SYNTHESIS, CHARACTERIZATION AND OPTIMIZATION OF NANOCOMPOSITE SCAFFOLD USING POLY LACTIDE/MULTI-WALLED CARBON NANOTUBES

by

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Thesis submitted in fulfillment of the requirements

for the degree of

Master of Science

May 2011

ACKNOWLEDGEMENT

In the name of Allah, the Most Beneficent, the Most Merciful.

I wish to express my gratitude to my beloved parents, brother and sister for their encouragement, support and patience throughout my life and education.

I would like to express my sincere and profound appreciation to my dedicated supervisor, Assoc. Prof. Dr. Sharif Hussein Sharif Zein for his excellent, generous and continuous guidance throughout my research. There are no words to articulate how grateful I am to have had the honour and pleasure to work under his supervision. His professionalism, experience, advice, constructive criticism and unconditional support have provided me opportunity to be success in my project. Besides, I would also like to extend my gratitude to my co-supervisor, Prof. Abdul Latif Ahmad. In addition I would like to thank my field supervisors, Dr. Tan soon Huat and Assoc. Prof. Dr. Hazizan Md Akil for their effort spent in guiding me throughout my study.

I would like to thank the administrative staff of School of Chemical Engineering at Universiti Sains Malaysia, especially our respected Dean, Prof. Azlina Harun @ Kamaruddin, Deputy Dean and all the technicians for their support during my research. I would also like to thank the financial support (Research Assistantship) during my research which was provided by the Universiti Sains Malaysia.

I would like to acknowledge and thank all of my friends, Mr.Rezaei, Mr.Mazaheri, Mr.Hassani and others for their help, support and significant contributions during my study.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ASTM	American Society of Testing and Materials
ATR-IR	Attenuated total reflectance infrared
β-BL	β- butyrolactone
CCD	Central composite design
CVD	chemical vapor decomposition
CNTs	Carbon nanotubes
CTE	Coefficient of thermal expansion
DOE	Design of experiments
DSC	Differential scanning calorimeter
DWNT	double-wall nanotubes
DXO	1,5-dioxepan-2-one
ε-CL	ε-caprolactone
FTIR	Fourier-transform infrared spectroscopy
F-value	Fisher's F value
KBr	Potassium bromide
MWCNTs	Multiwall carbon nanotubes
PCL	Poly (ε-caprolactone)
PBS	Phosphate buffer saline
PBPs	Petroleum-based polymers
PDI	polydispersity index
PE	Polyethylene
PEG	Poly(ethylene glycol)

PEO	Poly(ethylene oxide)
PP	Polypropylene
PS	Polystyrene
PGA	Polyglycolide or Polyglycolic acid
PLA	Polylactide
PLLA	Poly (L-lactide acid)
P(LLA-co-CL)	Poly (lactic-co- ε-caprolactone)
PLGA	Poly (lactic-co-glycolic acid)
PMMA	Poly(methyl methacrylate)
P-value	Probability value
ROP	Ring-opening polymerization
RSM	Response surface methodology
$Sn(Oct)_2$	Stannous octoate
SD	Standard deviations
SEM	Scanning electron microscope
SWCNTs	single-wall carbon nanotubes
TGA	Thermogravimetric analysis

LIST OF SYMBOLS

g	gram
h	Hour
Hz	Hertz
KPa	Kilo Pascal
Mn	Number average molecular weight
M_s	mass of swollen scaffold
M_d	dry scaffold
Mw	Weight average molecular weight
M	Molar
MPa	Mega Pascal
min	Minute
nm	nanometer
Tc	Cold crystallization temperature
T_g	Glass transition temperature
Tm	Melting temperature
V	volume of the obtained scaffold
V_p	volume of polymer in scaffold
wt%	Weight percentage
µm	Micron meter
°C	Degree Centigrade
W_d	surplus weight of scaffold after degradation
W_i	initial weight of scaffold before degradation

SINTESIS, PENCIRIAN DAN OPTIMASI SCAFFOLD KOMPOSIT NANO MENGGUNAKAN LAKTIDA POLI LAKTIDE/ NANO TIUB KARBON BERDINDING LAPISAN

ABSTRAK

Poli (L-laktide) (PLLA) scaffold telah banyak digunakan dalam kejuruteraan tisu dalam rangka untuk memperbaharui kulit, tulang, tulang rawan, ikatan sendi dan lain-lain. PLLA mempunyai kelebihan kebolehuraian biologi, kadar penguraian terkawal, sifat haba yang baik dan kesesuaian biologi. Ia dapat dihasilkan dari sumber yang diperbaharui, dan ia tidak beracun bagi manusia dan persekitaran. Walau bagaimanapun, sebahagian besar daripada scaffold tiga dimensi (3D) dibuat dari PLLA secara relatif memiliki sifat mekanik lemah dan mereka tidak mampu memenuhi keperluan untuk aplikasi tertentu. Sebuah kaedah umum untuk meningkatkan sifat mekanik sesuatu matrik polimer adalah dengan menggabungkan pengisi ke dalam polimer sebagai agen penguat. Nano Tiub karbon bermacam dinding (TNKBD) dianggap agen penguat unggul kerana sifat mereka yang unik. Gabungan TNKBD dalam matrik polimer mengarah kepada pembaikan sifat polimer yang luar biasa. Oleh itu, tujuan projek ini adalah untuk menyelidik sintesis, pencirian dan optimasi poli (L-laktida)/ nano tiub karbon bermacam dinding (PLLA / TNKBD) baru scaffold berliang disediakan oleh kaedah pengekstrakan-beku untuk aplikasi kejuruteraan tisu. Beberapa teknik pencirian seperti mikroskop imbasan elektron (SEM), analisis termogravimetri (TGA), calorimeter imbasan perbezaan (DSC) dan analisis spektroskopi Fourier-transform inframerah (FTIR) digunakan untuk menilai sifat morfologi, haba, struktur dan mekanikal scaffold . Scaffold yang diperoleh menunjukkan penyebaran yang baik dan struktur berliang yang saling

bersambungan dengan lebih daripada 80% keliangan dan saiz liang rata-rata sekitar 40 μm tersebar dalam saiz antara 50 dan 150 μm. Sebagai hasil dari interaksi antara muka yang tinggi antara PLLA dan TNKBD, scaffold telah mempamerkan peningkatan luar biasa pada sifat mekanik seperti modulus, kekuatan dan rentangan. Kajian penguraian invitro terhadap scaffold dinilai dengan merendam scaffold dalam penyangga fosfat salin (PBS) sehingga 24 minggu. Didapati bahawa penggabungan TNKBD dalam scaffold PLLA telah menurunkan kadar penguraian invitro. Dalam rangka untuk memiliki proses pembelajaran yang sistematik, corak eksperimen (DOE) perkakasan lembut disatukan dengan metodologi permukaan tindakbalas (RSM) dan corak komposit pusat (CCD) digunakan untuk menyelidik hubungan modulus, kekuatan dan rentangan scaffold dengan proses yang berbeza parameter, yang kemudiannya digunakan untuk proses pengoptimuman. Berdasarkan pengoptimuman tindakbalas bermacam, keadaan optimum untuk memiliki modulus maksimum (229.71 MPa), kekuatan (60.52 MPa) dan rentangan(10.72%) secara serentak diperoleh dengan penguatan 3.93% berat dari TNKBD, 157.62 ml kandungan pelarut, 5.10 jam tempoh pembekuan dan 2.81 hari tempoh rendaman.

SYNTHESIS, CHARACTERIZATION AND OPTIMIZATION OF NANOCOMPOSITE SCAFFOLD USING POLY LACTIDE/MULTI-WALLED CARBON NANOTUBES

ABSTRACT

Poly(L-lactide) (PLLA) scaffolds have been widely used in tissue engineering in order to regenerate the skin, bone, cartilage, ligament and etc. PLLA has the advantages of biodegradability, a controllable degradation rate, good thermal properties and biocompatibility. It can be produced from renewable resources, and it is nontoxic to humans and the environment. However, most of the three dimensional (3D) scaffolds made by PLLA have relatively poor mechanical properties and they are unable to meet the requirements for certain applications. A common method of improving the mechanical properties of a polymer matrix is to incorporate fillers into the polymer as a reinforcement agent. Multi-walled carbon nanotubes (MWCNTs) are considered to be an ideal reinforcing agent due to their unique properties. The incorporation of MWCNTs in a polymer matrix leads to remarkably improved properties of the polymer. Therefore, the aim of this project was to investigate the synthesis, characterization and optimization of the novel poly(L-lactide)/multi-walled carbon nanotube (PLLA/MWCNT) porous scaffolds prepared by the freeze-extraction method for tissue engineering application. Several characterization techniques such as scanning electron microscopy (SEM), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy analysis (FTIR) were used to evaluate the morphological, thermal, structural and mechanical properties of the scaffolds. The obtained scaffolds showed well-distributed and interconnected porous structures with more than 80% porosity

and median pore size around 40 μm distributed within a region between 50 and 150 μm in size. As a result of high interfacial interaction between PLLA and the MWCNTs, the scaffolds exhibited remarkable improvements in mechanical properties such as modulus, strength and elongation. *In vitro* degradation studies of the scaffolds were assessed by immersing the scaffolds in phosphate buffered saline (PBS) for up to 24 weeks. It was found that the incorporation of MWCNTs in PLLA scaffolds decreased the rate of *in vitro* degradation. In order to have a systematic process study, design of experiment (DOE) software coupled with response surface methodology (RSM) and central composite design (CCD) was used to investigate the relation of the modulus, strength and elongation of the scaffolds with different process parameters, which were then used for the optimization process. Based on the multi responses optimization, the optimum conditions for having the maximum modulus (229.71 MPa), strength (60.52 MPa) and elongation (10.72 %) simultaneously was obtained with reinforcing 3.93 wt % of MWCNTs, 157.62 ml solvent content, 5.10 hr freezing hours and 2.81 days immersing time.

CHAPTER 1

INTRODUCTION

1.1 Introduction

In the swiftly growing field of tissue engineering, novel biomaterials are being intensely examined. The use of polymeric biomaterials started in the 1940s during the Second World War (Castner and Ratner, 2002). Recent advances in polymeric biomaterials have been focused on tissue engineering towards solving problems of patients who have suffered tissue and organ loss or imperfection (Hu *et al.*, 2003). This is an indispensable step toward the application of scaffolds in tissue engineering. The development of polymeric biomaterials can be considered as an evolutionary improvement. A biomaterial is defined as a material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body (Williams, 1999). Reports on the applications of natural polymers as biomaterials date back thousands of years (Barbucci, 2002). Polymeric biomaterials are relatively easy to manufacture into products with various shapes, at reasonable cost, and with desirable mechanical and physical properties. However, the application of synthetic polymers to medicine is more or less a recent phenomenon.

Tissue engineering has emerged in the last decade of the 20th century as an alternative approach to circumvent the existent limitations in the current therapies. Many applications of tissue engineering which was intensively studied and reported include blood vessels (Vaz *et al.*, 2005), heart valves, bone, skin (Chen *et al.*, 2005) cartilage regeneration (Cohen *et al.*, 2003), nerves, liver and other organ systems

(Morita *et al.*, 2002). Other potential applications of tissue engineering include the replacement of worn and poorly functioning tissues; replacement of small caliber arteries, veins, coronary, and peripheral stents; replacement of the bladder and fallopian tube; and restoration of cells to produce necessary enzymes, hormones, and other bioactive products (Lanza *et al.*, 2007).

The principle of tissue engineering involves fabrication of new and functional living tissue using living cells, which are usually associated, in one way or another, with a matrix or scaffolding to guide tissue development. Thereby, tissue engineering has the potential to produce a supply of immunologically tolerant 'artificial' organ and tissue substitutes that can grow with the patient. This should lead to a permanent solution to the damaged organ or tissue without the need for supplementary therapies, thus making it a cost-effective treatment in the long term (Patrick Jr *et al.*, 1998; Hutmacher, 2000). Hence, many natural and synthetic polymeric biomaterials and their hybrid matrices have been developed to be used in tissue engineering applications (Agrawal and Ray, 2001; Li and Tuan, 2005 ; Smith *et al.*, 2009).

Bio based and biodegradable materials are the most well-known terms among the researchers in the world nowadays due to their sustainability and biodegradability (Stevens, 2002). The term "bio based polymers" refers to naturally occurring polymeric materials and/or natural substances that can be polymerized into high molecular weight polymers (Sudesh and Iwata, 2008). Therefore bio based polymers include biopolymers and their associated blends and composites and synthetic polymers made from renewable sources (Sudesh and Iwata, 2008). The term biodegradability refers to natural degradation in the surrounding environment. It should be noted that not all bio based polymers are biodegradable and vice-versa.

Polymeric scaffolds are often designed as temporary structures having the desired physical, chemical, and mechanical properties required for implantation. The use of degradable polymers is desirable because the need for surgical removal is obviated; however, care must be taken to ensure the compatibility of both intermediate and final degradation products, the timing of the degradation process, and how each of these affects the regenerative process. The rate and mechanism of degradation (surface erosion or bulk) will impact the mechanical properties of the scaffold: bulk eroding polymers maintain their physical structure until the molar mass of the polymer is sufficiently low for polymer dissolution in the aqueous surroundings, at which point there is a precipitous loss of mechanical properties; surface eroding polymers lose their shape and mechanical properties gradually over time. For both degradation mechanisms, the regenerative process will inevitably be negatively impacted if the degradation products are toxic to the tissue that has formed and/or if the integrity of the scaffold is lost prior to new tissue formation and integration with the host. This narrows the selection of polymers to those that degrade at rates slow enough for cell integration and tissue growth and to those that produce only biocompatible degradation products (Shoichet, 2009).

The development of synthetic biopolymers has benefited the design and development of three-dimensional (3D) templates or scaffolds that reinforce the tissue and in some cases, organize regenerating tissue for tissue-engineered products (Kim and Mooney, 1998; Mano *et al.*, 1999). Moreover, unlike natural biodegradable polymers, synthetic biopolymers can be easily mass-produced (Middleton and Tipton, 2000). Besides, polymeric scaffolds require high porosity with interconnected pores and desirable chemical properties (Madihally and Matthew, 1999). On the other hand, polymers have undoubtedly improved our lifestyle because

of their wide range of properties available at low cost and hence versatility in applications. It is unacceptable to avoid the use of polymeric materials and hence the need arises for the low cost, renewable resource polymeric materials which can provide the properties of a commodity polymer while minimizing any detrimental effects on the environment.

Biodegradable synthetic polymers (BSPs), such as poly (lactic acids)/ poly(L-lactide) (PLLA) can play a significant role in the commodity area if they possess desired qualities. PLA and its copolymers are part of a diverse group of poly(α-hydroxy acid)s used in biomedical applications since the 1970's. While initially studied for packaging and agricultural applications (Kricheldorf *et al.*, 1996; Sinclair, 1996) these polymers are nowadays mostly used in the biomedical and pharmaceutical industries as controlled drug delivery systems, as well as in the veterinary and agrochemical fields. The aliphatic polyesters such as PLLA are versatile biomaterials due to their biodegradability and biocompatibility (Moon *et al.*, 2000; Moon *et al.*, 2001; Shinoda *et al.*, 2003). PLLA is synthetic biodegradable polymer, which can be used as a pharmaceutical and biomedical material for drug delivery systems and tissue regeneration (Thomson *et al.*, 1995; Ikada and Tsuji, 2000).

The most frequently investigated and widely employed polymer in 3D scaffolds is PLLA (Zhou *et al.*, 2005; Gong *et al.*, 2007; Raghunath *et al.*, 2007; Gui-Bo *et al.*, 2010). PLLA has the advantages of biodegradability, a controllable degradation rate, good mechanical properties and biocompatibility. It can be produced from renewable resources, and it is nontoxic to humans and the environment (Sodergard and Stolt, 2002; Auras *et al.*, 2004). Porous scaffolds of PLLA have been widely used in tissue engineering to guide the regeneration of skin

(Zacchi *et al.*, 1998), bone (Ishaug *et al.*, 1997), cartilage (Li and Tuan, 2005) and ligament (Lin *et al.*, 1999).

Several techniques have been reported to produce polymeric porous scaffolds for tissue engineering such as solvent casting, particulate leaching (Reignier and Huneault, 2006), gas foaming (Nam *et al.*, 2000), micro-fabrication, pressure-activated microsyringing (PAM) (Mariani *et al.*, 2006), gravity spinning (Williamson *et al.*, 2006), 3D micro-printing (Hutmacher *et al.*, 2001), electro-spinning (Murphy and Mikos, 2007) and the freeze-extraction method (Ho *et al.*, 2004). Among these techniques, freeze-extraction is the most common, low-cost and high-yield technique with saving time and energy. This method is the easiest technique to scale up synthesising porous scaffolds with interconnected pore networks (Yang *et al.*, 2004). The unique advantage of this method lies in capability of designing 3D nanostructures by well-designed procedure without using any special equipment and porosity of the scaffold can be easily controlled or modified by this method (Ho *et. al.*, 2004).

The physical aspects of scaffold design, as with polymer choice, depend largely on the final application. The scaffold is meant to provide the appropriate chemical, physical, and mechanical properties required for cell survival and tissue formation. Essentially, the polymeric scaffold is designed to define the cellular microenvironment (cell niche) required for optimal function (Madlambayan *et al.*, 2005; Madlambayan *et al.*, 2006). Understanding the series of stimuli provided during development and/or healing is the guide to which tissue engineers most often turn when designing a scaffold. Typically, the scaffold is a 3D open-cell, interconnected porous structure, allowing facile communication between the biological cells dispersed in the scaffold. Depending on the intended use, these

structures are also conducive to cell proliferation, migration, and/or differentiation. The stimuli that define the cellular microenvironment include the chemical, physical, and mechanical properties of the scaffold as well as other cells and signaling molecules incorporated into the scaffold design. The 3D of the scaffold is key to its use in tissue engineering, where a 3D cell construct is meant to integrate into a 3D tissue. Determining the appropriate physical structure of the polymeric scaffold requires an understanding of the tissue into which it is being implanted. For example, polymeric scaffolds designed for implantation into the spinal cord have included elaborate designs of the gray and white matter tracts (Moore *et al.*, 2006) while implantations into bone have imitated the porosity of trabecular bone (Holy *et al.*, 2003).

The mechanical properties of the scaffold are dictated by the tissue into which it is implanted. For example, hard tissue, such as bone, necessitates a stiff polymeric scaffold (Ruhé *et al.*, 2005) whereas a soft tissue, such as nerve, requires a malleable polymeric scaffold (Belkas *et al.*, 2005; Katayama *et al.*, 2006; Clements *et al.*, 2009) and an elastomeric tissue, such as skin (or blood vessel), demands a flexible polymeric scaffold (Guan *et al.*, 2005; Guan and Wagner, 2005). In addition to the mechanical properties, the tissue engineered scaffold is designed for enhanced cell penetration and 3-dimensional tissue formation. This has been achieved by incorporating pores or cell-cleavable groups within the scaffold design. For many years, pores were introduced into scaffolds by a variety of processes involving salt leaching (Lu *et al.*, 2000; Lu *et al.*, 2000) phase inversion (Holy *et al.*, 1999; Holy *et al.*, 2000) and high-pressure gasification (Riddle and Mooney, 2004).

Today we understand that the mechanical properties of the scaffold can influence cell proliferation thus attention has refocused on the design of the biomaterial. Most

of the engineered biomaterial scaffolds are polymeric, and thus the opportunity to design polymers for applications in medicine is great. Importantly, our concept of a scaffold includes both the 3-dimensional traditional geometrically defined construct and the newer injectable material, which does not provide a distinct macroscopic architecture but still provides a controlled microenvironment for the cells. It is this microenvironment which is a key determinant of success and is comprised of cell interactions with other cells, soluble or matrix-bound growth factors and adhesion molecules, and the biomaterial itself through mechanical and chemical stimulus. The underlying strategy for the future is to understand the tissue sufficiently to design a polymeric biomaterial with the appropriate properties for success, whether the application is in vitro or in vivo degradation (Shoichet, 2009).

1.2 Problem Statement

Scaffolds with various porous structures, porosities, pore size and pore interconnectivity can be tailor-made. However, most of the scaffolds have relatively poor mechanical properties and are unable to meet the requirements for certain applications such as tissue engineering, integrity handling, implantation and tissue support during healing (Zhang and Ma, 1999; Zhang and Zhang, 2001; Kothapalli *et al.*, 2005). Hence, there is a need to fabricate 3D polymer scaffolds with improved mechanical properties. (Zhou, Gong et al., 2005). A common method of improving the mechanical properties of a polymer matrix is to incorporate fillers into the polymer as a reinforcement agent. The incorporation of nanometer-sized carbon nanotubes (CNTs) in a polymer matrix leads to remarkably improved properties of the polymer. Multi-walled carbon nanotubes (MWCNTs) are considered to be the ideal reinforcing agent due to their unique properties. CNTs are one of the most

promising candidates for the design of novel polymer composites (Spitalsky *et al.*, 2009). Polymer/CNTs composites could be used as scaffold materials for tissue engineering and bone cell proliferation (Zanello *et al.*, 2006). MWCNTs possess high mechanical strength, thermal conductivity and extraordinary optical properties (Kim *et al.*, 2007). They can be used in the reinforcement of fibers, as atomic force microscopy tips and in nanocomposites as well as in biomedical systems and devices such as sutures, orthopaedic fixation devices and tissue-engineering scaffolds (MacDonald *et al.*, 2005; Abarrategi *et al.*, 2008).

1.3 Objectives of research

The porous structural scaffolds with a suitable pore size, porosity and biodegradability and reinforced mechanical and thermal properties need to be synthesized for tissue engineering applications. Therefore, the objectives of this research were:

- To produce PLLA and PLLA/MWCNT scaffolds by incorporation of MWCNTs into the PLLA as a reinforcement agent.
- To characterize the synthesized PLLA and PLLA/MWCNT scaffolds.
- To study the biodegradability of the PLLA and PLLA/MWCNT scaffolds.
- To optimize the production of the PLA and PLLA/MWCNT scaffolds and evaluate the effect of different parameters using response surface methodology (RSM) by design of experiment (DOE) software.

1.4 Outline of the thesis

This thesis is organized into five chapters:

Chapter 1, commenced with some basic information on the definition of biomaterials, biodegradable polymers and MWCNTs followed by a brief introduction on the overview of the biodegradable polymers application in tissue engineering. The concerned issues, which generated the ideas and inputs for this research work, were also elaborated upon. The primary objectives and the general flow of the research program were also outlined.

Chapter 2, relates some background and classification on engineering polymeric biomaterials for biomedical applications and tissue engineering. Explications on the functions, requirements and available synthesis methods of scaffolds production with special focus on the interpenetrating PLLA as biodegradable polymer with MWCNTs by freeze extraction method and the applications of resulted scaffolds were also provided. Subsequently, a literature review was done on various published works on PLLA and MWCNTs based composite biomaterials and scaffolds for tissue engineering and biomedical applications particularly those that are closely related to this work. Finlay we have discussed about some statistical optimization methods which has been used in this work.

Chapter 3, details the experimental procedures employed in this research. Descriptions of lab equipments used as well as any other processing techniques utilized in generating any data that were used and presented in the research are reported.

Chapter 4, is actually the results and discussion chapter according to the research objectives. This chapter describes the synthesis and characterization of PLLA and PLLA/MWCNT porous scaffolds prepared by the freeze-extraction method. In addition, investigates the optimum condition of scaffolds production based on DOE and finally in vitro degradation studies of the scaffolds were assessed by immersing the scaffolds in phosphate buffered saline (PBS) for up to 24 weeks.

Chapter 5, provides a summary of the results obtained in this research and presents concluding remarks on the present work and also recommendations for future studies.

CHAPTER 2

LITERATURE REVIEW

2.1 Polylactide/poly(lactic acid)

2.1.1 Introduction

Polylactide or poly(lactic acid) (PLA) is a biodegradable aliphatic polyester known for biomedical and pharmaceutical applications and ecological benefits (Tsuji *et al.*, 2002). PLA can be produced from renewable resources such as starch and possess some comparable properties to petroleum-based polymers (PBPs) such as polyethylene, polypropylene and polystyrene yet is generally inferior as a semi-crystalline engineering thermoplastic (Tsuji *et al.*, 2002). The properties of PLA can be controlled by the type of lactic acid (L or D enantiomers) used, extent of branching, and length of the polymer chain (Kamm *et al.*, 2006). In general, PLA can be synthesized by the condensation of lactic acid, or by the ring-opening polymerization of lactide, a cyclic dimer of lactic acid (Tsuji *et al.*, 2002). Properties such as thermal, hydrolytic stability and rate of biodegradation can be modified by altering molecular characteristics such crystallinity and can be achieved in variety of ways including copolymerization, blending, and addition of additives. In addition to medical and pharmaceutical applications, PLA is showing great potential for commodity applications because of the recent decrease in the cost of its production and the increase in cost and instability of fossil feedstock.

The optically active lactic acid (2-hydroxy propionic acid) has two enantiomeric L and D- (S- and R-) forms. The isolation of lactic acid was reported back in 1780 (Holton *et al.*, 1971), however, the linear dimer (lactoyl lactic acid) from lactic acid was first reported in 1845 by Pelouze (Holton, *et al.*, 1971).

Carothers et al. (Carothers *et al.*, 1932) reported the two-step synthesis of high molecular PLA from the cyclic dimer (lactide) of lactic acid. The cyclic dimerization of lactic acids proceeds by condensation resulting in three different lactides. The chemical structure of lactic acid in two different optically active forms and its conversion in to L-, D-, and meso-lactides are illustrated in Figure 2.1 (Holton, *et al.*, 1971). The melting temperature of the lactic acids is 17 °C. The melting temperature of L- and D-lactides (97 °C) is higher than the meso-lactide (53 °C).

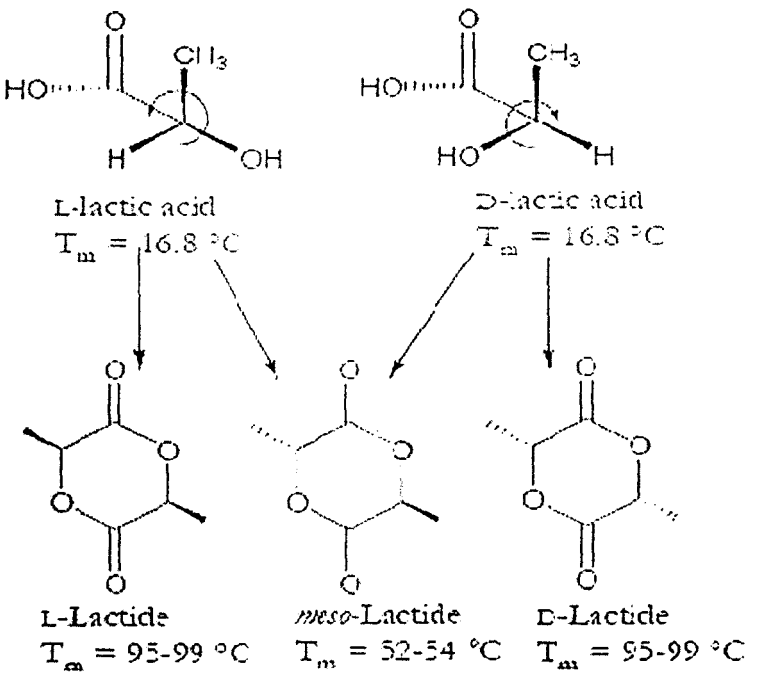

Figure 2. 1 Cyclic dimerization by condensation of L-, and D-lactic acids into L-, D-, and *meso*-lactides.

In addition to PLA, other aliphatic polyesters which have good mechanical properties, hydrolyzability, and biocompatibility are derived from glycolide (GA), β-butyrolactone (β-BL), ε-caprolactone (ε-CL), and 1,5-dioxepan-2-one (DXO) (Albertsson and Varma, 2003). Figure 2.2 illustrates the common lactones and their resultant polymer upon ring-opening polymerization. These polymers have shown

remarkable potential, both as homopolymers and their copolymers. Copolymerization has been used to improve the mechanical, hydrophilic, and biodegradation properties of these polymers by utilizing various architectures such as linear random and block copolymers along with complex architectures such as stars, brushes, cyclic, cross-linked, hyperbranched aliphatic polyesters (Choi *et al.*, 1998; Finne and Albertsson, 2002; Albertsson and Varma, 2003).

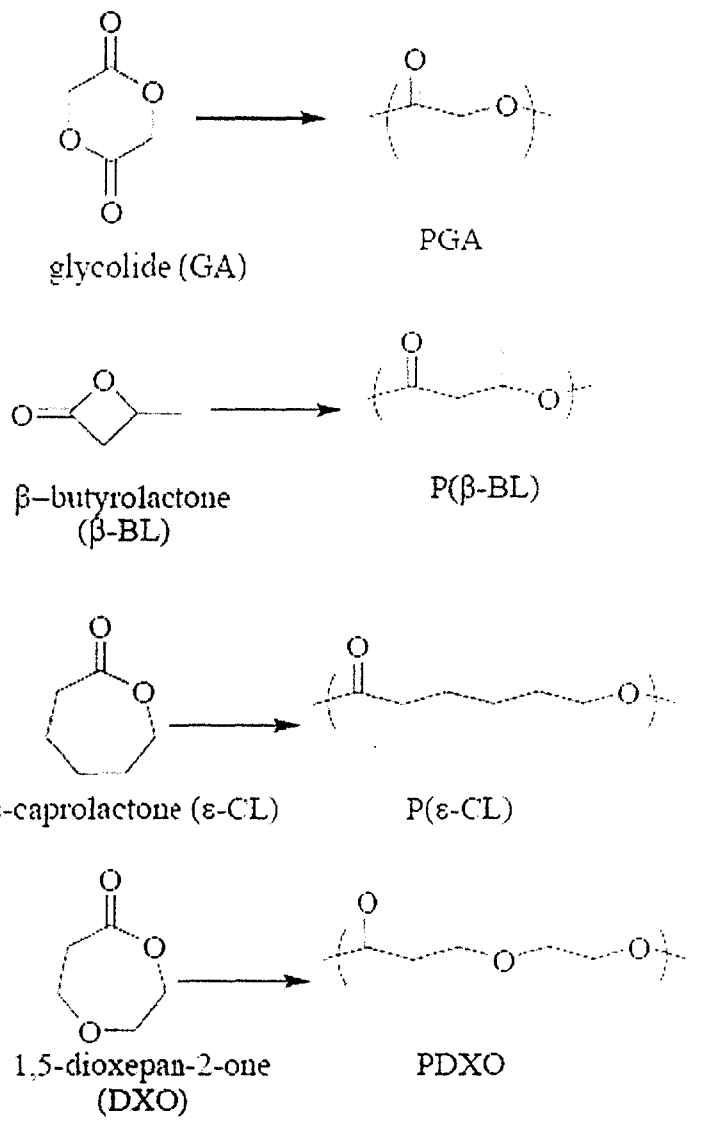

Figure 2. 2 Molecular structures of other lactones and aliphatic polyesters

2.1.2 Synthesis, Mechanisms, and Commercial Production

2.1.2.1 Synthesis

PLA polymer can be prepared by the direct condensation of lactic acid or by the ring-opening polymerization (ROP) of lactide, the cyclic dimer of lactic acid. Figure 2.3 shows a schematic of lactic acid production from starch and illustrates the synthesis of PLA by direct condensation and ring-opening routes.

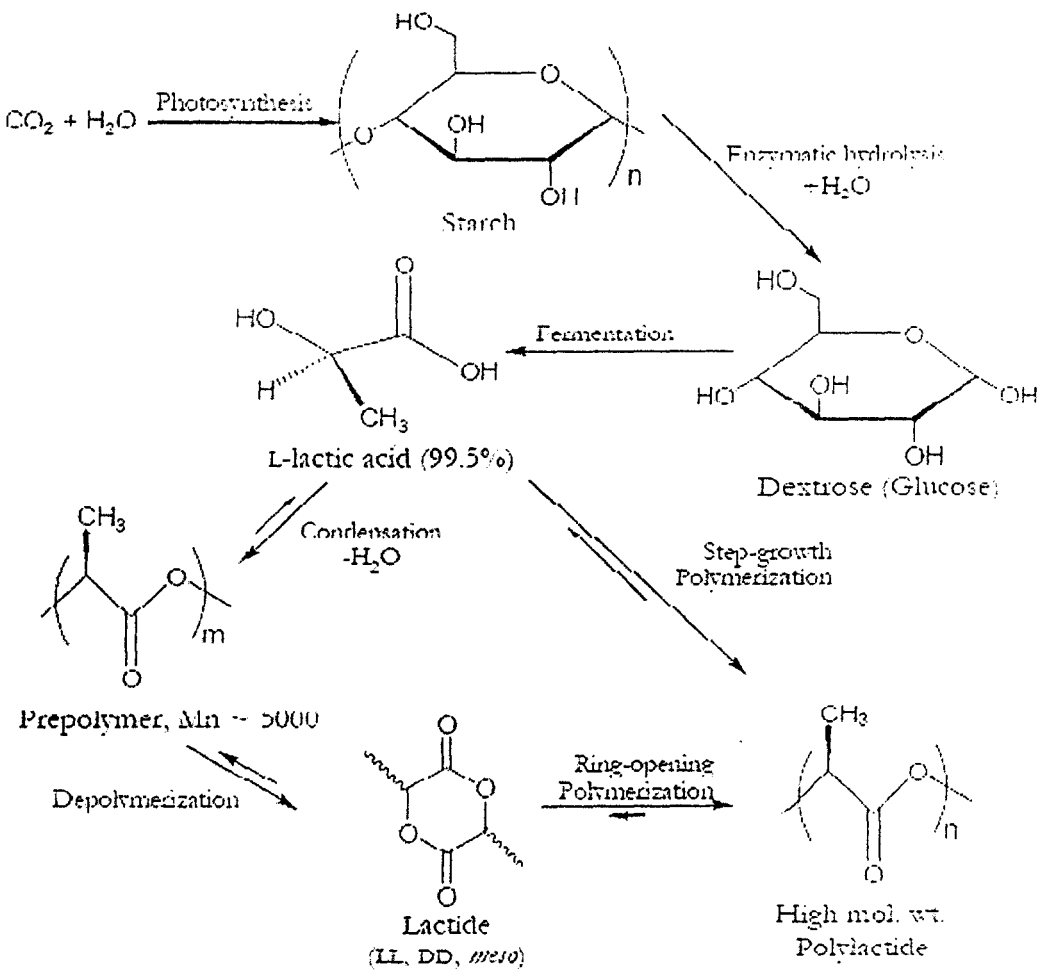

Figure 2. 3 Synthesis of lactic acid and poly(lactic acid) (Vink *et al.*, 2003; Mecking, 2004)

However, the condensation polymerization necessitates the removal of water even at traces level. Mitsui Toatsu Chemicals, Inc. invented a process for the efficient removal of water by azeotropic distillation and therefore, resulted in the production of high molecular weight (100-300 kD) PLA (Ajioka *et al.*, 1995). However, the feasibility of this process is greatly affected by the amount of catalyst, size of reactor, and the economical recovery of solvents. On the contrary, more versatile and efficient production of PLA is achieved by ROP of lactides in the presence of an initiator/catalyst. An efficient and versatile catalyst for the ROP of lactide is tin(II) 2-ethylhexanoate which is also known as stannous octoate ($Sn(Oct)_2$) (Albertsson and Varma, 2003). It offers benefits such as a very high catalytic activity, low levels of racemization, good solubility in the melt of lactide, and is an approved food additive (Ajioka, Enomoto et al., 1995). Stannous octoate does not initiate the polymerization of lactides and needs to be converted to tin(II)-alkoxides. The initiation requires presence of the hydroxyl or other nucleophilic species (Penczek *et al.*, 2000). For lactides, impurities such as water, lactic acid, and linear dimmers and trimers act as initiators (Zhang *et al.*, 1994). Figure 2.4 illustrates the ROP of lactide to PLA using alcohol and stannous octoate as catalyst system.

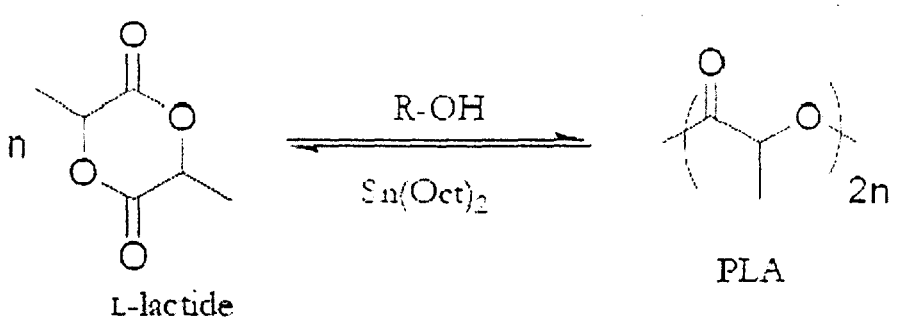

Figure 2. 4 Polymerization of lactide to PLA using R-OH/Sn(Oct)2 initiator/catalyst system

2.1.2.2 Mechanisms

The reaction mechanisms for ROP of lactides are dictated by the catalyst/initiator systems and cationic, anionic, coordination mechanisms are a few of the proposed mechanisms based on the kinetics of reaction, side reactions and the end group analysis (Stridsberg *et al.*, 2002). Complexes of tin, zinc, aluminum, lanthanides and strong bases such as metal alkoxides have been used as catalyst systems for ROP of lactides. However, tin(II) 2-ethylhexanoate is the most commonly used catalyst/initiator for ROP of lactides. As mentioned earlier, initiation requires an active hydrogen compound (Kafrawy and Shalaby, 1987). The polymerization proceeds by the coordination of lactide in the active species and the propagation progresses by insertion of lactide units into the tin-oxygen bond (Mecerreyes *et al.*, 1999). There are two major proposed mechanisms for the co-ordination insertion polymerization of lactides, that is the activated monomer mechanism (Veld *et al.*, 1997) and coordination insertion (Kowalski *et al.*, 2000) by formation of tin(II) alkoxides. In the activated monomer mechanism, $Sn(Oct)_2$ forms a donor-acceptor complex with the monomer and activates the monomers towards the nucleophilic attack by the alcohol leading to the insertion of monomer into Sn-O (metal-oxygen) bond (Du *et al.*, 1995). $Sn(Oct)_2$ is liberated at every propagation stage and Sn(II) atoms are not covalently bonded to polymer chains (Kowalski, Duda et al., 2000). Figure 2.5 shows the activated monomer mechanism for ROP of lactide and other lactones.

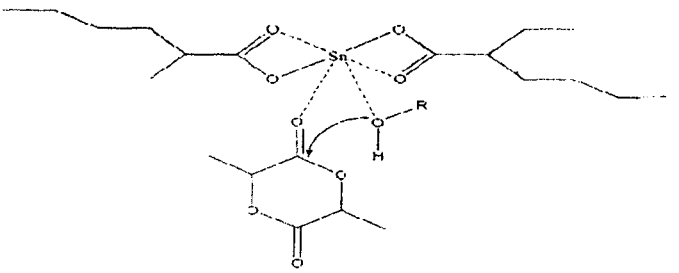

Figure 2. 5 Activated monomer mechanism for ROP of lactides using $Sn(Oct)_2$

Penczek and co-workers proposed the coordination insertion mechanism based on observations involving the dissociation of at least one 2-ethylhexanoate group from $Sn(Oct)_2$ as 2-ethylhexanoic acid. The polymerization is thought to be initiated by compounds containing hydroxyl groups (added intentionally or present as impurities), resulting in the tin(II) alkoxide, a true initiator prior to polymerization (Du, Lemstra et al., 1995; Kowalski, Duda et al., 2000) Based on polymerization of GA and DXO, Von schenck and co-workers (von Schenck *et al.*, 2002) suggested the nucleophilic attack of alkoxide (tin(II) alkoxide) on the carbonyl carbon of monomer followed by acyl-oxygen [C(O)--O]bond cleavage of the monomer which results in formation of R-O-C(O)-- and --O-Sn(Oct) end groups. The propagation proceeds by the addition of monomers to the Sn-O bond. Figure 2.6 illustrates the Sn(II) alkoxide complex initiated ROP of lactides.

This mechanism was also supported by the Kricheldorf and co-workers (Kricheldorf *et al.*, 1995; Kricheldorf *et al.*, 2000) who reported dynamic complex systems based on $Sn(Oct)_2$/Initiator and that the system responds to any change in reaction conditions by a change in the concentration and structure of active initiator species. Their conclusion was based on the observation of liberated octanoic acid during the reaction of alcohols and $Sn(Oct)_2$ and the presence of carboxylic acids decreases the reaction rate as it affects the equilibrium of complex formation. Also,

information about the relationship between the molecular weight and monomer/initiator ratio was provided.

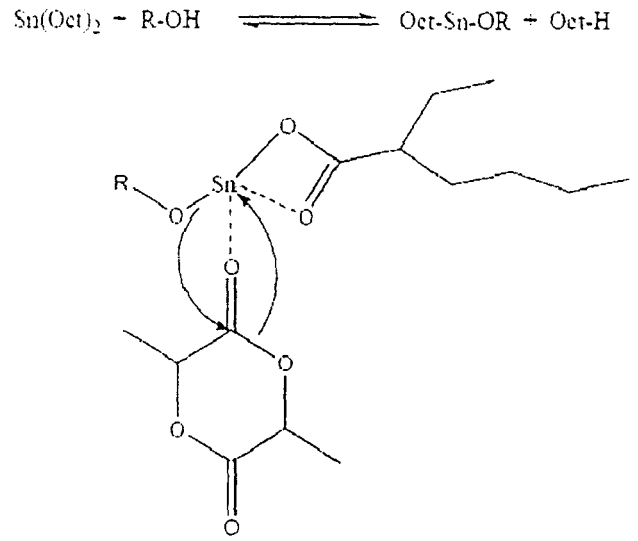

Figure 2. 6 Tin (II) alkoxide complex initiated ROP of lactides using Sn(Oct)2.

Cationic polymerization mechanisms for the bulk polymerization of lactides in presence of $Sn(Oct)_2$ were proposed by Nijenhuis et al.(1992) and Schwach et al.(1997). Pennings and co-workers (Nijenhuis *et al.*, 1992) proposed a nucleophilic attack of hydroxyl compounds (R-OH) on the lactone/Sn(Oct) complex as shown in Figure 2.7. The proposed mechanism is similar to transesterification mechanisms. The complex II generates a new species similar to complex I after reaction with lactones. It is proposed that the catalyst is not chemically bound to growing polymer chain ends and that the catalyst can polymerize larger numbers of polymer chains than the number of catalyst molecules. This effectiveness of catalyst could decrease the number average molecular weight and also broaden the molecular weight distribution.

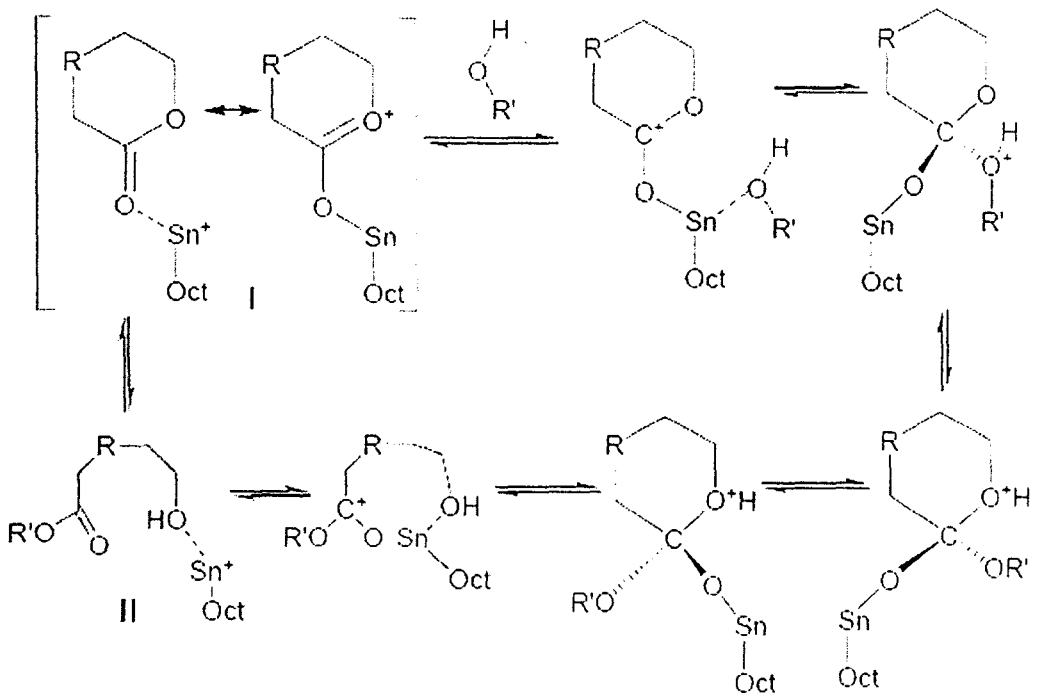

Figure 2. 7 Cationic polymerization mechanism for the $Sn(Oct)_2$/lactone system as Proposed by Nijenhuis *et al.*

Vert and co-workers (Schwach *et al.*, 1997) proposed a mechanism in which there is the formation of carbocations from lactones resulting after the reaction of the protonated catalyst, Sn+(Oct), on the carbonyl carbon of lactone. The cationic mechanism involving the co-initiation by lactic acid or octonoic acid is demonstrated in Figure 2.8.

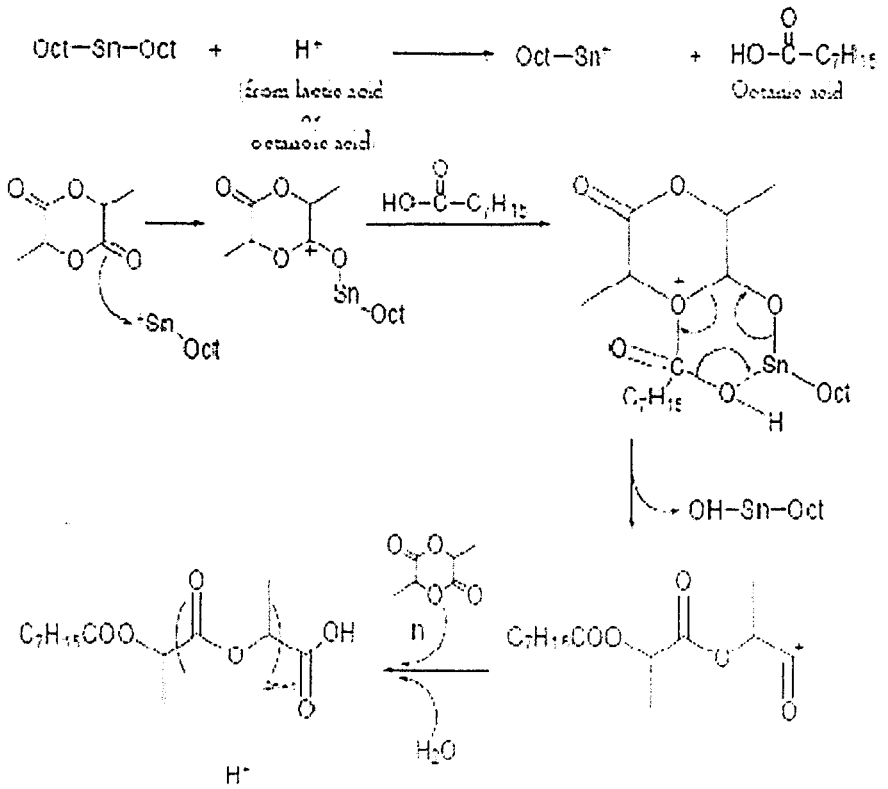

Figure 2. 8 Cationic polymerization mechanism for the Sn(Oct)2/lactide system as proposed by Schwach *et al.*

Numerous mechanisms such as cationic, anionic, enzymatic and co-ordination insertion exist for the ring opening polymerization using organocatalysts as reviewed by Kamber et al. (Kamber *et al.*, 2007) Many other metal complexes such as aluminum complexes (Montaudo *et al.*, 1996) iron complexes (O'Keefe *et al.*, 2001) zinc complexes (Williams *et al.*, 2002) have been explored as catalysts for lactide polymerization. Coordination insertion polymerization is the most widely accepted mechanism as it provides an explanation for the highly stereoregular polymers obtained with $Sn(Oct)_2$ (Ryner *et al.*, 2001). Hillmyer and coworkers reported a highly active metalloenzyme inspired dizinc catalyst for the controlled polymerization of lactide (Williams, Brooks et al., 2002).

2.1.2.3 Commercial Production of PLA

Commercial scale production of PLA has been successfully achieved by both methods, ROP of lactides and direct condensation of lactic acids (Kashima *et al.*, 1995). Direct condensation utilizes azeotropic distillation to remove water from the reaction system and hence drives the reaction to attain a reasonable mol. wt. of PLA (Enomoto *et al.*, 1994; Kashima, Kameoka et al., 1995). The properties of PLA produced from lactic acid were reported to be different compared to the PLA produced by ROP of lactides (Ajioka, Enomoto et al., 1995). PLA polymer demands the control of the L/D composition and rheology depending on the end use properties and processing of the polymer.

A process has been developed for the continuous production of PLA from lactic acid through lactide as an intermediate product. Details about poly(lactic acid) production and technology can be found in work published by Drumright, Gruber, and Henton (Drumright *et al.*, 2000). Dextrose obtained from the renewable resource (corn), was fermented to lactic acid. The lactic acid, then, condenses to pre-polymers (oligomers) in a continuous process. With the help of tin catalysts, the low molecular weight pre-polymers are converted into lactide with higher rates and selectivity. The lactide is purified by vacuum distillation and lactic acids and its linear esters are recovered and fed back to the lactic acid tank. The pure lactides are separated into enantionmers. Melt polymerization of lactides can be performed using tin catalyst without any need for solvent. Unconverted monomers are recycled back to the lactic acid stage (Kamm, Gruber et al., 2006).

Production of lactic acid is an important step for converting corn into PLA and is also the cost-determining step. Lactic acid can be obtained either by a chemical synthesis or a fermentation process. During fermentation, sugars

(sucrose/dextrose) can be broken down into lactic acid by using microorganisms on a commercially viable scale. Different microorganisms are used depending on the production, optical purity, and cost of lactic acid. These microorganisms require nutrients such as salts and vitamins to function. As the lactic acid is produced the pH of the fermentation reaction drops and therefore affects the production. Hence, lime ($Ca(OH)_2$) and chalk ($CaCO_3$) are used to control the pH between 5.0 and 6.8. The fermentation product is a lactate salt which upon acidification or salt splitting results in lactic acid. The crude lactic acid is then subjected to purification by removal of microorganisms, separation of by-products, nutrients and residual sugars and finally concentrated to 60-70% with >98% optical purity (Kamm *et al.* 2006).

2.1.3 PLA Properties

2.1.3.1 Thermal Properties of PLA

PLA obtained from L- and D-lactides is semi-crystalline (0-37%) and a relatively hard material with melting temperatures ranging from 170-190 °C and glass transition temperatures (T_g) ranging from 50-65 °C (Tsuji *et al.*, 2002). The melting temperatures can be as high as 220 °C and as low as 130 °C depending on the distribution of L- and D-lactides in the backbone (Farrington *et al.*, 2005). Witzke *et al.* reported that the melting temperatures decrease by 3°C for every 1% intial *meso*lactide concentration and almost no crystallinity with 18% *meso*-lactide (Witzke, 1997). PLA has relatively low thermal stability and above 190 °C, the molar weight decreases and thermal degradation (weight loss) can be observed in the range of 235-255 °C (Engelberg and Kohn, 1991) Because of the semi-crystalline nature of PLA, physical properties such as changes in the crystalline/amorphous ratio are strongly affected by the thermal effects (Celli and Scandola, 1992). The heat of

fusion for 100% crystalline PLA from L-lactides ranges from 93-203 J/g as reported in different researches as listed in Table 2.1. Crystallization of PLA has been thoroughly investigated such as by Fischer et al. (Fischer *et al.*, 1973) about melt and solution crystallization, Kalb and Pennings (Kalb and Pennings, 1980) about spherulitic growth from melt, Vasanthakumari and ennings (Vasanthakumari and Pennings, 1983) about crystallization kinetics and crystal growth, Cohn et al (Cohn *et al.*, 1987) about amorphous/crystalline morphology and Kishore et al. (Kishore *et al.*, 1984) about isothermal melt mechanism. Upon heating amorphous samples, crystallization rates increase with an increase in temperature (100 -160 °C) and reach a maximum before showing a decreasing trend (Tsuji *et al.*, 2005).

Table 2. 1 Thermal properties of PLA(Jamshidi *et al.*, 1988; Ikada and Tsuji, 2000; Tsuji, Miyase et al., 2005)

Properties	**Value**	**Units**
Degree of crystallinity, XC	0-37	%
Melting temperature, Tm	170-190	°C
Equilibrium melting temperature, Tm°	205-215	°C
Heat of fusion for 100% crystalline PLLA	93-203	J/g
Glass transition temperature, Tg	50-65	°C
Decomposition temperature, Td	235-255	°C

The crystallization is strongly affected by the optical purity of PLA. The crystallization time for PLLA increased 40% with the incorporation of 1% meso-lactide (Kolstad, 1996). Iannace and Nicolais reported a maximum crystallization rate at 105 °C and the overall rate of bulk crystallization (Iannace and Nicolais, 1997). According to the rate that the chains are deposited on the crystal surface, Hoffman divided the melt crystallization kinetics in three regimes (Sperling, 1986). As the temperature is lowered through regimes A, B, and C, the crystallization rate

becomes larger than the nucleation rate. In PLA, the transition of crystallization kinetics from regime B to regime A was observed above 163 °C by Vasanthakumari and Pennings whereas the transition of crystallization kinetics from regime C to regime B was observed around 115 °C by Iannace and Nicolais (Iannace and Nicolais, 1997). Di Lorenzo reported the transition of the crystallization kinetics from regime C to regime B at 120 °C by the Hoffman and Lauritzen theory. Spherulitic growth rate was found to be function of crystallization temperature and molecular weight (Vasanthakumari and Pennings, 1983). Growth rate (G) was observed to increase with a decrease in molecular weight. According to Vasanthakumari and Pennings, a viscosity-average molecular weight change from 150,000 g/mol to 690,000 g/mol reduces the growth rate from 5 μm/min to 2.5 μm/min (Vasanthakumari and Pennings, 1983) Di Lorenzo and He et al. reported the growth rate of 6.7 and 9.1 μm/min, respectively for PLA isothermally crystallized at 130 °C (Di Lorenzo, 2001).

2.1.3.2 Physical Properties of PLA

The important physical properties of PLA are summarized in Table 2.2. The density of PLA was reported to be in the range of 1.25 to 1.29 g/cm^3 and the refractive index between 1.35-1.45 (Tsuji, 2005). Solubility parameter (δ) of PLA was reported in the range of 19.0-20.5 (J/cm^3)$^{0.5}$ and PLA is reported to be soluble in dioxane, chloroform, methylene chloride, dichloroacetic acid, and acetonitrile (Kharas *et al.*, 1994). Crystalline PLA is not soluble in tetrahydrofuran, ethyl acetate, or acetone. PLA is insoluble in water, alcohols and alkanes and hence precipitate in alcohols and alkanes. The reported surface energy of PLA ranges from 35.9-43.9 mN/m depending on the processing and detailed information can be found in the