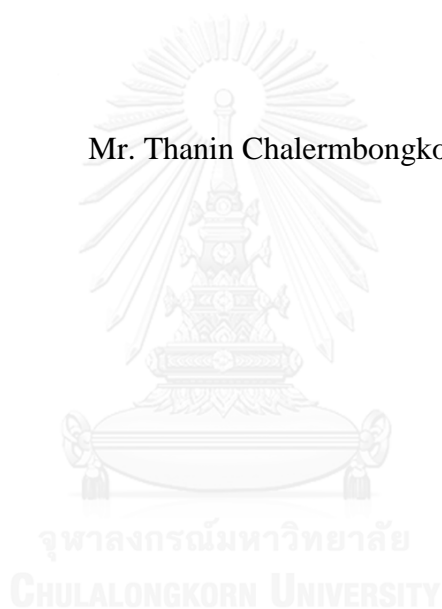สาขาวิชาเคมี ภาควิชาเคมี
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SYNTHESIS OF POSITIVELY CHARGED POLY(LACTIC ACID) FOR PREPARATION OF ELECTROSPUN FIBER

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ปัน (SYNTHESIS OF POSITIVELY CHARGED POLY(LACTIC ACID) FOR
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พอลิแล็กติดแอซิดเป็นพอลิเอสเทอร์ที่สามารถย่อยสลายได้เองตามธรรมชาติและเข้ากับ
สิ่งมีชีวิตได้ดีจึงทำให้พอลิแล็กติกแอซิดจัดเป็นพอลิเมอร์ที่มีศักยภาพในการใช้เป็นวัสดุทดแทนในงาน
บรรจุภัณฑ์และวัสดุชีวภาพ อีกทั้งยังใช้อย่างกว้างขวางในงานด้านต่าง ๆ แต่ด้วยความไม่ชอบน้ำของพอลิ
แล็กติกแอซิดทำให้สามารถนำไปใช้งานในงานที่ต้องสัมผัสน้ำโดยตรงได้ไม่เต็มประสิทธิภาพ เช่น การ
นำส่งยา หรือ งานวิศวกรรมเนื้อเยื่อ ดังนั้นเพื่อที่จะเพิ่มความชอบน้ำให้กับเส้นใยอิเล็กโทรสปันพอลิแล็ก
ติกแอซิด พอลิแล็กติกแอซิดที่มีปลายทั้งสองด้านเป็นประจุบวกจึงถูกสังเคราะห์ขึ้นโดยการทำปฏิกิริยา
ระหว่างพอลิแล็กติกแอซิดกับสารไกลซิดิลไตรเมทิลแอมโมเนียมคลอไรด์ (GTMAC) เพื่อให้ตัวพอลิ
เมอร์มีความชอบน้ำมากขึ้น หลังจากนั้นจึงนำมาผสมกับพอลิแล็กติกแอซิดทางการค้าโดยแปรปริมาณที่
ใช้และน้ำหนักโมเลกุลของพอลิแล็กติกแอซิดที่มีประจุบวกแล้วนำสารละลายไปผลิตเป็นเส้นใยอิเล็กโท
รสปันเพื่อเพิ่มความชอบน้ำให้กับแผ่นเส้นใยอิเล็กโทรสปันของพอลิแล็กติกแอซิดให้มากขึ้น ขนาดเส้น
ผ่านศูนย์กลางของเส้นใยอิเล็กโทรสปันที่ได้จากสารละลายผสมระหว่างพอลิแล็กติกแอซิดทางการค้ากับ
พอลิแล็กติกแอซิดที่ปลายทั้งสองเป็นประจุบวกนั้นพบว่ามีขนาดลดลงจาก 462 nm เป็น 208 nm คิดเป็น
55% เมื่อเพิ่มปริมาณที่ใช้จาก 10 wt% เป็น 40 wt% อีกทั้งเส้นใยยังมีขนาดลดลงจาก 650 nm เป็น 462
nm คิดเป็น 28% เมื่อลดน้ำหนักโมเลกุลของพอลิแล็กติกแอซิดที่มีประจุบวกจาก 15 kDa เป็น 4 kDa
โดยค่าที่วัดได้จากเครื่องจุลทรรศอิเล็กตรอนแบบส่องกราด (SEM) นอกจากนี้แล้วขนาดเส้นใยจะลดลง
เมื่อเพิ่มปริมาณพอลิแล็กติกแอซิดที่มีประจุบวกแล้ว ความชอบน้ำของเส้นใยพอลิแล็กติกแอซิดที่วัดโดย
วิธี air-water contact angle measurement ยังเพิ่มขึ้นด้วยโดยดูจากค่าที่วัดได้ลดลงจาก 137° เป็น 123°
($p < 0.05$) อย่างมีนัยสำคัญเมื่อผสมพอลิแล็กติกแอซิดประจุบวก 10 wt% รวมไปถึงค่า contact angle
ลดลงด้วยเมื่อเพิ่มปริมาณพอลิแล็กติกแอซิดที่มีประจุบวก จากผลที่ได้สรุปได้ว่างานวิจัยนี้สามารถเตรียม
เส้นใยอิเล็กโทรสปันที่มีขนาดเส้นใยลดลงและมีความชอบน้ำเพิ่มมากขึ้นได้ ซึ่งเส้นใยนี้สามารถนำไปใช้
ในงานต่าง ๆ ที่ต้องใช้งานร่วมกับน้ำได้อย่างมีประสิทธิภาพ

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THANIN CHALERMBONGKOT: SYNTHESIS OF POSITIVELY CHARGED POLY(LACTIC ACID) FOR PREPARATION OF ELECTROSPUN FIBER. ADVISOR: ASST. PROF. VARAWUT TANGPASUTHADOL, Ph.D., CO-ADVISOR: ASST. PROF. WORAWAN BHANTHUMNAVIN, Ph.D., 54 pp.

Poly(L-lactic acid) (PLLA), a biodegradable and biocompatible polyester, has been widely investigated as a very promising replacement for packaging and biomaterials in many applications. The high hydrophobic character of PLLA posed a challenge in developing a biodegradable polymer having 'positive' charges in its structure. In this work, electrospun fiber mats fabricated from commercial-grade PLLA doped with low molecular weight PLLA having two positively charged end groups (PLLAdi+) were prepared with the aim to develop a nano-fiber mat that possessed positive charges in the fibers. PLLAdi+ with molecular weight ranged from 4 to 15 kDa were synthesized by incorporating glycidyl trimethylammonium chloride (GTMAC) into the PLLA chain ends. Up to 55% reduction (from 462 to 208 nm) of fiber diameter was achieved when increasing the PLLAdi+ (4 kDa) content mixed in the commercial PLLA fiber from 10 to 40 wt%. In addition, the fiber diameter also decreased from 650 to 462 nm (28% reduction) when decreasing the molecular weight of PLLAdi+ used from 15 to 4 kDa, as determined by SEM. Besides the size reduction of the fiber when added PLLAdi+, the hydrophilicity of PLLA fiber mat as determined by air-water contact angle was decreased significantly from 137° to 123° ($p < 0.05$) when 10 wt% PLLAdi+ was added compared to the non-doped mat. The contact angle was also decreased when the PLLAdi+ content increased. The thin and hydrophilic PLLA fibers were successfully prepared and could potentially be used in applications related to aqueous environment.

Department:	Chemistry	Student's Signature	
Field of Study:	Chemistry	Advisor's Signature	
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LIST OF ABBREVIATIONS

26G	needle tip gauge size 0.45 mm (No.26)
$CDCl_3$	deuterated chloroform
Da	dalton
DC	direct current
DCM	dichloromethane
DI	deionized
DMSO	dimethyl sulfoxide
DS	degree of substitution
equiv.	equivalent
EtOH	ethanol
GPC	gel permeation chromatography
GTMAC	glycidyl trimethylammonium chloride
LA	L-lactic acid
MeOH	methanol
M_n	number-average molecular weight
M_w	weight-average molecular weight
NMR	nuclear magnetic resonance spectroscopy
PDI	polydispersity index, dispersity index
PLLA	poly(L-lactic acid)
PLLAdi+	positively charged PLLA synthesized from PLLAdiCOOH
PLLAdiCOOH	dicarboxyl chain end PLLA
*p*TSA	*para*-toluene sulfonic acid
SA	succinic acid
SEM	scanning electron microscope
$SnCl_2.2H_2O$	tin (II) chloride dihydrate
THF	tetrahydrofuran

CHAPTER I

INTRODUCTION

1.1. Statement of problem

In recent years, poly(L-lactic acid) (PLLA) has received much attention due to its biodegradable and biocompatible properties, which provide important economic benefits. PLLA is a biopolymer and renewable polyester, which has been widely used in several applications such as packaging materials, biomedical materials, and fibers. However, PLLA is highly hydrophobic which provide less efficiency when used in biomedical and biomaterial field that related to aqueous media. Therefore, PLLA attached with positively charged parts is one way to enhance the hydrophilicity of PLLA and derive their benefits particularly such as bactericidal properties as well.

Electrospinning is a key versatile method to produce the non-woven nanofibers providing high surface area. Using electrospinning technique, smaller size and high surface area fibers were electrospun, which are applied to various applications, for example, scaffold, water filter, and wound dressing bandage.

The PLLA nanofibers were used in several applications as mentioned above. Nevertheless, hydrophobicity of the PLLA was a drawback for many works. In order to provide more hydrophilicity, the PLLAdi+ was performed as PLLA dopant to enhance its hydrophilicity. Therefore, the PLLA attached with positive charges were electrospun to nanofibers which will be provided more hydrophilic and be suitable for used as scaffolds in tissue engineering field, for example.

Consequently, in this work, the PLLA was positively charged by reacting both polymer chain ends with quaternary ammonium containing molecule, GTMAC, via ring opening reaction of its epoxide ring. Subsequently, the PLLAdi+ was mixed with PLLA and electrospun into the non-woven nanofibers. Polymer concentration, amounts of PLLAdi+ dopant, and molecular weights of PLLAdi+ were varied to assess the effect on electrospinning and morphology of the fibers.

1.2. Objective

1) Synthesize and characterize the Mw-tunable PLLA containing positively-charged end or PLLAdi+.

2) Prepare the PLLA/PLLAdi+ nanofiber using electrospinning technique

3) Investigate the effect of parameters on morphology and hydrophilicity of electrospun fiber.

CHAPTER II

LITERATURE REVIEW

2.1. L-Lactic acid, poly(L-lactic acid) and synthesis

2.1.1. L-Lactic acid

L-Lactic acid is a featured monomer of the promising polyester poly(L-lactic acid). This monomer is commercially produced by the enzymes in the anaerobic fermentation pathway of some bacteria and molds, which sugar, starch, or molasses were converted into lactic acid molecules. This made the lactic acid, their derivatives, and their polymers reproducibly from renewable natural resources, and also environmental friendly. The lactic acid molecule has a hydroxyl and carboxyl functional group, which probably result in intermolecular and intramolecular esterification reactions [1]. For the intermolecular reaction, the condensation reaction could proceed to yield higher oligomers or polymers which promoted by water removal. On the other hand, for intramolecular esterification, the cyclic dimer, lactide, is formed.

Moreover, the lactic acid is the simplest α-hydroxy acid with a chiral carbon atom that exhibit in two enantiomeric forms, as L- or D- form isomers with different characteristics. Besides, the carboxyl and hydroxyl groups of lactic acid participate in a broad variety of chemical reactions [2].
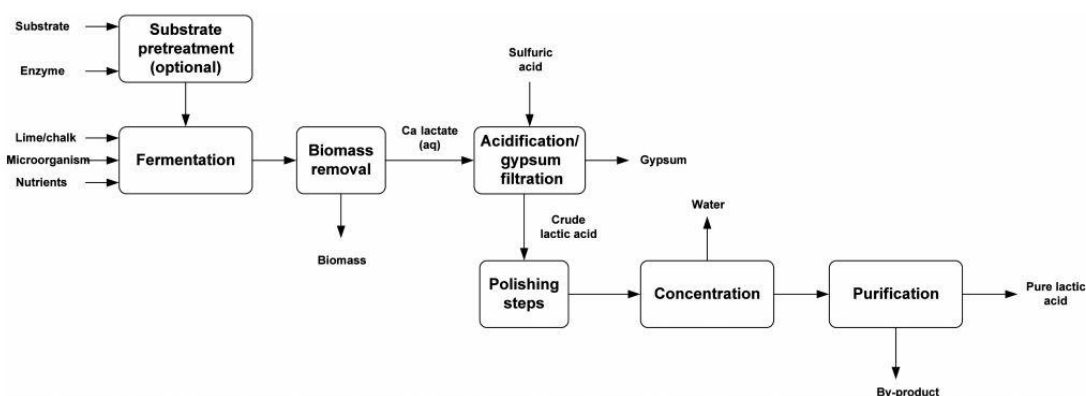
Figure 2.1 Simplified scheme of traditional lactic acid production process [1]

2.1.2. Poly(L-lactic acid) and synthesis

Poly(L-lactic acid) (PLLA) is a featured polyester that can be reproduced by the renewable resources available in the country. PLLA has received much attention due to its biodegradable character via the ester hydrolysis and biocompatible properties, which can be recognized as promising biomaterial replacement and engineering material used in several applications such as packaging, fiber, and biomaterials [3-5].

PLLA can be synthesized by condensation polymerization directly from its building block lactic acid (**Figure 2.2**), which is derived from fermentation of sugars from carbohydrate sources such as corn, sugarcane, or tapioca [1]. On the other hand, ring opening polymerization of the lactide monomer, lactic cyclic dimer, took place to provide higher molecular weight of PLLA and less impurities compared to the condensation. However, the condensation yielded the low molecular weight of PLLA but this method is cheap and can be further improved by using difunctional chain extender to get higher molecular weight of PLLA [6]. Due to two enantiomers of lactic acid subunit, the PLLA were observed in three stereoisomers as syndiotactic (heterotactic), isotactic, and atactic (see **Figure 2.3**) that are different in chemical structure and properties.
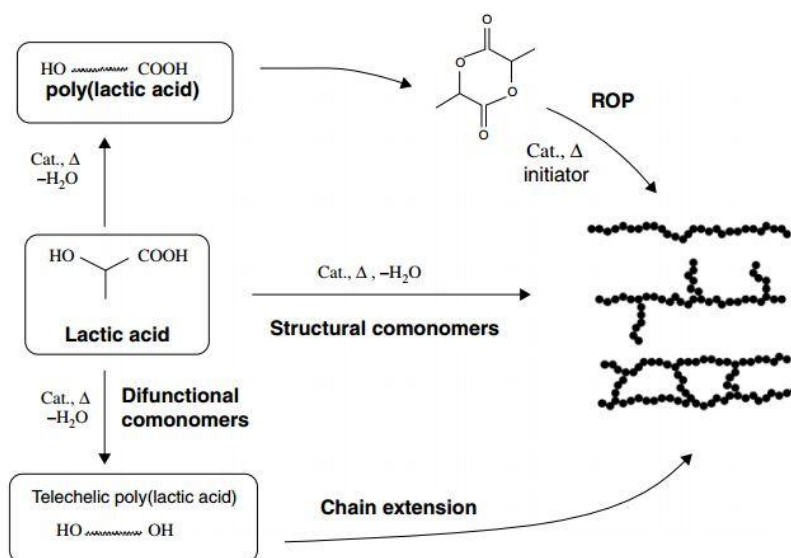
Figure 2.2 Manufacturing routes for lactic acid based polymers [6]
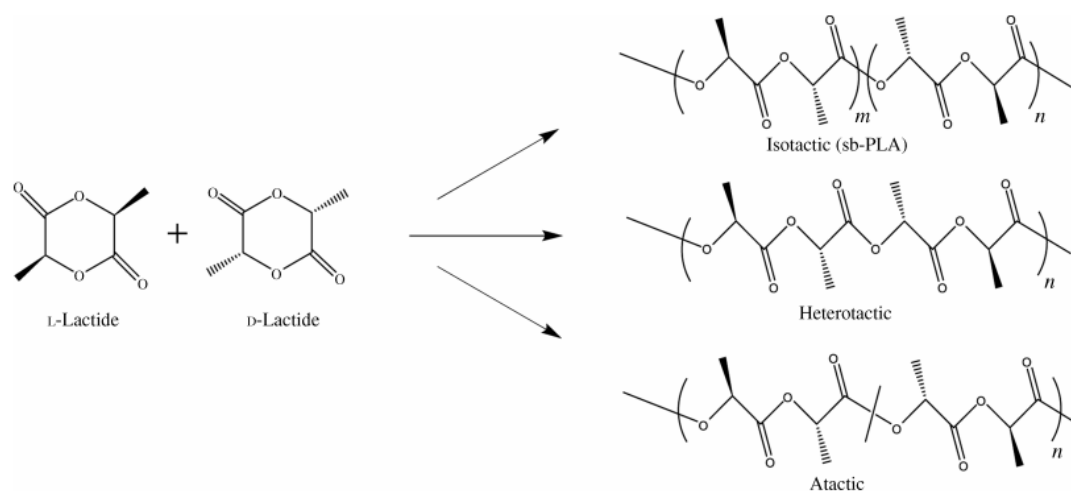
Figure 2.3 Stereoisomers of poly(lactic acid) synthesized from the L-, D-lactide [7]

PLLA has been recognized as green polymer due to its promising properties such as biodegradability, biocompatibility, renewability, and non-petroleum based polymer. Nevertheless, the properties of PLLA such as thermal stability and impact resistance are inferior to those of conventional polymers used for thermoplastic applications [8]. Especially, the high hydrophobicity of the PLLA provides less efficiency when used in biomedical and biomaterial field that related to aqueous media.

In order to improve the hydrophobicity of PLLA and increase its potential applications, copolymers, blended polymers, and nanocomposites of lactic acid and other functional hydrophilic monomers have been developed considerably. In addition, using the positively charged molecules, cationic molecules or polymers would improve the hydrophilicity of the PLLA effectively.

Therefore, in this research, the method that introduces the positive charge molecules or polymers would be interesting to induce the more hydrophilic PLLA and derive their other useful benefits.

2.2. Electrospinning

Electrospinning technique has been widely investigated as a versatile method to produce nanoscale non-woven fiber mat that has high surface area to volume ratio, which the solidified fabricated mats can be produced directly from the variety of

polymer solutions [9]. The basic electrospinning setup consists of three major components, e.g. high DC voltage power supply, electrically conducting spinneret, and collector separated at a desired distance. In a typical process, positive electrode from the power supply is attached to the needle containing the spinning solution to charge the polymer solution and the other is attached to an opposite collector. While the voltage was applied, the surface of fluid droplet was electrostatically charged. The fluid droplet elongated into a conical liquid as known as the Taylor cone due to the electrostatic interactions [10], as shown in **Figure 2.4**. Once the intensity of the electric field reached critical value, the polymer solution droplet was ejected from the tip of Taylor cone into liquid jet when repulsive electrostatic force overcomes against the surface tension and finally deposited as a non-woven fabric mat on a grounded metal collector. During fibrous jet travels through the air, the liquid jet elongates, the solvent evaporates, leaving behind a nonwoven nanofibrous polymer mat on the collector [11].
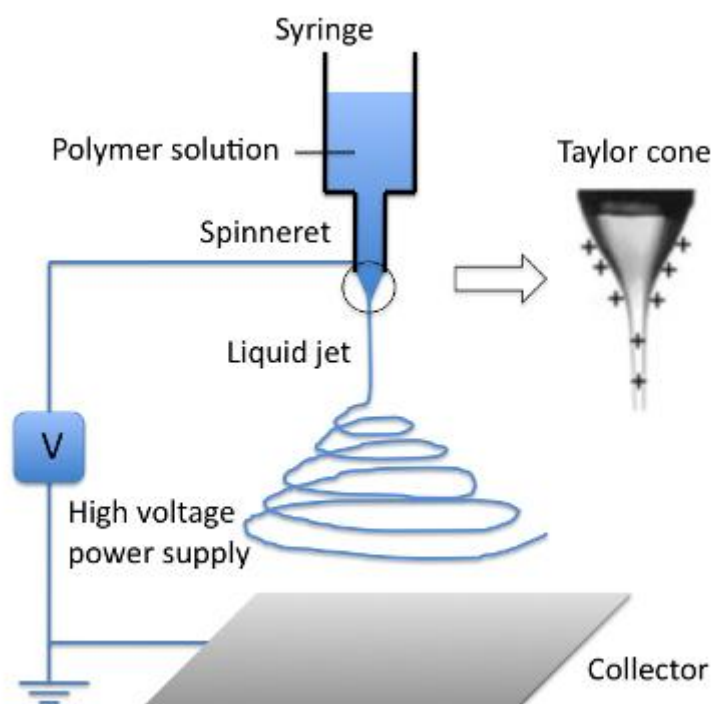
Figure 2.4 Schematic illustrate of electrospinning process and Taylor cone [12]

Among all factors, the fiber diameter and the nanostructured morphology depended on either intrinsic properties of the polymer solution such as viscosity,

surface tension, and conductivity, or by the processing parameters such as applied electric field strength, solution flow rate, and working distance [11].

Conductivity is one of the most important intrinsic parameters that affect the overall process of electrospinning and it can easily control the fiber morphology. This parameters can be increased effectively by adding the cationic molecules into the electrospinning solution or using high polarity solvent, which the electrospun fiber morphology would be enhanced to yield the reduced fiber diameter.

In recent years, there are some research publications which reported the electrospun PLLA usage for several applications, especially in tissue engineering. However, the hydrophobicity of the PLLA bring its electrospun nanofiber to be more hydrophobic that can be the obstacle for many applications. Therefore, adding some cationic molecules can help improve the overall conductivity that the fiber morphology could be improved.

2.2.1. The cationic molecules: the effect on fiber morphology
2.2.1.1 Inorganic and organic salts

In 2006, Qin et al. [13] studied the influence of different salts on the electrospun polyacrylonitrile (PAN). One weight percentage of inorganic salts (LiCl, $NaNO_3$, NaCl, and $CaCl_2$) were added into the PAN 4–10 wt% solution dissolved in DMF before electrospinning. It was found that the salts can improve the solution conductivity and reduced the electrospun fiber diameter that affect the fiber morphology.

In 2002, Zong et al. [14] investigated the structure and process influence on electrospun PLLA bioabsorbable nanofiber membranes. The polymer 20-40 wt% solutions of PDLA in DMF and PLLA in dichloromethane/DMF were added by 1 wt% inorganic salts NaCl, NaH_2PO_4, and KH_2PO_4 before electrospinning process. The results found that concentration and salt addition have relatively effects on the fiber diameter, higher charge density from salts favored the formation of uniform nanofibers, and salts addition dramatically changed the electropun PDLA morphology to form the uniform fibers and decrease their diameter.

2.2.1.2 Surfactant

In 2005, Tong Lin et al. [15] investigated the effect of polymer concentration and cationic surfactant on the morphology of the polyacrylonitrile (PAN) nanofibers. The PAN concentration 1-11 wt% were dissolved in DMF to produce the polymer solution. Traces of dodecyl trimethylammonium bromide (DTAB), a cationic surfactant, was then added into the PAN solution for electrospinning. From the results, the fiber morphology evolved from a beads on string structure to uniform fibers with increasing fiber diameters. Moreover, addition of cationic surfactant, the formation of beads was suppressed and the average diameter of the PAN nanofibers were reduced. For the another of his work in 2004 [16], the charge effect of cationic surfactants on the elimination of beads in electrospun polystyrene was investigated. The small amount of cationic surfactant efficiently eliminated the beads because of higher charge density and also improve the polymer solution conductivity.

In 2009, Kriegel et al. [17] studied the influence of three surfactant types (anionic, cationic, non-ionic) and concentration on chitosan-poly(ethyleneoxide) (CS-PEO) blend nanofibers. The 3:1 CS-PEO blend polymer solution was prepared in acetic acid and then added by the surfactants to determine its influences. From the results, addition of PEO and increasing surfactants concentration induced spinnability and yield larger fibers, which surfactants affected its morphology.

For more cationic amphiphiles, Lin et al [18] investigated the effective method to control fiber diameter and variability of the fiber prepared from poly(ethersulfone), poly(caprolactone), and poly(caprolactone-co-ethyl ethylene phosphoester) by doping polymer solutions with cationic amphiphiles, octadecyl rhodamine (R18) or octadecyltrimethylammonium bromide (OTAB). From the results, the fiber diameter reduction was observed when the amphiphiles used due to the combination effect of positive charges that increased the solution conductivity and surface tension reduction.

2.2.1.3 Non-ionic and ionic polymer

In 2005, Mincheva et al. [19] prepared the electrospun polyelectrolyte-containing nanofibers in the presence of a non-ionic water-soluble polymer. *N*-carboxyethylchitosan served as a major component along with polyelectrolytes

(PAMPS and P(AMPS-co-AA)) with addition of non-ionic polymer (polyacrylamide and polyvinylalcohol), which hypothesized that the non-ionic polymer would facilitate the nanofibers formation and suppressed the repulsion forces between ionogenic groups of polyelectrolyte that impede the fiber formation. The results showed that the formation of fiber was performed by hydrogen bond between the polyelectrolytes and non-ionic polymer, and also the fiber diameter was significantly decreased with increasing the polyelectrolyte content due to increasing in solution conductivity.

2.3. Hydrophilicity and morphology improvement in electrospun PLLA nanofibers

Nowadays, there are several research work on the hydrophilic improvement of PLLA nanofiber obtained from both lactic acid and lactide dimer.

In 2006, Bhattarai et al. [20] developed the hydrophilicity of electrospun nanofibrous polylactide and evaluated the fibroblast cell proliferation, morphology, and cell-matrix interactions. The hydrophilicity of electrospun PLLA fiber was improved by doping small amount of low molecular weight poly(ethylene glycol) (PEG) into the polymer solution before getting electrospun. Furthermore, it was found that the fiber diameter and the air-water contact angle were decreased with increasing the PEG blend ratio. The best polymer solution ratio is 80/20 PLLA/PEG that favored the activity of fibroblast cell.

According to the previous study of our lab, Nutjarin Pansomabat, a member of Tangpasuthadol group used GTMAC to react with PLLA. She reported in her Master's thesis that carboxyl (–COOH) chain end effectively reacted with GTMAC than the hydroxyl chain end did and the hydrophilicity of the polymer was improved as measured by the air-water contact angle.

As can be seen in previous researches, all cationic surfactants and molecules contained the positive charges from quaternary ammonium group $(+N(CH_3)_3)$ as same as the GTMAC molecules. Therefore, this work focused on the influence of positive charges derived from the GTMAC molecules to improve the fiber morphology and hydrophilicity of the electrospun nanofibrous PLLA mats.

CHAPTER III

EXPERIMENTAL

3.1. Materials

L-Lactic acid solution (LLA, 88 wt%) and granular calcium chloride ($CaCl_2$) was supplied from Carlo Erba Reagent, France. Tin (II) chloride dihydrate ($SnCl_2.2H_2O$), succinic acid (SA), triethylamine (Et_3N), *para*-toluene sulfonic acid (*p*TSA), and glycidyl trimethylammonium chloride (GTMAC) were obtained from Sigma-Aldrich, USA. Methanol (MeOH), ethanol (EtOH), sodium chloride (NaCl), and chloroform-d were purchased from Merck, Germany. Cetyl trimethylammonium bromide (CTAB) was purchased from Fluka, Switzerland. Chloroform, tetrahydrofuran (HPLC grade), and dimethyl sulfoxide (DMSO) were purchased from RCI Labscan Limited, Thailand. Commercial PLLA, M_w 103 kDa (Ingeo™ 4043D), was provided by NatureWorks LLC. All reagents were used as received without further purification or modification, except for GTMAC that was dehydrated by high vacuum pump before used to reduce its water content.

3.2. Instrumentations

3.2.1. Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR spectra of the synthesized polymer samples dissolved in $CDCl_3$ were performed on a Varian, Mercury-400 nuclear magnetic resonance spectrometer (USA) operated at 400 MHz and a Bruker Advance-III 300 operated at 300 MHz at room temperature. Proton chemical shifts were expressed in parts per million (ppm) relative to tetramethylsilane (TMS) or the residual deuterated solvent resonance as a reference.

3.2.2. Gel Permeation Chromatography

Molecular weight of the synthesized polymers were obtained by Waters 600 controller chromatograph equipped with three HR (Waters) columns (HR1 or HR2, and HR4) (MW resolving range = 100-500,000 Da) at internal column temperature

35 °C and a refractive index detector (Waters 2414). THF, HPLC grade, was used as a solvent for the polymers and as an eluent for GPC with a flow rate of 1.0 mL/min. Sample injection volume was 50 µL. The retention time was calculated for polymer molecular weight reported in Dalton relative to polystyrene standards (molecular weight of 7 polystyrene standards: 996 – 188,000 Da).

3.2.3. Ramé-Hart Contact Angle Goniometer

Hydrophilicity of the polymer fibers and films were determined by the static, advancing & receding air-water contact angle measurement using a Ramé-Hart Contact Angle Goniometer (USA), model 100-00 equipped with Gilmont syringe and 24-gauge blunt needle tip. A droplet of DI water was place on the sample surface by the syringe. Silhouette images of water droplet were then measured for the contact angle.

3.2.4. Scanning Electron Microscope

To assess the fiber size and diameter, Fiber mat topography and morphology were characterized using a JEOL JSM-6610LV field emission scanning electron microscope operated at 15 kV with magnification of 2,000, 5,000, and 9,000×. The samples were coated with thin layer of gold by an ion-sputtering device for 4 minutes before the analysis. The resulting fiber diameters were determined directly by SemAfore version 5.21 software using a scale bar given from the micrograph as calibration scale. The diameters were reported as mean ± standard deviation and averaged from 80 different measuring points from two SEM micrographs.

Nevertheless, the electrospun fibrous mats were not flattened before the analysis which caused the depth of field in the image that may affect the fiber size determination. However, this effect would be declined apparently by averaging the fiber from several measured areas.

3.2.5. Differential Scanning Calorimeter

Differential scanning calorimeter (DSC) was performed on DSC 204F1, NETZSCH (Germany). DSC thermograms were received by heating samples from room temperature to 240°C at heating rate of 10°C/min under a nitrogen atmosphere.

Two heating cycles were performed to discard the heating history appeared on the first cycle; thus, all DSC thermograms were expressed from the second heating cycle.

3.3. Methods

To improve and modify the hydrophilicity of PLLA, lactic acid was first polymerized to dicarboxyl ends PLLA (PLLAdiCOOH) using succinic acid (SA) as an initiator. Then GTMAC was finally introduced to the PLLAdiCOOH using ring opening reaction in order to obtain PLLAdi+ (see **Figure 3.3**).

3.3.1. Synthesis of PLLAdiCOOH
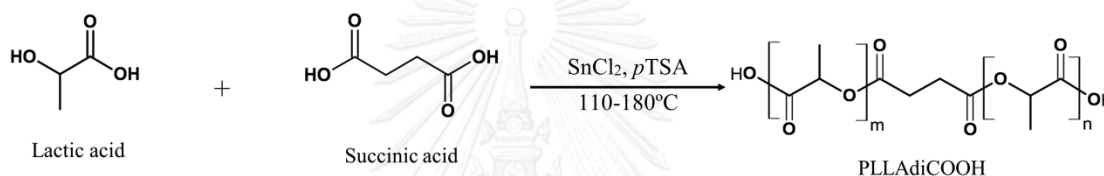
Figure 3.1 Synthesis scheme of PLLAdiCOOH

PLLAdiCOOH was polymerized via condensation by binary catalyst system of $SnCl_2$ and pTSA as catalyst and co-catalyst, respectively (**Figure 3.1**). The polymers were prepared using a revised synthesis method as described in previous work [21]. Firstly, 88 wt% L-lactic acid solution, various amount of succinic acid (0.05 to 4 mol% of L-lactic acid), and first half-portioned pTSA were weighed and put into 50 mL three-neck round bottom flask equipped with magnetic stirrer and distillation apparatus. The reaction was firstly carried out at 110ºC with partial reduced pressure for 0 to 10 hours. This purpose of the 'dehydration' or 'oligomerization' step was to reduce water content and grow lactide oligomers. Heating was further increased to 140ºC and 160ºC for 1 and 2 hours, respectively, with step-wise reducing the pressure to remove water by-product from the reaction. The polymerization was further carried out at 180ºC for 4 to 16 hours in high vacuum while $SnCl_2.2H_2O$ (0.4 wt % of L-lactic acid) and the remaining half-portioned pTSA (overall pTSA is 1 equiv. mole of $SnCl_2.2H_2O$) were added into the flask. After the reaction was finished, the crude polymer was cooled down and dissolved in

dichloromethane before precipitating in cold ethanol for purification. The crude polymer was precipitated and filtrated twice, then fully dried in vacuum. Finally, the white powder of PLLAdiCOOH was collected and then characterized by nuclear magnetic resonance (NMR) using $CDCl_3$ as deuterated solvent and gel permeable chromatography (GPC) and finally calculated for their molecular weight and yield.

3.3.2. Synthesis of PLLAdi+

In order to improve the hydrophilicity of the PLLA, PLLAdiCOOH was introduced with positively charged quaternary ammonium group to form the PLLAdi+, which GTMAC was attached to the free carboxyl ends of PLLAdiCOOH via nucleophilic substitution reaction, S_N2 (**Figure 3.2**). PLLAdiCOOH was weighed into a three-neck round bottom flask with excess amount of GTMAC (3 equiv. mole of PLLAdiCOOH), 1 equivalent mole of triethylamine based on PLLAdiCOOH, and DMSO as solvent. The flask was mounted with magnetic stirrer and $CaCl_2$ tube to trap the moisture from the reaction. The substitution reaction was performed at 70ºC for 24 hours. The resulting polymer was precipitated in 2M NaCl solution and was centrifuged at 5,000 rpm for 5 minutes. The polymer was washed with DI water and then centrifuged repeatedly 3 – 6 times in order to purify the polymer and remove the unreacted GTMAC before freeze-dried. Finally, the white powder of PLLAdi+ was collected and then characterized by NMR.
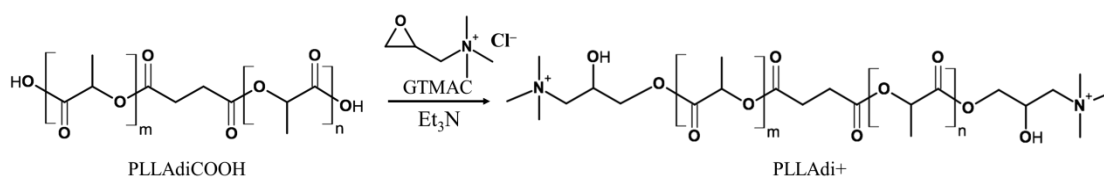
Figure 3.2 Synthesis scheme of PLLAdi+
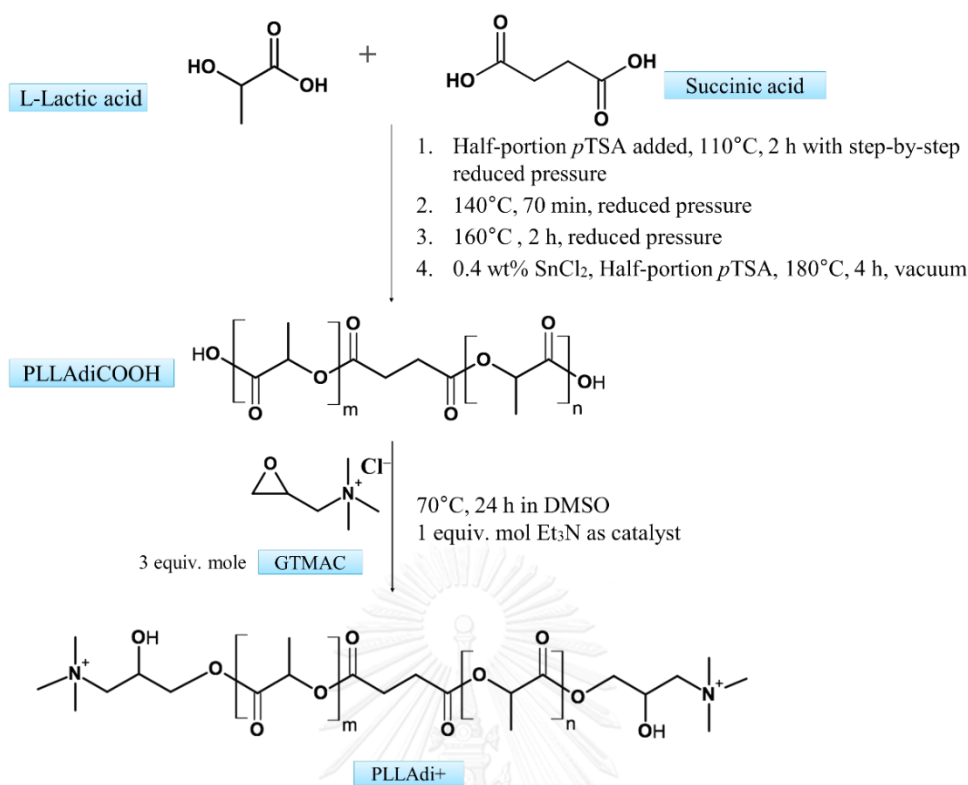
Figure 3.3 Overall synthesis procedure of PLLAdi+

3.3.3. Electrospinning

To produce a PLLA nanofibers mat, the electrospinning technique was performed properly with suitable required parameters. Commercial PLLA served as the major component of the polymer blends due to its high molecular weight and consequent high viscosity to afford the chain entanglements and the ability to be electrospun efficiently. The PLLAdi+, were used as the PLLA dopants for the electrospinning. All polymers were thoroughly dissolved in chloroform/methanol mixture (3:1) under stirring for 90 minutes before loading into a 5 mL plastic syringe equipped tightly with disposable 26G blunt needle tip (0.45 mm diameter). The syringe was immediately placed into the syringe pump and the solution was electrospun horizontally at flow rate 1 mL/h (Kd scientific syringe pump), 20 kV voltage applied from a DC high voltage power supply (Gamma High Voltage Research DES30PN/M692), and tip-to-collector distance was 20 cm. As shown in **Figure 3.4**, the electrospun fiber jet was collected onto a 10 cm x 10 cm aluminum foil as a ground collector. Electrospun mats were carefully left to dry at ambient

temperature overnight and further dried via high vacuum for 4 hours to remove the residual solvent completely before investigation.
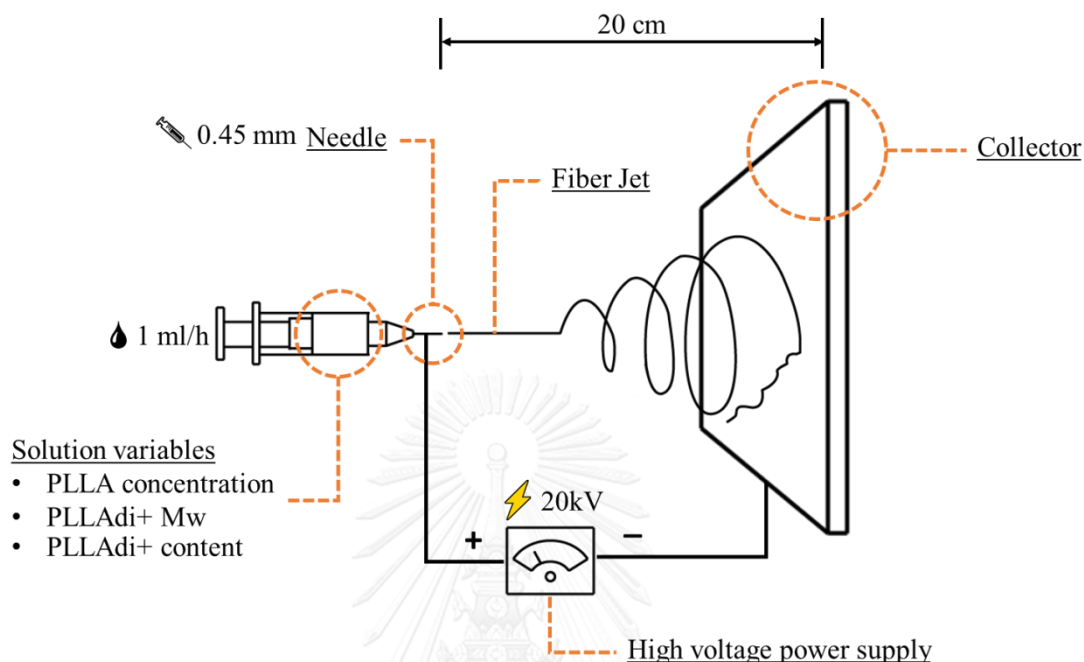
Figure 3.4 Schematic artwork of electrospinning set-up and parameters

3.3.4. Characterization of synthesized polymers

The synthesized PLLAdiCOOH and PLLAdi+ were characterized by NMR and GPC. ^{1}H NMR integrations of the methylene protons of succinic acid and PLLA methine protons were compared to determine the repeating unit and molecular weight as well.

3.3.5. Film casting

The polymer film was cast for use as reference specimens for air-water contact angle measurement in order to determine the hydrophilicity of the PLLA/PLLAdi+ blends. The polymers were dissolved in chloroform and then allowed to dry at room temperature for 2-3 days in a 4 cm Petridish glass plate.

3.3.6. Air-water contact angle measurement

In order to determine the wettability and hydrophilicity of prepared PLLA electrospun mats and films, static and dynamic contact angle were measured using Ramé-hart™ standard goniometer. The water droplets were carefully controlled to be equal in each measuring. The reported contact angle is an average of 5 measuring points on different surface area.

3.3.7. Statistical analysis

All data obtained from the air-water contact angle measurement were presented as mean ± standard deviation. The IBM® SPSS® statistics version 22 software was carried out in this statistical analyzes to provide the descriptive statistics and determine the mean differences. Comparison of mean differences were performed by one-way analysis of variance (one-way ANOVA) with the Fisher's Least Significant Difference (LSD) and Duncan's method that used for post hoc analysis. A calculated value of p less than 0.05 ($p < 0.05$) was considered to be statistically significant.

```
┌─────────────────┐        ┌─────────────────┐
│  L-Lactic acid  │────────│  Succinic acid  │
└─────────────────┘        └─────────────────┘
                   │
                   │  pTSA
                   │  SnCl₂.2H₂O
                   │  110°C-180°C
                   │  Vacuum
                   ▼
         ┌──────────────────┐
         │  Polymerization  │
         └──────────────────┘
                   │
                   ▼
         ┌────────────────────┐
         │ Crude PLLAdiCOOH   │
         └────────────────────┘
                   │
                   ▼
      ┌──────────────────────────────┐
      │ Purification by reprecipitation │
      └──────────────────────────────┘
                   │
                   │  CH₂Cl₂
                   │  Chilled EtOH
                   │  Vacuum dried
                   ▼
┌─────────┐   ┌──────────────┐   ┌──────────────────┐   ┌──────┐
│ GTMAC   │───│  PLLAdiCOOH  │───│ Characterization │───│ NMR  │
└─────────┘   └──────────────┘   └──────────────────┘   └──────┘
         │                                     │
         │  Et₃N                               └───────│ GPC  │
         │  70°C                                       └──────┘
         │  24 h
         ▼
 ┌──────────────────┐
 │  Crude PLLAdi+   │
 └──────────────────┘
         │
         ▼
 ┌──────────────┐
 │ Purification │
 └──────────────┘
         │
         │  2 M NaCl
         │  DI water
         ▼
 ┌──────────────┐   ┌──────────────────┐   ┌──────┐
 │  PLLAdi+     │───│ Characterization │───│ NMR  │
 └──────────────┘   └──────────────────┘   └──────┘
```
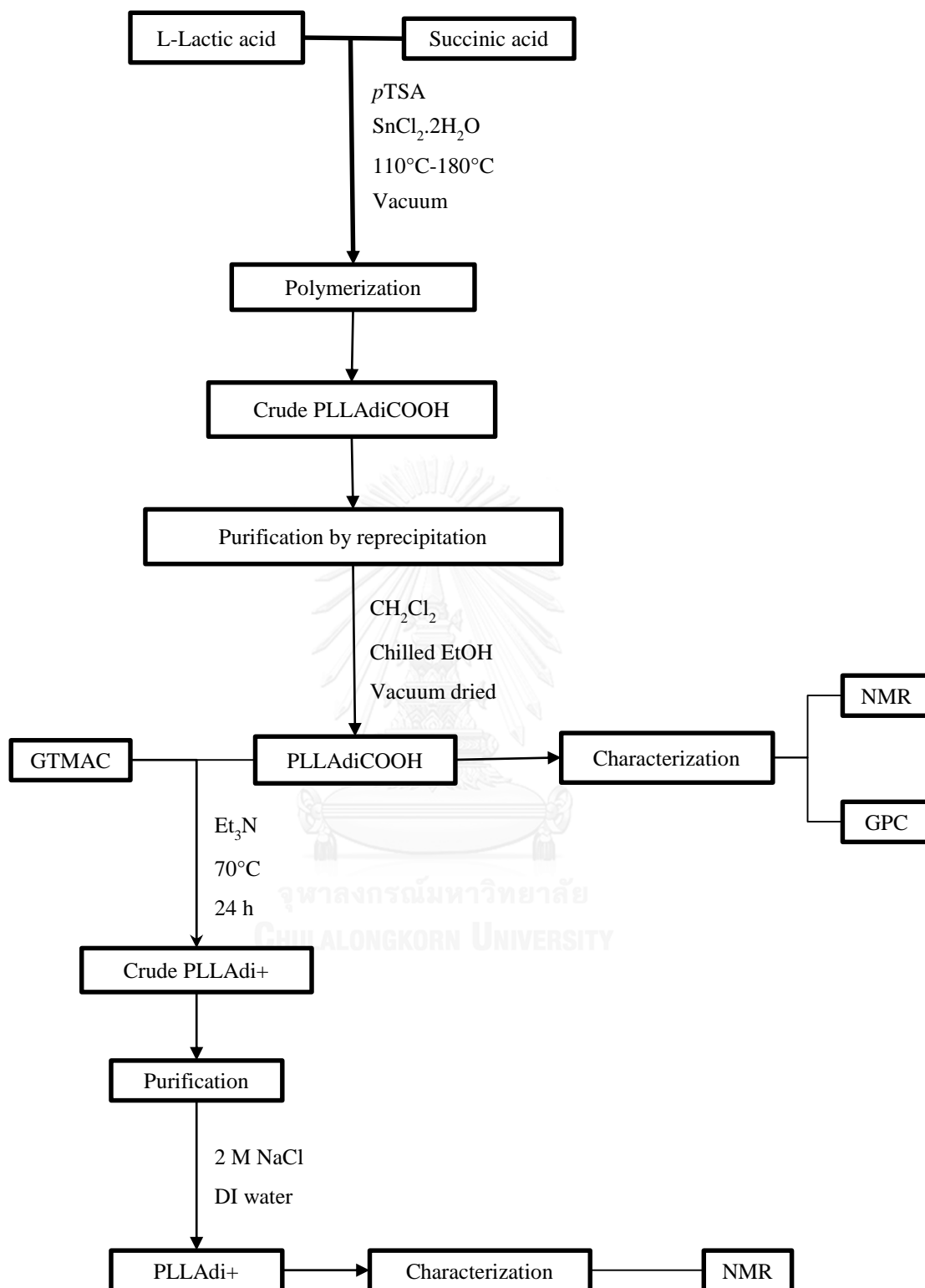
Figure 3.5 Experimental flow chart
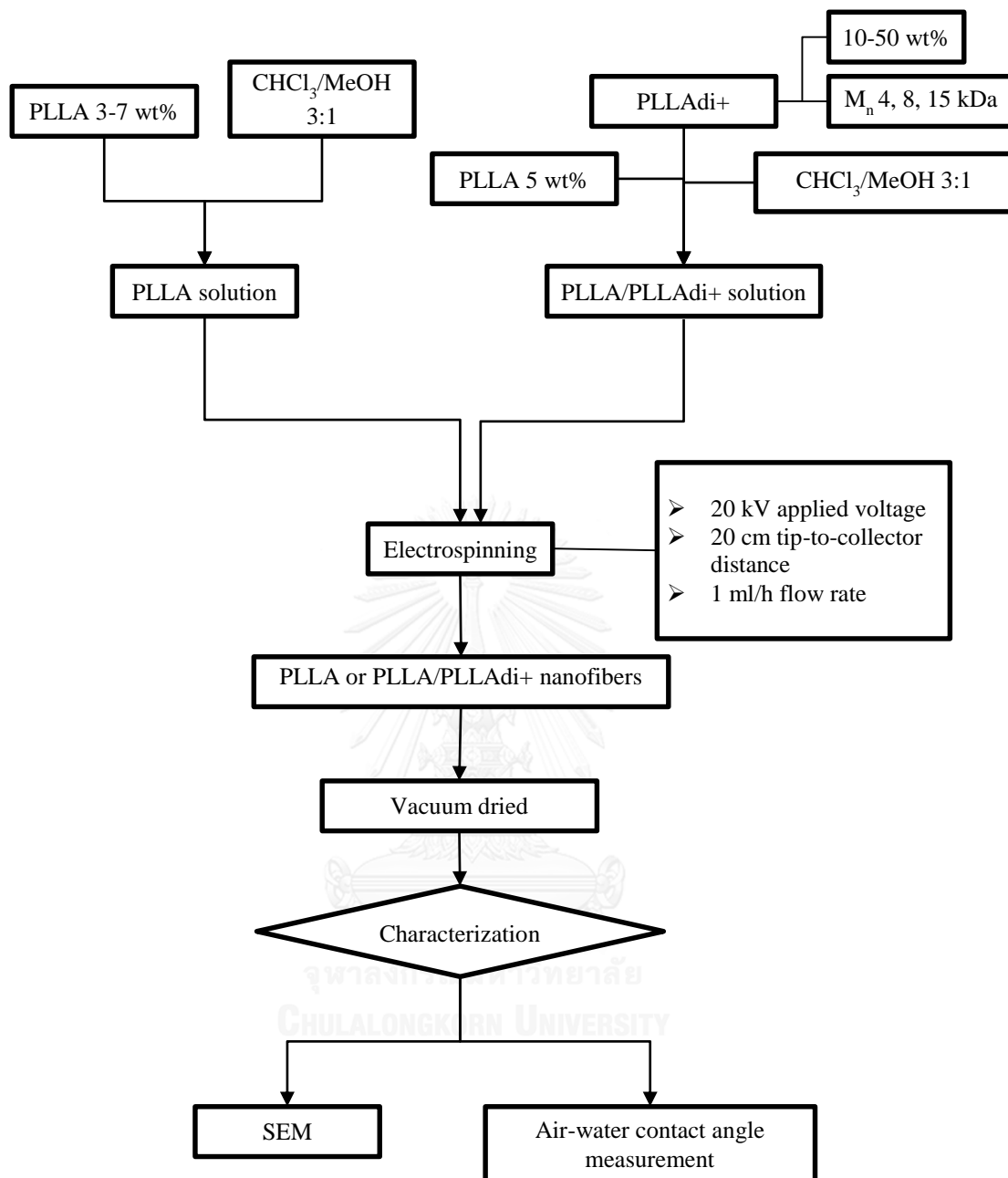
Figure 3.6 Experimental flow chart (continued)

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1. Synthesis and characterization

4.1.1. Synthesis and characterization of PLLAdiCOOH

As shown in **Figure 3.1**, a small amount of a dicarboxylic acid molecule, succinic acid (SA), was added into the reaction as the initiating molecule for growing PLLA chain, resulting in PLLA with two carboxylic ends or PLLAdiCOOH. The ester bonds formed on both sides of SA molecules by reacting with the hydroxyl group of lactic acid. On the other hand, the lactic acid molecules could be esterified to PLLA chain and acted as initiating polymer chain for growing PLLAdiCOOH chain via hydroxyl group that would be reacted with carboxyl group of succinic acid. Yield percentage of dried PLLAdiCOOH products were calculated based on weight of dried polymer to theoretical weight ratio as shown in Eq. 1 below.

(Eq. 1)

$$\text{yield}(\%) = \frac{\text{weight of dried PLLAdiCOOH powder}}{(\text{mole of succinic acid} \times 84.09\,\text{g}) + (\text{mole of L}-\text{lactic acid} \times 72.05\,\text{g})} \times 100$$

The dicarboxyl ends PLLAs, PLLAdiCOOHs, were synthesized in good yield from lactic acid monomer and succinic acid at 110ºC to 180ºC using tin-catalyzed polycondensation (**Figure 3.1**). Dehydration time, mol% of succinic acid added into reaction, and polymerization time were adjusted to produce PLLAdiCOOHs with varying molecular weight as confirmed by ^{1}H NMR and GPC analysis.

Comparison of peak integrations between the methylene proton (-CH$_2$-) of succinic acid and methyne (-CH-) proton of the lactic unit in PLLA backbone were used to calculate the molecular weight as shown in **Figure 4.1**. The proton signals at 2.8 ppm were assigned to methylene protons (c) of succinic acid in PLLAdiCOOH backbone. The methyne (d) and methyl proton signals of lactic unit in the polymer chain appeared at δ 5.2 and 1.5 ppm, respectively. Nevertheless, a small amount of undesired normal PLLA was also observed as low intensity peak of methyne proton

signal in the hydroxyl end unit appeared at δ 4.3 ppm (peak *b*). This normal PLLA occurred when low content (< 1 mol%) of SA was used in the polycondensation. This reveals that the low amount of SA provides less opportunity to react with lactic acid molecules, letting the lactic acid to react with each other producing the normal PLLA chains. For its molecular weight, the number-average molecular weight ($\overline{M}_n$) of PLLAdiCOOH can be calculated based on the relative intensities of proton signals as described below (see Eq. 2).

(Eq. 2)

$$\overline{M}_n \text{ of PLLAdiCOOH} = (\frac{72.05\,\text{g mol}^{-1} \text{ of lactic unit} \times {}^dH \times ({}^cH/4)}{{}^bH + ({}^cH/4)}) + M_{PC}$$

where ${}^bH$, ${}^cH$, ${}^dH$ are intensity of methyne proton of normal PLLA chain, methylene proton of succinic acid, and methyne proton of lactic unit in PLLAdiCOOH backbone, respectively. M_{PC} equals 262 g/mole which referred to the total sum of molecular weight of succinic unit (84 g/mole) in the polymer backbone and lactic unit at both chain ends (178 g/mole) that contained the carboxyl group.
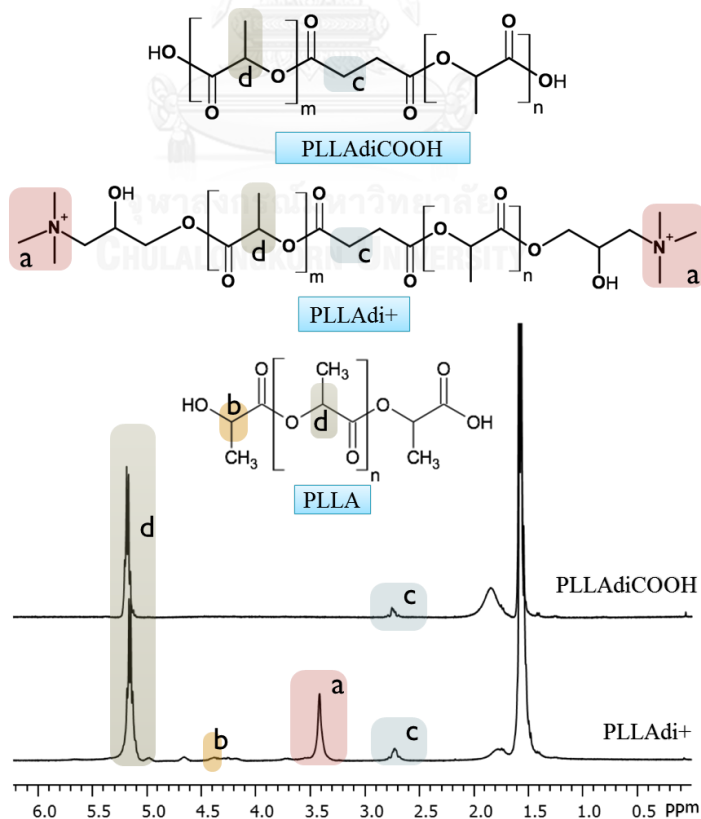
Figure 4.1 ^{1}H NMR spectra of PLLAdiCOOH and PLLAdi+ in CDCl$_3$

In the first step of overall reaction, the dehydration time at 110ºC was varied from 0 to 10 h as can be seen in **Table 4.1**. While the dehydration time increases, yield and molecular weight of each sample was not significantly different. This indicates that water removal in the dehydration step (at 110℃) was insufficient to increase the amount of product for this reaction equilibrium.

Table 4.1 Effect of dehydration time on PLLAdiCOOH molecular weight

Entry	Dehydration time (h)	Polymerization time (h)	mol% SA	Yield (%)	$\overline{M}_n$ NMR	$\overline{M}_n$ GPC	PDI
PLLAdiCOOH-01	0	4	2	60	5,522	7,076	1.29
PLLAdiCOOH-02	2	4	2	64	5,017	6,526	1.31
PLLAdiCOOH-03	5	4	2	70	4,657	7,791	1.31
PLLAdiCOOH-04	10	4	2	73	5,306	6,988	1.32

For polymerization step at 180ºC, polymerization time was varied from 4–16 h (**Table 4.2**) to produce polymer with molecular weight > 10 kDa. Increasing the polymerization time increases the polymer molecular weight as expected due to the polymers have more time to bond with other polymer chains.

Table 4.2 Effect of polymerization time on PLLAdiCOOH molecular weight

Entry	Dehydration time (h)	Polymerization time (h)	mol% SA	Yield (%)	$\overline{M}_n$ NMR	$\overline{M}_n$ GPC	PDI
PLLAdiCOOH-05	10	4	0.5	82	25,336	10,738	1.50
PLLAdiCOOH-06	10	8	0.5	78	14,240	15,318	1.53
PLLAdiCOOH-07	10	12	0.5	80	27,281	14,342	1.52
PLLAdiCOOH-08	10	16	0.5	78	20,148	15,402	1.62

Mole percentage of succinic acid (mol% SA) was varied from 0.05 to 4% (**Table 4.3**) as hypothesized that the lower SA added gave PLLAdiCOOH with higher M_w since less SA added, the more lactic chain length attached with the succinic acid

initiator. From the results, the polymer molecular weight increased as the mol% SA decreased as expected. Unfortunately, in PLLAdiCOOH-09, molecular weight from NMR could not be determined because the weak intensity of methylene peak was lower than interference signals in the spectrum (see Appendix).

Table 4.3 Effect of mole percentage of succinic acid on PLLAdiCOOH molecular weight

Entry	Dehydration time (h)	Polymerization time (h)	mol% SA	Yield (%)	$\bar{M}_n$ NMR	$\bar{M}_n$ GPC	PDI
PLLAdiCOOH-09	2	4	0.05	79	N/A	10,253	1.62
PLLAdiCOOH-10	2	4	0.5	71	15,814	8,352	1.54
PLLAdiCOOH-11	2	4	1	78	10,854	8,432	1.40
PLLAdiCOOH-12	2	4	2	64	5,017	6,526	1.31
PLLAdiCOOH-13	2	4	4	52	3,865	3,995	1.26

After dealing with many synthesis tasks (**Table 4.4**), three synthesized dicarboxyl ends PLLA, PLLAdiCOOH–13, –01, and –06, were selected to further produce positively charged PLLA (PLLAdi+), due to their molecular weight range and their consistent in molecular weight results from both NMR and GPC analysis. Therefore, the molecular weight of PLLAdiCOOH could be tuned or controlled as you desired when used parameters such as dehydration time, polymerization time, and mol% SA from this condition table.

Table 4.4 Selected PLLAdiCOOHs for synthesis of PLLAdi+s

Entry	Dehydration time (h)	Polymerization time (h)	mol% SA	Yield (%)	$\bar{M}_n$ NMR	$\bar{M}_n$ GPC	PDI
PLLAdiCOOH-13	2	4	4	52	3,865	3,995	1.26
PLLAdiCOOH-01	0	4	2	60	5,522	7,076	1.29
PLLAdiCOOH-06	10	8	0.5	78	14,240	15,318	1.53

4.1.2. Synthesis and characterization of PLLAdi+

The chain ends of PLLAdiCOOH were further modified to the positively charged ends to improve the overall hydrophilicity of the PLLA by reacting with quaternary ammonium containing molecule, GTMAC (**Figure 3.2**). After the products were purified and lyophilized, the polymers were weighed and collected for determining the percentage yield (Eq. 3) and molecular weight (Eq. 4) as shown below:

(Eq. 3)

$$\text{yield}(\%) = \frac{\text{weight of dried PLLAdi} + \text{powder}}{(\text{mole of succinic acid} \times 84.09\,\text{g}) + (\text{mole of L}-\text{lactic acid} \times 72.05\,\text{g})} \times 100$$

$$\overline{M}_n \text{ of PLLAdi+} = (\frac{72.05\,\text{g mol}^{-1}\text{of lactic unit} \times {}^d\text{H}}{{}^c\text{H}/4}) + M_{PP} \qquad \text{(Eq. 4)}$$

where ^{c}H, ^{d}H are integral of methylene proton of succinic acid and methyne proton of lactic unit in PLLAdiCOOH backbone, respectively (see **Figure 4.2**). M_{PP} equals 565 g/mole which referred to the total sum of molecular weight of succinic unit (84 g/mole) in the polymer backbone, lactic unit at both polymer ends (144 g/mole) that attacked the epoxide ring of GTMAC, and molecular weight of GTMAC attached to the PLLAdiCOOH chain (337 g/mole).

After substitution of GTMAC at both polymer chain ends, the methyl proton signal (*a*, **Figure 4.2**) of quaternary ammonium group in GTMAC appeared at δ 3.4 ppm as shown in **Figure 4.1**. Low intensity proton signal surprisingly showed at 4.4 ppm which related to the methyne proton signal of the hydroxyl ends in normal PLLA. This unexpected finding suggested that the ester bonds in the polymer backbone were partially hydrolyzed probably due to traces of remaining water in GTMAC, although attempts to completely dehydrate the reagent were carried out in high vacuum.
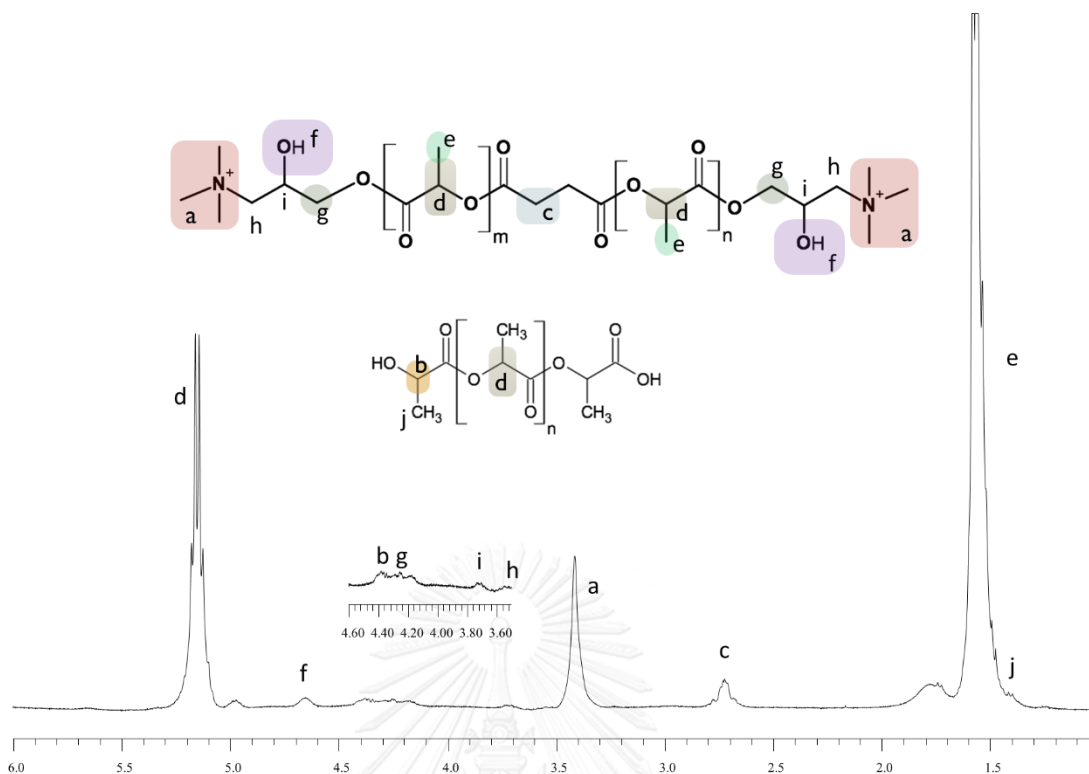
Figure 4.2 ^{1}H NMR spectrum of PLLAdi+ in CDCl$_3$

PLLAdi+ with molecular weight of 4, 8, and 15 kDa, namely P1, P2, and P3, respectively. Thus, there are 3 PLLAdi+ species that difference in polymer chain length to be used in electrospinning step. Subsequently, degree of substitution (DS%) of GTMAC on PLLAdiCOOH was calculated using the proportion of the GTMAC amount on the polymer chain to total amount of chain ends, which could be derived from the intensity of the methyl proton signal of GTMAC and methylene proton signal of SA as shown in the Eq. 5 below:

$$DS\% = \frac{^aH/9}{2(^bH)+2(^cH/4)} \qquad \text{(Eq. 5)}$$

where a is methyl proton signal of quaternary ammonium group, b is methyne proton signal of normal PLLA, c is methylene proton signal of succinic acid.

The DS of PLLAdi+s were 99% (P1), 56% (P2), and 64% (P3). P1 has highest DS since P1 has the shortest polymer chain; it therefore has the highest numbers of carboxyl chain ends, creating more opportunity for the carboxyl group to react with GTMAC.

Table 4.5 Three molecular weights of PLLAdi+ and their degree of substitution

PLLAdi+ Abbrev.	PLLAdiCOOH entry	DS%	Yield (%)	M_n-NMR (Da)
P1	PLLAdiCOOH–13	99	90	4,550
P2	PLLAdiCOOH–1	56	86	6,955
P3	PLLAdiCOOH–6	64	83	16,802

For PLLAdi+ synthesis, both carboxyl group of PLLAdiCOOH react with epoxide ring of GTMAC to generate the PLLAdi+ using triethylamine as basic catalyst. The reaction was proceeded via substitution reaction (S_N2) which epoxide ring of GTMAC was cleaved by carboxylate ion ($-COO^-$) of PLLAdiCOOH.

As can be seen in **Figure 4.3**, the mechanism shows that basic catalyst, triethylamine (Et_3N), deprotonates both carboxyl end groups of PLLAdiCOOH to carboxylate ions ($-COO^-$) in order to raise the nucleophilicity of the polymer chain end groups due to the negative charge density of carboxylate ion. The carboxylate ions then attacked the less hindered carbon of GTMAC epoxide ring and the oxygen of epoxide ring will be finally protonated. Finally, PLLAdi+ that had two quaternary ammonium ends was obtained. Triethylamine can act as base to abstract the carboxyl protons; however, its steric structure would not let the side reaction proceeds easily.
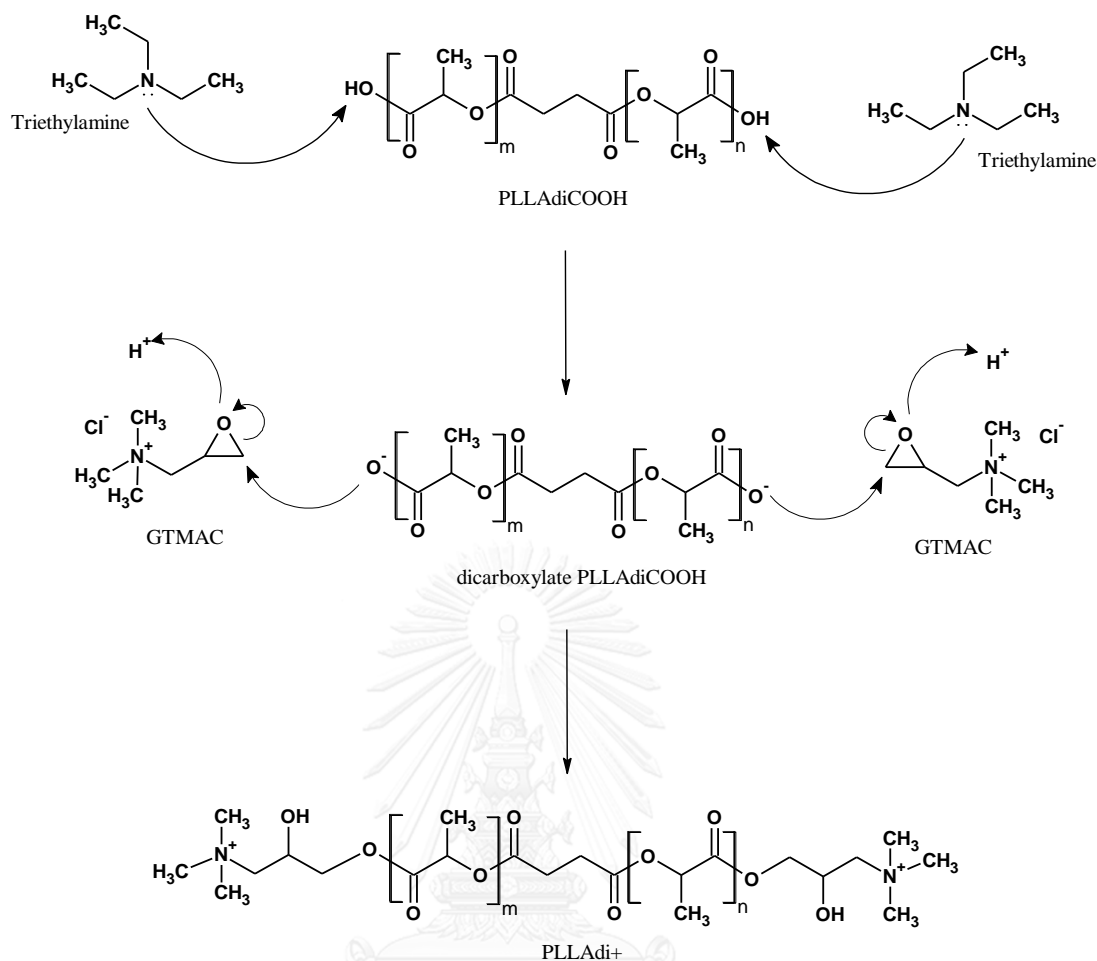
Figure 4.3 Mechanism of base-catalyzed substitution reaction in PLLAdi+ synthesis

4.1.3. DSC analysis

Thermal properties of three selected PLLAdiCOOHs (-13, -1, and -6) and synthesized PLLAdi+s (P1 – P3) were determined using DSC technique as shown in the **Figure 4.4** and **Figure 4.5**, respectively. For the first thermograms, PLLAdiCOOH-6 showed the highest T_g and T_m (ca. 50°C and 150°C respectively) due to its high crystallinity caused by interactions between high molecular weight polymer chain while PLLAdiCOOH-13 expressed the lowest T_g and T_m because of it had low crystallinity resulted from their low molecular weight. Similarly, the thermograms in the **Figure 4.5** presented the T_g and T_m of synthesized PLLAdi+s (P1 – P3). The highest molecular weight PLLAdi+, P3, showed the high crystallinity while the other showed no crystalline structure and had lower T_g noticeably. However, the T_g and T_m of PLLAdi+s were quite low compared to the corresponding precursor PLLAdiCOOHs because of its low crystallinity.

Figure 4.4 DSC thermograms of synthesized PLLAdiCOOHs at various molecular weight
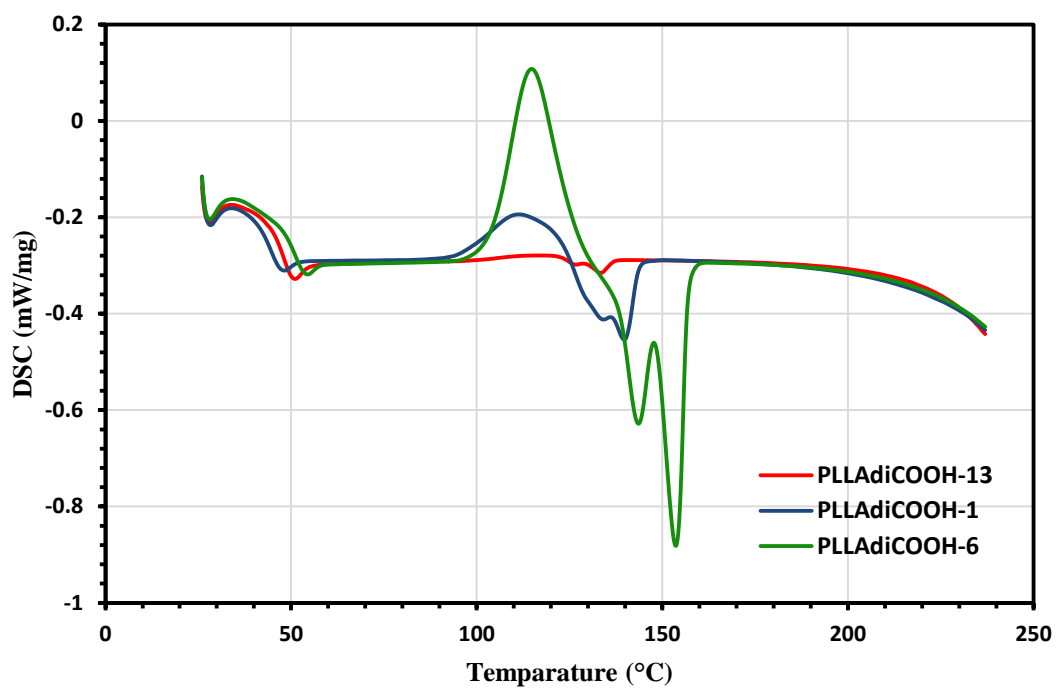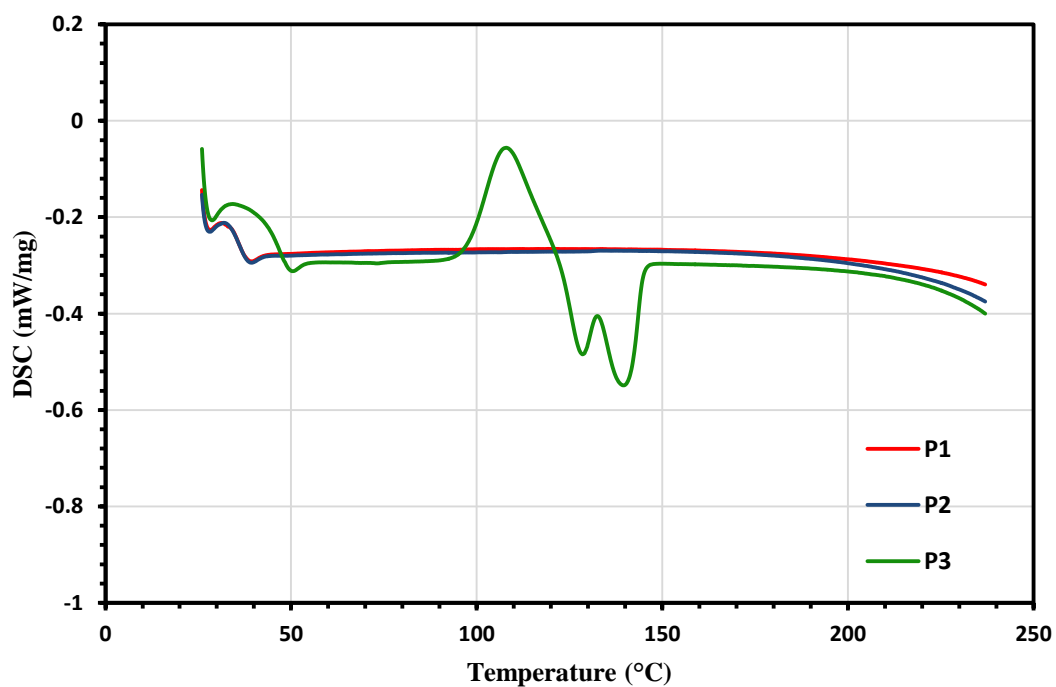

Figure 4.5 DSC thermograms of synthesized PLLAdi+s at various molecular weight.

4.2. Electrospinning

Many kinds of polymer solutions were fabricated into the fiber mats via electrospinning technique as a novel tool for producing nanofibrous scaffolds for various promising applications. As you known, the fiber morphology of nanofibers can be influenced both by the intrinsic properties of polymer solutions such as conductivity and viscosity, and by processing parameters such as applied voltage, spinning distance, and flow rate [22-24]. In this work, all electrospinning works were performed with identical parameters; therefore, the fiber diameter and morphology of fibrous mats would be affected closely by the intrinsic properties of the polymer solutions. Both PLLA solution and PLLA doped with PLLAdi+ solution were electrospun to produce the desirable hydrophilic electrospun PLLA fibers. For the best result, the required parameters must be optimized fully and controlled to enhance the fibrous mat morphology as well as their properties.

In the solvent selection, chloroform was chosen to dissolve PLLA and PLLAdi+ because both polymers can be dissolved in completely and it would be evaporated easily. Methanol is usually used as PLLA precipitation reagent; however, in this case, it was used to enhance the conductivity of the polymer solution because its high polarity and can be evaporated easily than other high polar solvents [9, 25]. DMSO and DMF, which have higher polarity and dielectric constant than methanol, were also used in many works to improve the conductivity of the polymer solution but they were hard to be evaporated completely from the fiber mat. As shown in **Figure 4.6**, the fiber mat electrospun from chloroform/methanol 3:1 shows dramatically decreasing in fiber diameters from ca. 3 to 1 μm compared with electrospun fiber mat using chloroform as solvent. This result reveals that higher polarity of the solvent causes the conductivity of the polymer solution increased, which smaller fiber diameter would be obtained.
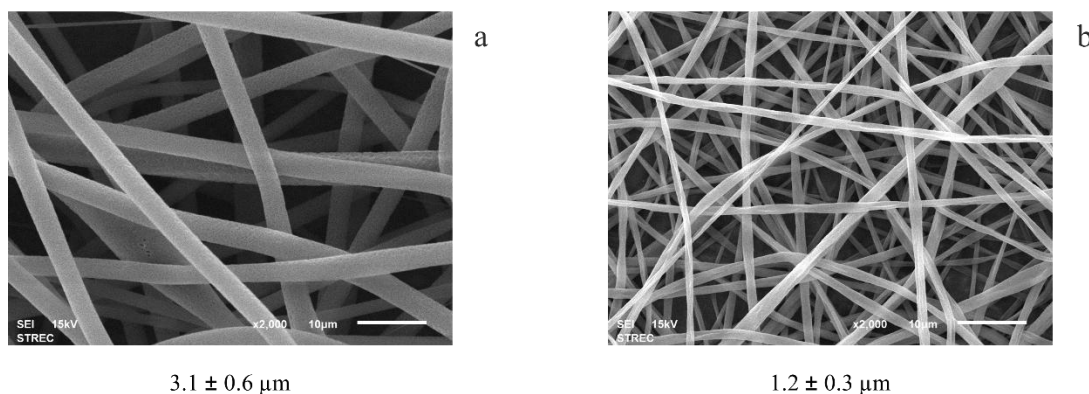
3.1 ± 0.6 µm   1.2 ± 0.3 µm

Figure 4.6 SEM images of commercial PLLA electrospun fiber mats with different
        solvents used at magnification 2,000×: (a) chloroform, (b)
        chloroform/methanol 3:1

The polymer concentration usually plays the promising role on the properties
of polymer solution which affect the electrospinning and fiber morphology as well.
The concentration of commercial PLLA solution was varied from 2% to 40 wt% in
order to obtain fibers without beads. It was found that the polymer concentration
higher than 20 wt% could not be electrospun to fiber mats. Due to its high viscosity,
the polymer solution stuck at the needle tip and some part did go through the needle to
form the large diameter fibers. On the other hand, the smaller fiber diameter was
obtained from the polymer solution at concentration below 10 wt%, which was
consistent with the previous work by Deitzel and coworkers [26, 27]. As a result, the
PLLA concentration from 3 – 7 wt% would be used in further electrospinning step
because this condition gave acceptable fiber diameters (≤ 1 µm). This parameter will
be further discussed below in section 4.2.1.

Moreover, PLLAdi+s were also tried to be electrospun directly as single
component in the 3:1 chloroform/MeOH solution. Unfortunately, small polymer
particles and beads were found along with the PLLAdi+ fibers (**Figure 4.7**; for
PLLAdi+, M_n = 15 kDa, sample *P3*). This result suggested that molecular weight of
the synthesized PLLAdi+s were insufficient to lead the entanglement between the
polymer chains. Consequently, the PLLAdi+ would be used as dopants in commercial
PLLA solution to afford the enhancement of hydrophilic PLLA in the fibrous mats.
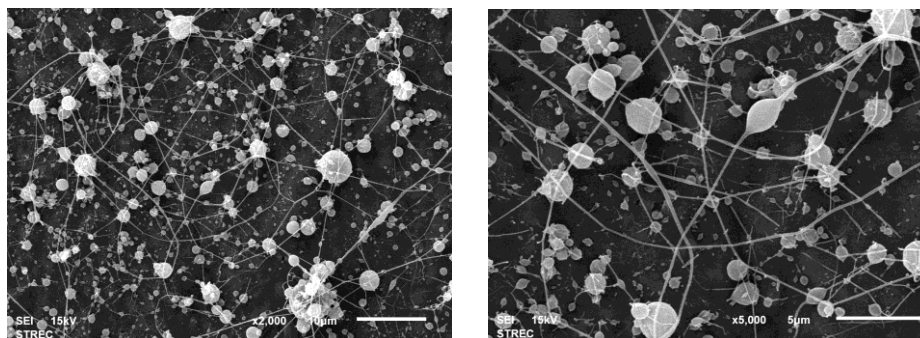
Figure 4.7 SEM micrograph of electrospun neat PLLAdi+ P3 (M_w 15 kDa) as PLLAdi+ representative obtained from 10 w/v% solution in chloroform/MeOH 3:1.

Consequently, the PLLAdi+s were added into polymer solutions, before loading into syringes, as dopants, to enhance the PLLA hydrophilicity. Electrospining samples were prepared as described in **Table 4.6** using chloroform/methanol 3:1 as solvent. Methanol was added to the polymer solutions in order to raise their conductivity for providing the smaller diameter fibers. Specifically, this work investigated how the PLLA concentration, molecular weight of dopant, and dopant concentration affected the fiber diameter and wettability of the fiber mats.

4.2.1. Effect of PLLA concentration on fiber morphology

At the beginning, this research assumed that decreasing the polymer concentration would decrease the fiber diameter to obtain the smaller fibers that improve the surface area and can be used efficiently in many applications. According to **Figure 4.8**, SEM images of electrospun fibers reveal that the fiber diameter decreased apparently as the polymer concentration was reduced, due to the low polymer content compared with others was induced to form the smaller fiber. This indicated that fiber diameter and morphology was influenced significantly by the polymer concentration, which was consistent with results obtained in previous studies [28]. As a result, PLLA concentration at 5 wt% (entry 2) was chosen for further testing because it gave acceptably small fiber diameter which no beads or any defects was found.

Table 4.6 Electrospinning solution condition

Entry	PLLA concentration (wt%)	PLLAdi+ dopant[a] (wt%)	PLLAdi+ species/types	Solvent
1	3			
2	5	0	–	
3	7			
4	5		*P1*	
5	5	10	*P2*	
6	5		*P3*	
7	5	20		CHCl$_3$:MeOH 3:1
8	5	30	*P1*	
9	5	40		
10	5	50		
11	3		*P1*	
12	3	10	*P2*	
13	3		*P3*	
14	7		*P1*	
15	7	10	*P2*	
16	7		*P3*	

[a] based on weight of commercial PLLA added to the solution

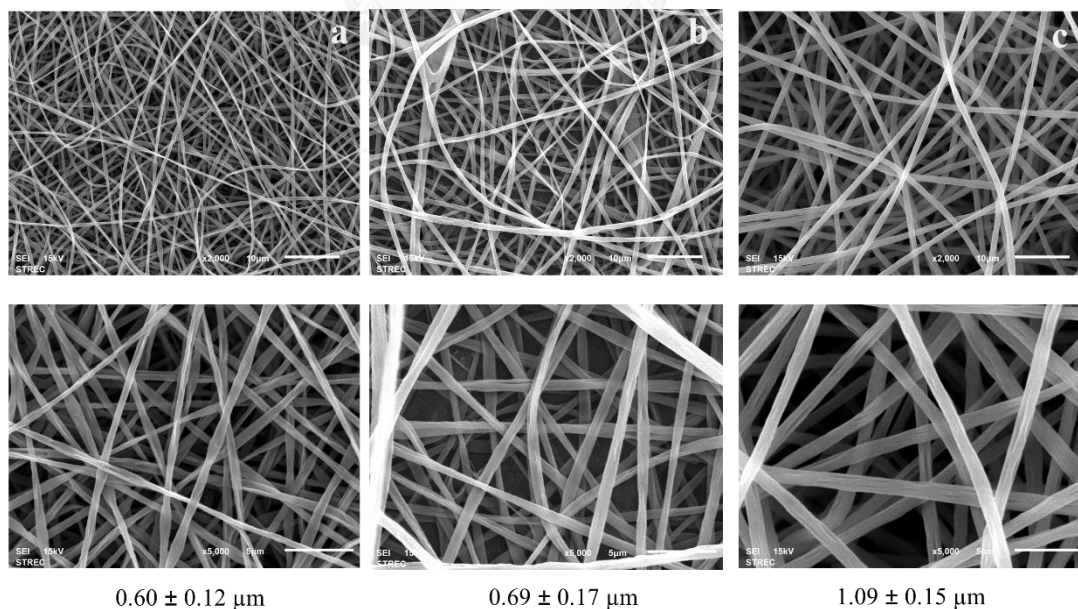| 0.60 ± 0.12 μm | 0.69 ± 0.17 μm | 1.09 ± 0.15 μm |

Figure 4.8 SEM images of PLLA fibers show the effect of concentration on the fiber diameters at magnification of 2,000× and 5,000×. (a) Entry 1, 3% PLLA; (b) entry 2, 5% PLLA; and (c) entry 3, 7% PLLA.

4.2.2. Effect of PLLAdi+ molecular weight on fiber morphology

Dealing with dopant species, the PLLAdi+ dopants selected for electrospinning with PLLA were the PLLAdi+ P1, P2, and P3, which were different in polymer chain length and consequent molecular weight. In particular, we supposed that electrospinning of PLLA doped with varying species of PLLAdi+s will alter the hydrophilicity of the material that afford fibers with more hydrophilic characteristics, and will reduce the fiber diameter as well. As shown in **Figure 4.9**, the PLLA/P1 mat shows the smallest fiber diameter whereas PLLA/P2 fiber showed the largest fiber diameter. This could be attributed to the fact that P1 was the positively-charged PLLA with the lowest M_w in this work. The amount of positive charges in P1 was therefore highest that induced the highest charge density compared to other PLLAdi+. The high charge density in PLLA/P1 would improve the conductivity of the polymer solution, resulting in smaller fiber diameter.

0.46 ± 0.11 μm

0.73 ± 0.18 μm

0.65 ± 0.15 μm
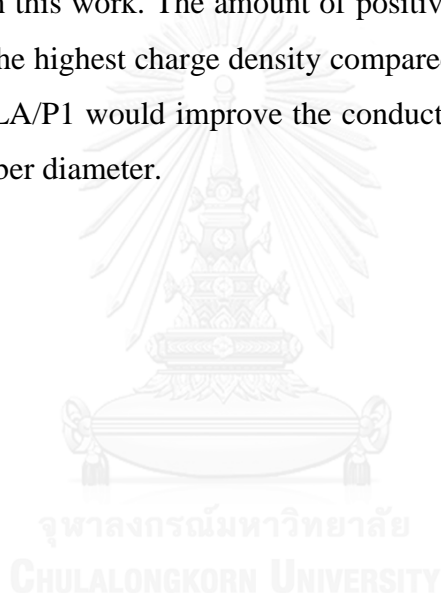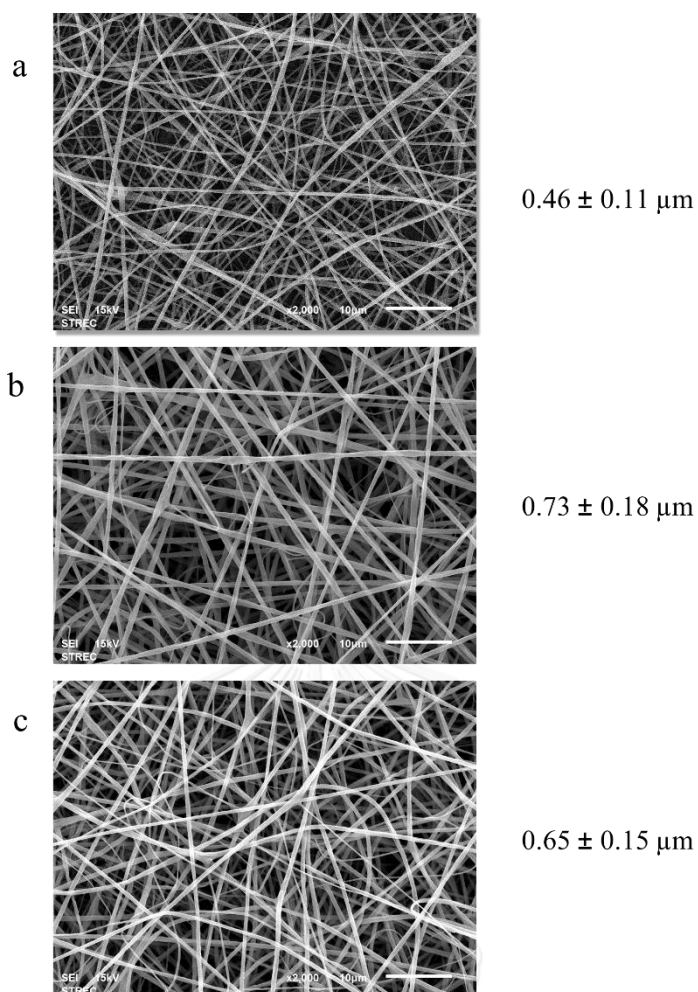
Figure 4.9 SEM images of PLLA/PLLAdi+ electrospun fiber–The influence of PLLAdi+ dopant species on fiber diameters at 2,000× magnification: (a) PLLAdi+ P1 (4 kDa), (b) PLLAdi+ P2 (8 kDa), (c) PLLAdi+ P3 (15 kDa).

4.2.3. Effect of PLLAdi+ contents on fiber morphology

For the PLLAdi+ dopants studies, it was hypothesized that increasing amount of dopants would reduce the apparent fiber diameter to provide the smaller size fibers and would improve PLLA hydrophilicity as well. The PLLAdi+ P1 (4 kDa) was selected and used as the dopant in this section, because its high positive charge density. The P1 doping content was altered from 0% to 50% and then doped in commercial PLLA. As illustrated by **Figure 4.10**, the fiber diameter was reduced while the percentage of dopant increased. These results indicate that increasing

amounts of PLLAdi+ decrease the fiber diameter because higher dopants contents induced the higher conductivity of the polymer solution, therefore, the solution jet was purged out at a faster rate to obtain small fiber diameter on the collector [14]. As shown in **Figure 4.11**, the graph was plotted as a function of the amount of PLLAdi+ dopants on the diameter of nanofibrous mats which showed decreasing curve of fiber diameter from ca. 700 nm to 200 nm when the percentage of dopants increase.

0.69 ± 0.17 μm   0.46 ± 0.11 μm   0.29 ± 0.08 μm

0.24 ± 0.07 μm   0.21 ± 0.05 μm   0.23 ± 0.06 μm
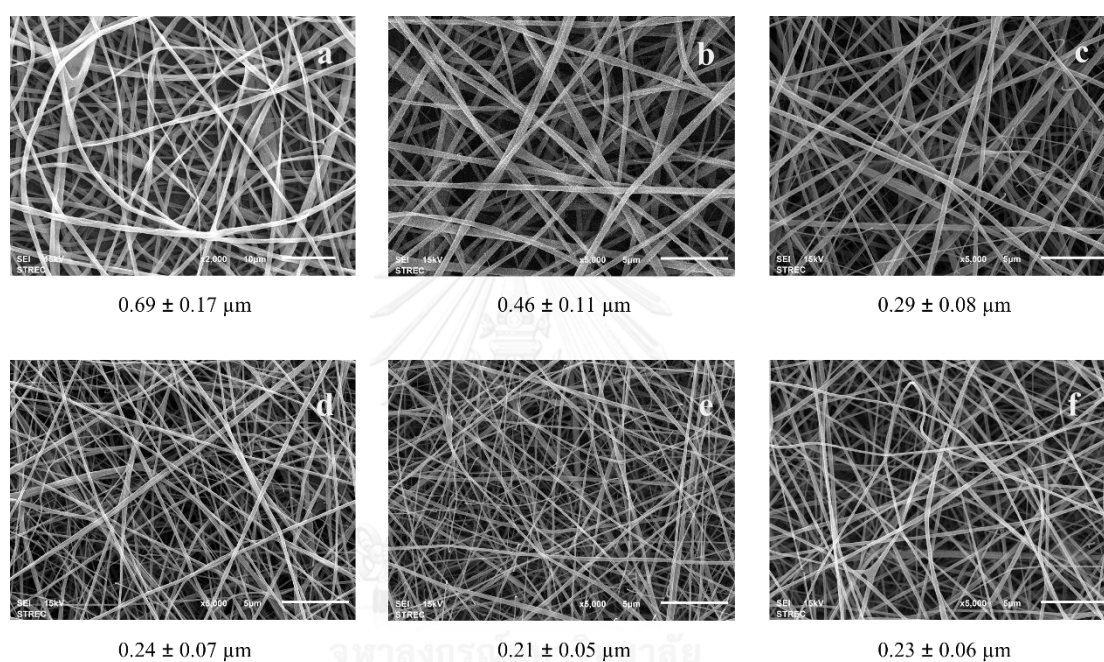
Figure 4.10 SEM images of PLLA/PLLAdi+ electrospun fibers which percentage of
dopants were varied from 0%(a), 10%(b), 20%(c), 30%(d), 40%(e) to
50%(f). The figure shows that the fiber diameter was reduced while the
%dopant increased. All figures were presented at 5,000× magnification.
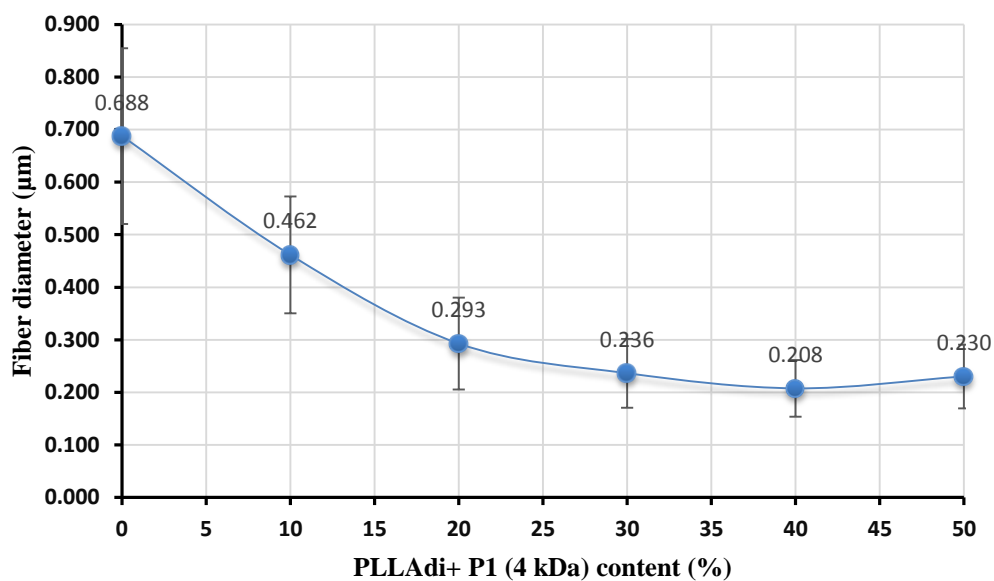
Figure 4.11 Average diameters (μm) of electrospun PLLA/PLLAdi+ fiber mats at various PLLAdi+ *P1* (4 kDa) contents.

Figure 4.12 Average diameters (μm) of electrospun PLLA/PLLAdi+ fiber mats at various PLLAdi+ *P2* (8 kDa) contents.

To support the results documented previously, PLLAdi+ *P2* which has molecular weight of 8 kDa was mixed with commercial PLLA and was further processed in the same condition as the PLLA/*P1* fiber did. As plotted in **Figure 4.12**, the fiber diameter was found to decrease from 688 nm to 206 nm with increasing the amount of PLLAdi+ dopant from 0 – 50%.
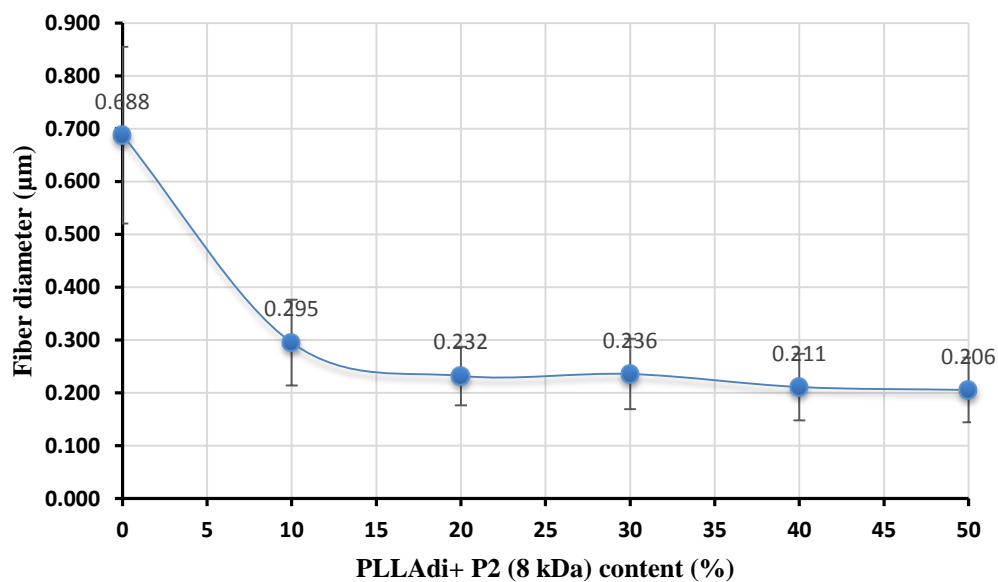
Likewise, the effects of PLLAdi+ dopants on the fiber diameter were further determined in the compared results of *P1* and *P2*, as illustrated in **Figure 4.13**. The graph showed that both *P1* and *P2* dopants can reduce the fiber diameter of the electrospun fiber significantly as can be seen that PLLAdi+ dopants show promising reduction in the diameter from 688 nm to ca. 300 – 500 nm when *P1* and *P2* contents as low as 10 wt% was used. The diameter decreased continuously when both dopants used were in 30 – 50 wt% but at a lesser extent.
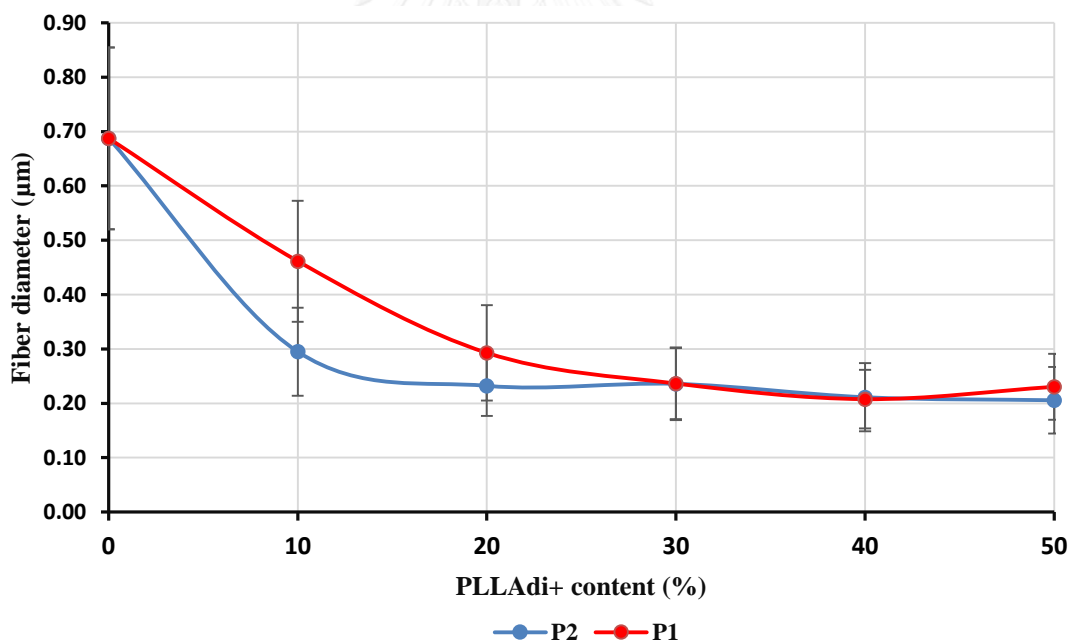

Figure 4.13 Average diameters (μm) of electrospun PLLA/PLLAdi+ fiber mats at various PLLAdi+ contents, which compare the diameters between P1 and P2.

4.2.4. Effect of PLLAdiCOOH on fiber morphology

A plasticizer is a low molecular weight material added into polymers to improve their flexibility. The plasticizer was inserted among the polymer chains and keeps them apart which reduces their interaction forces between the polymer chains which makes the material more flexible. The polymer doped with plasticizer would probably have reduced strength and stiffness but the material will be more useful where flexibility is required [29]. According to the definition above, the unreacted PLLAdiCOOHs that might be left in the PLLAdi+ material could also reduce the fiber size. Therefore, the plasticizer effect of PLLAdiCOOH in electrospinning of PLLA solution was determined as well (**Table 4.7**).

Table 4.7 Electrospinning solution condition of PLLA doped with PLLAdiCOOH

Entry	PLLA concentration (wt%)	PLLAdiCOOH dopant[a] (wt%)	PLLAdiCOOH (M_w)	Solvent
17	5		4 kDa	
18	5	10	8 kDa	$CHCl_3$:MeOH 3:1
19	5		15 kDa	

[a] based on weight of commercial PLLA added into the solution

According to **Figure 4.14**, the SEM images showed the fibrous electrospun nanofiber obtained from the polymer solution contained PLLAdiCOOH as dopant. Referring to the figure below, the fiber diameter were reduced comparing to the blank (no dopant added) since PLLAdiCOOH would penetrate among the PLLA chains. This effect resulted in dispersion of the polymer chain which viscosity of the polymer solution was consequently reduced and increase polymer chain mobility that bring the solution to be electrospun easily that afford the smaller fiber diameter.
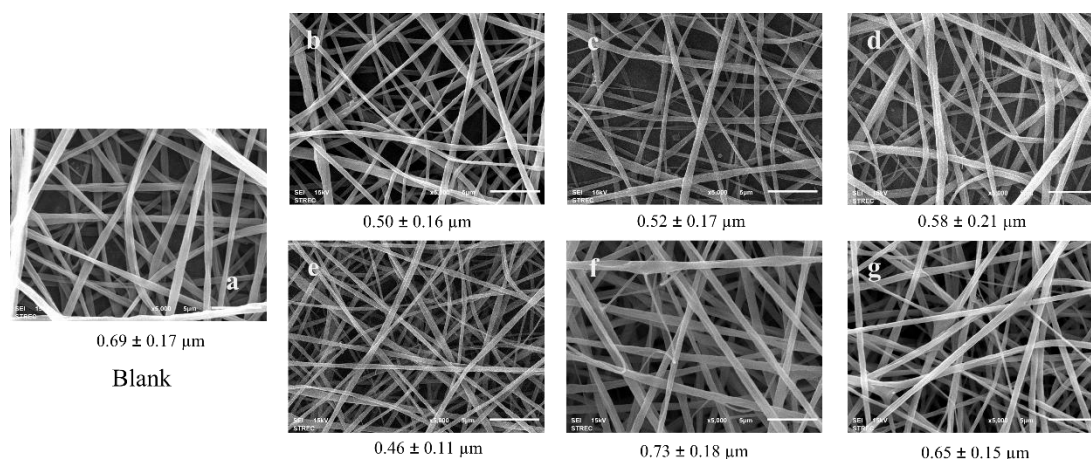
0.50 ± 0.16 μm 0.52 ± 0.17 μm 0.58 ± 0.21 μm

0.69 ± 0.17 μm
Blank

0.46 ± 0.11 μm 0.73 ± 0.18 μm 0.65 ± 0.15 μm

Figure 4.14 SEM micrographs and average fiber diameters (μm) of electrospun PLLA/PLLAdiCOOH fiber mats (b – d) at various PLLAdiCOOH molecular weight compare with PLLA (a) and PLLA/PLLAdi+ fiber mats (e – g).

Referring to **Figure 4.14**, the PLLA/PLLAdi+ electrospun fibers (e – g) were compared with fibrous mat obtained PLLA/PLLAdiCOOH (b – d). The similar results in decreasing the fiber diameter were obtained from the fibers that fabricated from PLLA solution doped with both PLLAdi+ and PLLAdiCOOH. For varying molecular weight of dopants, 4(*b*), 8(*c*), and 15(*d*) kDa; the effect of molecular weight of the PLLAdiCOOH dopants on the fiber morphology were not different significantly as shown in the figure above.

4.2.5. Effect of cationic surfactant on fiber morphology

As described above, the fiber morphology can be influence by intrinsic properties of polymer solution, especially in conductivity which related closely to cationic surfactant. The conductivity of polymer solutions could be increased by adding small amount of the surfactant, which lead to observe in smaller fiber diameter.

Cetyl trimethylammonium bromide, an amine based cationic quaternary surfactant, was widely used in antiseptic agent and synthesis of gold nanoparticles and silica nanoparticles as well; thus, it would be used to investigate the cationic surfactant effect comparing with PLLAdi+. According to the **Figure 4.15**, CTAB and

PLLAdi+ were similar partially in quaternary ammonium group that containing the nitrogenous positive charge; however, their molecular weight were totally different as compared 364 Da of CTAB and 4 kDa of P1.

To investigate the effect of the cationic surfactant, 10 wt% of CTAB was added into the commercial PLLA solution as dopant and then further electrospinning proceed using identical parameters similar to PLLAdi+ dopants condition.
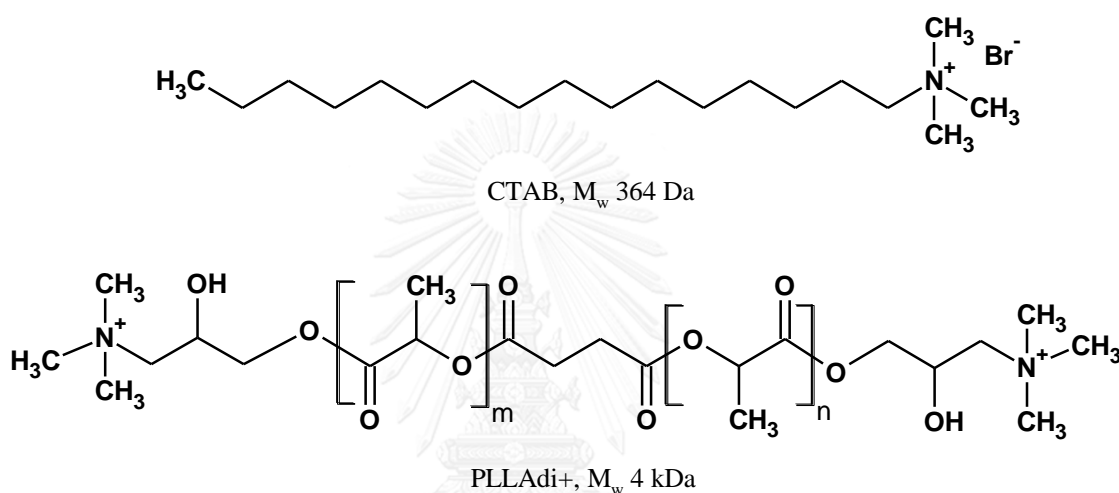
Figure 4.15 Cetyl trimethylammonium bromide (CTAB) and PLLAdi+ structure

From the results, the fiber diameter of PLLA/CTAB nanomats was 391 nm compare to the fiber diameter obtained from PLLAdi+ P1 at 462 nm as shown in **Figure 4.16**. There is no significant difference in morphology between the CTAB and P1 doped nanofibers. Thus, this result can be proved that PLLAdi+ has efficiency to reduce the fiber diameter as same as the cationic surfactant, CTAB.

Comparing to the blank, both conditions that dopants were used reduced the fiber diameter efficiently from 688 nm to about 400 nm. This result indicated that the conductivity of the polymer solution was raised by the positive charge from quaternary ammonium group of both CTAB and P1 to yield the smaller fibrous diameter.

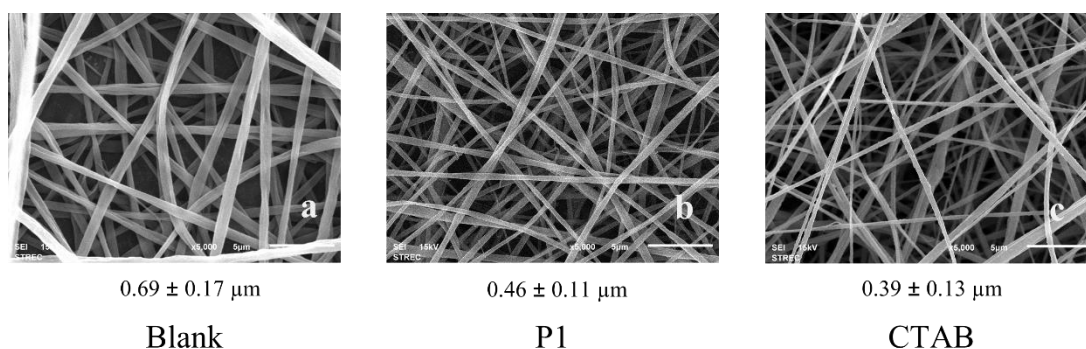| 0.69 ± 0.17 μm	0.46 ± 0.11 μm	0.39 ± 0.13 μm
Blank	P1	CTAB

Figure 4.16 SEM images of PLLA, PLLA/PLLAdi+, PLLA/PLLAdiCOOH electrospun fiber mats at 5,000 magnification.

Lastly, Additional electrospinning conditions (entry 11-16) were carried out to fabricate their nanofibers. The PLLA polymer concentration was in range of 3-7 wt%. The PLLAdi+ dopants used was *P1, P2*, and *P3* which were different in their molecular weights (**Figure 4.17**). According to far left micrograph of the second row in **Figure 4.17**, the electrospun material obtained from the 3 wt% PLLA doped with 10 wt% P1 (entry 11) could not be fabricated as fiber probably because the concentration of commercial PLLA was so low that the PLLAdi+ dopant size should be large (high in M_w) enough to hold the polymer in fiber form together by chain entanglement. Consequently, the lowest PLLA concentration with 10 wt% P1 that the fiber would be electrospun was 5 wt%.

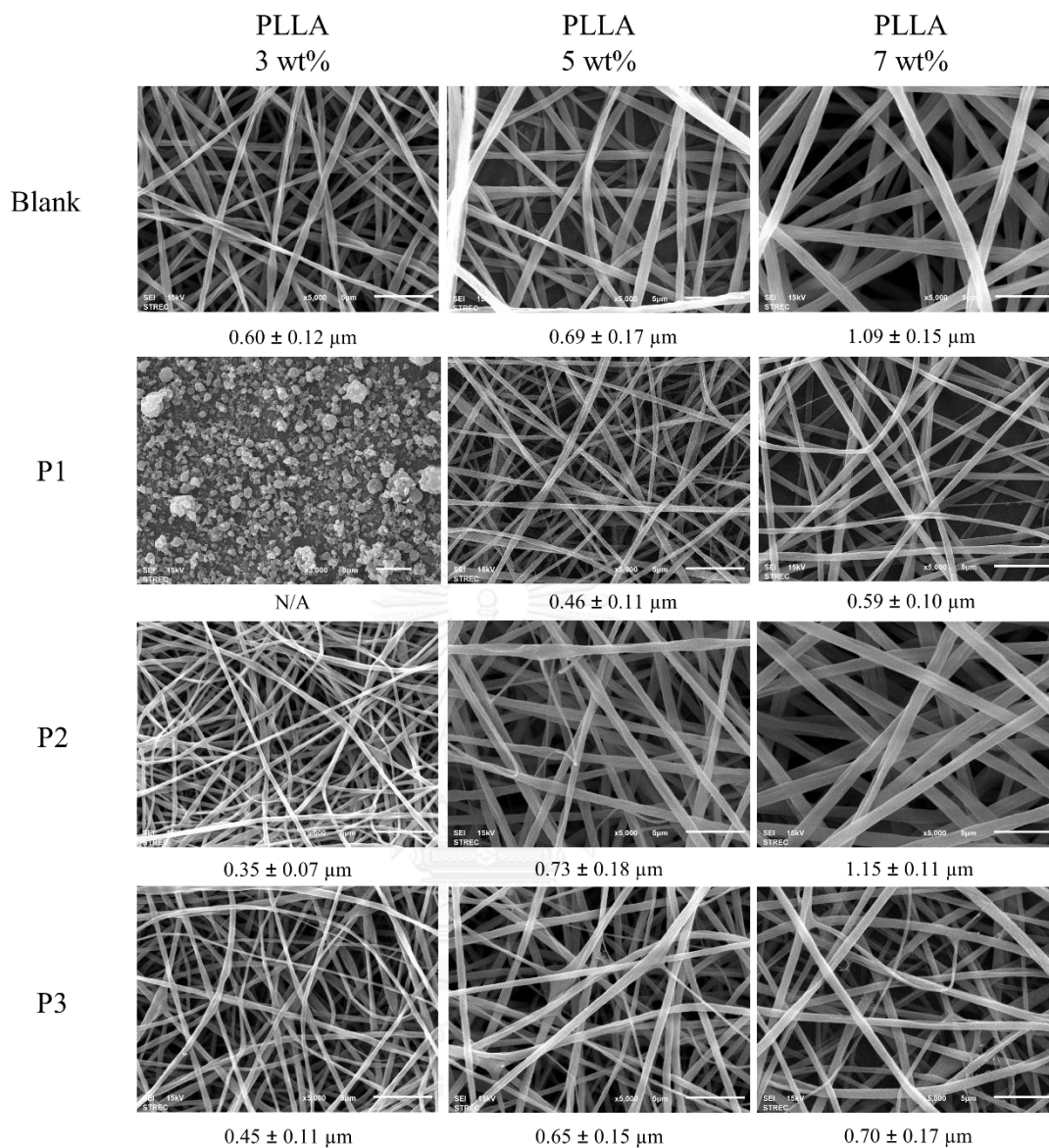|	PLLA 3 wt%	PLLA 5 wt%	PLLA 7 wt%
Blank	0.60 ± 0.12 µm	0.69 ± 0.17 µm	1.09 ± 0.15 µm
P1	N/A	0.46 ± 0.11 µm	0.59 ± 0.10 µm
P2	0.35 ± 0.07 µm	0.73 ± 0.18 µm	1.15 ± 0.11 µm
P3	0.45 ± 0.11 µm	0.65 ± 0.15 µm	0.70 ± 0.17 µm

Figure 4.17 SEM micrographs of electrospun PLLA and PLLA/PLLAdi+ fiber mats from different PLLA concentration (3 – 7 wt%) and molecular weights of PLLAdi+ (4–15 kDa). All figures were shown in magnification of 5,000× except for fiber mat from 3% PLLA doped with *P1*, which was in 3,000×.

4.3. Hydrophilicity

Air-water contact angle measurement was performed on the fiber mats and cast films of PLLA and PLLA/PLLAdi+ to investigate how hydrophilic the samples were. This work hypothesized that the positive charge of PLLAdi+ would induce the hydrophilic property of the fiber mat.

4.3.1. Effect of PLLAdi+ and PLLAdiCOOH molecular weights on hydrophilicity

As mentioned above, the hydrophilicity of the fibers can be measured by water contact angle measurement. This work hypothesized that decreasing molecular weight of PLLAdi+ dopant species would reduce the apparent water contact angle to afford hydrophilic fibers. The PLLA, PLLA/P1, PLLA/P2, and PLLA/P3 fiber as entry 2, 4, 5, and 6, respectively, were measured for air-water contact angle using the goniometer (see **Table 4.8**). According to the results, the lowest to the highest contact angle was entry 4, 5, 6, and 2, respectively. This result reveals that the presence of PLLAdi+ in the polymer blend helps increasing the hydrophilicity of the fiber mats with extent depending on the charge density of the PLLAdi+. As described before, the P1 has low M_W, it would therefore provide the highest positive charge density when all dopant (P1 to P3) were added in equal amounts. These results supported that the hydrophilicity of the fabric PLLA non-woven mats were successfully improved.

Table 4.8 Air-water contact angle of PLLA/PLLAdi+ and PLLA/PLLAdiCOOH fibrous nanomats shows the effect of various molecular weights of PLLAdi+ and PLLAdiCOOH

Entry	Dopant (Abbrev./M_w)	Contact Angle[1]($°$)
2	No dopant	137 ± 6^a
4	PLLAdi+ P1 /4 kDa	123 ± 12^b
5	PLLAdi+ P2 /8 kDa	130 ± 4^c
6	PLLAdi+ P3 /15 kDa	135 ± 7^a
17	PLLAdiCOOH /4 kDa	130 ± 3
18	PLLAdiCOOH /8 kDa	132 ± 23
19	PLLAdiCOOH /15 kDa	140 ± 10

[1] Means in the same column with different letters are statistically different at $p < 0.05$

For another results, the contact angle of entry 4 was surprisingly low compared with others PLLA/PLLAdi+ fiber, entry 5 and 6. This is due to the density of positive charge from PLLAdi+ added to the PLLA solution, especially in case of PLLA/P1 fiber, P1 has low molecular weight, thus, the density of the charge increased where all dopants added to the solution is equally added in 10 wt% based on PLLA amounts.

In contrast, the results from electrospun PLLA/PLLAdiCOOH as shown as entry 17-19 appeared that the hydrophilicity of the fiber mats were not improved efficiently compared to the contact angle obtained from the electrospun PLLA mats doped with PLLAdi+. This is due to the conductivity of the polymer solution could not be enhanced by adding the PLLAdiCOOH; nevertheless, the conductivity can be improved dominantly when the PLLAdi+ added because of its quaternary ammonium positive charges.

4.3.2. Effect of PLLAdi+ contents on hydrophilicity

For this section, the PLLAdi+ contents in the solution were altered on the hope that higher blending ratios would bring the PLLA fiber mats more hydrophilic by reducing the contact angle. The P1 was chosen to help improve the hydrophilicity of PLLA mats due to its low molecular weight that comes with high charge density. According to **Figure 4.18**, the contact angles of various P1 dopant contents electrospun fiber were recorded. From the results, the contact angle was decreased from 137° to 123° while using 10 wt% dopant. After that, the contact angle continued to increase surprisingly from 123° to 140° when the dopant increased from 10 to 50 wt%, respectively. This unexpected finding was probably resulted from reducing the fiber diameter with increasing dopant contents, which lead to decrease the polymer fill fraction of the mat surface and increase the air fraction exposed at the surface [30]; therefore, the fibrous mat surface roughness was raised by decreasing fiber diameter. Although positive charges of the P1 dopant would attract more water molecules to improve the hydrophilicity, their influences was insufficient to overcome the surface roughness.
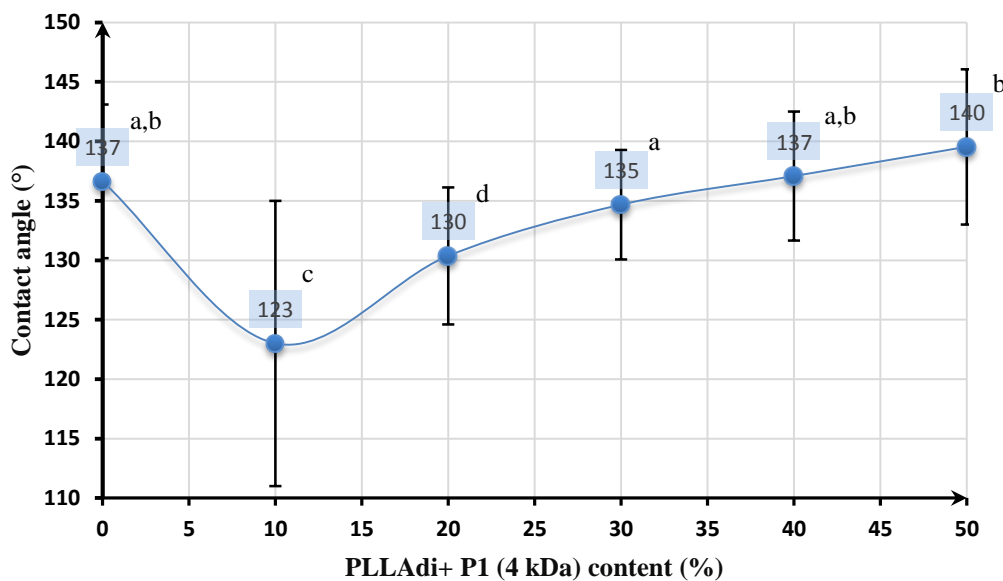
Figure 4.18 Air-water contact angle of the PLLA/P1 fiber mat at various P1 contents. Means in the graph with different letters are statistically different ($p < 0.05$).

Likewise, the amount of dopants P2 were varied in the same condition as the dopants P1 did. From the results, the contact angles were increased with increasing the dopant content which was similar to the resulting contact angle in P1 dopant, as can be seen in **Figure 4.19**. The angles were increased continuously from 137° to 146° for increasing dopant to 30 wt%; however, in 50 wt% dopant content, the angles were decreased which indicated that the effect of positive charges can overcome the surface roughness at this point but the forces were not sufficient to overcome the roughness to yield more hydrophilic fiber mats.

Furthermore, the contact angle measured from the nanofibers (**Figure 4.18**) were higher than corresponding films (**Figure 4.20**) due to the fiber mats have highly rough surface compared with their films, as previously documented [11]. The figure showed the trend of contact angle of PLLA/PLLAdi+ films that the angle would be reduced when the PLLAdi+ content increased, which were not consistent conversely with the results from fibrous mats.
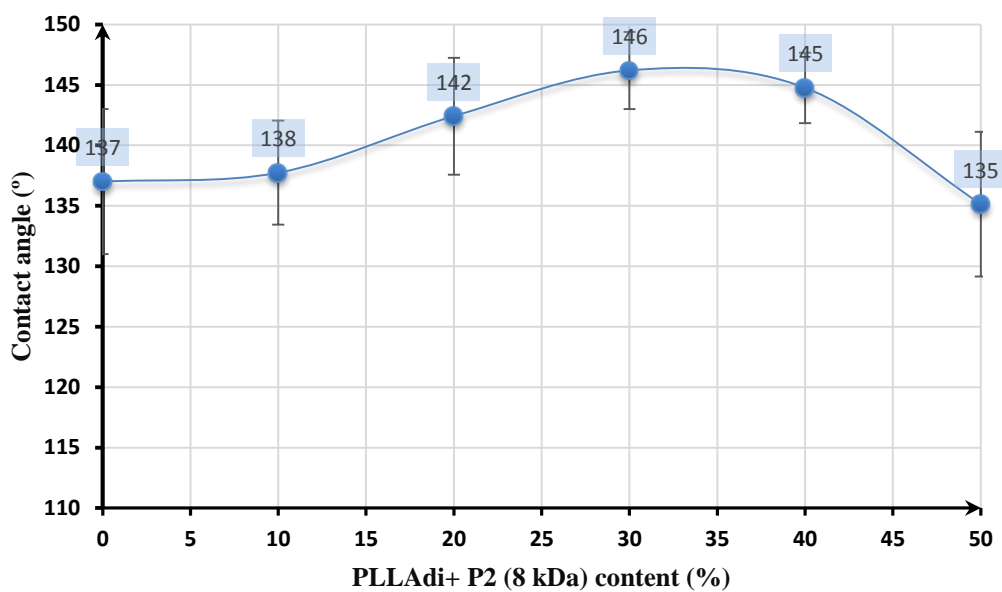
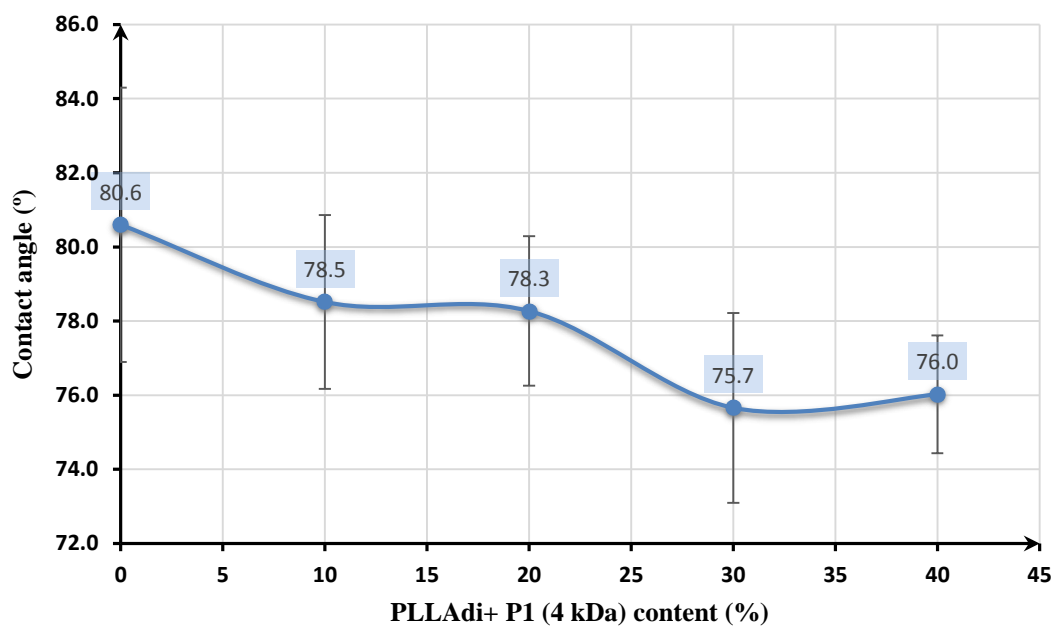**Figure 4.19** Air-water contact angle of the PLLA/P1 fiber mat at various P2 contents.

Figure 4.20 Air-water contact angle of the PLLA/P1 corresponding films

CHAPTER V

CONCLUSION

5.1. Summary

Poly(L-lactic acid), a promising biodegradable polyester, has been studied as biomaterial replacement in several applications. However, the high hydrophobic character of PLLA was a challenged issue for developing a more versatile material. As previous discussed, PLLA with two carboxyl chain ends (PLLAdiCOOH) were successfully synthesized via polycondensation of lactic acid and succinic acid. For synthesis of PLLAdiCOOH, mole percentage of succinic acid and polymerization time played important role in controlling the molecular weight and polymer chain length. Using SnCl2 and pTSA as binary catalysts, the molecular weight increasing was achieved by decreasing the relative mole of succinic acid used or increasing the polymerization time; however, the M_w was not affected by increasing dehydration time (at 110ºC). Three PLLAdiCOOHs with MW of 4, 8, and 15 kDa were synthesized and were used to prepare PLLA with two positive charges at the polymer chain ends (PLLAdi+) by the ring-opening reaction of the carboxylic chain ends and GTMAC. The degree of GTMAC substitution as high as 99% was achieved for 4 kDa PLLAdiCOOH.

Afterwards, PLLAdi+ was used as dopant in the electrospinning process of commercially available PLLA. The fiber diameter was increased moderately by raising the concentration of commercial-grade PLLA used which served as major component in the polymer blend. Increasing the relative amount of PLLAdi+ dopants and using the low M_w PLLAdi+ led to a reduction in the fiber diameter. The fiber diameter was reduced from 650 to 462 nm when decreasing the molecular weight of PLLAdi+ used from 15 to 4 kDa, and from 688 to 208 nm when raising the PLLAdi+ content from 10 to 40 wt%.

The hydrophilicity of PLLA fiber mat as determined by air-water contact angle was decreased significantly from 137º to 123º at the confidence level 95% ($p < 0.05$) when 10 wt% of 4 kDa PLLAdi+ was added compared to the non-doped mat

because of increasing positive charge in the polymer matrix. The angle grew, however, from 123º to 140º when increasing the PLLAdi+ content. It was possible that the surface roughness of electrospun fiber mat overcame the influence of PLLAdi+ on the contact angle. Although the natural surface roughness of electrospun fiber occurred, the thin and hydrophilic PLLA fibers were successfully prepared and could potentially be used in applications related to aqueous environment.

5.2. Future works

To enhance the effect of the dopants on the fiber properties, introducing a greater amount of positive groups by using branched PLLA structure would be quite interesting. Thus, the tricarballylic acid, tricarboxylic acid, is appropriate molecules that would increase the number of polymer chain ends to 3 and 4 carboxylic end groups which can be further derivatized to 3 and 4 quaternary ammonium containing molecules (**Figure 5.1**).
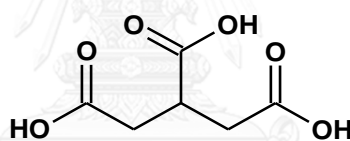
**Figure 5.1** Chemical structure of tricarballylic acid

Biological responses, such as antibacterial activity and cytotoxicity, for this type of PLLA fiber mat should also be investigated in order to obtain initial data for its biomedical application.

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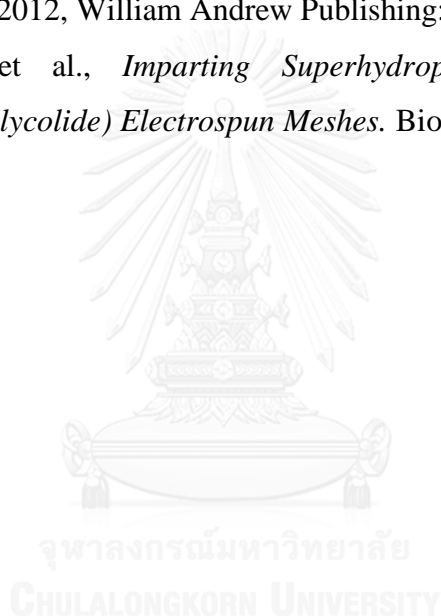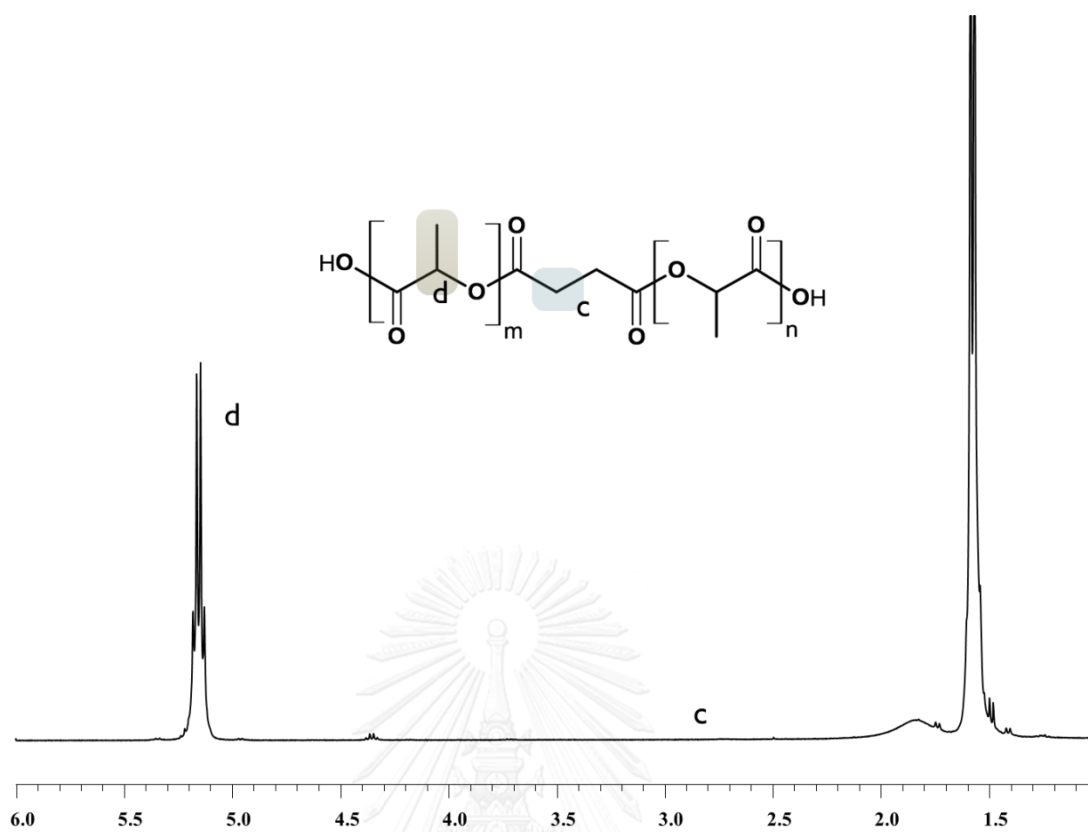
APPENDIX

Figure S.1 ^{1}H NMR spectrum of PLLAdiCOOH-09 showed the methylene proton signal of succinic unit in polymer chain (c) was interfered by noise.
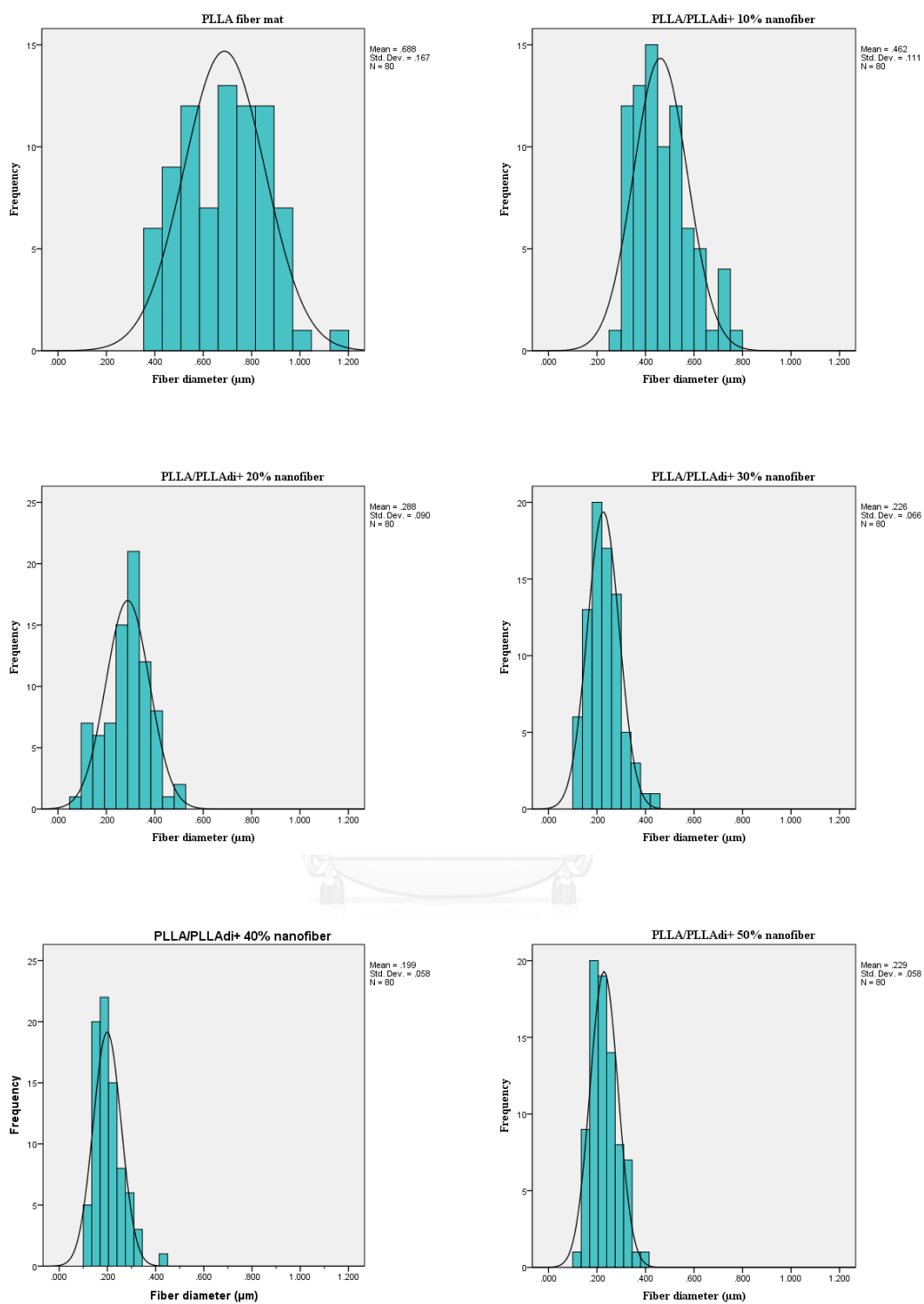
Figure S.2 Histograms of PLLA and PLLA/PLLAdi+ electrospun nanofiber with various PLLAdi+ content: 0 − 50 wt% P1.

VITA

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