ABSTRACT

SINGHI, BHAVYA. Development of Solvent Spinning Process for Bacterial Polyester. (Under the direction of Dr. Martin W. King)

Polyhydroxyalkanoates (PHAs), also known as bacterial polyesters, have emerged in the last decade as viable materials for biodegradable products. These aliphatic polyesters are produced by bacterial synthesis of carbon. There is a wide range of hydroxyalkanoate units with varying mechanical and structural properties. Currently PHAs are being processed via melt spinning at temperatures as high as 200 °C. Extruded filaments exhibit a high degree of polymerization and command high molecular weights. In the past few years, PHAs have been utilized for biomedical applications such as sutures, wound dressings and films due to their biocompatibility and resorbability. They have also been studied for controlled release applications involving a variety of chemicals and drugs, some of which are thermally sensitive. Due to their high melt spinning temperatures, the incorporation of chemicals and thermally sensitive drugs has involved a lower temperature post spinning process. Such an approach can potentially cause non-uniform absorption and lead to an uneven and unpredictable release profile. This study aims at resolving this issue by developing a low temperature spinning process for bacterial polyesters. After testing multiple solvents, methylene chloride was chosen as a suitable solvent for dissolving poly-3-hydroxybutyrate-4-hydroxybutyrate (P34HB). The polymer was regenerated as films on microscopic slides in a cold methanol bath. Numerous tests were performed on the polymer solution as well as the regenerated polymer films to determine an optimum concentration for the spinning dope and a preferred temperature for the coagulation bath. Further work on this project will involve

laboratory scale multifilament extrusion using the developed process as well as translating the process to a pilot scale.

Development of Solvent spinning Process for Bacterial Polyester

by
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APPROVED BY:

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DEDICATION

To my family and friends

To my advisor Dr. Martin W. King

BIOGRAPHY

Bhavya Singhi was born on 7th September 1992 and hails from New Delhi, India. She completed her schooling from Mount Carmel School, Dwarka, New Delhi and pursued a Bachelor's degree in Fibers and Textile Processing Technology from the Institute of Chemical Technology, Mumbai, India. She undertook summer internships and training programs at Vardhman Polytex. (Ludhiana, India), Bombay Rayon Fashions Ltd. (Tarapur, India) and Bombay Textile Research Association (Mumbai, India). She completed her undergraduate program in May 2014.

She joined North Carolina State University in August 2014 to pursue a Master of Science in Textile Chemistry program. She interned for two months at RTI International (Raleigh, NC). She served as the President of the Textile Association of Graduate Students (TAGS) and the Chair of the Teaching Effectiveness Committee in NCSU Graduate Student Association. She expects to graduate in the Fall 2016 and will continue onto a PhD in Fiber and Polymer Science.

Apart from academics, she actively participates in events around the campus. She enjoys cooking and watching movies.

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CHAPTER 1

INTRODUCTION

1.1. Motivation and Significance

Polyhydroxyalkanoates (PHAs), also commonly known as bacterial polyesters, are considered to be novel polymers because of their biodegradability. A wide range of hydroxyalkanoate units, such as butyrates and valerates, are produced by bacterial synthesis. These units can be polymerized and copolymerized with varying mechanical and structural properties. Many companies have started replacing polyester and polyethylene with PHAs for short term plastic applications, such as packaging and food storage. Owing to the versatility of PHAs along with their biocompatibility and resorbability, they are also being used in biomedical applications such as sutures and wound dressings. They have also been explored for controlled release applications involving a variety of chemicals and drugs, some of which are thermally sensitive.

Currently, PHAs are melt spun and drawn into filaments for their various applications at high processing temperatures which can reach to 200 °C, inhibiting the possibility of incorporating drugs within the polymer during the spinning stage as most drugs cannot sustain such high temperatures and hence require a post spinning incorporation process. This raises the need for a low temperature solvent spinning process for bacterial polyesters that will address the major drawbacks associated with incorporating drugs post melt spinning. Examples of such disadvantages include non-

uniform absorption and uneven and unpredictable release profiles. Although solvent spinning includes dry, wet and gel spinning, dry spinning is also an unsuitable method since it requires hot air to remove the volatile solvent.

Our work will focus on studying the parameters of the solvent spinning process and developing an optimum solvent spinning method for bacterial polyester. Our regenerated polymer can be potentially used for controlled release applications in any field including drug delivery (internal and external), wound dressings and infection control (release of antimicrobial agents), and agrotextiles (insect repellant meshes).

1.2. Goals and Objectives

The ultimate goal of this project is to develop a low temperature spinning process for bacterial polyester to produce continuous filaments on a pilot scale level. However, before fulfilling the ultimate goal, our main objective is to study the feasibility of developing a solvent spinning process for bacterial polyester by developing a process for solvent cast polymer films, as both processes share similar parameters.

Our study has three specific aims:

1. Finding a suitable solvent for dissolution of bacterial polyester.

   This is the key step in order to carry out this study as without a suitable solvent system that can dissolve the polymer efficiently we cannot develop a solvent spinning process. Four solvents – tetrahydrofuran, methylene dichloride,

chloroform and dioxane were compared for their rate of dissolution, toxicity and flammability.

2. Determining an optimum concentration for the spinning dope.

   Spinning dopes of multiple concentrations (5%, 15%, 30% and 40%) were prepared to evaluate them on the basis of their viscosity and degree of dissolution. Optical microscopy under polarizing light was used to capture images of the solution before and after filtration. The images were analyzed to obtain the size of the particles present on the microscope slide.

3. Identifying a suitable temperature of the coagulation bath for polymer regeneration.

   The spinning dopes were regenerated in the form of films at room temperature and -25 °C to identify which temperature would provide better properties for the regenerated polymer films. Gel permeation chromatography (GPC) and thermal gravimetric analysis (TGA) was carried out to determine the level of degradation during dissolution while differential scanning calorimetry (DSC) was performed to compare the thermal properties and degree of crystallinity of the regenerated polymer films.

1.3. Thesis Outline

The thesis is divided into five chapters. The first chapter introduces this study and the motivation behind it. It also lists the objectives and specific aims pertaining to the study. The second chapter provides relevant literature regarding bacterial polyesters, alternative spinning techniques, the mechanisms of polymer dissolution and polymer regeneration. The third chapter covers the experimental processes undertaken during the study, including the materials and equipment used, processing methodology and testing procedures. The fourth chapter discusses the results obtained from all the tests, such as the comparative solvent study, optical microscopy, GPC, DSC and TGA. Finally, the fifth chapter summarizes the findings of the study. It lists the conclusions and proposes the scope for further work.

CHAPTER 2

LITERATURE REVIEW

2.1. What are Polyhydroxyalkanoates?

Polyhydroxyalkanoates (PHAs) represent an important group of biodegradable linear polyesters produced in nature by bacterial fermentation of sugar or lipids. They are produced in many grades, differing in composition, molecular weight and other properties [1, 2] to store carbon and energy. The formation of a particular variety of polymer, either a homopolymer or a copolymer, depends on the type of bacteria. But even more important are the conditions of polymer formation, mainly the substrate used for feeding the bacteria and the conditions of their growth. More than 150 different monomers can be combined within this family to give materials with extremely different properties [3].

PHAs can be either thermoplastic or elastomeric materials, with melting points ranging from 40 to 180 °C. The mechanical properties and biocompatibility of PHAs can also be changed by blending, modifying the surface or combining PHAs with other polymers, enzymes and inorganic materials, making it possible for a wider range of applications. The simplest PHA is poly-3-hydroxybutyrate (P3HB), whose molecular structure is shown in Figure 2.1.

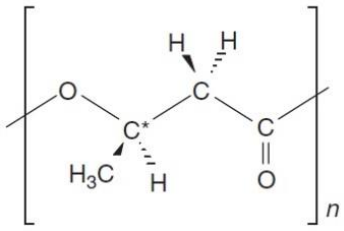

Figure 2.1. Molecular structure of P3HB [3]

2.1.1. Types of Polyhydroxyalkanoates

Polyhydroxyalkanoates are biopolyesters with various side chains and fatty acids with hydroxyl groups at either the 3- or 4- position. There are three types of polyhydroxyalkanoates [5]:

(a) Short chain length hydroxyalkanoic acids (PHA_{SCL}) with an alkyl side chain, which are produced by *Ralstonia eutropha* and many other bacteria. PHA_{SCL} contain 3–5 carbon atoms, for example, poly-3-hydroxybutyrate (P3HB), poly-4-hydroxybutyrate (P4HB) and poly-3-hydroxyvalerate.

(b) Medium chain length hydroxyalkanoic acids (PHA_{MCL}) with alkyl side chains that are produced by *Pseudomonas oleovorans* and other *Pseudomonas sensu stricto*. PHA_{MCL}.. They contain 6–14 carbon atoms.

(c) Long chain length (PHA_{LCL}) obtained from long chain fatty acids, which contain more than 14 carbon atoms.

2.1.2. Blends of Polyhydroxyalkanoates

Various PHA blends have been developed to improve their performance and to offset their high price of manufacturing. It also offers more scope to expand their range of applications [6]. P3HB with poly (lactic acid) (PLA) is one of the most studied blends, which exhibits mechanical properties that are intermediate between the individual components [7]. Although PLA and P3HB are biodegradable polymers synthesized from renewable resources, their potential applications can be hampered due to brittleness and the formation of spherulites depending on the blend ratio [8]. Blending of polycaprolactone (PCL) and P3HB offers a good option to improve the mechanical performance of both homopolymers [9]. Another PHA polymer which is studied extensively is a copolymer of P3HB and P4HB, poly (3-hydroxybutyrate-4-hydroxybutyrate), also known as P34HB [10].

2.1.3. Properties & Applications

Two main areas of applications for PHAs are considered to be healthcare and packaging. They have found a large market as an eco-friendly biodegradable plastic for products such as grocery bags, food containers, bottles, etc., because of their hydrophobicity, oxygen permeability and ultra-violet resistance [11].

A combination of resorbability and biocompatibility, together with other properties makes them suitable materials for medical product applications [12].

Polyhydroxybutyrate (PHB) composites with apatite can be used as biodegradable bone fracture fixation devices and as bone repair materials, which takes into account the piezoelectric properties of PHB [13] which are similar to those of bone. A variety of PHA blends are being researched for application in tissue engineering scaffolds [14]. Another potential application for PHA involves the controlled release of drugs [15].

2.2. Spinning Processes

The conditions used during the spinning process depend highly on the type of polymer being spun. Usually, the exact procedures have to be developed for each polymer as small deviations in spinning or drawing from the optimum process may be detrimental to obtaining the desired properties of the spun and drawn filaments [16].

Traditionally the fiber forming processes for polymers are melt, dry, and wet spinning. All three methods involve the formation of continuous filament strands by forcing the liquid material through small dies or holes in a spinneret. Melt spinning involves cooling of the molten strands to form the solid filaments, whereas dry and wet spinning involves removal of a solvent in order to form solid filaments. A fourth spinning process, called dry jet-wet spinning, commonly known as gel spinning, is also utilized for certain polymers. These three processes are together referred to as solvent spinning.

2.2.1. Melt Spinning

Melt spinning is a preferred method of synthesis for thermoplastic polymeric filaments due to high rate of production and lower costs [17]. The molten polymer is first pumped through a filter, called a spin-pack, which removes any particulate matter that would otherwise be trapped in the tiny spinneret holes. The polymer is then pressurized through the spinneret to form continuous strands of polymer filaments. A cooling gas reduces the temperature of the filaments so that they solidify and a drive roll controls their initial take-up speed. The filaments may undergo subsequent heating and stretching to impart additional molecular orientation and crystallinity. Stretching of the fibers in both the molten and solid states provides for orientation of the polymer chains along the axis. Finally, the filaments have a surface finish (lubricant) applied and are wound up onto bobbins at a constant speed, with a tension control device to maintain the rate of rotation and the uniformity of the package build [17].

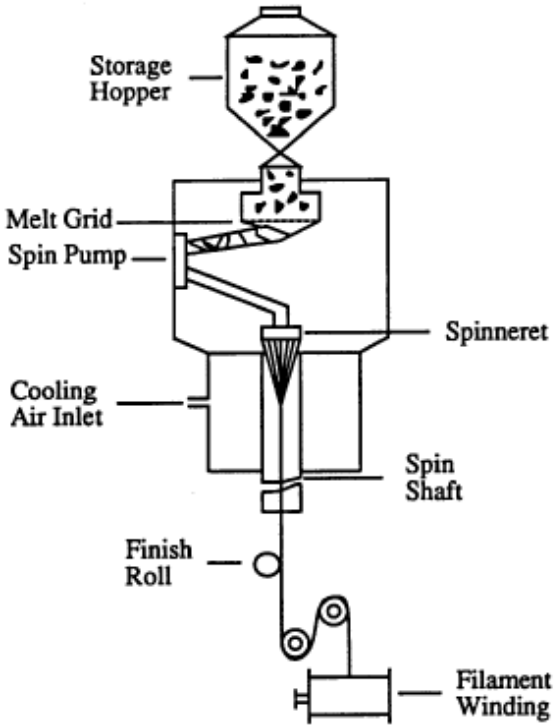

Figure 2.2. Schematic Diagram of Melt Spinning [18]

The surface of melt-spun fibers consists of many large spherulites due to the high rate of cooling, especially in polyethylene fibers. After drawing the fibers have a fibrillar structure and their surface is fairly smooth. Annealing under tension leads to the formation of a more perfect fibrillar structure in the core, and this effect seems to increase with a rising tension load during annealing [19].

2.2.2. Solvent spinning

Solvent spinning consists of three different spinning methods – dry, wet and gel spinning. In all three processes the polymer is dissolved in a suitable solvent, preferably at a low temperature. The polymer solution called "the spinning dope" is pressurized through the spinneret holes and the solvent is extracted. The method of solvent extraction is what differentiates the three spinning processes. In dry

spinning (Figure 2.3) the solvent is evaporated while in wet spinning (Figure 2.4) the solvent diffuses into a liquid bath. During gel spinning the fibers are first air dried and then immersed into a cold liquid bath to extract the solvent [20].

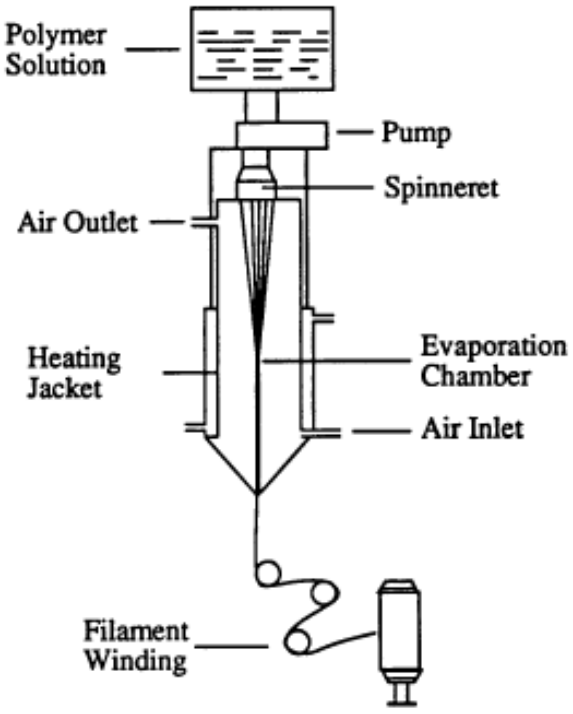

Figure 2.3. Schematic Diagram of Dry Spinning [18]

Dry spinning requires hot air to evaporate the solvent and solidify the polymer to form filaments. The extruded filaments can be drawn at slightly elevated temperature and then wound onto bobbins.

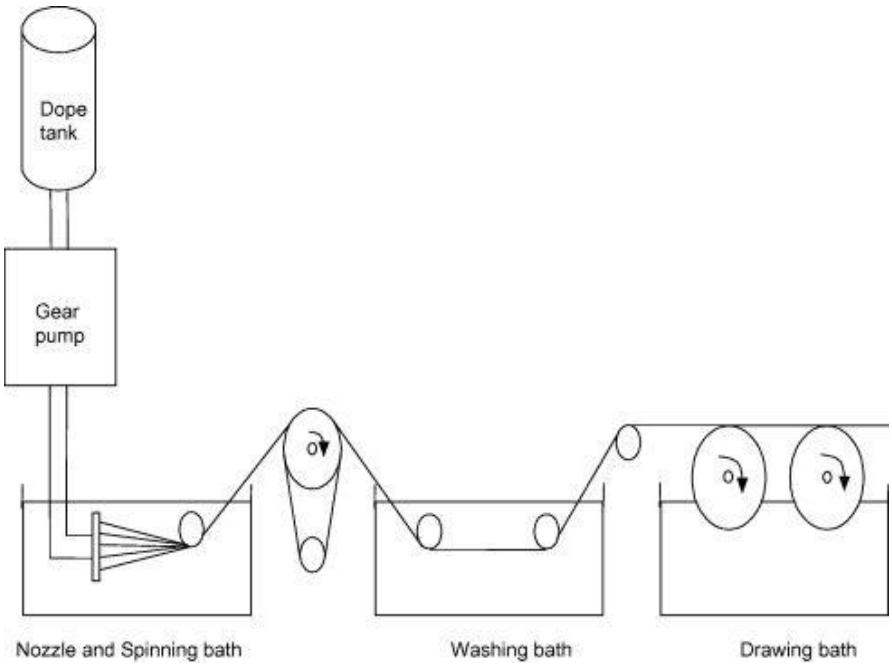

Figure 2.4. Schematic Diagram of Wet Spinning [21]

Wet spinning is a low temperature process where the polymer is regenerated from the spinning dope when it enters the coagulation bath. The coagulant solution has to be a non-solvent for the polymer so that continuous filaments are generated in the coagulation bath and are then taken up on rollers for washing and drawing [22].

Gel spinning (Figure 2.5.) also requires a spinning dope and a coagulation bath. It is distinguished from wet spinning by the presence of an air gap between the spinneret and the coagulation bath. The coagulation bath is maintained at a temperature much below that of the spinning dope, which allows the polymer strands to form a stretchable gel like structure. They are then taken up by the rollers and wound onto a spool. One of the advantages of having the air gap is so the temperature at the spinneret can be significantly higher than the temperature of the coagulation bath. Also, the air gap prevents clogging of the spinneret during extrusion. [23]

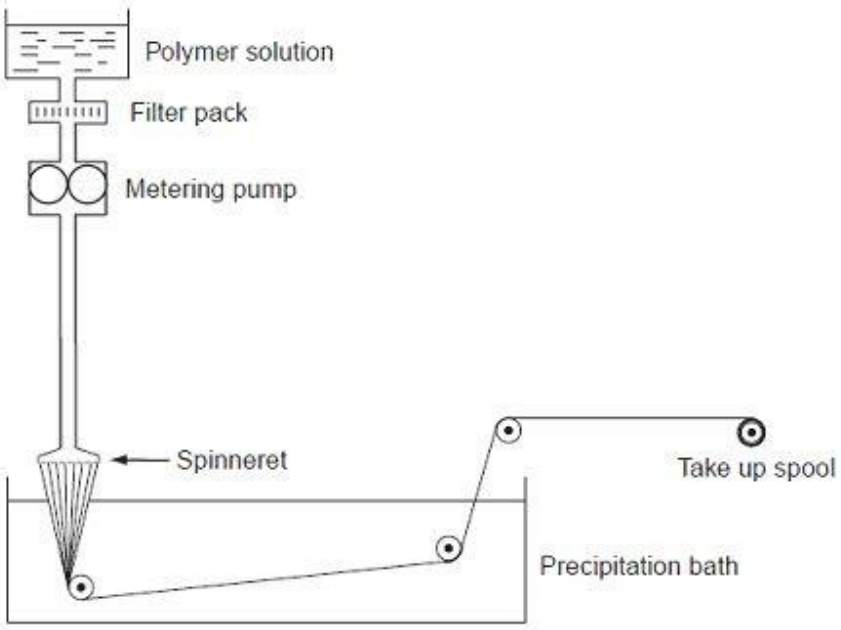

Figure 2.5. Schematic Diagram of Gel Spinning [21]

As inferred from the above descriptions of the spinning processes, wet and gel spinning have a lower rate of spinning compared to melt and dry spinning due to the fluid drag forces on the filaments in the coagulation bath, which leads to high yarn tension. The coagulation baths need to be long in order for the solvent to have time to diffuse out from the extruded filaments. And for long baths, the tension can become sufficiently high that the filaments may break, as their tensile strength is exceeded by the shear stress on the filaments [24].

An advantage for wet and gel spinning over dry and melt spinning is the number of spinneret holes. The total number can approach 60,000 in a single spinneret plate, if the spinning is done directly into a coagulation bath or with liquid extraction. Because of the liquid diffusion process, each filament forms an outer "skin" or sheath almost immediately the polymer dope enters the coagulation bath, and so the potential for the filaments to touch each other and fuse together is practically eliminated compared with dry or melt spinning [22].

Table 2.1. Comparison of Spinning Processes

Processing Stage	Melt Spinning	Dry Spinning	Wet Spinning	Gel Spinning
Polymer state before extrusion	Molten	Solution	Solution	Solution
Temperature before extrusion	High (Above polymer melt temperature)	Low	Can be high or low depending on solvent volatility	Can be high or low depending on solvent volatility
Temperature during extrusion	High	High	Low	Low
Extrusion Process	Molten polymer filaments solidify	Filaments regenerate after solvent evaporates	Filaments regenerate directly in coagulation bath	Filaments extruded in air and form gel like structure in coagulation bath
Solvent Type	--	Volatile	Can be volatile or non-volatile	Can be volatile or non-volatile
Rate of Spinning	High	Moderate	Low	Low
Number of Spinneret Holes	Low (Avoid polymer fusion)	Low (Avoid polymer fusion)	High	High
Filament Crystallinity / Orientation	Moderate	Moderate	High	High

2.3. Solvent Cast Polymer Films

Solvent cast process for polymer is one of the oldest technologies to manufacture thin films. These films possess uniform thickness, high quality and dimensional stability. The key elements of the process are dissolution of the polymer in a suitable solvent that can form stable and viscous solution, formation of a homogeneous layer of polymer film on a casting line and removal of the solvent [25]. The dissolved polymer solution is filtered and spread homogeneously onto a mould. The mould is either subjected to heat for removal of volatile solvent or is immersed in a trough of coagulant to allow a solvent-non solvent exchange. The film is then extracted from the mould, washed and dried in order to remove any residual solvent.

It can be clearly seen that the process for solvent cast polymer film is very similar to solvent spinning process. This similarity is taken into account while developing the low temperature spinning process for bacterial polyester. A process for solvent cast film is developed which can be translated to a process for solvent spinning.

2.4. Polymer Dissolution

Polymer dissolution or the process of dissolving a polmer in a solvent system is an important area of interest due to its application in solvent spinning. A thorough understanding of the dissolution process as well as factors involved in polymer dissolution is necessary to produce a suitable spinning dope. Polymer dissolution is also vital within the field of controlled drug delivery and time-release applications. An

ideal drug delivery system provides the drug only when and where it is needed, and at the required dosage level so as to elicit the desired therapeutic effect [26].

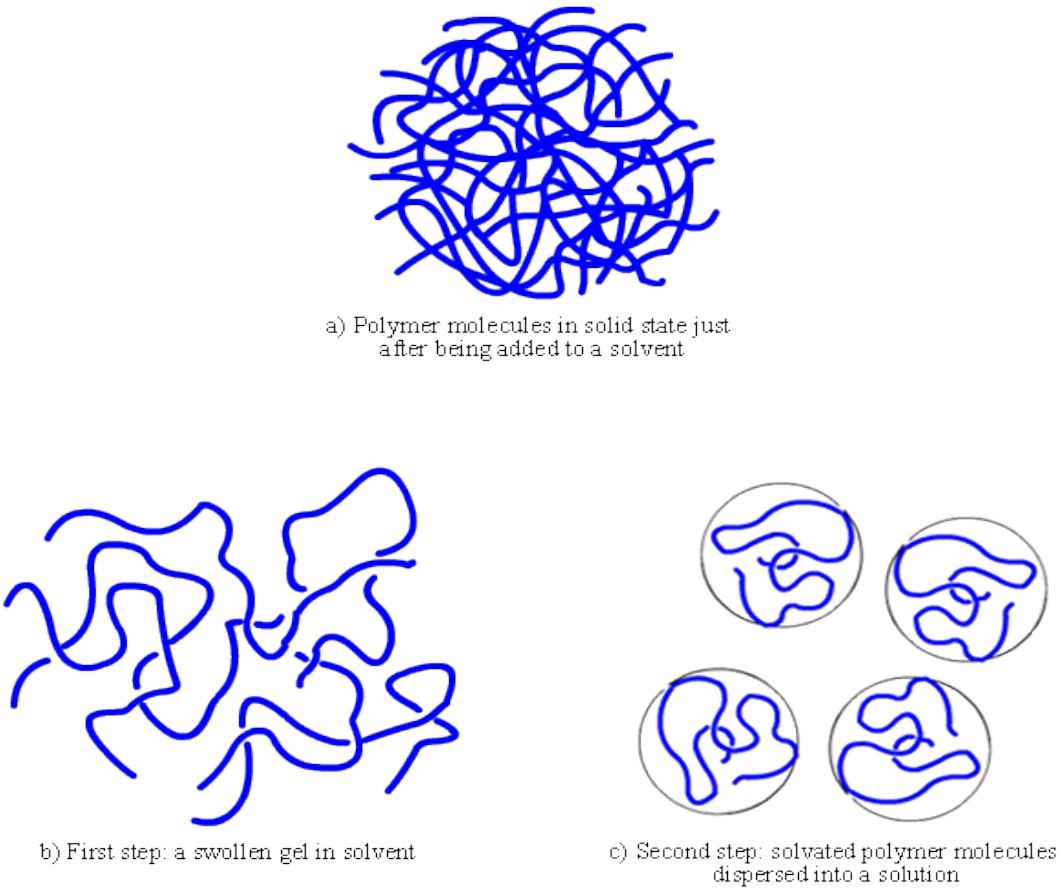

Figure 2.6. Schematic Diagram of Polymer Dissolution [27]

2.4.1. Process of Dissolution

The dissolution of a polymer in a solvent involves two transport phenomena, the penetration of the solvent into the polymer, followed by disentanglement of the polymer chains [27]. When a un-crosslinked, solid state polymer is in contact with a thermodynamically compatible liquid (solvent), the latter diffuses into the polymer. A gel-like layer is formed adjacent to the solvent-polymer interface due to plasticization of the polymer by the solvent. This results in swelling of the polymer and deeper penetration of the solvent. After an induction period of time, the polymer becomes dissolved in the solvent. A schematic diagram of solvent diffusion and polymer dissolution is shown in Figure 2.6.

2.4.2. Factors affecting Polymer Dissolution [28]

a) ***Polymer molecular weight and polydispersity.*** High molecular weight polymers usually have lower rates of dissolution due to their high degree of chain entanglement and low diffusion coefficient.

b) ***Polymer structure, composition and conformation.*** Based on the crystallinity and polymer structure, the penetration of the solvent will differ. High crystallinity results in slower penetration and a decreased rate of dissolution.

c) ***Type of solvents and additives.*** Each solvent system has its own diffusivity, which depends on its polarity, strength, molecular size, and swelling power. The

rate of dissolution will decrease with increasing size of the solvent molecule, indicating that the dissolution rate is limited by the rate at which solvent molecules diffuse and penetrate.

d) ***Environmental parameters and processing conditions.*** External parameters, such as the degree of agitation, the temperature as well as radiation exposure, can influence the dissolution process. The rate of dissolution increases with agitation and the stirring frequency of the solvent due to a decrease in the surface layer thickness. The dissolution rate approaches a limiting value if the pressure or shear rate of the solvent against the surface of the polymer increases.

2.5. Polymer Coagulation

2.5.1. Process of coagulation

Polymer coagulation is the process where a polymer solution is exposed to a non-solvent, leading to solvent-nonsolvent exchange and polymer precipitation. This process constitutes the most important step in the formation of filaments during the wet and gel spinning processes [29].

Polymer coagulation is a non-equilibrium process which is controlled by the rate of solvent-coagulant exchange at the polymer interface. A dense polymer "skin" or outer sheath layer is usually formed at the onset of immersing a polymer solution into a coagulant bath.

2.5.2. Factors affecting Polymer Coagulation [30]

a) ***Solvent/coagulant miscibility.*** The miscibility of the solvent and the coagulant is controlled by an interaction energy parameter. As the miscibility of the solvent and coagulant decreases it has been observed that the development of filaments becomes easier and faster.

b) ***Coagulant strength.*** When the coagulant is weak, a partial dissolution of the polymer into the coagulating bath is observed. As a result there is limited or no "skin" formation at the polymer/coagulant interface.

c) ***External factors.*** Process conditions such as the temperature, the linear rate of extrusion through the spinneret and the take-up speed can also affect the rate of polymer coagulation.

CHAPTER 3

MATERIALS AND METHODS

3.1. Dissolution of Bacterial Polyester in a Suitable Solvent

The first step in this study was to determine a suitable solvent system for the dissolution of poly(3-hydroxybutyrate-4-hydroxybutyrate) (P34HB) polymer sample, which was supplied by Shenzhen Ecomann Biotechnology Co. Ltd (China). The polymer was obtained in the form of semi-crystalline cylindrical pellets and placed in a vacuum desiccator to keep it stored in a dehydrated condition. Based on previous literature, four potential solvents were identified – tetrahydrofuran (THF), methylene dichloride (MDC), chloroform and dioxane (Table 3.1) [31].

Table 3.1. Possible Solvents for P34HB

Solvent	Obtained From	Chemical Grade
Tetrahydrofuran	BDH	ACS Grade
Methylene dichloride	Sigma Aldrich	ACS Reagent, ≥ 95.5%
Chloroform	Fisher Scientific	ACS Grade
Dioxane	Fisher Scientific	ACS Grade

The primary criterion to compare the performance of each solvent was to measure the rate of polymer dissolution in each one. A stopwatch was used to measure the time taken to dissolve 1 gram of dehydrated P34HB in 10 ml of each solvent stirring at 600 rpm. Apart from the rate of dissolution, the solvents were also judged on the basis of their volatility, toxicity, flash point and flammability. Methylene dichloride was identified as a suitable solvent to conduct the preliminary spinning studies.

3.2. Identifying Optimum Concentration of Spinning Dope

Methylene dichloride was used to make spinning dopes at room temperature with P34HB at four concentrations – 5%, 15%, 30% and 40%. They were then filtered using 10ml slip tip syringes (Becton Dickinson & Company, Franklin Lakes, NJ) with 0.80 μm pore size syringe filters (Corning Life Sciences, Durham, NC). The filtered and unfiltered dopes were then subjected to optical microscopy under polarizing light in order to determine the extent of solubility and the average undissolved particle size. These test results were used to identify an optimum concentration for the spinning dope that would provide the best results for regeneration of the polymer. Since the 40% solution had large quantities of visible undissolved polymer pellets, it was decided that the filtrate obtained from it would not be at the desired concentration, and hence the 40% dope was neither filtered nor carried though to the next coagulation step.

3.2.1. Optical Microscopy

To measure the undissolved particle size in the dopes, optical microscopy was carried out using polarizing light at 20x magnification with a Nikon Labophot-Pol microscope fitted with a Tucsen camera. The IS Capture software was used for capturing and processing the images. All the microscopic slides in this process were thoroughly cleaned before use. Multiple images were taken to analyze and calculate the average size of the undissolved polymer particles in the spinning dopes, both before and after filtration. Images of a control (blank) microscope slide were also captured in order to obtain a realistic understanding of the dust and other contaminants present on the microscope lens and slides.

Figure 3.1. Nikon Labophot – Pol Optical Microscope [32]

3.3. Determining preferred Regeneration Temperature in Methanol

Since methanol was determined to be an appropriate coagulant from the literature [33], ACS grade methanol was obtained from Fisher Scientific. To regenerate the P34HB polymer in film form, the filtered and unfiltered spinning dopes were cast onto microscope slides and immersed in a methanol bath. The process was repeated to regenerate the P34HB films at room temperature and -25 °C. The temperature of the methanol bath was lowered below room temperature using dry ice. The temperature of the inner bath was measure using a digital thermometer probe (Fisher Scientific).

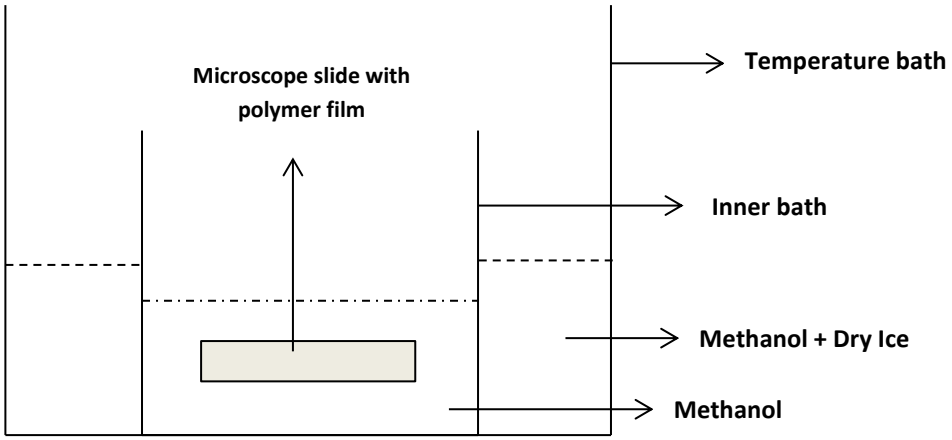

Figure 3.2. Schematic diagram showing P34HB Polymer Film Regeneration

A number of polymer properties were measured on the original as received polymer as well as on the regenerated polymer films before and after the dissolution and regeneration processes. They included the molecular weight distribution by gel permeation chromatography (GPC) and thermal analysis using differential scanning

calorimetry (DSC) and thermal gravimetric analysis (TGA). The results were analyzed to obtain the number and weight average molecular weights, the polydispersity index, the thermal degradation profiles, the melting temperature, glass transition temperature and crystallization temperature. This was essential in order to determine to what extent the polymer experienced degradation during the dissolution and regeneration processes.

3.3.1. Gel Permeation Chromatography (GPC)

Filtered and unfiltered polymer films regenerated at room temperature were subjected to a gel permeation chromatography in a THF column on a Waters Alliance High Performance Liquid Chromatograph (HPLC) to assess their molecular weight distribution and polydispersity index.

Figure 3.3. Waters Alliance HPLC Equipment [34]

The polymer films were dissolved in THF at room temperature to make a 1mg/ml solution for GPC testing. An injection volume of 50 µl was used for the test. Since the GPC data did not yield any major differences between the filtered and unfiltered polymer films, only the filtered films together with the original as received polymer were used for the thermal analysis.

3.3.2. Differential Scanning Calorimetry (DSC)

A Perkin Elmer Diamond DSC instrument was used to perform thermal analysis on the original and regenerated polymer samples. Individual specimens were prepared by weighing between 3-5 mg of materials in a crimped aluminum pan. A dummy cycle was carried out by heating from 25 °C to 180 °C to rid the specimen of any impurities and moisture. It was then cooled to -40 °C using liquid nitrogen and heated again to 180 °C. The heating cycle ended when the specimen temperature was brought back down to 25 °C. The program cycle was held at the isothermal temperature of 180 °C and -40 °C for two minutes so as to ensure that the whole specimen reached the same temperature. The temperature was raised and cooled at the rate of 20 °C/min.

Figure 3.4. Perkin Elmer Diamond DSC Instrument [35]

3.3.3. Thermal Gravimetric Analysis (TGA)

A Perkin Elmer Pyris 1 TGA instrument was used to obtain thermograms of the control and the filtered 5%, 15% and 30% polymer samples. A nitrogen atmosphere was used to determine the degradation profiles based on loss of weight over temperature. The data was also used to verify the GPC results which measured the level of degradation during the process of polymer dissolution. Since the regeneration process did not have any impact on polymer degradation, only the samples regenerated at room temperature were tested by TGA.

Figure 3.5. Perkin Elmer Pyris 1 TGA Instrument [36]

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Selection of a Suitable Solvent

As it can be seen in Table 4.1 a rapid rate of dissolution and low toxicity were the prime factors in choosing methylene dichloride for the study. The absence of a flash point indicated that it is a non-flammable solvent that is safer and less toxic to use.

Table 4.1. Comparison between Solvents

Solvent	**Properties**			**Rate of Dissolution** (approx.)
	Boiling Point	**Flash Point**	**Toxicity**	
Methylene Dichloride	39.6 °C	None	Low	8 min
Tetrahydrofuran	66 °C	-14 °C	Moderate	26 min
Dioxane	101.1 °C	12 °C	Low	20 min
Chloroform	61.2 °C	None	Moderate	34 min

4.2. Identifying the Optimum Concentration of Spinning Dope

Visual inspection of the four solutions (5%, 15%, 30% and 40%) resulted in the elimination of the 40% solution because it had a high concentration of undissolved pellets and had too high a viscosity. The 5% solution on the other hand had too low a viscosity, which made it difficult to control the flow during extrusion. The 30% unfiltered solution contained large visible undissolved particles. This led to a higher viscosity and high shearing forces for the spinning dope, which led to the formation of crystals at the edges. All the solutions were evaluated along with 15% and 30% concentrations to determine the optimum level for solvent spinning.

4.2.1. Optical Microscopy

Based on the microscopic images of the filtered and unfiltered solutions, average particle size data has been tabulated in Table 4.2. It can be seen that the filtered solutions had an average particle size close to that on the control slide. This implies that most of the particles seen in the images of the filtered solutions were due to dust and contamination.

Table 4.2. Average Particle Size in Spinning Dopes

5% Unfiltered	26.30 μm	**5% Filtered**	18.65 μm
15% Unfiltered	32.12 μm	**15% Filtered**	16.31 μm
30% Unfiltered	33.57 μm	**30% Filtered**	19.87 μm
40% Unfiltered	36.36 μm	**Control Slide**	21.12 μm

Figure 4.1. (a-h) Optical Microscope Images [Magnification 20x]

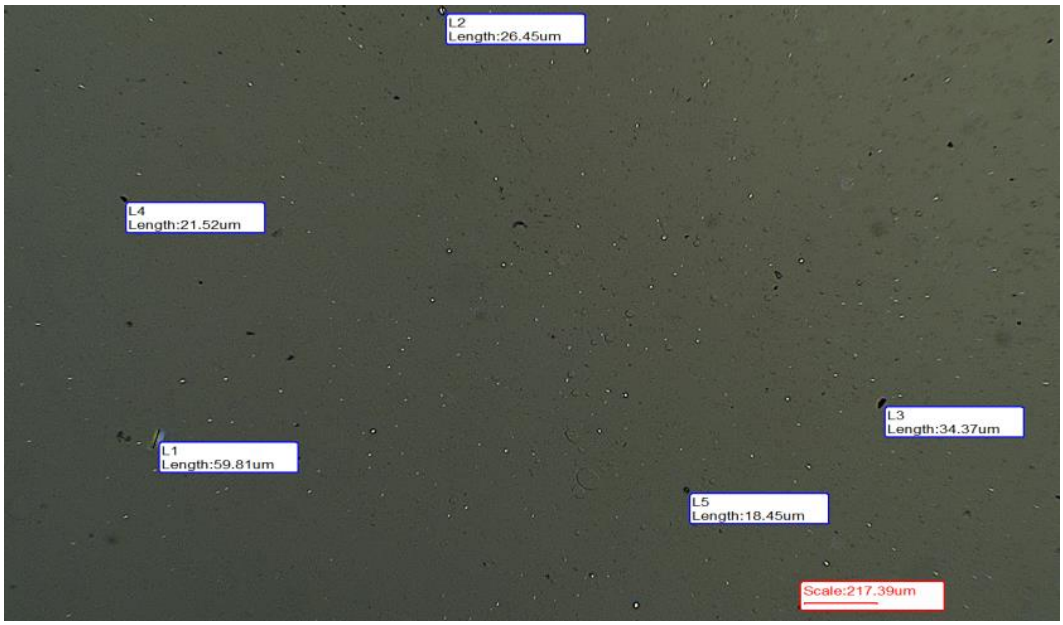

Figure 4.1.(a) Optical View of 5% Unfiltered Solution

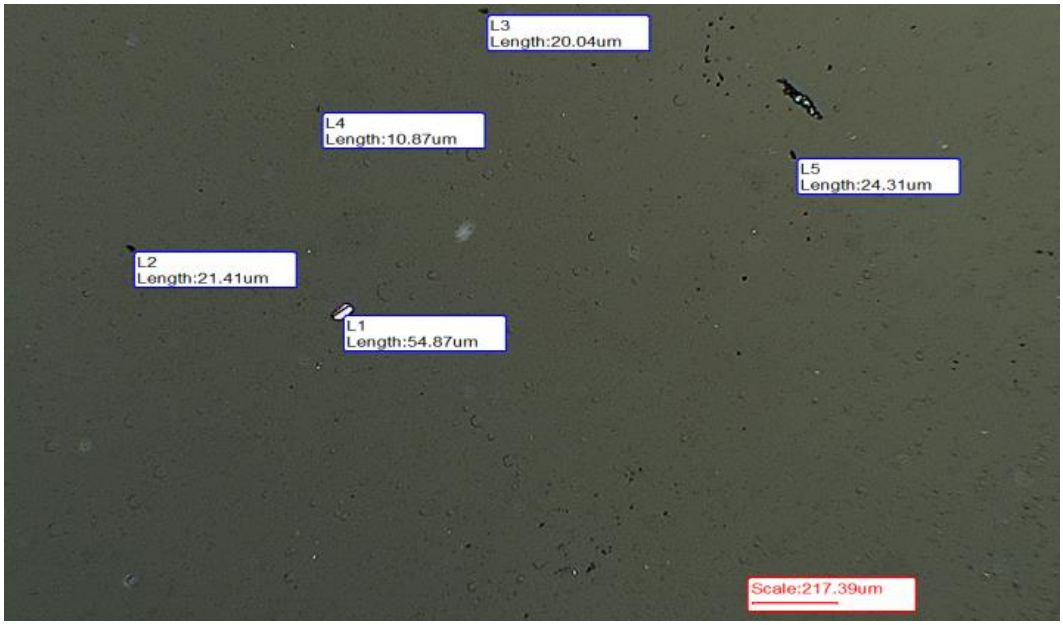

Figure 4.1.(b) Optical View of 5% Filtered Solution

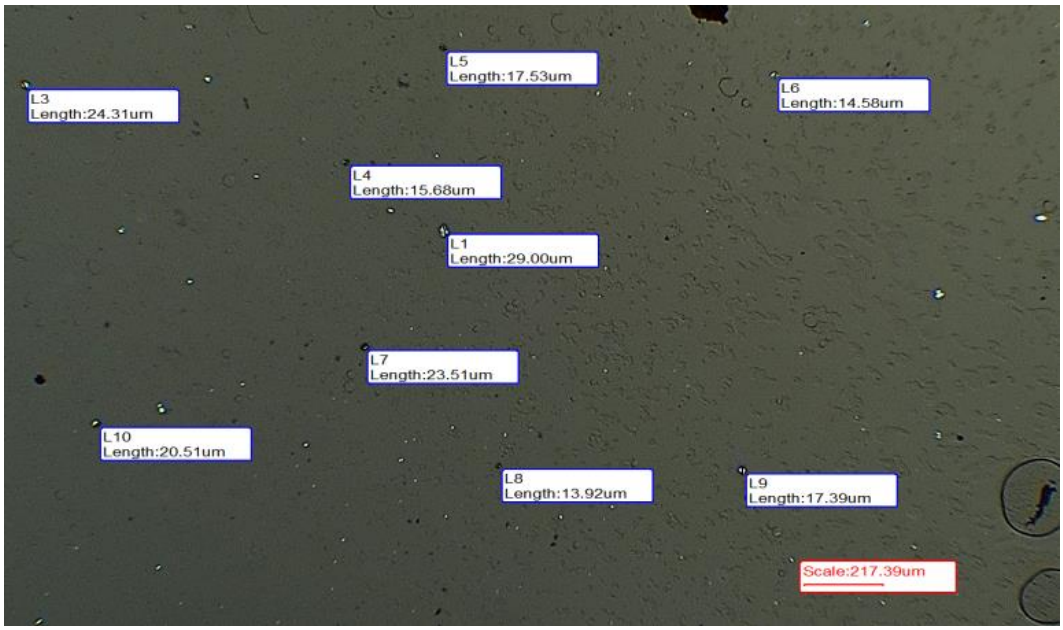

Figure 4.1.(c) Optical View of 15% Unfiltered Solution

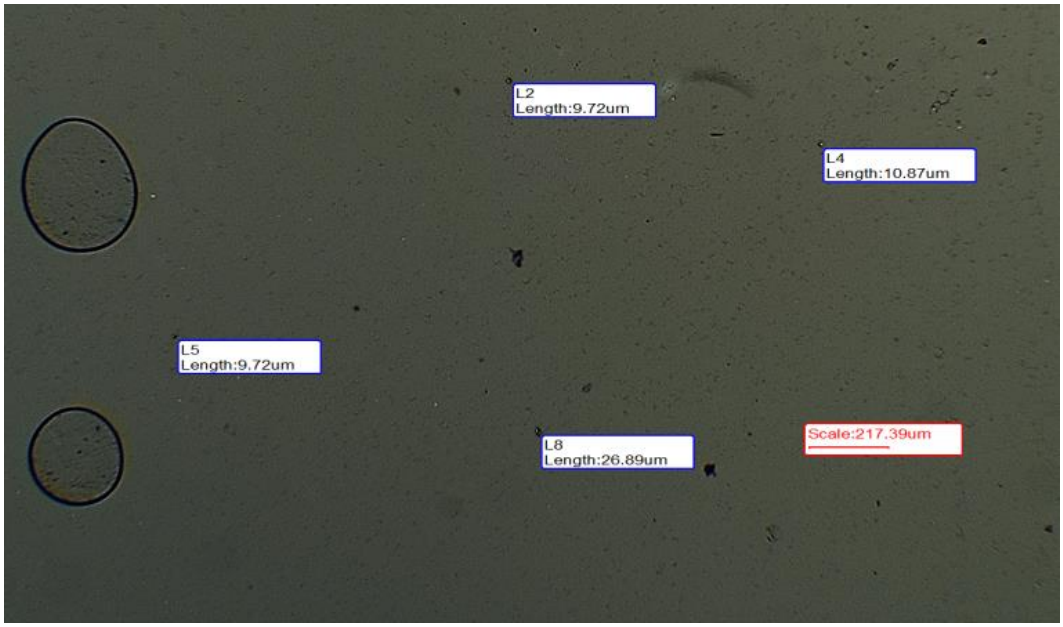

Figure 4.1.(d) Optical View of 15% Filtered Solution

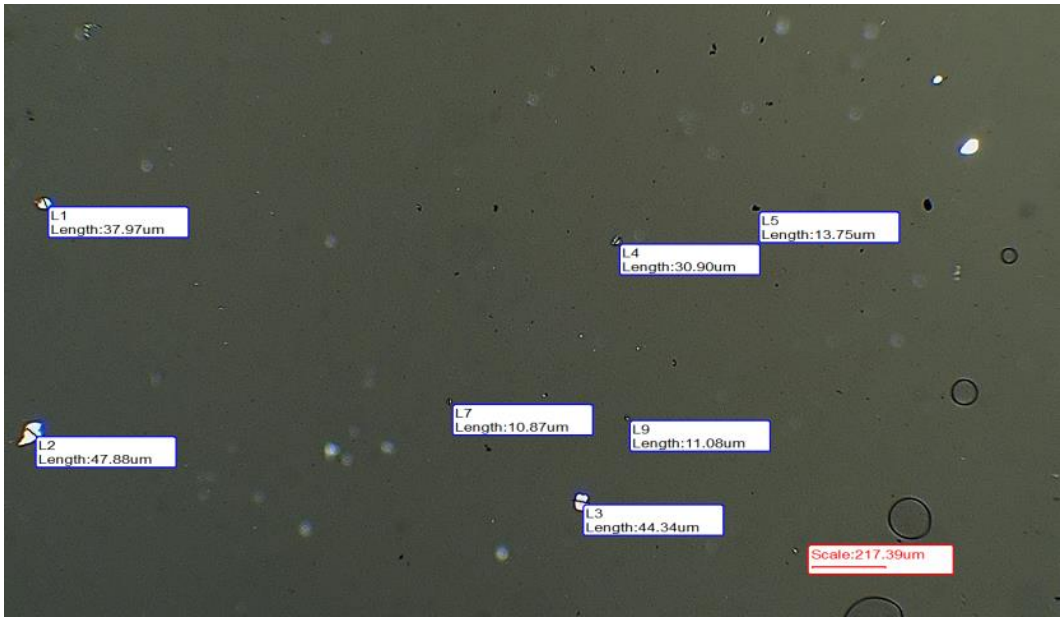

Figure 4.1.(e) Optical View of 30% Unfiltered Solution

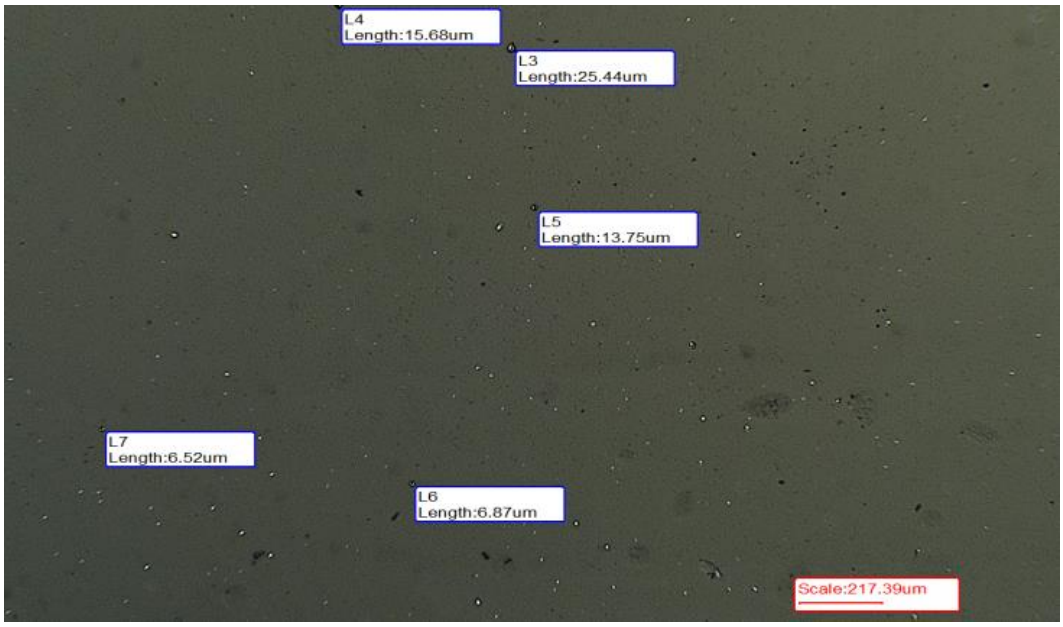

Figure 4.1.(f) Optical View of 30% Filtered Solution

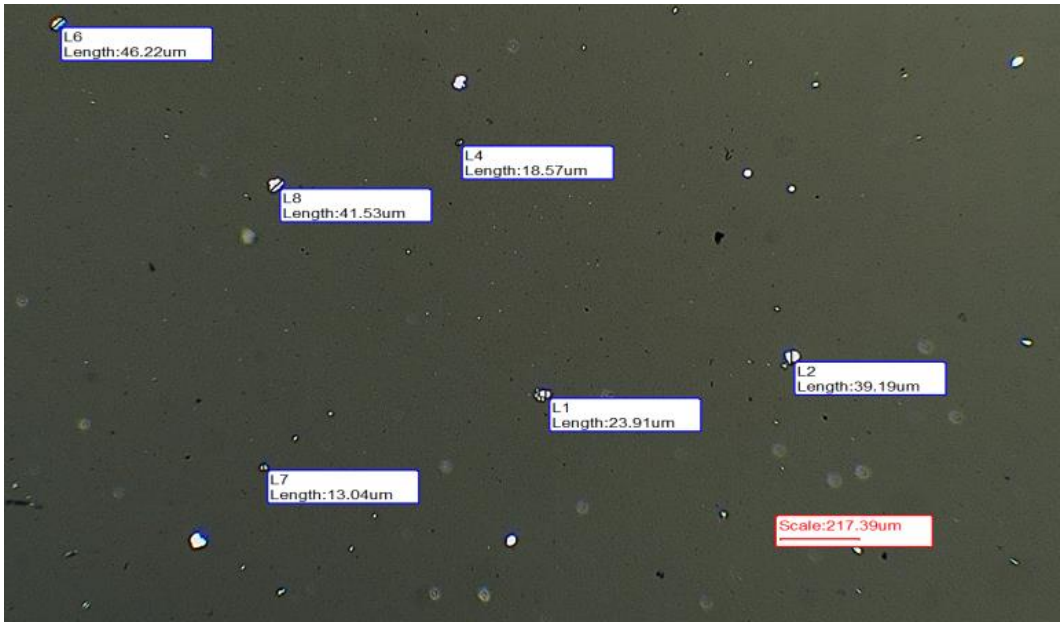

Figure 4.1.(g) Optical View of 40% Unfiltered Solution

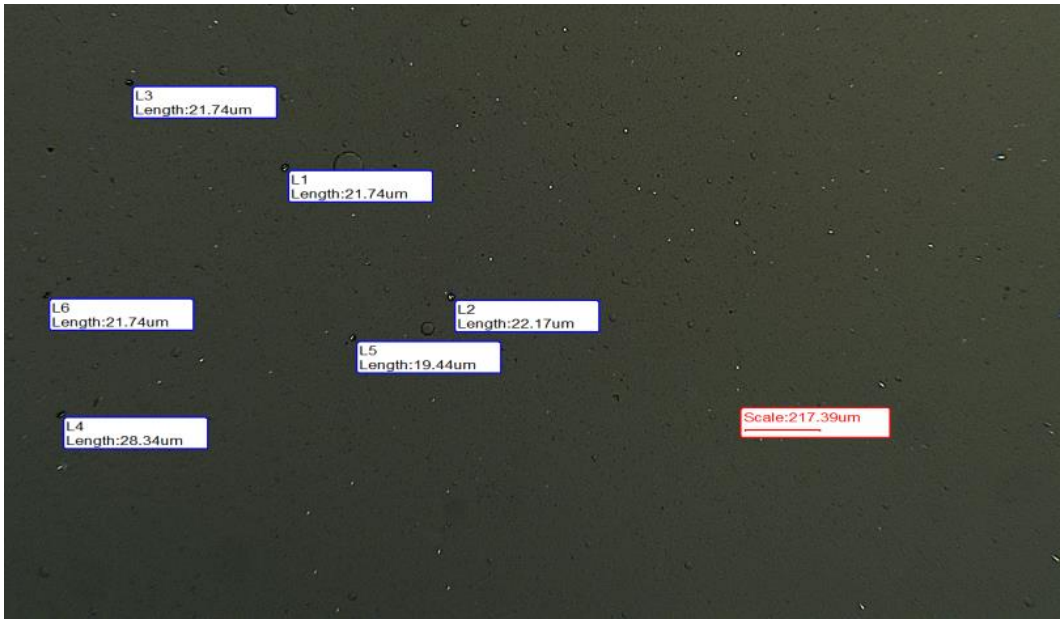

Figure 4.1.(h) Optical View of Control Slide

4.2.2. Gel Permeation Chromatography (GPC)

As seen below in Figure 4.2 (a), the bar graph of polydispersity index (PDI) indicates that the polymer is suitable for spinning as all of them have PDI values close to 2. The 5% and 15% filtered samples have a lower PDI as there were only a few undissolved particles. On the other hand, the 30% solution had a higher PDI for the filtered sample because of incomplete dissolution. This had led to a decrease in dissolved polymer concentration for the filtered dopes. Also, because the samples prepared in THF were filtered before testing by GPC, the concentration of polymer which was eventually present in the 30% filtered solution was very low.

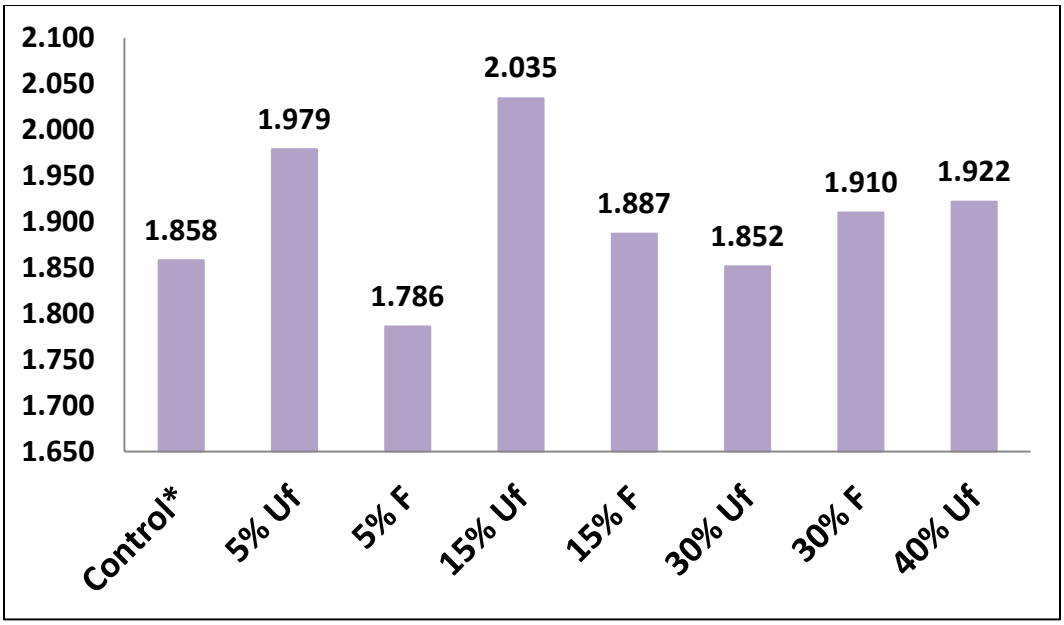

Figure 4.2. Polydispersity Index (PDI)

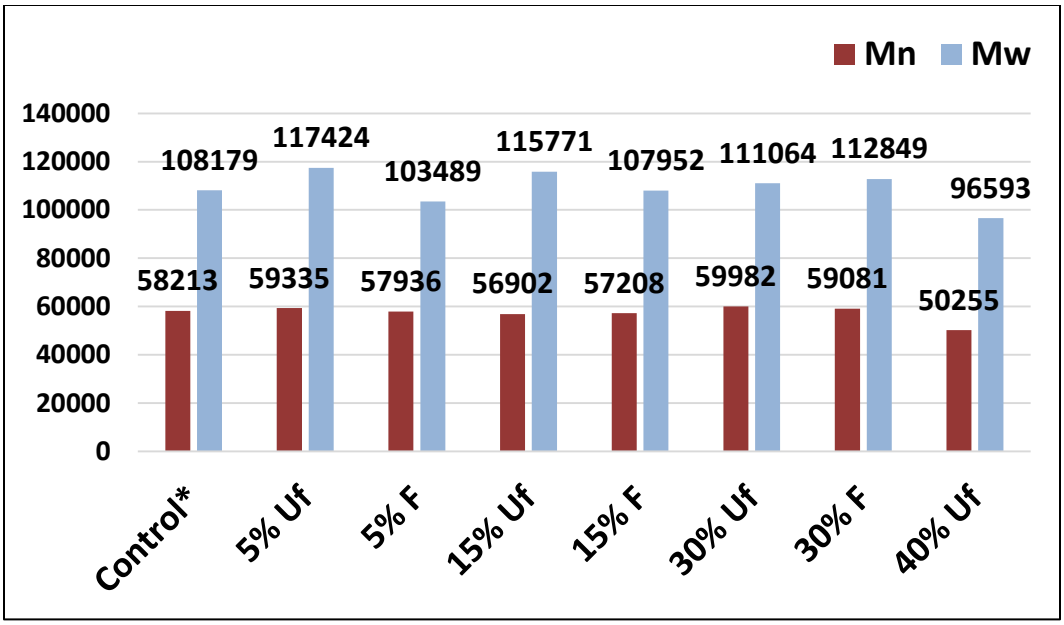

Figure 4.3. Weight and Number Average Molecular Weight Distributions

The molecular weight distribution in Figure 4.2 (b) is uniform. Mn and Mw values for the regenerated polymer did not differ significantly from the control sample. This implies that there was minimal degradation during polymer dissolution in methylene dichloride.

Hence, based on the results from optical microscopy and GPC, it can be concluded that 15% is an optimum concentration for solvent spinning of this particular P34HB polymer.

4.3. Determining the Thermal Properties of the Regenerated Polymer

4.3.1. Differential Scanning Calorimetry (DSC)

The DSC curves for the regenerated polymer at room temperature and at -25 °C were compared to the control polymer sample. They were also analyzed for their thermal properties, such as the glass transition temperature (T_g), the crystallization temperature (T_c) and the melting temperature(T_m). Calculations were made to estimate the percent crystallinity from the size of the melt endotherm peak and the crystallization endotherm peak using the following formula-

$$\% \, Crystallinity = \frac{[\Delta H_m - \Delta H_c]}{\Delta H_m^\circ} \times 100$$

Where,

ΔH_m : Heat of melting

ΔH_c : Heat of crystallization

ΔH_m° : Theoretical heat of melting for 100% crystalline P34HB, which is 120.6 J/g [37]

Figure 4.4. DSC Curves for Control Sample

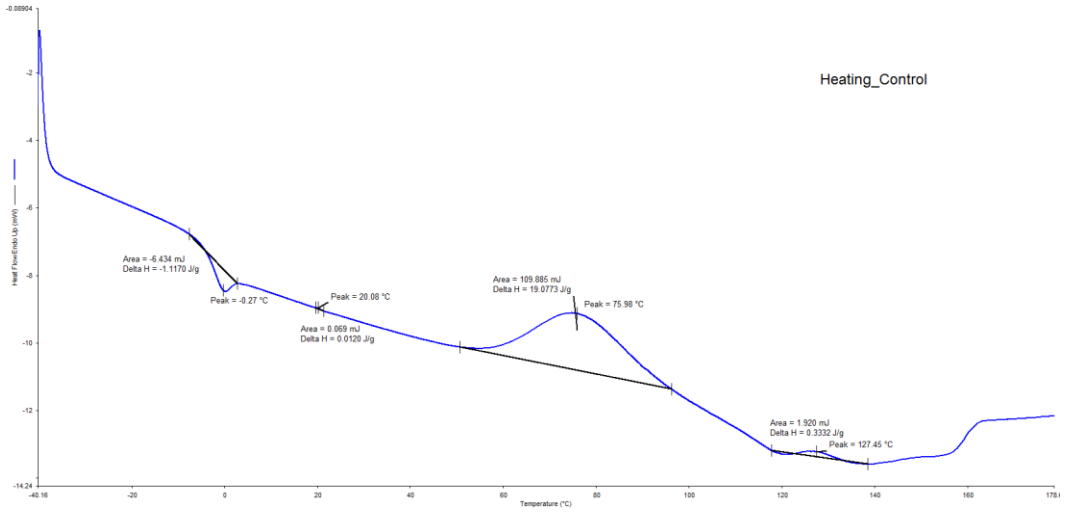

Figure 4.4.(a) Heating Curve for Control Sample

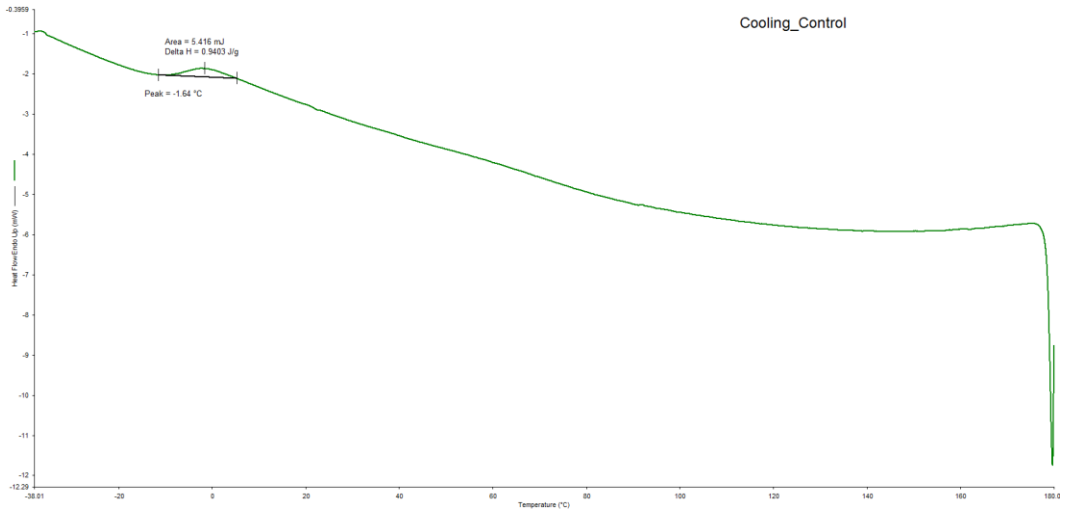

Figure 4.4. (b) Cooling Curve for Control Sample

As seen in the heating curve above, there are two visible peaks of T_c and T_m. This can be attributed to the polymer sample being a copolymer with a non-uniform distribution and unequal composition of the two monomers. The stark difference in the range of the enthalpy values for the two melting and crystallization peaks signifies a large difference in the relative composition of the monomers. The wide peaks can also be attributed to a difference in Mw and Mn values. As the DSC equipment had been calibrated to go to a low temperature of -50 °C, the T_g was not determined from this plot. Instead, the cooling curve provided us with a cooling crystallization peak which was in the same range as the heating crystallization endotherm peak.

Figure 4.5. DSC Curves for 5% Polymer Solution

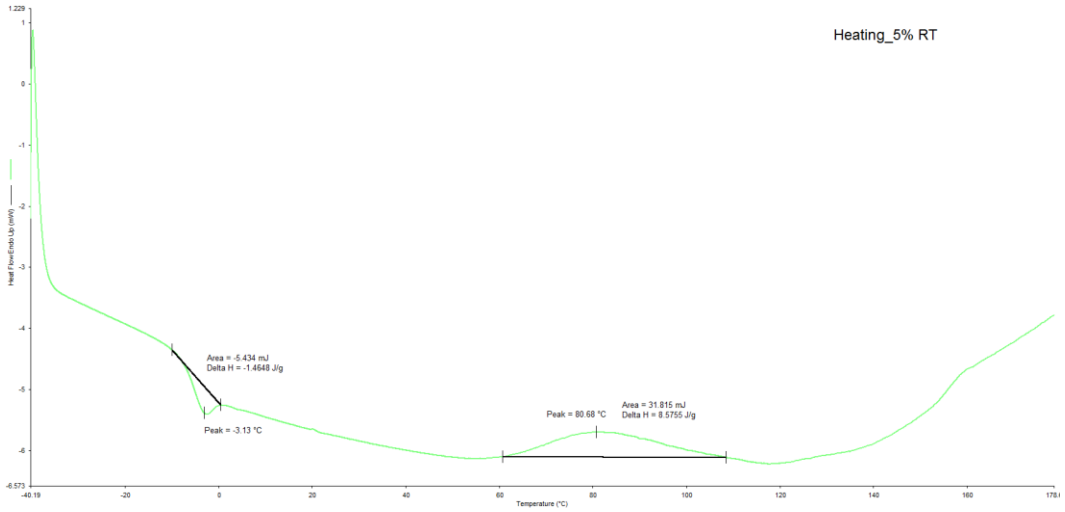

Figure 4.5 (a) Heating Curve for 5% Polymer Solution Regenerated at Room Temperature

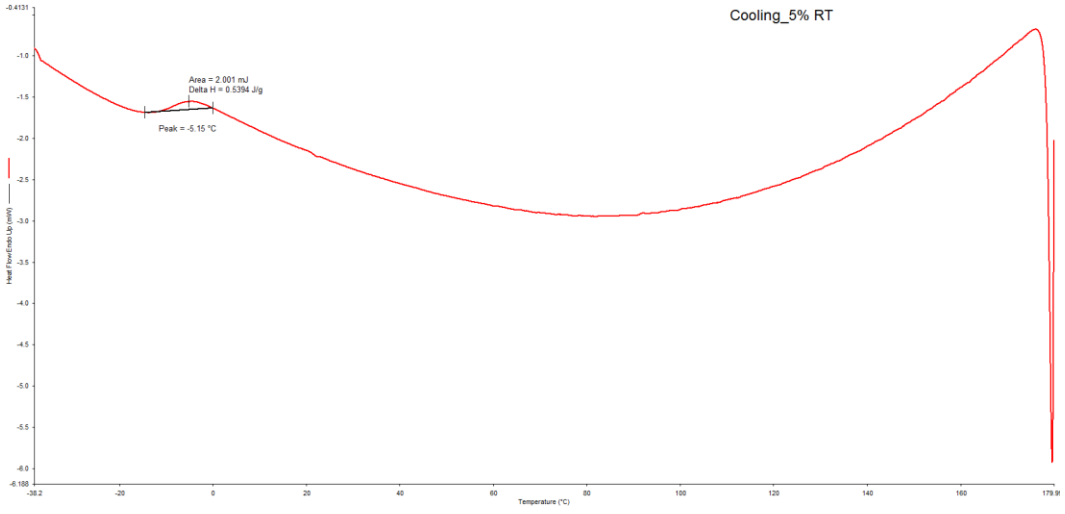

Figure 4.5. (b) Cooling Curve for 5% Polymer Solution Regenerated at Room Temperature

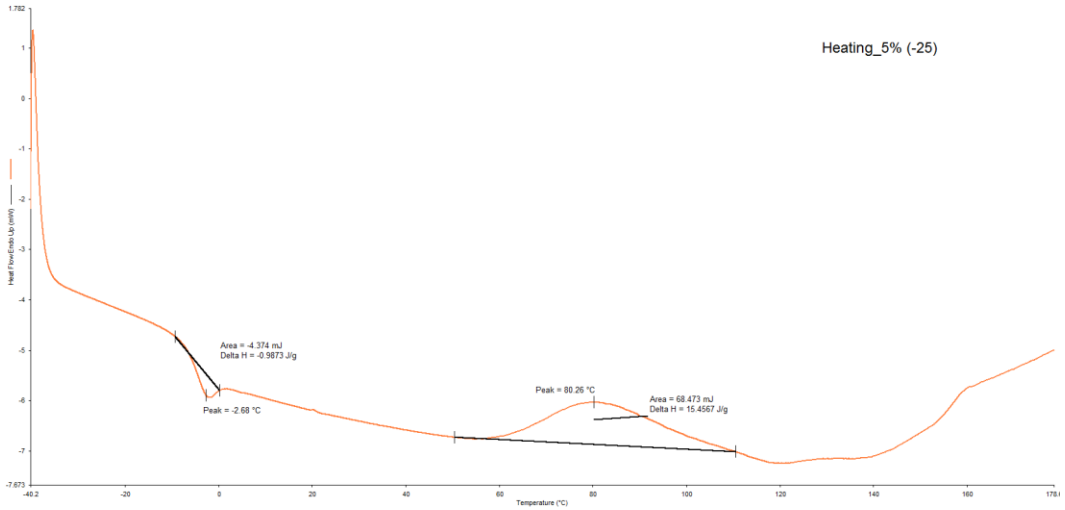

Figure 4.5. (c) Heating Curve for 5% Polymer Solution Regenerated at -25 °C

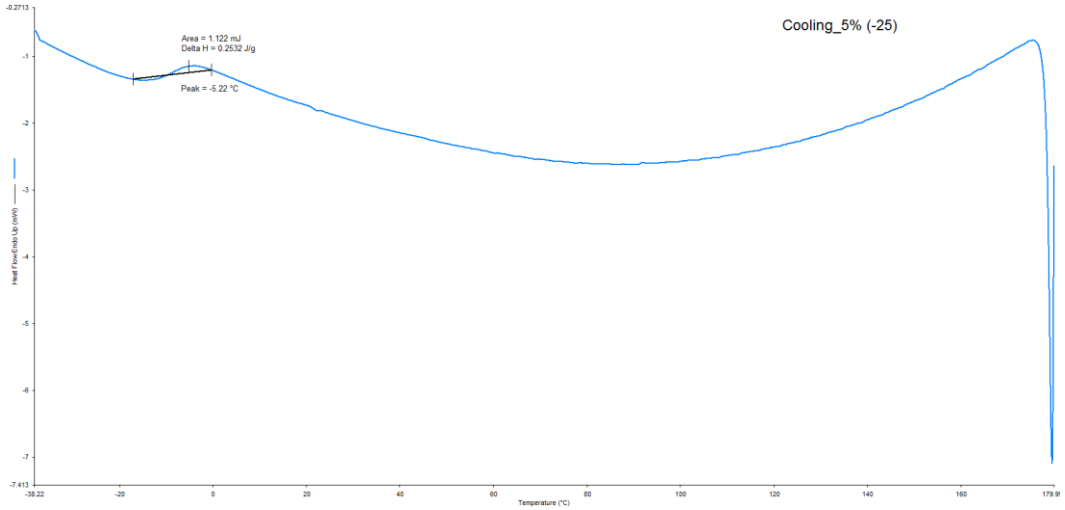

Figure 4.5. (d) Cooling Curve for 5% Polymer Solution Regenerated at -25 °C

The presence of only single endotherm peaks for crystallization and melting can be attributed to the low concentration of the polymer in the spinning dope. At 5%, the effect of the lower composition monomer is negligible. Also, with 5% being such a low concentration, the temperature of the coagulation bath has no significant effect on the regenerated polymer.

Figure 4.6. DSC Curves for 15% Polymer Solution

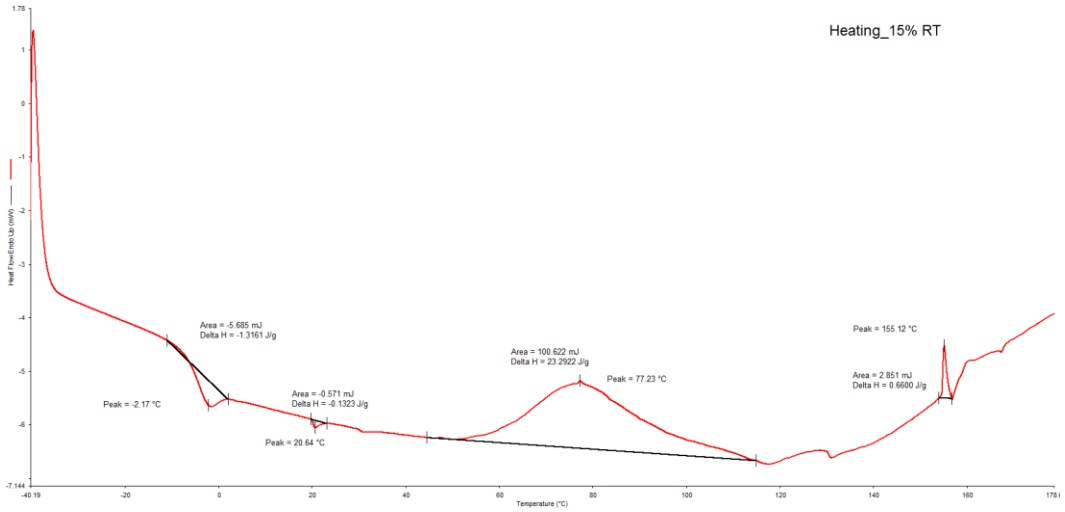

Figure 4.6.(a) Heating Curve for 15% Polymer Solution Regenerated at Room Temperature

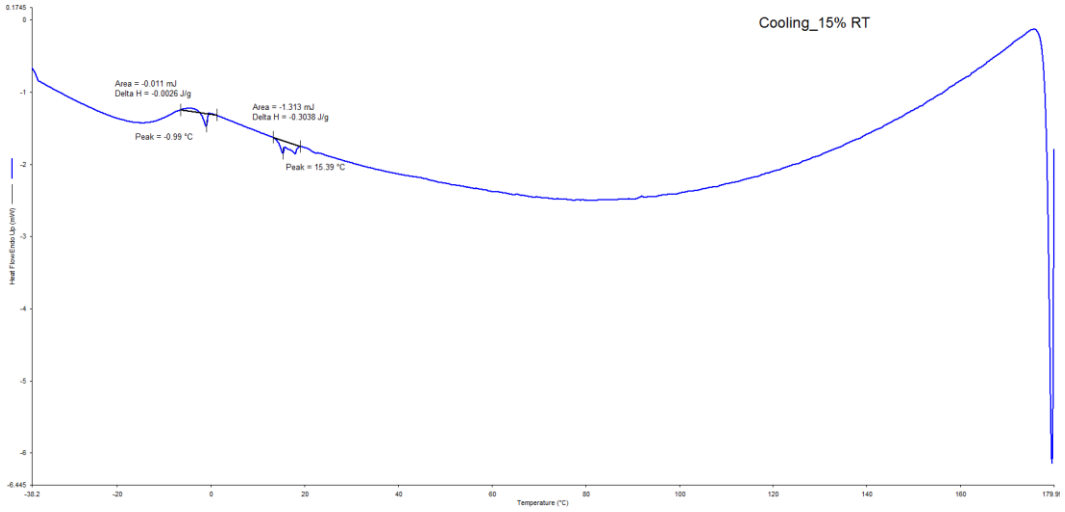

Figure 4.6.(b) Cooling Curve for 15% Polymer Solution Regenerated at Room Temperature

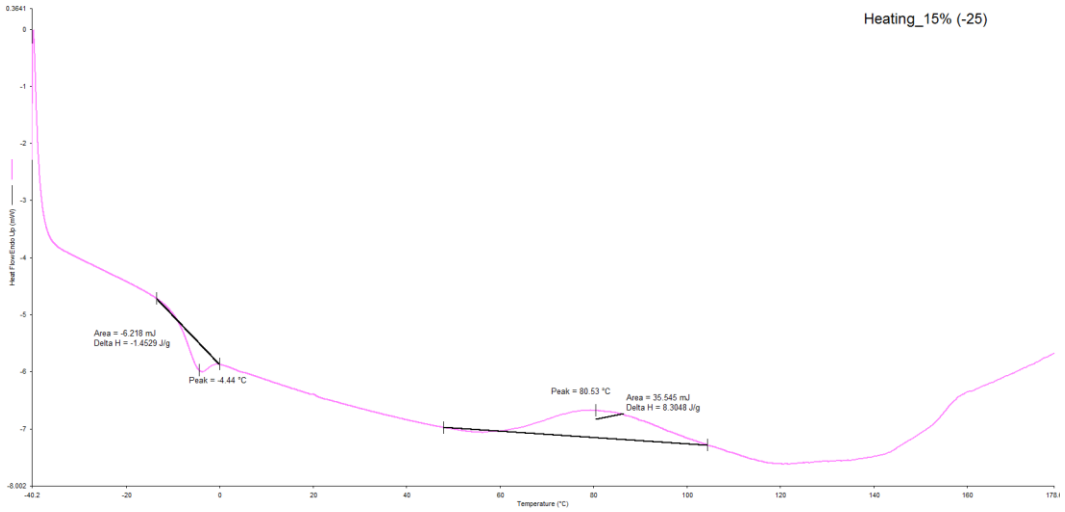

Figure 4.6.(c) Heating Curve for 15% Polymer Solution Regenerated at -25 °C

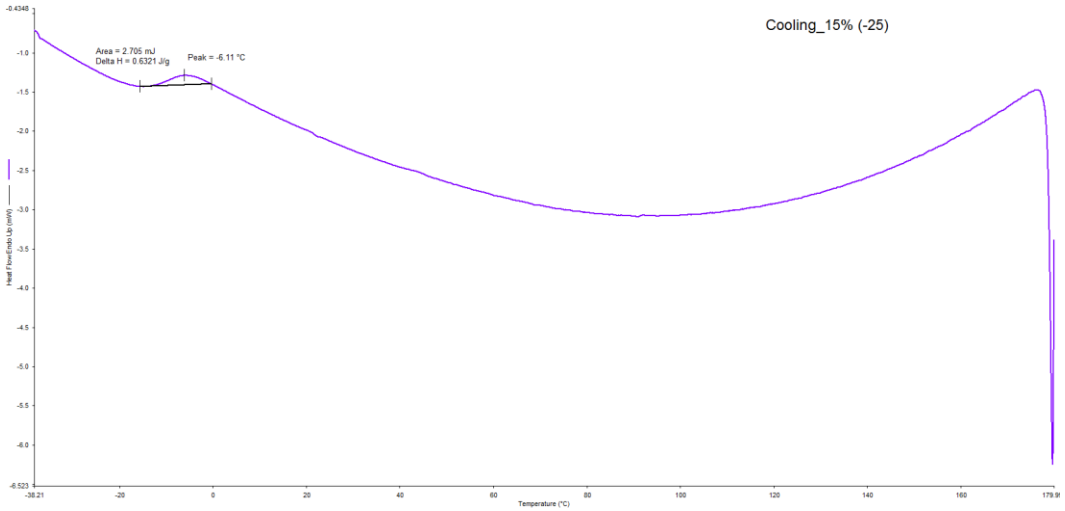

Figure 4.6.(d) Cooling Curve for 15% Polymer Solution Regenerated at -25 °C

The DSC curves of the 15% solution samples regenerated at room temperature were similar to those of the control P34HB polymer sample. There were two distinct endotherm peaks for both melting and crystallization of the samples regenerated at room temperature, while those regenerated at -25 °C they both had single peaks. This implies that the polymer regenerated at -25 °C had better chain distribution and uniformity. This is a significant finding for controlled release applications as the polymer will not have separate properties based on its monomeric composition. It will perform with predictably uniform release and rate of degradation.

Figure 4.7. DSC Curves for 30% Polymer Solution

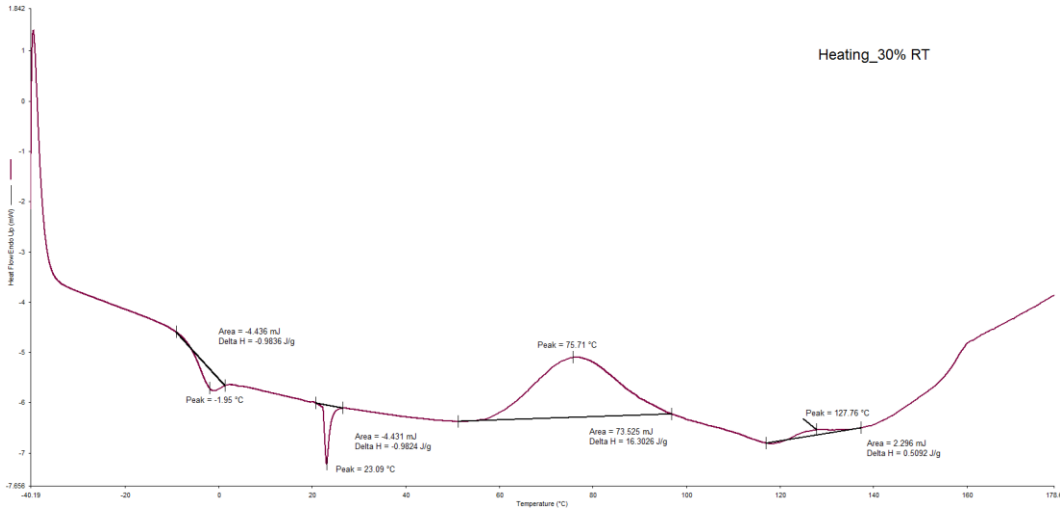

Figure 4.7.(a) Heating Curve for 30% Solution Regenerated at Room Temperature

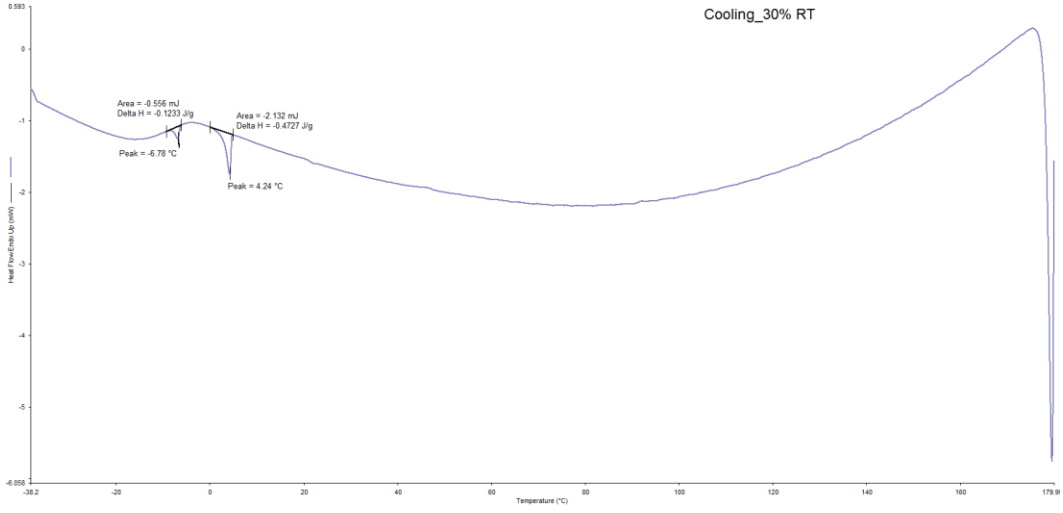

Figure 4.7.(b) Cooling Curve for 30% Solution Regenerated at Room Temperature

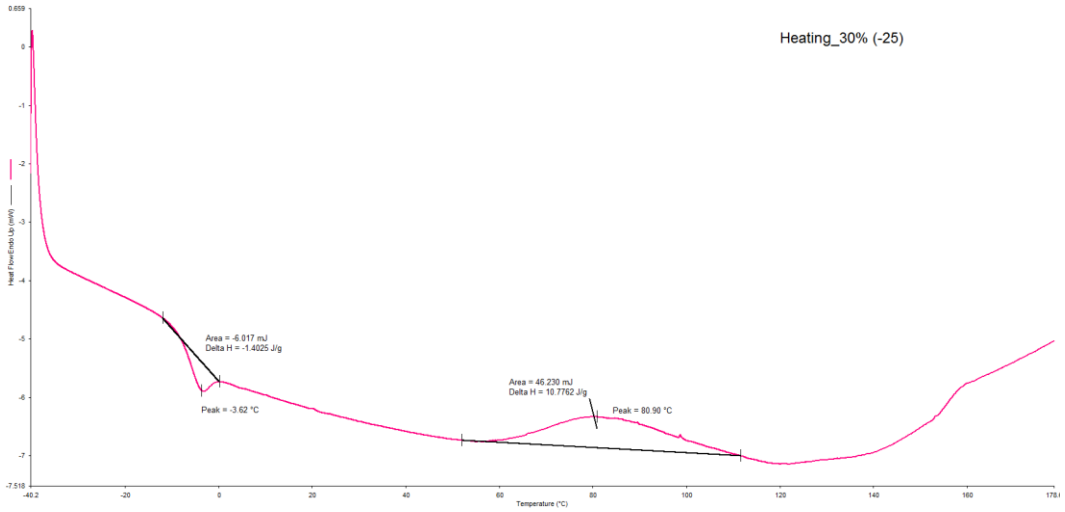

Figure 4.7.(c) Heating Curve for 30% Solution Regenerated at -25 °C

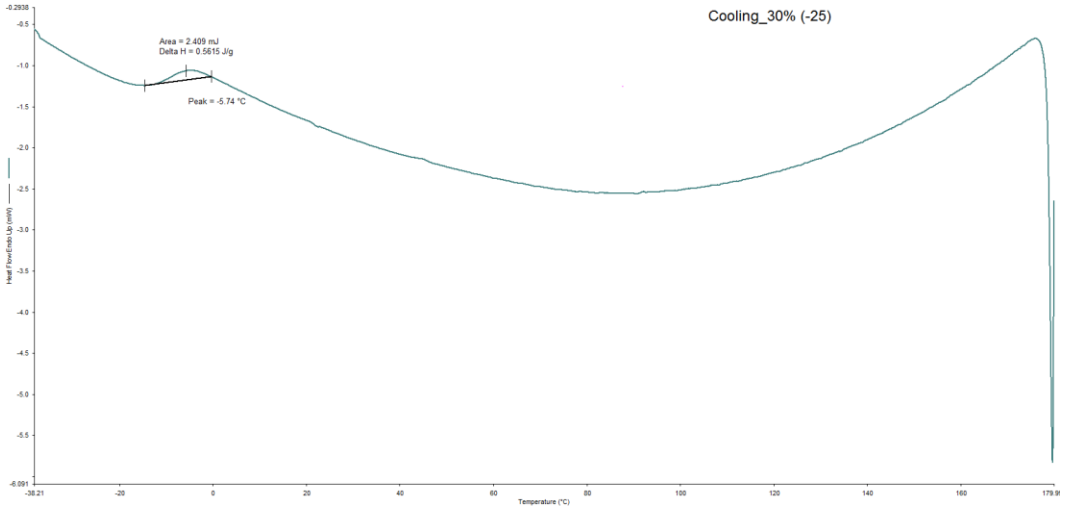

Figure 4.7.(d) Cooling Curve for 30% Solution Regenerated at -25 °C

The DSC curves for the 30% samples can be explained in the same way as for the 15% samples in Figure 4.5. It can be seen that the samples regenerated at -25 °C have a more uniform curve and a single endotherm peak for both melting and crystallization. From these findings it can be concluded that the optimum concentration for P34HB spinning dope should be either 15% or 30%, and the temperature for regeneration should be -25 °C.

Table 4.3. Percent Crystallinity of Polymer Samples

Sample	Percent Crystallinity
Control	16.8 %
5% (Room Temperature)	8.3 %
5% (-25 °C)	13.6 %
15% (Room Temperature)	20.4 %
15% (-25 °C)	8.1 %
30% (Room Temperature)	14.3 %
30% (-25 °C)	10.1 %

The values of % crystallinity as calculated above for polymer films are remarkably similar to those seen in the research article by Krzysztof Moraczewski [33]. As the extrusion process imparts better orientation, these values are expected to increase for filaments. Additionally, as the overall crystallinity is not too high for the polymer itself, there is scope for carrying out drawing of the filaments which would further enhance the alignment of polymer chains in the filaments.

4.3.2. Thermal Gravimetric Analysis (TGA)

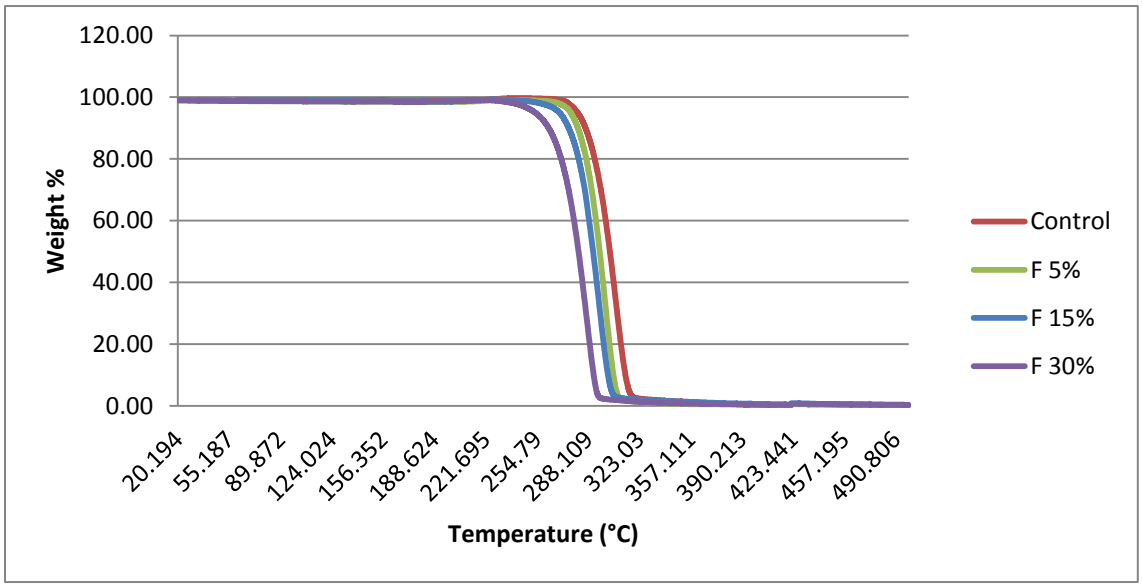

Figure 4.8. TGA Curves of Control, 5%, 15% and 30% Samples

As can be seen from Figure 4.7, there was no significant change in the degradation profiles of the regenerated samples compared to the original control polymer. This supports the finding of the GPC tests which also confirmed that the dissolution process did not increase the level of polymer degradation. Additionally, since the test was performed under nitrogen and the final weight % was close to zero, it can be inferred there were no inorganic components present in the control polymer sample. Also, the regenerated samples did not contain any residual chemicals from the dissolution process, which suggests that this solvent system would be suitable for medical applications such as controlled delivery of drugs and growth factors.

CHAPTER 5

CONCLUSIONS AND FUTURE SCOPE

5.1. Conclusions

As mentioned in Chapter 1, the goal of this study was to study the feasibility of a solvent spinning process for bacterial polyester by developing a process for solvent cast polymer films. Based on this goal, three specific aims were established in order to achieve the broad objective of the study. The results in Chapter 4 have successfully fulfilled the three aims. They are summarized as follows:

1. The four solvents: tetrahydrofuran, methylene dichloride, chloroform and dioxane, were compared and it was found that methylene dichloride was the most suitable solvent for the particular type of bacterial polyester sample we were working with.

2. The optimum concentration for the solvent cast was identified as a 15% solution. This was because the 5% solution had too low a viscosity, while the 30% solution had a reasonably high viscosity with quite a few undissolved polymer particles. The 40% solution was eliminated due to its high viscosity and poor dissolution performance.

3. The adequate temperature of the coagulation bath was determined at -25 °C based on the results from the DSC tests. The heating curves had single peaks for the

crystallization and melt endotherms which indicated uniform distribution of the polymer chains.

5.2. Future Scope

Based on our ultimate goal for this study, and as specified in Chapter 1, the results obtained so far have pointed to two recommendations for future work.

1. To translate this process from polymer films into continuous filaments in the laboratory. This can be carried out using syringe needles and a big glass coagulation bath. The filaments will need to be tested for their mechanical and thermal properties.

2. Convert the monofilament process into a pilot scale process for continuous multifilament extrusion. The larger scale process will require optimizing the process developed previously in the lab to make it suitable for bigger, faster and wider equipment.

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