MACROMOLECULAR DESIGN OF HYDROXYL FUNCTIONAL LINEAR AND STAR-SHAPED L-LACTIDE AND ε-CAPROLACTONE BIODEGRADABLE POLYESTERS UTILIZING BIOSAFE CATALYSTS FOR BIOMEDICAL APPLICATIONS

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ABSTRACT

MACROMOLECULAR DESIGN OF HYDROXYL FUNCTIONAL LINEAR AND STAR-SHAPED L-LACTIDE AND ε-CAPROLACTONE BIODEGRADABLE POLYESTERS UTILIZING BIOSAFE CATALYSTS FOR BIOMEDICAL APPLICATIONS

In the present study, macromolecular design of homo and copolymers of lactide (LA) and ε-caprolactone (CL) in different structures by the use of biocompatible catalysts and co-initiators were performed to satisfy a need of tailor-made bioassimilable polymeric structures without any hazardous metal contaminants for various medical applications. Linear and star shaped (di, tetra and hexa functional) poly(L-lactide) (PLLA) and poly(ε-caprolactone) (PCL) homo/copolymers were synthesized by using bismuth(III)acetate (Bi(III)Ac) and creatinine as biosafe catalysts and ethylene glycol, pentaerythritol and myo-inositol as co-initiators.

The effect of catalyst type on polymer properties was observed by differences in crystalline structure. Crystalline and amorphous linear and star shaped PLLAs were obtained by using Bi(III)Ac and creatinine as catalysts, respectively. The activity of creatinine was very low comparing to Bi(III)Ac and $SnOct_2$ catalysts. The reactivity of LA monomer was found to be higher than that of CL monomer. The high molecular weight polymers having low PDI values were obtained by using Bi(III)Ac catalyst contary to creatinine catalyst. The decrease in glass transition temperatures and molecular weights of synthesized PLLA and PCL homo/copolymers were observed with the increase in amount of co-initiators due to the decrease in chain length and disruption of crystal formation.

The cytotoxicity properties of the catalysts and synthesized linear and functional homo/co PLLAs and PCLs were carried out according to MTT assay. Cytotoxicity of Bi(III)Ac was found as lower than that of $SnOct_2$. Creatinine and the synthesized polymers did not show any cytotoxic properties. The observation of no cytotoxic effect of creatinine catalyst results in the biosafe usage of creatinine catalyst instead of toxic $SnOct_2$ for the synthesis of moderate or low molecular weight homo/co PLLAs and PCLs in bioapplications.

ÖZET

HİDROKSİ FONKSİYONLU DOĞRUSAL VE YILDIZ ŞEKİLLİ L-LAKTİD VE ε-KAPROLOKTAN BİYOBOZUNUR POLİESTERLERİN BİYO GÜVENLİ KATALİZÖRLER KULLANILARAK BİYOMEDİKAL UYGULAMALARA YÖNELİK MAKROMOLEKÜLER DİZAYNI

Bu çalışmada, farklı medikal uygulamalarda ihtiyaca yönelik biyo polimerik yapıların sağlanması için tehlikeli metal kontaminasyon içermeyen biyo uyumlu katalizörler ve yardımcı başlatıcılar kullanılarak L-laktid (LA) ve ε-kaprolakton (CL) homo ve kopolimerlerinin makromolekül dizaynı gerçekleştirilmiştir. Lineer ve yıldız şekilli (iki, dört ve altı hidroksi fonksiyonlu) poli(L-laktid) (PLLA), poli(ε-kaprolakton) (PCL) homo/kopolimerleri biyo uyumlu bizmut(III)asetat (Bi(III)Ac) ve kreatinin katalizörleri ve yardımcı başlatıcı olarak etilen glikol, pentaeriltritol ve myo-inositol kullanılarak sentezlenmiştir.

Sentezlenen polimerlerin kristal yapılarında katalizör çeşidinin etkisiyle farklılıklar gözlenmiştir. Kristal ve amorf yapıda linear ve yıldız şekilli PLLA'lar sırasıyla Bi(III)Ac ve kreatinin katalizörleri kullanılarak elde edilmiştir. Kreatinin aktivitesi Bi(III)Ac ve $SnOct_2$ katalizörlerine göre oldukça düşüktür. LA monomerinin reaktivitesi CL monomerinin reaktivitesinden daha yüksek olduğu saptanmıştır. Yüksek molekül ağırlıklı ve düşük PDI değerlerine sahip polimerler kreatinin katalizörünün aksine Bi(III)Ac katalizörü kullanılarak elde edilmiştir. Kullanılan yardımcı başlatıcı miktarı arttıkça kristal yapı oluşumunun bozulması ve zincir uzunluğunun azalmasından dolayı, sentezlenen PLLA ve PCL homo/kopolimerlerinin camsı geçiş sıcaklıklarında ve molekül ağırlıklarında azalma gözlenmiştir.

Katalizör ve sentezlenen linear ve fonksiyonel homo/ko PLLA ve PCL polimerlerinin sitotoksik özellikleri MTT test ile belirlenmiştir. Bi(III)Ac'nin sitotoksitesi $SnOct_2$'ye göre daha düşük olduğu bulunmuştur. Kreatinin ve sentezlenen tüm polimerler herhangi bir sitotoksik etki göstermemiştir. Kreatinin katalizörünün sitotoksik etki göstermemesi, kreatinin biyo güvenli olarak toksik $SnOct_2$ katalizörü yerine düşük ve ortalama molekül ağırlıklarında homo/ko PLLA ve PCL polimerlerinin sentezinde biyo uygulamalara yönelik kullanılabileceği sonucuna varılmıştır.

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CHAPTER 1

INTRODUCTION

Homopolymers and copolymers of lactide and ε-caprolactone synthesized by stannous(II) ethylhexanoate commonly known as stannous octoate ($SnOct_2$) catalyst are intensively used in variable biomedical and pharmaceutical applications ranging from medical implants, scaffolds and resorbable sutures for tissue engineering to controlled drug delivery systems (Andreopoulas et al. 2000, Avgoustakis et al 1993, De Jong et al. 2005, Middleton et al. 2000, Murphy et al. 2000 and Odelius et al. 2008). Although $SnOct_2$ is a highly efficient catalyst used for ring opening polymerization of lactide and ε-caprolactone, the impossibility of complete removal of tin contamination in these biopolymers requires some constraints for the medical applications (Fernandez et al. 2013, Schwach et al. 1997 and 2002, Stjerndahl et al. 2007 and 2008, Tanzi et al. 1994). The toxicity of tin compounds has been deduced in vitro and in vivo studies (Ahmed and Tsuchiya, 2006, Cooney and Wuertz, 1989, Yamada et al. 2008). The presence of tin compounds as residual catalyst in polymers leads to the toxic influence on the inhibition of cell growth, DNA damage, neurotoxicity and etc. (de Mattos et al. 2000, Silva et al. 2002, Tsuji et al. 2010, Yamada et al. 2008). The possibility of penetration of trace amounts of tin compounds especially to brain nervous system requires much attention in the synthesis of PLAs and PCLs with tin based catalysts for biomedical applications (Floera and Büsselberg 2005, Tsuji et al. 2010, Yamada et al. 2008, Yasuaki 2000). Schwach et al. (2002) determined the increase of residual tin concentration from 306 to 795 ppm during hydrolytic degradation of PLA synthesized by $SnOct_2$. The achievement of high residual tin concentration in this study is confronted to the toxicity requirements related with the biomedical applications of PLA. Albertsson group studied the controlled ring opening polymerization and the minimization of residual tin content in the polymerization of lactide and ε-caprolactone (CL) by using $SnOct_2$, and also different cyclic tin alkoxides. Controlled ring opening polymerization and reduction in residual tin content were established by using cyclic tin alkoxides and decrease of $SnOct_2$ amount used in the polymerization or treatment with 1,2-ethanedithiol, respectively. Acceptable residual tin content for biomedical

applications is less than 5 ppm. It was recommended that the required monomer/initiator ratio should be equal or less than 10000 for the achievement of acceptable $SnOct_2$ residue in the synthesis of polycaprolactone (Ryner et al. 2001 and Stjerndahl et al. 2007, 2008). In addition, the synthesis of biopolymers by using monomer/catalyst ratio of 10000 in the industry is not feasible due to the long reaction time. Furthermore, controlled ring opening polymerization is quiet difficult in the presence of trace amounts of water or other impurities consisting hydroxyl group due to the high affinity of $SnOct_2$ for transesterification reactions.

The demand in development of efficient biocompatible catalyst instead of $SnOct_2$ has been increasing in polyester based polymer synthesis intended for biomedical applications. As an alternative to $SnOct_2$, less or non-toxic metal catalysts available (Ca, Fe, Mg, Zn(II) salts) or unavailable (Bi, Ge, La, Zr,) in body and also nontoxic metal free catalysts available in body (guanidinum salts such as creatinine or enzymes such as lipase) or not (boric acid, salicylic acid, N-hetereocyclic olefins) has been used in PLA and PCL synthesis. In addition, mostly low molecular weight PLAs have been obtained by using alternative catalysts due to their lower catalytic efficiencies compared to the toxic $SnOct_2$ and many other toxic metal based catalysts (Chen et al. 2007, Dobrzynski et al. 2001, 2002 and 2006, Finne et al. 2003, Hege and Schiller 2014, Hsiao and Lin 2013, Kricheldorf and Boetcher 1993, Kricheldorf and Damrou 1998, Kricheldorf et al. 2000, 2005, Li et al. 2004, Matsumura 2006, Naumann et al. 2016, Srivastava and Albertsson et al. 2006 and 2007, Ren et al. 2016, Xu et al. 2014, Wei et al. 2009, Wheaton et al 2009 and Zhong et al. 2001).

Kricheldorf et al. studied the synthesis of poly(L-lactide) or poly(ε-caprolactone) by using different types of bismuth based catalysts such as bismuth(III) hexanoate ($BiHex_3$), bismuth-2-mercaptoethanol complex ($BiMe_2$), bismuth halides ($BiCl_3$, $BiBr_3$, BiJ_3, BiF_3), bismuth triacetate (Bi(III)Ac), triphenyl bismuth (Ph_3Bi), diphenyl bismuth bromide (Ph_2BiBr) and diphenyl bismuth ethoxide (Ph_2BiOEt). The reactivity ratios of Ph_2BiOEt, Ph_2BiBr, $BiMe_2$, $BiHex_3$, $BiBr_3$ and Ph_3Bi in poly(ε-caprolactone) synthesis could be arranged in an order from high to low. In the first time, the reactivity of Ph_2BiOEt as a non toxic catalyst was found as higher than that of $SnOct_2$ in the polymerization of ε-caprolactone at 90 ^oC (Kricheldorf et al. 2004, 2005, 2006, and 2008, Lahcini et al. 2008). Duval et al (2014) synthesized poly(D,L-lactide-co-glycolide) (PLGA) copolyesters by using three different catalysts; toxic $SnOct_2$, and

low toxic zinc lactate and bismuth subsalicylate. They concluded that the ring-opening polymerization of PLGA with $SnOct_2$ was eight times faster than that of PLGA with bismuth subsalicylate and 19 times than that of PLGA with zinc lactate. Although PLGA copolymers synthesized with bismuth subsalicylate and $SnOct_2$ have similar sequence structures, the hydrolytic degradation of PLGA copolymers synthesized with bismuth subsalicylate is slower than that of PLGA copolymers synthesized with $SnOct_2$.

Reliability of bismuth catalyst as a biosafety material can be deduced from the variable utility of Bi compounds such as bismuth citrate, bismuth phosphate, bismuth subcarbonate, bismuth subgallate, bismuth subsalicylate, bismuth subnitrate, ranitidine bismuth citrate in pharmaceuticals for the treatments of skin leisure, stomach ulcer, Parkinson's disease, hypothyroidism, ulcerative colitis, gastrointestinal diseases, Helicobacter pylori infections, and also radiotherapeutic agents (Desoize 2004, Lambert and Midolo 1997, Price et al. 2012, Sun et al. 1999 and Sadler et al. 1994, Tilman et al. 1996, Udalova et al. 2008, Zhang et al. 2006). However, Recklinghausen et al. (2008) demonstrated that the toxicity of bismuth compounds depends on the structure of bismuth compound. While bismuth citrate and bismuth gluathione have no toxic effect, methylated bismuth induces cyto-and genotoxic effects in human cells in vitro.

Creatinine can be utilized as guanidine salts available in body such as erythrocytes and all secretions such as bile, sweat, and gastrointestinal fluids (Chuma et al. 2008 and Narayanan and Appleton 1980). Creatinine is produced from dehydration of creatine and it is filtered out of blood in kidneys. 95 % of creatine is stiutated in muscle and the others in liver, kidney, brain, pancreas and testis. Creatine as known as a responsible molecule for energy production in muscles, is also found in some foods such as meat and fish. For that reason, it taken as dietary supplements for athletes. Creatine analogs such as phosphonylcreatine (PCR) and cyclocreatine are used as anticancer agents during chemotherapy (Wyss M. and Kaddurah-Daouk 2000). Li et al. (2004) and Wang et al. (2004) only studied the synthesis of homo polylactides by using guanidine based catalysts and creatinine, respectively.

The tailoring of molecular structure of biopolymers by means of biosafe catalysts and co-initiators has attracted a great importance in biomedical applications. Enhancement of degradation and physical properties of PLAs or PCLs can be optimized by incorporation of branch structure using different co-initiators. Incorporation of complex macromolecular architecture to PLA or PCL biopolymers by means of coinitiators lead to additional improvement of its properties such as increase in

hyrophilicity, solubility, decrease in melt viscosity and crystallinity (Atkinson and Vyazovkin 2013, Choi et al. 2005, Schömer and Frey 2011 and Zhu et al. 2010). Especially, crystalline and hydrophobic structure of PLA or PCL biopolymers limit their medical applications. For that reason in this study, synthesis of linear and star shaped PLAs and PCLs with variable architecture were carried out by using Bi(III) acetate and creatinine as biocompatible catalysts and three different co-initiators; ethylene glycol (EG), pentaerythritol (PENT) myo-inocitol (MYO) and dipentaerythritol (DIPENT) to extend the medical applications of PLA and PCL by providing enhancement of hydrophilicity and decrease in crystallinity. Although no study about the toxic effect of Bi(III)Ac and creatinine is available, cytotoxic properties of Bi(III)Ac, creatinine and $SnOct_2$ catalysts were investigated according to MTT assay in this study. The presence of creatinine in human metabolizm and the use of different bismuth compounds in drugs deduce more safety by comparing to the commonly used toxic $SnOct_2$ catalyst. The synthesis of high molecular weight polyesters is possible by using Bi(III)Ac catalyst superior to enzymes. The role of reaction conditions such as catalyst ratio, co-initiator type and temperature on the polymerization of L-lactide by using highly efficient and commercial available Bi(III)Ac catalyst and creatinine and also final polymer properties was focused. The initiation activities of co-initiators on Bi(III)Ac and creatinine were compared. The molecular weights, glass transition and melting temperatures of the polymers can be controlled by the type and amount of co-initiators. The intended use of creatinine available also in human metabolism as a catalyst is the extinction of the metal accumulation in body for bioapplications of PLAs synthesized by toxic metals. This synthesized PCL and PLA prepolymers can also be used for further synthesis of branched amphiphilic copolymers composed of PCL or PLA as hydrophobic group and PEG as hydrophilic group for gene delivery, tissue engineering and drug delivery applications.

In the literature, many studies about the syntheses of various L-lactide based ABA type block or multiblock or random copolymers comprising of glycolide, ε-caprolactone (CL), 1,5-dioxepan-2-one (DXO) or trimethylene carbonate (TMC) are available. Hovewer, the number of studies about the effect of functional groups on PLA synthesis is limited (Cohn and Salomon 2005, He et al. 2004, Huang et al. 2004, Kim et al. 2001, Kricheldorf and Rost 2005 a, Prospiech et al. 2005 and Stridsberg and Albertsson 2000). Especially, hydroxy telechelic PLAs synthesized by using different co-initiators have been used as intermediate polymers for further synthesis of various

kinds of poly(ester-urethane)s (Hiltunen et al. 1997 and Nakayama et al. 2008). He et al. (2004) studied the synthesis and cell affinity of functionalized poly(L-lactide-co-β-malic acid). They found that the cell adhesion of PLLA increased by the functionalization of PLLA with malic acid. Di, tri or tetrafunctional lactide or caprolactone based polymers can be prepared by using various co-initiators such as diethylene glycol, tetraetylene glycol, 1,1,1 tri(hydroxy methyl propane), glycerol or pentaerythritol (Baimark and Molloy 2005, Kricheldorf et al. 2004 a,b and Quian et al. 2000). Kricheldorf et al. (2004 a,b) suggested that the synthesis of di, tri or tetra functional lactide based polymers having different numbers of –OH end groups can lead to the preparation of building blocks of more complex architectures such as triblock or multiblock copolymers or networks. Lemmouchi et al (2007) and Cheng et al. (2008) carried out novel synthesis of star-shaped poly(ethylene glycol)-block-poly(lactide) copolymers and star-shaped poly(ε-caprolactone)-block-poly(ethyl ethylene phosphate) for drug delivery applications or tissue regeneration. Cheng et al. concluded the suitability of these synthesized star-shaped copolymers especially for delivery of hydrophobic drugs. Sriputtirat et al. (2012) synthesized low molecular weight P(LA-co-CL) (90/10) copolymers by using $SnOct_2$ and different co-initiators such as 1,4-butanediol, pentaerythritol or D-sorbitol. They also demonsrated the combined use of P(LA-co-CL) copolymer having pentaerythritol with chitosan at the ratio of 70/30 for further development into the wound dressing device by considering film properties and the release rate of tetracycline hydrochloride used for protection of tissue from bacterial infection.

1.1. Scope of the Thesis

In the scope of this study, the use of biocompatible catalysts in synthesis of lactide and ε-caprolactone based homopolymers and copolymers is the main focus. The catalyst type is an important criteria in the synthesis of lactide or ε-caprolactone based biopolymers used in various medical applications due to the impossibility of complete removal of catalysts during purification of these biopolymers. The requirement of biosafe catalysts in synthesis of PLA or PCL based biopolymers used for medical applications will be obligatory for future strategy.

The other criterion in this thesis is synthesis of innovative lactide and ε-caprolactone based biodegradable polymers by using biocompatible catalysts. The variable structured such as linear and branched lactide and ε-caprolactone based homopolymers and copolymers were synthesized. Synthesis of variable structured homopolymers and copolymers leads to the enhancement of degradability and biocompatibility properties of the homopolymers and copolymers by virtue of different type of co-initiators and biocompatible catalysts, respectively. Based on the relationship between the structure and degradation properties of the polymers, the design of di, tetra, penta or hexa- functional lactide or ε-caprolactone based homopolymers and copolymers having variable molecular weight and thermal properties by considering the type and amount of co-initiator and catalyst was taken as a primary goal of this work. To reach this target, the effects of different initiators on structure, molecular weight, and thermal properties of lactide based homo polymers and copolymers by using less or nontoxic catalysts were investigated. In theory, the increase in branching number accelerates degradation of polymers. The structure effect on degradation properties of the synthesized homopolymers and copolymers can be verified in future studies.

In the first time, linear and star shaped copolymers of poly(L-lactide-co-caprolactone) by using bismuth(III) acetate or creatinine and linear and star shaped homo poly(ε-caprolactone) polymers by using creatinine were synthesized and also the kinetic and thermodynamic factors affecting the ring-opening polymerization of L-LA by using Bi(III)Ac or creatinine were determined in this study. Especially, functional lactide based homopolymers and copolymers with controlled architecture providing fast degradation rate or cell proliferation are needed to use in medical fields such as resorbable implant materials and controlled drug delivery systems. The increase of encapsulation efficiencies of hydrophilic drugs by using star shaped PCL homo or copolymers will be possible by providing increment in their hydrophilicity. For that reasons, it is crucial significance to deduce less or no cytotoxic effect of bismuth(III) acetate or creatinine as catalysts instead of $SnOct_2$ and to synthesize linear and star shaped lactide based homopolymers and copolymers by using EG, PENT, or MYO as initiators in our study for different medical applications. Sequential polymerization method was applied to control the block length during the copolymerization. The use of biocompatible metal-free systems as catalysts especially creatinine provides no possibility of metal contamination in the polymer for the synthesis of homo and coPLAs

and also improves the cell viability grown on homo and coPLAs. Furthermore, the special structures of branched lactide or ε-caprolactone homopolymers and lactide-ε-caprolactone copolymers were observed to have lower crystallinity, lower glass transition temperatures and higher hydrophilic properties than that of pure linear PLA. As a result of these changes, the utilization of these synthesized homo polymers and copolymers will be possible for applications requiring controlled degradation rate such as drug releasing implants, absorbable sutures, nerve guides, resorbable prostheses or tissue regeneration such as wound healing or artificial dura mater. These synthesized homo polymers and copolymers in this study can also be used for further synthesis of polyurethanes having shape-memory properties for variable medical applications ranging from surgical implants, scaffolds or cardiovascular prostheses such as intra-aortic balloons, meniscus implants. Also for future studies, injectable polymeric gels for cancer thearapy can be prepared by copolymerization of branched PLLAs or PCLs with hydrophilic glycolide monomer or low molecular weight polyethylene glycol by using non-toxic catalyst and cancer drugs such as doxorubicin, paclitaxel or irinotecan.

1.2. Outline of the Thesis

This chapter includes a brief introduction for the research area and the research objectives of this thesis titled as synthesis and characterization of polylactide based homo/copolymers for biomedical applications. In the second chapter, literature review about PLA and PCL as biodegradable homopolymers and copolymers was reported. The introduction about polymerization methods, mechanisms, kinetics and biocompatibility of PLA and PCL homopolymers and copolymers were described through this chapter. In the third chapter, detailed experimental procedures for the synthesis and characterization of the all homopolymers and copolymers have been implemented. Synthesis and characterization results of OH functional PLLA and PCL homopolymers and poly(L-lactide-co-ε-caprolactone) P(LL-co-CL) copolymers were reported in Chapter four. Also, the effects of catalyst type on polymerization of homopolymers and copolymers were investigated in this chapter. Cytotoxicty results of Bi(III)Ac and creatinine as catalysts and cell adhesion studies of homopolymers and copolymers were reported. Finally, the major achievements of the present work and the suggestions for future study were presented in Chapter five.

CHAPTER 2

LITERATURE REVIEW

The state of current knowledge about polylactide (PLA) and poly-ε-caprolactone (PCL) as biodegradable homopolymers and copolymers are reported in this chapter. The studies about synthesis, chracterization, bicompatibility and bioapplications of PLA and PCL homopolymers and copolymers are condidered. Fistly, the introduction about biodegradable PLA and PCL polymers is given. In the second part, synthesis of PLA and PCL polymers is reported by considering polymerization methods, polymerization mechanisms and polymerization kinetics. In the third part, biocompatibility of biodegradable polymers is described by appproaching prerequisites for biomedical applications.

2. 1. Biodegradable Polymers

Biodegradable polymers are defined as polymers that are degraded and catabolized, eventually carbon dioxide and water as non-toxic degradation products, by microorganisms under the natural environment (Okada 2002). In recent years, biodegradable polymers such as poly(lactide) (PLA), poly□□(ε-caprolactone) (PCL) and poly(glycolide) (PLG) have been received increasing attentions because of their wide applications as medical materials. The achievement of a controlled degradation for an assigned function is possible by designing chemical structure, copolymer composition and molecular weight of the polymer. The general introduction about PLA and PCL polymers are given in this chapter by considering their basic features.

2.1.1. Polylactide

Polylactide (PLA) belongs to a group of aliphatic polyesters as known as biodegradable thermoplastics polymers. Lactic acid as a basis of PLA also defined as 2-hydroxy propionic acid ($HOCH(CH_3)COOH$) occurs naturally in animals,

microorganisms, many natural foods and fermented foods such as milk, corn, cane molasses, potato, whey, barley and beet sugar (Maharana et al. 2009, Stevens 2002,Wee et al. 2006).

Lactic acid is the simplest hydroxy acid with a symmetric carbon atom and exists in two optically active forms. Stereoforms of lactic acid depends on the production method used as shown in Figure 2.1. Commercial lactic acid is mostly produced by bacterial fermentation of renewable resources such as carbohydrates. Fermentative production of lactic acid provides a great advantage for producing optically pure L- or D-lactic and also DL-lactic acid, depending on the strain selected for fermentation (Dumbrepatil et al. 2008). The strain of Lactobacilli is mostly used in the fermentation process to produce stereoregular L-lactic acid. However, a racemic mixture of D and L-lactic acid isomers are produced by the chemical process. In addition, chemical process route is not preferred due to the limited supply of petrochemical resources such as acetaldehyde (Gupta and Kumar 2007, Wee et al. 2006).

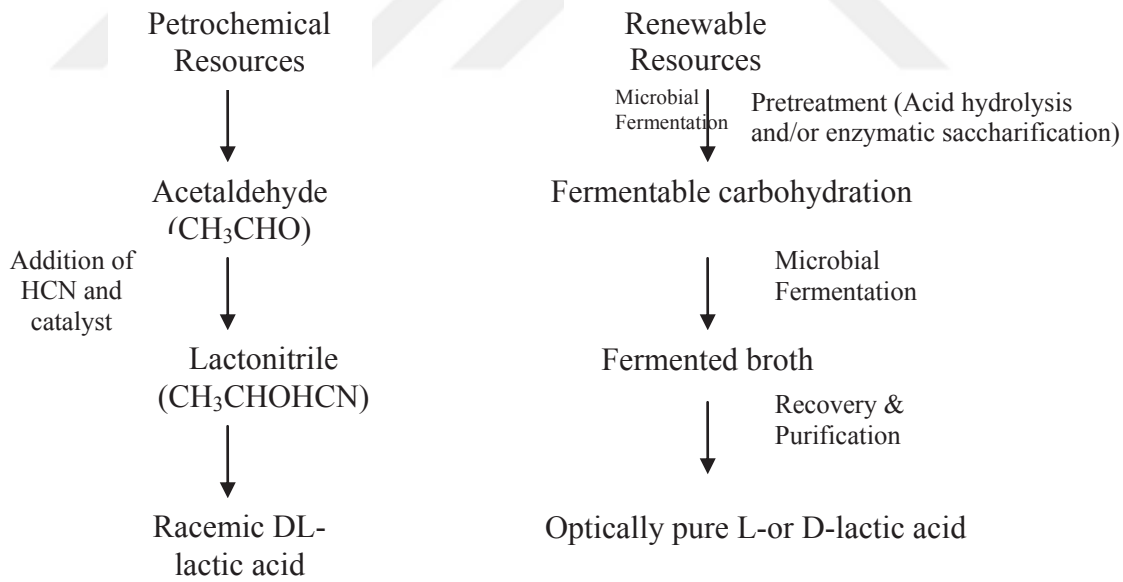


Figure 2.1. Production methods of lactic acid
(Source: Wee et al. 2006).

Lactide (3,6-dimethyl-1,4-dioxane-2,5-dione) obtained from dimerization of lactic acid can be in four different forms depending on the configurations of the lactic acid. Stereoisomers of lactic acid and lactide are shown in Figure 2.2. The optically

active D- lactide, L-lactide and the optically inactive meso or D,L-lactide and racemic lactide are obtained due to the two different stereoforms of lactic acid. L-lactide and D-lactide consist of two molecules of L-lactic acid and D-lactic acid, respectively. Meso-lactide consists of one molecule of L-lactic acid and one molecule of D-lactic acid. Racemic lactide is formed from the equimolar (50:50) mixture solutions of L-lactide and D-lactide (Mobley, 1994 and Lou et al. 2003).

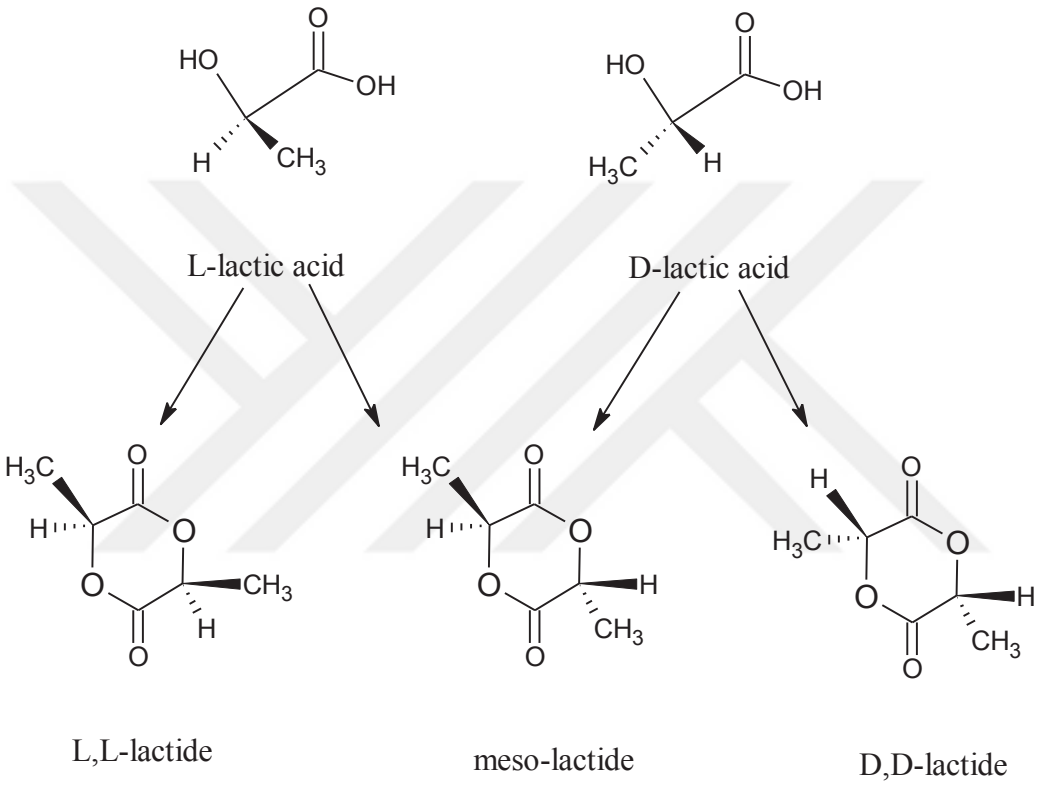


Figure 2.2. Stereoisomers of lactic acid and lactide.

The physical and chemical properties of PLA polymers such as morphology (amorphous or crystalline structure), mechanical, and degradation properties are dependent on the choice of the stereoisomer and monomer type. Isotactic crystalline PLA and PDLA are synthesized only by the polymerization of optically pure L-LA and D-LA, whereas rac-LA (a 1:1 mixture of L-LA and D-LA) and meso-LA lead to the formation of atactic amorphous poly(rac-lactide) and poly(meso-lactide) also known as PDLLA, respectively. PLAs are synthesized using two different polymerization methods defined as condensation and ring opening polymerizations according to the used monomer structure. As shown in Figure 2.3, condensation and ring opening

polymerizations are carried out from lactic acid and lactide, respectively. The existence of both a hydroxyl and a carboxyl group in lactic acid enables the formation of low molecular weight PLA polymers *via* condensation polymerization. Condensation polymerization requires the removal of the water during the reaction. The presence of water leads to the formation of low molecular weight PLA polymers. However, high molecular weight PLA polymers are synthesized from the ring opening polymerization of lactide as known as a cyclic diester of lactic acid. The purity of lactide is also a critical parameter in the formation of high molecular weight polymers. For that reason, lactide monomer should be highly purified by distillation, recrystallization or both before the ring opening polymerization of lactide (Mehta et al. 2005).

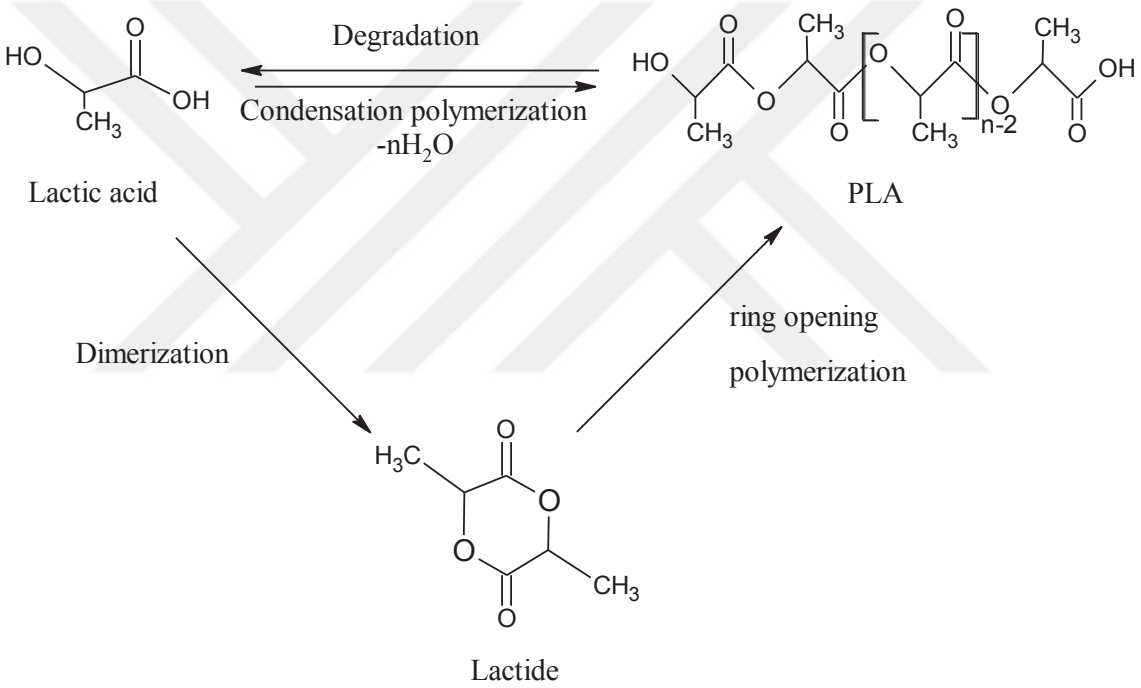


Figure 2.3. PLA synthesis.

The vital property of PLA is its biodegradability and leading to the formation of non-toxic degradation products for living organisms during degradation. As shown in Figure 2.3., the high molecular weight PLA can be degraded to lactic acid in the presence of water. For that reason, PLA, is one of the most important synthetic biodegradable polymers investigated for a wide range of biomedical and pharmaceutical applications such as controlled drug delivery, resorbable medical materials (sutures, wound covers, stents), medical implants (screws, pins), and scaffolds for tissue

engineering, and also food packaging applications due to the allowance of nontoxic products (Andreopoulos et al. 2000, De Jong et al. 2005, Middleton and Tipton 2000, Murphy et al. 2000, Nef et al. 2017, Wildemann et al. 2005 and Wu and Ding 2004).

Scaffolds are playing an increasingly important role in coronary interventions. Bioresorbable PLA based scaffolds can also be used alternative to metal stents. Stents can be made of aliphatic polyesters, such as poly(L-lactic acid) (PLLA), poly(glycolic acid)(PGA), and poly(ε-caprolactone) (Brugaletta et al. 2012, Kraak et al. 2014, Nef et al. 2017, Yuan et al 2015) . Bioresorbable stents have been reported for a variety of applications. Ye et al. (1998) developed a tubular PLLA/PCL microporous stent for delivering gene transfer vectors to the arterial wall. Biodegradable drug eluting stents based on lactide, glycolide, caprolactone homopolymers or copolymers were described to prevent bacterial adhesion in ureteral applications (Liatsikos et al. 2009, Ma et al. 2016)

The application areas of PLAs are significantly dependent on the choice and distribution of stereoisomers within the polymer chains and also molecular weight of the polymer due to the their effects on physical, mechanical and degradation properties. For example, isotactic poly(L-lactide) (PLLA) is a highly crystalline material with melting and glass transition temperatures of 170 °C and 56 °C, respectively. This high glass transition temperature leads to the low biodegradation rate of crystalline PLA at body temperature. PLA exhibit high tensile strength and low elongation and have a high modulus that makes them more applicable than amorphous polymers especially for load-bearing applications such as in orthopedic fixation. Atactic poly(rac-LA) is amorphous and subject to a comparatively fast degradation. The polymer made from an optically inactive racemic mixture of D and L enantiomers, poly-DL-Lactide (PDLLA) is an amorphous polymer. This material has lower tensile strengths and higher elongation and much faster degradation time making it more suitable as a drug delivery system. (Bendix 1998 and Mehta et al. 2005).

The physical properties of PLA homo polymers even for the amorphous PDLLA as mentioned above such as: high glass transition temperature, high stiffness, hydrophobicity and long degradation time restrict their bioapplications, especially requirements for controlled degradation. Many studies are carried out to overcome the problems of PLA by physical blending with poly(lactide-co-glycolide) (PLGA), PCL, PEG etc., copolymerization with caprolactone, glycolide, polyethylene glycol etc. or homopolymerization with initiators having variable numbers of hydroxyl groups such as

glycerol, dietyleneglycol, pentaerythritol, hyperbranched PEG, etc. (Dobrzynski et al. 2002, 2006, Hu and Liu 1994, Jiang and Schwendeman 2001, Kim et al. 2003, Nabid et al. 2011, Tsuji 2000, Schömer and Frey 2011, Tsuji and Ikada 1998, Wu and Ding 2004). The use of different initiators having variable numbers of hydroxyl groups is applied for the synthesis of branched polymers. Especially, thermal, mechanical, rheological and degradation properties of branched polymers are significantly different from the corresponding linear polymers. Branched PLA polymers have lower melt viscosity, crystallinity, glass transition and melting temperatures and higher solubility in solvents, and also higher degradability properties than that of linear PLA (Atkinson and Vyazovkin 2013, Schömer and Frey 2011). Meanwhile, the last two methods branching and copolymerization in the few studies are used together to modify the properties of PLA significantly. Nabid et al.(2011) synthesized self assembled micelles of pentaerythritol-centered amphiphilic star-block copolymers based on PCL and PEG for hydophobic drug delivery such as anticancer drug known as quercetin. Dailey et al. (2005) synthesized three major groups of branched polyesters based upon poly(vinyl alcohol)-grafted poly(lactic-co-glycolic acid) (PVA-g-PLGA) namely, the neutrally charged PVA-g-PLGA, negatively charged sulfobutyl-modified PVA-g-PLGA and positively charged amine modified PVA-g-PLGA for the development of a variety of drug delivery vehicles. They also emphasized that the rate of biodegradation can be manipulated through polymer modification to achieve half-lives ranging from several hours to several weeks. Wang et al. (2012) also synthesized pentablock and multibranched copolymers bearing PEG, hyperbranched polyglycidol and PLA by using $SnOct_2$. They also demonstrated that the use of this synthesized branched copolymer in controlled drug delivery systems by carrying out the release studies of anticancer drug doxorubicin loaded micelles at various pH conditions.

2.1.2 Poly(ε-caprolactone)

Poly(ε-caprolactone) (PCL) belongs to a group of aliphatic biodegradable polyesters like PLA. ε-Caprolactone monomer as known as 6-hexanolactone or 2-oxepanone is used in the production of PCL. Baeyer Villiger oxidation method of cyclohexanone by peracetic acid is mostly used for the commercial production of ε-caprolactone monomer as shown in Figure 2.4. In addition, ε-caprolactone monomer

and 6-hydroxyhexanoic acid are also obtained as intermediate products in the production of adipic acid from oxidation of cyclohexanol by using different type of microorganisms such as Acinetobacter sp.strain (Burgard et al. 2013, Labet and Thielemans 2009, Thomas et al. 2002). Minami and Kozaki (2003) have invented the alternative production of ε-caprolactone from renewable starch sources. ε-Caprolactone monomer is a clear, colourless liquid at room temperature and has a melting temperature of -2 ^{o}C. Figure 2.5 shows the PCL synthesis from ring opening polymerization of ε-caprolactone. The repeating unit of PCL consists of five nonpolar methylene groups and one polar ester group. The presence of ester group in the structure provides the biodegradability of PCL due to the hydrolytic unstability of aliphatic ester linkage (Perrin and English 1997).

Figure 2.4. The commercial production of ε-caprolactone monomer from Baeyer Villiger oxidation method (Source: Labet and Thielemans, 2009).

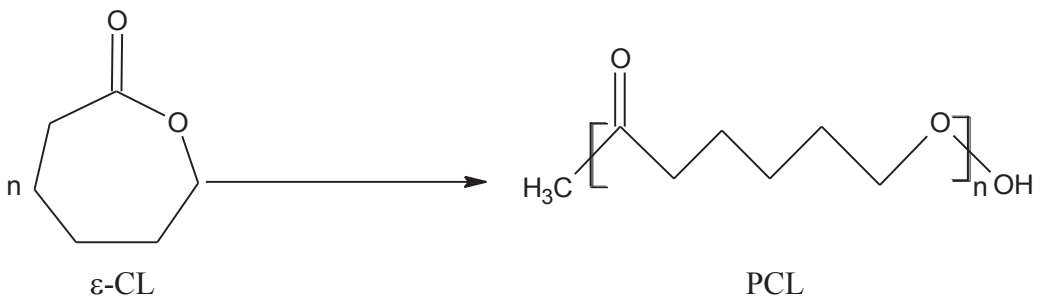


Figure 2.5. Synthesis of PCL by ring opening polymerization of ε-caprolactone.

PCL is a tough, flexible and semicrystalline polymer having a glass transition temperature of -60 ^{o}C and relatively low melting temperature of 55-60 ^{o}C and a high decomposition temperature of 350 ^{o}C. PCL has been extensively used as a homopolymer or constituent of various copolymers in biomedical applications due to its good biocompatibility, biodegradability and high drug permeability. PCL degrades

more slowly than PLLA and is thus suitable for the long term degradation in medical applications. PCL are used especially for long term drug delivery system due to its good drug permeability, high crystallinity and low degradation rate. Pitt et al. (1981) defined that the in vivo degradation of poly(D,L-lactide) was 2.8 times faster than the PCL under the same conditions. Degradation time of PCL depending on its molecular weight is given between 2 and 3 years (Nair and Laurencin 2007, Middleton and Tipton 2000). Dash and Konkimella (2012) reviewed the PCL based formulations for drug delivery and tissue engineering applications considering different preparation methods such as microspheres, scaffolds, nanoparticles, micelles, films and hydrogels. They concluded that the hydrophobicity of PCL is the major drawback responsible for its limited use in pharmaceutical formulations. For that reason, different approaches for incorporation of hydrophilic structure to PCL such as copolymerization or blending have been used. PCL blends consisting of poly(ethylene glycol) (PEG) or poly(D,L-lactide-co-glycolide) (PLGA) and PCL co-polymers synthesized with glycolide, lactide, 1,5-dioxepan-2-one poly(ethylene oxide) (PEO) or PEG provide the enhancement of degradation rate of PCL. The increase in degradation rate of PCL by blending or copolymerization can be explained by the changes in morphological properties such as reduction in crytallinity and increase in hydrophilic structure (Andronova et al. 2003, Dash and Konkimella 2012, Feng et al. 2009, Gou et al. 2009, Grijpma et al. 1994, Jiang et al. 2011, Lemmouchi et al.1998, Li et al. 2003, Mundargi et al. 2007, Perrin and English 1997, Wang and Dong 2006 and Wei et al. 2008).

The another method for enhancement of hydrophilicity and degradation of PCL alternative to preparation of PCL blends and copolymers is the synthesis of branched PCL polymers. Branched polymers increase the hydrophilicity and degradation of the polymer due to the increase in the number of functional end groups. Many types of branched polymers are prepared by using two different approaches called as core-first approach and arm-fist approach according to the structure of initiator used. The core-first approach is based on a multifunctional core used as the initiator such as poly(amidoamine) dendrimer (PAMAM-OH), polyethyleneimine (PEI), phosphazene, porphyrazine (Celik et al. 2009, Cui et al. 2004, Liu et al.2006, Miao et al. 2006). The arm-first approach is based on the coupling of linear living polymers with a multifunctional initiator such as trimethylolpropane, pentaerythritol, dipentaerythritol, etc. The degree of crystallinity, melting and degradation temperatures of star-shaped PCLs are lower than that of linear PCL with equivalent molecular weight and they are

decreased with an increase in arm numbers. (Choi et al. 2005, Wang et al. 2005). Choi et al (2005) also determined the radii of gyration of star-shaped PCLs using small-angle X-ray scattering (SAXS) to investigate the effect of the branching structure on molecular dimension. They calculated the branching ratio (g) from the ratio of the mean-square radii of gyration between a star-branched polymer $(R_g^2)_{branched}$ and its linear polymer $(R_g^2)_{linear}$ with similar molecular weight by using the Equation 2.1. The g values obtained between 0.74 and 0.89 show a decreasing trend with an increase in the degree of branching. This result also confirmed that star-branched polymers occupy less volume in solution than linear polymers of the same molecular weight.

$$g = \frac{(R_g^2)_{branched}}{(R_g^2)_{linear}} \qquad \text{Equation 2.1}$$

Many studies were carried out for improvement of degradability of PCL by decreasing its crytallinity. Yuan et al. (2005) synthesized hexaarmed star-shaped poly(ε-caprolactone)-b-poly(D,L-lactide-co-glycolide) by using cyclotriphosphazene and $SnOct_2$ as initiator and catalyst, respectively. The melting temperature and crystallinity of linear PCL found as 61 ^oC and 54.7 % decreased to 38.7 ^oC and 3.1 % for star shaped copolymer, respectively. The decrease in melting temperature and crystallinity was explained by the presence of amorphous D,L-PLAGA segments and short chain length of star-shaped PCL arms leading to crystalline imperfection.

The important criteria in the synthesis of valuable PCL polymers possessing biocompatibility, biodegradability and drug permeability is the catalyst type used. $SnOct_2$ is the most widely used catalyst in PCL synthesis similar to the PLA synthesis. The other metal catalysts including tin, aluminium, zirconium, lanthanides, zinc, iron are used in polymerization of ε-caprolactone. The complete removal of the catalyst from the polymer is not possible. For that reason, these biocompatible PCL polymers for the medical applications should not be synthesized by using toxic metal catalysts. Some of the biocompatible metal free catalysts used in PCL synthesis are acid based catalysts such as fatty acid derivatives (formic acid, oxalic acid, succinic acid and fumaric acid), heteropolyacid, organic catalysts (N-heterocyclic carbenes (NHC)s) and enzyms such as lipase (Cheng et al. 2010, Kamber et al. 2009, Kobayashi 2010, Oledzka and Narine

2010, Wang et al. 2011). The effects of catalyst type on the synthesis are introduced in the next chapter by considering reaction mechanisms.

2.2. Synthesis of Biodegradable Polymers

In polymer synthesis, monomer structure, catalyst type, temperature, solvent type and reaction time effect the polymerization significantly. Polymerization reaction mechanism and kinetics are the important topics that are commonly investigated for the understanding of polymerization reaction. In this chapter, literature review about the polymerization methods, mechanisms and kinetics of biodegradable PLA and PCL homopolymers and copolymers and the effect of catalysts or initiator on polymerization mechanism are given.

2.2.1. Polymerization Methods of PLA and PCL

Polycondensation polymerization (step growth polymerization) and ring opening polymerization (chain growth polymerization) in the synthesis of PLA and PCL polymers have been described in the literature as two main polymerization methods. In the condensation polymerization, PLA and PCL are synthezised from polycondensation of lactic acid and 6-hyroxycaproic (6-hydroxyhexanoic) acid determined as hydroxyacids, respectively. In the ring opening polymerization, PLA and PCL are synthezised from lactide and ε-caprolactone determined as six and seven membered cyclic lactones, respectively (Ajioka et al. 1998, Garlotta 2001, Labet and Thielemans 2009).

The polymerization methods starting from the manufacturing of lactic acid with the fermentation of carbohydrates (starch, corn or sugar cane) as well as depolymerization of PLA is given in Figure 2.6. Condensation polymerization can be divided into different sections according to the polymerization conditions such as direct condensation, azeotropic or melt-solid condensation (Gupta and Kumar 2007, Maharana et al. 2009). Low molecular weight PLA and PCL oligomers are obtained from lactic acid or 6-hydroxyhexanoic acid by removal of water in direct condensation polymerization, respectively. The molecular weight of PLA oligomers was determined

less than 5000 Da (Erwin et al. 2003). Depolymerization of oligomers takes place to give a thermodynamically favored lactide in the thermal cracking process. The properties of crude lactide monomer depend on the depolymerization reaction parameters and structure of polylactic acid oligomer. Crude lactide monomer generally consists of water, lactic acid, lactic acid oligomers and residual catalyst as if used in the polymerization of lactic acid (Inkinen et al. 2010). Before the polymerization, lactide monomer should be purified to produce high molecular weight PLA by ring-opening polymerization. Hyon et al. (1997) synthesized low molecular weight PLAs (Mw: $1.6x10^3$-$1.6x10^4$) by conventional condensation polymerization of lactic acid without catalyst and high molecular weight PLAs (Mv: $2xl0^4$ and $6.8x10^5$) by ring opening polymerization of lactide with using $SnOct_2$ catalyst.

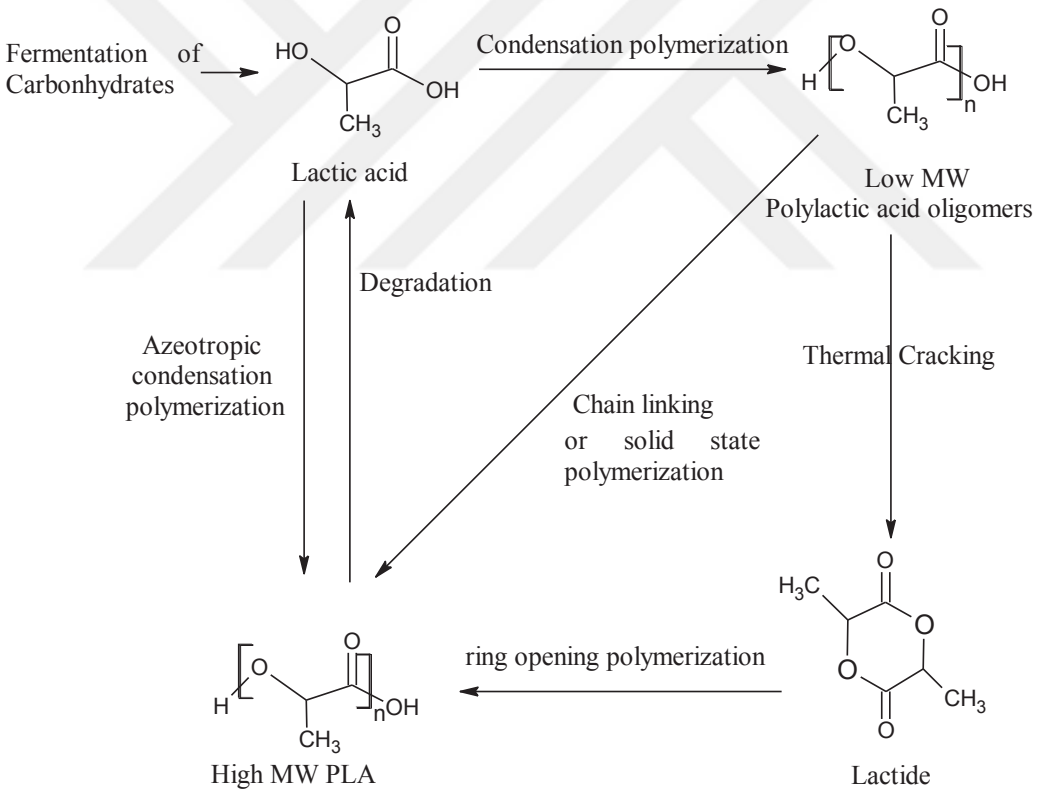


Figure 2.6. Polymerization methods and depolymerization of PLA.

High molecular weight PLA and PCL can not be obtained by using conventional condensation polymerization of lactic acid and 6-hydroxyhexanoic acid, respectively (Garlotta 2001, Labet and Thielemans 2009, Sisson et al. 2013, Okada 2003). The degrees of polymerization was determined as less than 100 in conventional direct

condensation polymerization of lactic acid. Hovewer, high molecular weight PLA is obtained by azeotropic condensation of lactic acid, solid state polymerization of lactic acid oligomers or using chain linking agents such as hexametylene diisocyanate. The removal of water from the reaction mixture is enhanced to obtain high molecular weight PLA in other condensation polymerization methods. Water formed in azeotropic or solid state condensation polymerization is removed by using an appropriate azeotropic solvent at below melting point of polymer or water tolerant hydrophobic catalysts such as distannoxanes and water absorber as molecular sieve above glass transition temperature under inert atmosphere, respectively (Gupta and Kumar 2007, Inkinen et al. 2011, Maharana et al 2009, Otera et al. 1996, Södergard and Stolt 2002).

2.2.1.1. Synthesis of PLA and PCL by Condensation Polymerization

Synthesis of PLA and PCL by condensation polymerization requires the use of the lactic acid or 6-hydroxyhexanoic acid due to the presence of hydroxyl and carboxyl group in the monomer as shown in Figure 2.7., respectively. Polycondensation reactions are usually performed in bulk with or without a catalyst, while vacuum and temperature are progressively increased (Hiltunen et al. 1997a, Moon et al. 2000, Hyon et al. 1997). Since synthesis of PCL by polycondensation polymerization is not prefferred due to the rare availability of expensive 6-hydroxyhexanoic acid monomer (Scullian and Zinck 2012). In this section, polycondensation polymerization of lactic acid is reported extensively. Reaction pathways in polycondensation of lactic acid are shown in Figure 2.8. Dehydration equilibrium reactions for esterification in Figure 2.8.a and b and ring-chain equilibrium reaction for depolymerization of PLLA into L-lactide in Figure 2.8.c are carried out during polycondensation of lactic acid (Moon et al. 2000). These reactions should be controlled by considering the removal of water to obtain high molecular weight PLA. For that reason, high vacuum and high temperature are required to remove the water during polycondensation of lactic acid. However, water in the polymerization mixture is not completely removed from the highly viscous reaction mixture (Gupta and Kumar 2007). Also, the high vacuum and temperature induces the depolymerization reaction due to the evaporation of lactide (Moon et al. 2000). For that reasons, low molecular weight polylactic acid is obtained by using direct condensation polymerization method. Also, the streoregularity can not be controlled during the

polymerization of lactic acid in this method. Although high molecular weight polyesters with good mechanical properties are not easy to obtain, the properties of lactic acid oligomers can be controlled by the use of different catalysts and functionalization agents, as well as by varying the polymerization conditions. Chain coupling agents (hexamethylene diisocyanate, 1-4 butane diisocyanate), bi/multi-functional hydroxyl compounds (1,4 butanediol, glycerol, pentaerythritol) and bi/multifunctional carboxylic acids (adipic acid, succinic acid, maleic acid) are used to modify and increase the molecular weight of polylactic acid (Gupta and Kumar 2007, Hiltunen et al. 1997b, Seppala et al. 2004, Shen et al. 2011).

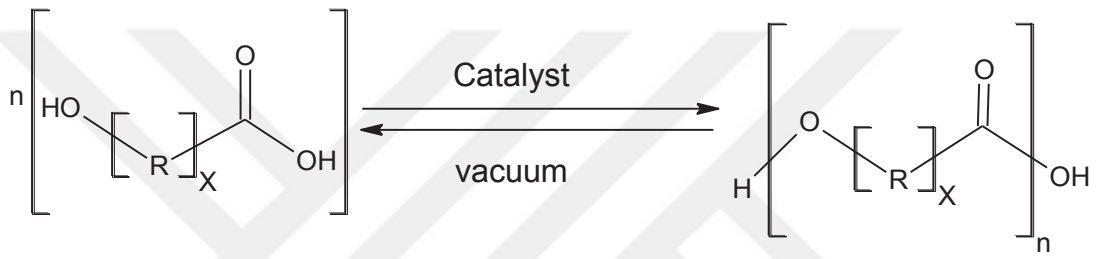


Figure 2.7. Direct condensation polymerization of the α-hydroxy acids
(R: $(CH_2)_2$ for lactic acid; R: $(CH_2)_5$ for 6-hydroxyhexanoic acid).

Acidic catalysts such as boric or sulfuric acid are usually used in the direct polycondensation of lactic acid. These catalysts accelerate the esterification and transesterification reactions, and also catalyze side reactions at temperatures above 120 ^{o}C. Therefore, the molecular weight of poly(DL-lactic acid) obtained from polycondensation is never higher than 3000 (Mehta et al., 2005). Ajioka et al. (1995) and Moon et al. (2000) investigated the effect of different catalysts in the azeotropic polycondensation of lactic acid in diphenyl ether and melt polycondensation of lactic acid. They determined the tin compounds (Sn, $SnCl_2$, SnO) and protonoic acids such as para toluenesulfonic acid (TSA) as effective catalysts in the synthesis of high molecular weight polylactic acid. Molecular weight of PLA was increased to $3x10^5$ and $5x10^5$ by using azeotropic polycondensation with diphenyl ether or melt-solid state polycondensation methods, respectively (Ajioka et al. 1995, Moon et al. 2001). Moon et al. (2001) carried out polymerization in two step by using binary catalysts system comprising tin dichloride hydrate and TSA. Although the use of binary catalyst system or high boiling point solvent in the polycondensation provides the increament in the

molecular weight of the polymer, the use of solvent and different catalysts and also purification step of the crude polymer increase the production cost in the preparation of pure PLA polymers.

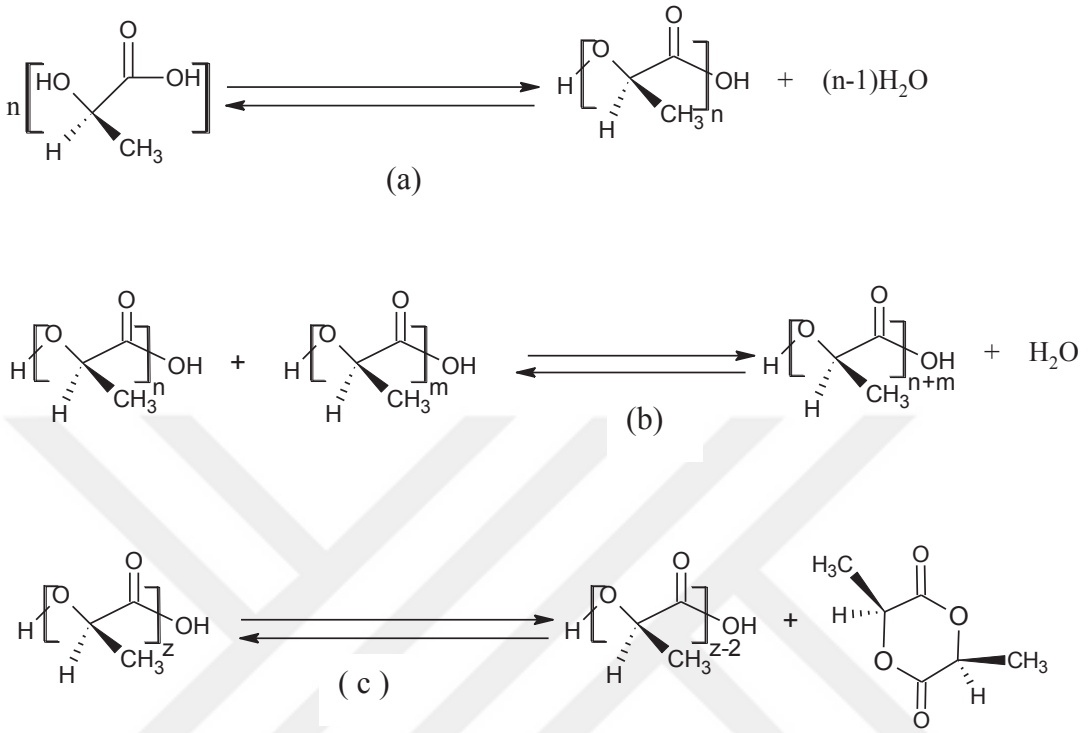


Figure 2.8. Reaction pathways in condensation polymerization of lactic acid (Source: Moon et al.2000).

The structure control of the copolymers or functional polymers synthesized by condensation polymerization is very difficult (Stridsberg et al. 2002). Aijoka et al. (1995, 1998) synthesized different type of homo/co PLA polymers by using direct polycondensation and ring opening polymerization methods. Although they obtained high molecular weight PLA polymers by using direct polycondensation method, they observed differences in thermal properties of homo/co PLA polymers synthesized according to two methods due to the difference in polymer sequence. They also deduced that random copolymers of D-lactic acid and L-lactic acid are only formed by using direct polycondensation method.

Melt polycondensation method is generally used in the synthesis of copolymers. Zhiyong et al. (2003, 2004) synthesized the new polyesteramides based on lactic acid or ε-caprolactone and aminoundecanoic acid (AU) by melt polycondensation

method. Figure 2.9 shows the synthesis of P(LA/AU) copolymers. Polyesteramide based on lactic acid or ε-caprolactone were prepared using a different polymerization procedure. This method requires high polymerization temperature. Aminoundecanoic acid and D,L-lactic acid or caprolactone, titanium dioxide, tetrabutyl titanate were kept under nitrogen atmosphere. The reaction mixture gradually heated to from 110 ^{o}C to 240 ^{o}C. The resultant melt was poured out into a steel plate to obtain P(LA-AU) or P(CL-AU) copolymer. Although the melting temperature and crystallinity values increase with the AU content, the decrease in degradation rate and water absorption were observed.

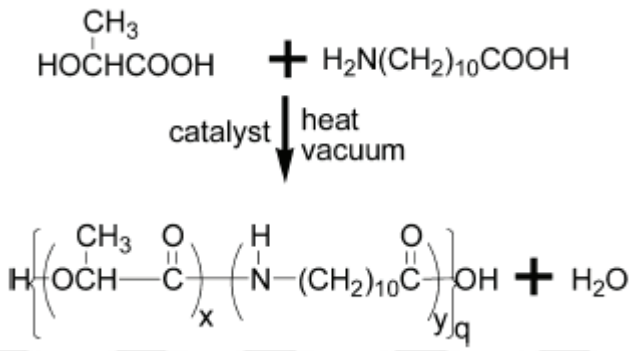


Figure 2.9.Synthesis of polyesteramide with D,L-lactic acid and aminoundecanoic acid (Source: Zhiyong et al. 2003).

2.2.1.2. Synthesis of PLA and PCL by Ring-Opening Polymerization

Ring-opening polymerization (ROP) is the most effective and versatile method in the synthesis of PLAs and PCLs due to the possibility of control of polymerization. Many different type of metal complexes as catalysts/initiators (alkoxides, carboxylates, oxides) (Sn(II), Sn(IV), Fe, Al, La, Mg, Ti, Zn etc.), organic based catalysts (4-dimethylamino)pyridine (DMAP), 4-pyrrolidinopyridine (PPY), N-heterocyclic carbenes (NHCs), organic amino calcium, methane- and trifluoromethane- sulfonic acid), organic acids (tartaric acid, citric acid) and biocatalysts (enzymes (lipase PS), guanidine derivatives) have been used in the ring opening polymerization of PLAs or PCLs (Casas et al 2004, Darensbourg and Karroonnirun 2010, Dechy-Cabaret et al. 2004, Dove 2012, Dubois et al. 1996, Eguiburu et al. 1999, Kim et al. 2001, Li et al. 2004, Libiszowski et al. 2002, Nederberg et al. 2001, Numato et al. 2007, Piao et al.

2003, Save et al 2002, Schwach et al. 2002, Slomkowski et al. 1997, Wang et al. 2004, Wu et al. 2005). Reaction pathway in ring-opening polymerization of lactide is shown in Figure 2.10. Cyclic lactide monomer is capable of opening the ring during polymerization due to its favorable thermodynamic property such as negative Gibbs free energy change (ΔG). ROP of lactide involves an acyl-oxygen bond scission with the presence of metal catalyst (M-O-R). The ease of polymerization of cyclic any polymer depends on thermodynamic and kinetic factors (Chanda 2000). Especially, polymerization occurs in the case of negative Gibbs free energy change (ΔG).

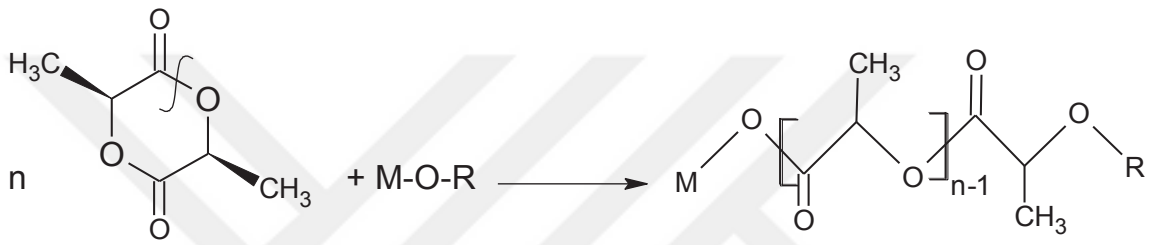


Figure 2.10. Reaction pathway in ring opening polymerization of lactide. M-O-R symbolize initiator or catalyst.

The selection of the catalyst/initiator in ring opening polymerization is an important criterion affecting the properties of synthesized polymer such as molecular weight, thermal and mechanical properties. For that reasons, catalytic efficiency, abundance, selectivity and toxic properties of catalyst should be taken account in the selection of catalyst for the ring opening polymerization of lactide. Among the various catalysts, tin(II) 2-ethylhexanoate ($SnOct_2$) is the most widely used catalyst for the synthesis of homo/coPLAs. The high reactivity of $SnOct_2$ in ring opening polymerization of lactide is the most important feature leading to the synthesis of high molecular weight PLA. In addition, the use of metal based catalysts in polymerization of biopolymers requires some limitations. The use of metal based catalysts can result in residual metal contamination in the polymer produced in the reaction, which can lead to a toxic effect due to the release of metals during biodegradation of polymers. Contamination of catalyst residue on PLA based biopolymers used for medical and pharmaceutical applications can lead to undesirable effects. It is possible that the active

metal leaches into solution to some degree during the polymerization (Kricheldorf and Saunders 1998 and Kricheldorf 2001, Kricheldorf et al. (2004 a,b), Piao et al. 2003, Rashkov et al. 1996 and Schwach et al. 2002). For that reason, the catalysts used in the synthesis of PLA based polymers for medical applications should be selected properly. $SnOct_2$ as a commonly used catalyst for polymerization of lactide, has high reactivity property and also a high cytotoxicity effect against a broad variety of microorganisms. Generally, biocidal properties of tin compounds lead to the use of them as antifouling agents across fungicides, bactericides and insecticides for different industrial and agricultural applications such as paint, textile or food. Especially, organotin compounds used in boat paints are responsible for contamination of marine and freshwater ecosytems (Cooney and Wuertz 1989, Okoro et al. 2011, White and Tobin 2004). Although $SnOct_2$ has been admitted by the American FDA as a stabilizing food additive, the present of $SnOct_2$ residue in the synthesized polymers used for medical or pharmaceutical applications can lead to undesirable effects due to its cytotoxicity effect for living cells (Schwach et al. 1997, Stjerndahl et al. 2007, Tanzi et al. 1994). Alternative catalysts having less toxic properties such as calcium, magnesium and zinc compounds had been tried instead of $SnOct_2$. However, the efficiencies of these alternative catalysts were low compared to $SnOct_2$ (Kricheldorf and Damrau 1998, Kricheldorf et al. 2000 a, Schwach et al. 1994, Wu et al. 2005, Zhong et al. 2001).

Casas et al (2004) performed the ring opening polymerization of ε-caprolactone by using different types of organic acids with the presence of benzyl alcohol as alternative catalysts. L-Tartaric acid, citric acid, L-lactic acid, hexanoic acid, propionic acid, glycine, L-serine and L-proline were used in the ring opening polymerization of ε-caprolactone. The highest molecular weight of PCL was obtained as 2730 Da by using tartaric acid. They ordered the catalytic efficiency of the metal free organic catalysts in the ring opening polymerization of ε-caprolactone as: tartaric acid>citric acid>lactid acid>proline. Low molecular weight PCLs were obtained by using nontoxic organic acid catalysts having low catalytic efficiency.

Leenslag and Pennings (1987) studied the polymerization of L-lactide as a function of polymerization temperature (100-140 ^{o}C), time and $SnOct_2$ concentration. PLA with the highest value of intrinsic viscosity ([η] = 13 dl/g, Mv $\approx 10^6$) was synthesized at a low catalyst concentration (0.015 wt%) and at 100 ^{o}C. Hyon et al. (1997) also studied the polymerization of L-lactide by using $SnOct_2$ catalyst in the

concentration range from 0.003 to 0.8 wt% at 130 ^{o}C for 72 h. Figure 2.11. shows the effect of $SnOct_2$ concentration on the viscosity average molecular weight of PLA and monomer conversion for bulk polymerization of L-LA at 130 ^{o}C for 72 h. As seen in the figure, the maximum molecular weight (M_v: 6.8x10^5) was obtained at concentration of $SnOct_2$ 0.05 wt%. The maximum M_v obtained from the study of Hyon et al. is lower than that of the study of Leenslag and Pennings. The decrease in Mv had been explained by the thermal depolymerization of the resultant polylactides at prolonged polymerization and higher polymerization temperature. Also, the presence of intra- and inter-molecular transesterification reactions due to the high reaction temperature and long reaction time affects the molecular weight.

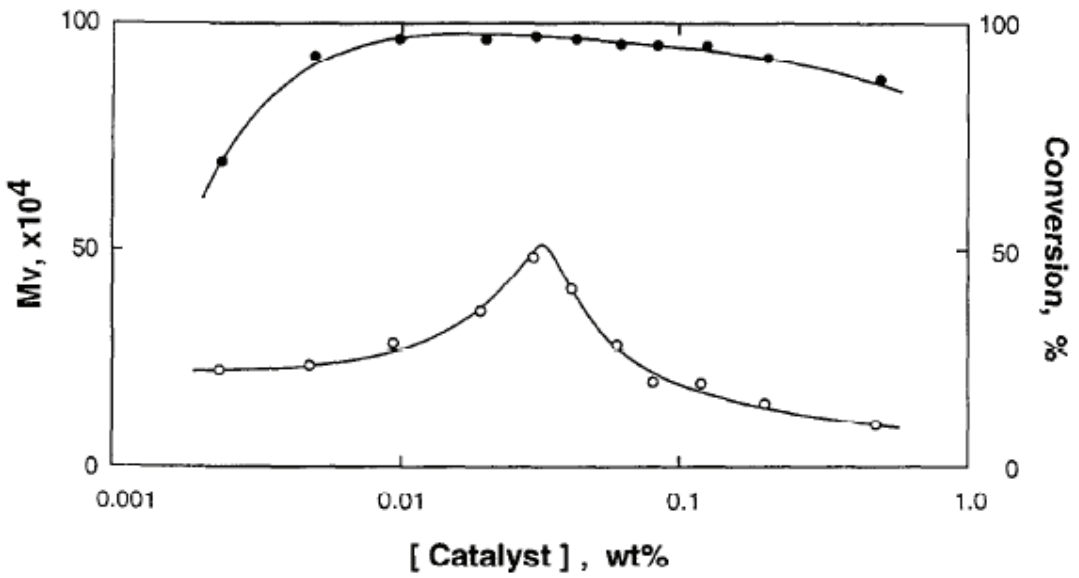


Figure 2.11. Effect of the $SnOct_2$ concentration on the viscosity average molecular weight (M_v) of PLA and monomer conversion for bulk polymerization of L-LA at 130 ^{o}C for 72 h. °: Mv •: conversion (Source: Hyon et al. 1997).

Another important criterion in ring opening polymerization is the purity of lactide or ε-caprolactone monomer. Inkinen et al. (2011) mentioned that it is possible to produce a wide range of molecular weights by controlling the purity of lactide. In previous studies, the lactide monomer was used as received (Stevels et al. 1996). However, purification of lactide is carried out by recrystallization with dry solvents like

toluene or ethyl acetate prior to use in the studies of Albertsson group and Kricheldorf group, respectively. Purification procedure of ε-caprolactone monomer is different than that of lactide monomer. ε-caprolactone is purified by drying over calcium hydride and distilling under reduced pressure (Kricheldorf and Hauser 1998, Kricheldorf et al. 2008, Stjerndahl et al, Zhong et al. 2001) Silanization of glassware is also required in ring opening polymerization of lactide and ε-caprolactone to obtain higher molecular weight polymer (Finne and Albertsson 2002, Finne et al. 2003, Kricheldorf and Damrou 1998, Kricheldorf et al. 2004b, 2005 a-d, Kricheldorf and Lee 1995)

The choice of initiator system, co-initiator as chain control agent, catalyst concentration, monomer-to-initiator ratio, polymerization temperature and time significantly affect the polymer properties such as the molecular weight, degree of crystallinity, thermal and mechanical properties (Duda et al. 2000, Prego et al. 1996, Vert et al. 1995, Zhao et al. 2002).

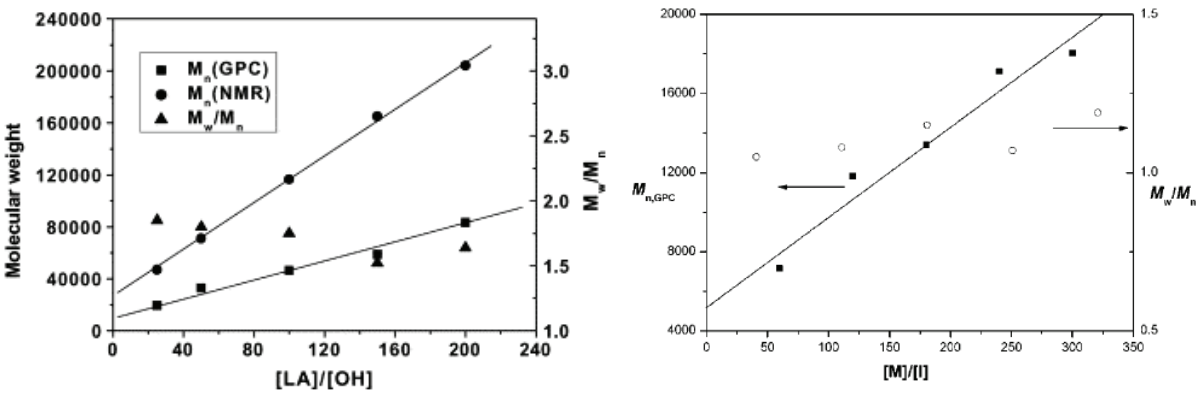
Wang et al.(2005) and Zhao et al. (2002) studied the effects of initiator and catalyst on the molecular weight of star-shaped PCLs and PLAs, respectively. They confirmed that catalyst amount ($SnOct_2$) had no apparent influence on molecular weights of branched PCL or PLA polymers synthesized with dipentaerythritol or starburst PAMAM-OH dendrimer as initiators, respectively. However, the molecular weight of the polymers linearly increased with the molar ratio of the monomer to the initiator ([M]/[I]). The results of Wang et al. (2005) and Zhao et al. (2002) were given in Table 2.1., Table 2.2 and Figure 2.12 to show the dependence of molecular weight on the ratios of [M]/[$SnOct_2$] and [M]/[I]. As seen in Table 2.1 and Table 2.2, the amount of $SnOct_2$ catalyst almost has no influence on the molecular weight of the resulting star-shaped PLAs and PCLs, respectively. However, molecular weights of star-shaped PLAs are significantly higher than those of star-shaped PCLs due to the usage of high molecular weight dendrimer in PLA instead of low molecular weight DIPENT in PCL. As seen in the figure, the number-average molecular weights of the star-shaped PLAs and PCLs linearly increase with the molar ratio of monomer to initiator ([M]/[I]), which indicates the role of the hydroxyl groups in initiator as effective propagation centers. They demonstrated that the hydroxyl-terminated dendrimer and hydroxyl functional dipentaerythritol as the initiators control the molecular weight of the star-shaped PLAs and PCLs, respectively.

Table 2.1. Results of bulk polymerization of L-lactide with various amounts of $SnOct_2$ catalyst at 130 ^oC and [LA]/[I] = 50 (Source: Zhao et al. 2002).

[LA]/[$SnOct_2$]	Rxn Time (h)	Conversion (%)	M_n (GPC)	M_w/M_n
100	12	84.8	28760	1.84
200	12	83.6	30150	1.72
400	12	81.4	30630	1.69
600	12	80.4	30100	1.65
1000	24	82	30760	1.64
2000	24	81.5	31840	1.6

Table 2.2. Results of bulk polymerization of caprolactone with various amounts of $SnOct_2$ catalyst at 120 ^oC (Source: Wang et al. 2005).

[CL]/[$SnOct_2$]	[CL]/[I]	Yield (%)	M_n (GPC)	M_w/M_n
250	122	88.4	10230	1.24
500	122	91.4	11420	1.17
1000	122	89.4	11820	1.08
2000	112	93.5	9160	1.12



(a) (b)

Figure 2.12. Dependence of molecular weight on the molar ratio of monomer to initiator (a)[LA]/[$SnOct_2$]=100/0.15 (Zhao et al. 2002) (b)[CL]/[$SnOct_2$]=1000/1 (Source: Wang et al. 2005).

The structure of PLA polymers depends on the alcohols used as co-initiators. (Karidi et al. 2013, Korhonen et al. (2001) used alcohols with different numbers of hydroxyl groups as co-initiators. 1,4 Butanediol, pentaerylthritol, polyglycerine-06 and polyglycerine-10 having OH groups 2, 4, 8 and 10 were used, respectively. Mono and difunctional alcohols yield linear polymers, while alcohols with hydroxyl functionality higher than two give star- or comb-shaped polymers. The high molecular weight polymers were obtained due to the presence of alcohols having high numbers of hydroxyl groups.

Kricheldorf et al. (2004 b) studied the effect of initiator structure on the polymerization of L-Lactide by using bismuth(III) acetate (Bi(III)Ac) as a catalyst. Tetra(ethylene glycol), 1,1,1 –tri(hydroxyl methyl)propane and pentaerythritol were used as initiators. Telechelic polylactides having two, three and four CH-OH end groups were obtained by using tetra(ethylene glycol), 1,1,1-tri(hydroxy methyl)propane (THMP) and pentaerythritol as co-initiators, respectively. The linear two functional and branched star-shaped poly(L-lactide)s having three and tetra functional structure were obtained as a result of the usage different initiators. The chain lengths were varied via the monomer/initiator ratio. The results of this study demonstrated that Bi(III)Ac is slightly less reactive initiator than tin(II) 2-ethylhexanoate. Kricheldorf et al. (2004 a, 2004b) suggested that –OH functional telechelic and star shaped PLAs or PCLs may be used as building blocks of more complex architectures such as ABA triblock copolymers, multiblock copolymers or networks.

2.2.1.2.1. Synthesis of Block or Random P(LA-co-CL) Copolymers by Ring-Opening Polymerization

Block and random copolymers play a central role in the incorporation of characteristic and intermediate properties of parent homopolymers, respectively. These differences between the properties of block and random polymers such as mechanical and thermal properties can allow us for making right decision about the use of block or random copolymers regarding to the the property requirements in different applications. The studies about ring opening polymerization of block or random lactide based copolymers with other biocompatible monomers such as ε-caprolactone (CL), glycolide, 1,5-dioxepan-2-one (DXO) or trimethylene carbonate (TMC) has been increasing due to

the effects of monomer sequencing and monomer composition on the degradation, thermal or mechanical properties of final biopolymer (Contreras and Davilla 2006, Darensbourg et al. 2007, Darensbourg and Karroonnirun 2010, Gruvegard et al. 1998, Nalampang et al. 2007, Ryner et al. 2001, Södergard and Stolt 2002, Stridsberg et al. 2002).

The type of the polymerization technique used is an important parameter in thermal and structural properties of the copolymers. Ajioka et al. (1998) synthesized high molecular weight PLCL copolymers around 120000 Da by using three different methods as shown in Figure 2.13; i) direct polycondensation of L-lactic acid and 6-hydroxyhexanoic acid; ii) ring opening polymerization of L-lactide and ε-caprolactone and iii) sequential polymerization of L-lactic acid and ε-caprolactone. As shown in the figure, the used polymerization method directly effects the melting and glass transition temperatures of the copolymers due to the differences in sequence of the copolymers. Amorphous PLCL copolymers synthesized by direct polycondensation of L-lactic acid and hydroxyhexanoic acid and ring opening polymerization of L-lactide and ε-caprolactone were obtained. Hovewer, crystalline PLCL copolymer was obtained in sequential polymerization. In this method, ring opening polymerization of ε-caprolactone was carried out after the polycondensation of L-lactic acid. The observation of melting temperature indicates the formation of crystalline block PLCL copolymer by using sequential method.

Considerable interests exist about the synthesis of block and random copolymers of L-Lactide and ε-caprolactone due to the biodegradable and biocompatible properties of these monomers. While PCL homopolymers are elastic and good permeability properties, PLLA homopolymers have fast biodegradability and brittle properties. However, block and random P(CL-LA) copolymers possess permeability, elasticity and biodegradable properties. Also, these properties of block or random copolymers are manipulated by using different copolymer compositions (Lemmouchi et al. 2008, Vanhoorne et al. 1992, and Quian et al. 2000).

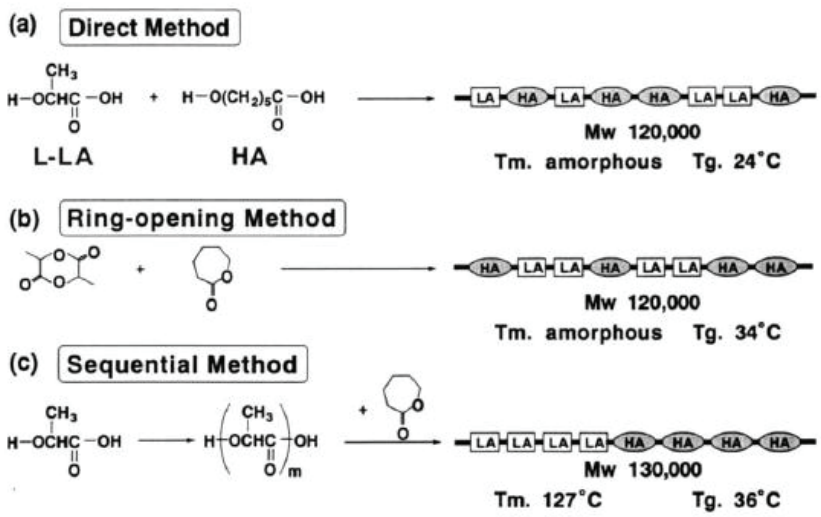


Figure 2.13. Different polymerization procedures used in PLCL copolymers a) direct polycondensation b) ring-opening polymerization c) sequential polymerization (HA denotes 6-hydroxyhexanoic acid).(Source: Aijoka et al 1998).

Quian et al. (2000) synthesized ABA block copolymer of lactide and ε-caprolactone by preparing di-OH functional PCL prepolymer and studied the effects of copolymer composition on thermal, mechanical, and water absorption properties of these polymers. Di-OH functional PCL prepolymer were synthesized by using $SnOct_2$ and ethylene glycol as catalyst and co-initiator, respectively. The ABA block copolymer were prepared in two step polymerization reactions as shown in Figure 2.14. $SnOct_2$/ε-caprolactone and $SnOct_2$/(lactide and prepolymer) mole ratios were kept constant as 5/10000. In the case of prepolymer synthesis, co-initiator/ ε-caprolactone mole ratio was used as 0.005. The yields of ABA copolymers were obtained as higher than 92.8 %. The molecular weights of ABA block copolymers having different LA/CL ratios such as 90/10, 80/20, 70/30, 60/40, 50/50 and 30/70 were found in the range of 156000 and 63700 from GPC analyses. The properties of the copolymers are significantly dependent on composition of the copolymers. The melting temperatures of the CL and LA unit also change with the composition in the range of 44-54 ^{o}C and 149-155 ^{o}C, respectively. As the L-LA content decreased from 90/10 to 30/70, the maximum maximum strains increased considerably from brittle structure to 791 %. The low water absorption properties of the copolymers and homopolymers are obtained around 1 %. They mentioned that block copolymers with higher LA content lead to higher water absorption, faster degradation and good permeability due to the higher ester group content and low crytalline properties.

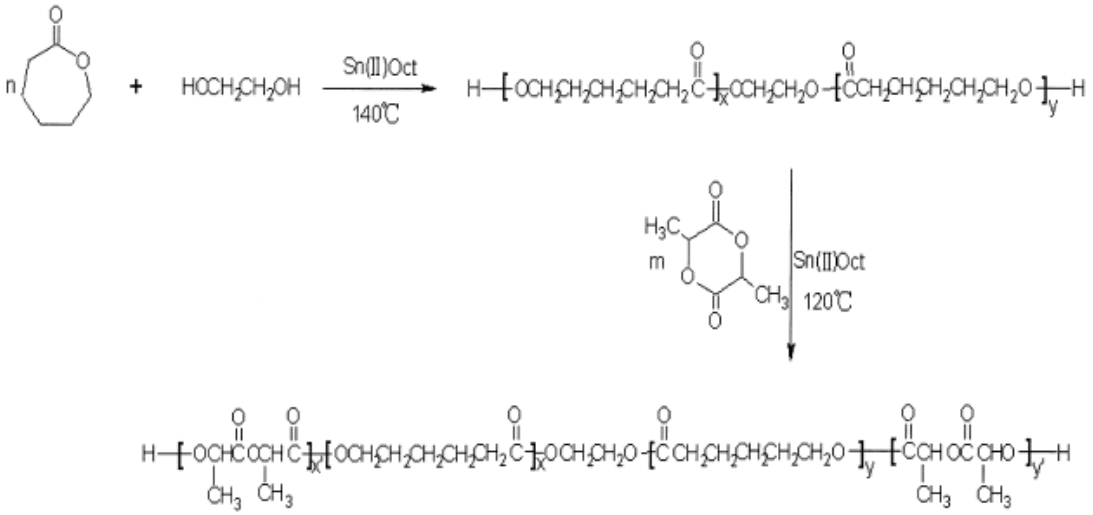
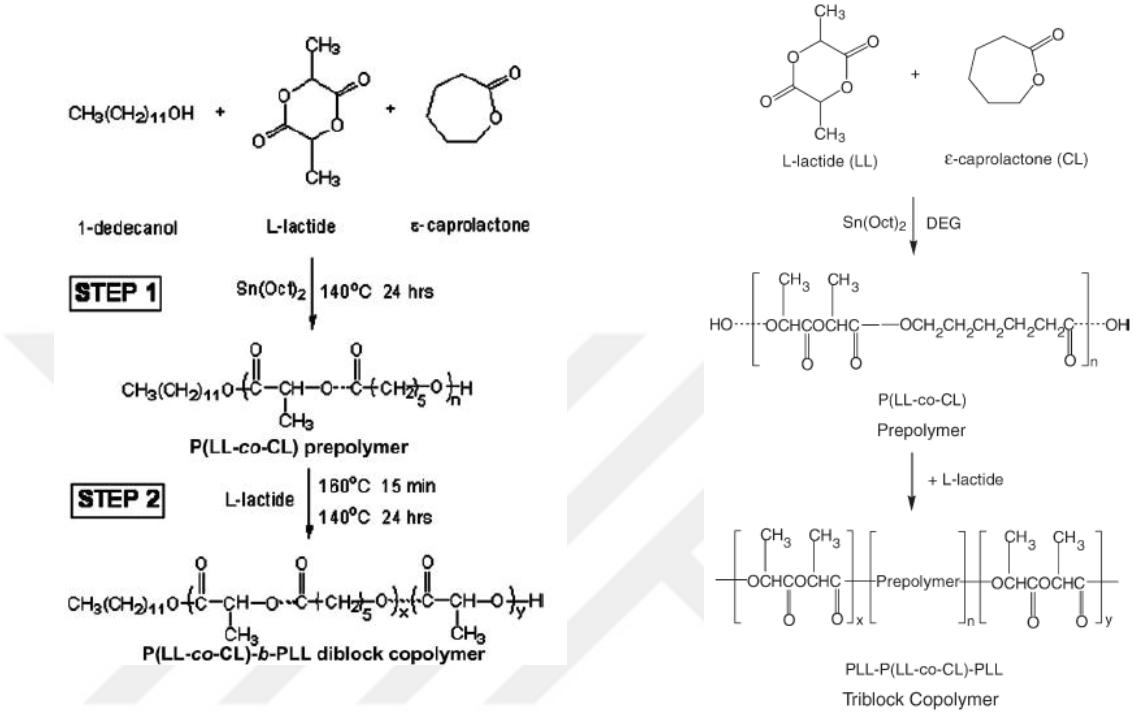


Figure 2.14. The schematic preparation of ABA block copolymers of L-lactide and ε-caprolactone (Source:Quian et al. 2000).

Niamsa and Baimark (2007) and Baimark and Molloy et al. (2005) synthesized AB diblock and ABA triblock copolymers of poly(L-lactide-co-caprolactone) and poly(L-lactide) by using mono-OH and di-OH functional poly(L-lactide-co-caprolactone) prepolymers, respectively. Mono-OH and di-OH functional P(LL-co-CL) prepolymers having 50:50 mol % as LL:CL ratio were synthesized by using dodecanol and diethylene glycol as co-initiators, respectively. The AB diblock and ABA triblock copolymers were prepared in two step polymerization reactions by using only $SnOct_2$ catalyst in the first step. The schematic preparation of AB diblock and ABA triblock copolymers is shown in Figure 2.15. In the case of P(LL-co-CL) prepolymer synthesis, the molecular weights of the prepolymers prepared by using different (ε-caprolactone and L-lactide)/co-initiator mole ratios as 125, 312.5 and 625 were measured as 15900, 37100 and 85300 g/mol, respectively. In the case of the synthesis of AB diblock and ABA triblock copolymers, no additional catalyst were used in the second step. The ABA block copolymers having 75/25 or 80/20 were prepared from P(LL-co-CL) prepolymers with low M_n as found as 15900, 37100 g/mol. The molecular weights (M_n) of ABA block copolymers having 80/20 as LA/CL ratios were found as 42400 and 57000 g/mol from GPC analyses. Baimark's group indicated that the synthesis of AB and ABA type polymers can be possible by using prepolymer as a macroinitiator without using another catalyst such as $SnOct_2$ during the second step reaction of block copolymers. Baimark's group also indicated that the thermal properties of the AB diblock copolymers determined from the DSC thermograms are controlled by the

amorphous block length of P(LL-co-CL) prepolymer and the molecular weight of the copolymer as shown in Figure 2.16. The observation of similar melting and crystallization temperatures and enthalpy values of samples b and c having different molecular weight was explained by the inhibition of the crystallization of PLL block due to the presence of long P(LL-co-CL) block.



(a) (b)

Figure 2.15. The schematic preparation of (a) AB diblock and (b) ABA triblock copolymers of poly(L-lactide-co-ε-caprolactone) and poly(L-lactide) (Source: Niamsa and Baimark 2007 and Baimark and Molloy, 2005).

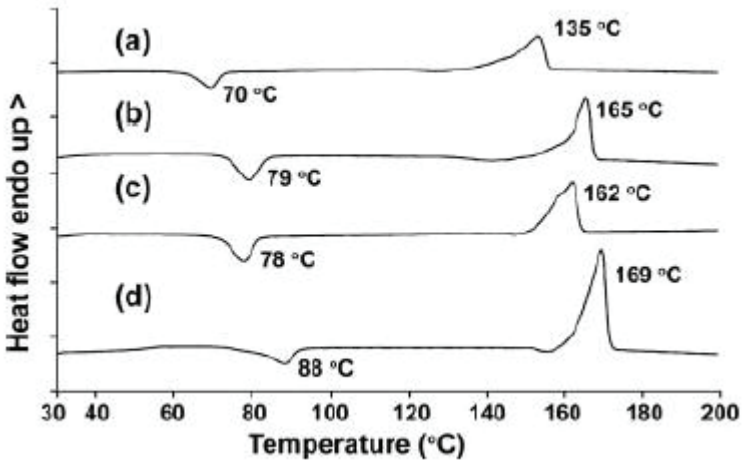


Figure 2.16. DSC thermograms from the second heating scans for diblock copolyesters of poly(L-lactide-co-ε-caprolactone) and poly(L-lactide) with variable molecular weight and block length of CL (a) 11400 (2.1), (b)17300 (2.3), (c) 31700 (2.0), and (d) 42400 (1.9) (Source: Niamsa and Baimark 2007).

Block or random structure of P(LL-co-CL) copolymers depends on the monomer addition sequence during polymerization. In't Veld et al. (1997) investigated the effect of monomer addition sequence in copolymerization of ε-CL and L-LA at 110 ^{o}C by using $SnOct_2$ and ethanol as catalyst and initiator, respectively. AB block copolymers of ε-CL and L-LA had been obtained when ε-CL was polymerized first. In addition, random copolymers of ε-CL and L-LA had been obtained when L-lactide was polymerized first. The formation of random copolymers is due to transesterification reactions which were caused by the-ε-CL derived hydroxyl end groups generated during the copolymerization of ε-CL with prepolymers of L-LA. The L-LA derived hydroxyl end-groups are less reactive compared to the ε-CL derived hydroxyl end groups. For that reason, the block copolymers of ε-CL and L-LA were obtained when transesterification reactions do not occur in the case of L-LA addition secondarily.

Monomer addition sequence plays also a significant role on the determination of crystal structure of the block or random P(LL-co-CL) copolymers. Nalampang et al.(2007) investigated the effects of monomer sequence on the chain microstructure of the copolymers prepared by one-step and two-step bulk ring-opening polymerization of LA and CL using $SnOct_2$ and 1-hexanol. Figure 2.17 indicates the schematic preparation of P(LA-co-CL) copolymers according to the sequential two-step polymerization. As shown in the figure, PCL prepolymer was polymerized first to 70 % conversion and LA was added in the second step. The microstructure of the copolymers synthesized by the two-step procedure was distinctly different from that of the one-step procedure. The average sequence lengths of lactidyl and caproyl units of the copolymers synthesized by the two-step procedure were significantly longer than that of the corresponding one-step procedure. Less transesterification and lower degree of randomness were obtained in the two-step procedure. Blocky structure of the copolymers synthesized by the two-step procedure led to the formation of semi-crystalline structure, whereas the copolymers synthesized by the one-step procedure were led to the formation of mainly amorphous structure.

Lemmouchi et al.(2007) have synthesized the novel star-shaped poly(ethylene glycol)-block-poly(lactide) copolymers by ring opening polymerization of lactide using commercial three or four-armed poly(ethylene glycol) as an inititator and potassium hexamethyldisilazide as a catalyst. Polymerizations were carried out in toluene at room

temperature.The decrease in molecular weight, glass transition and melting temperatures of the copolymers were observed by decreasing the rate of [LA]/[PEG].

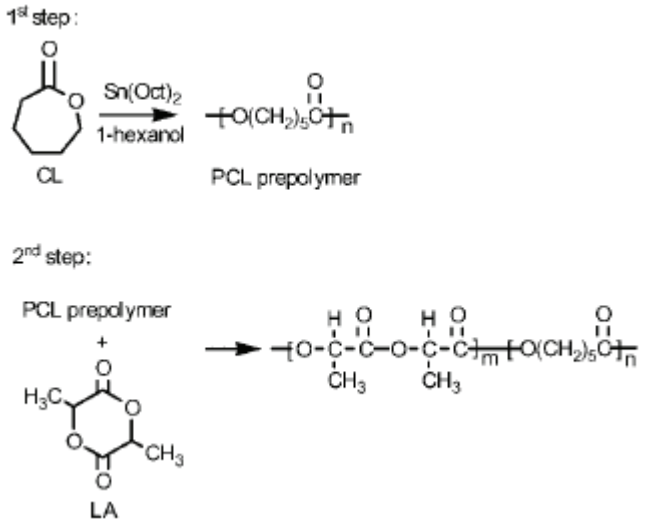


Figure 2.17. The schematic preparation of P(LA-co-CL) copolymers according to the sequential two-step polymerization (Source: Nalampang et al. 2007).

Nakayama et al. (2008, 2007) studied the synthesis of homo and multiblock poly(ester urethane)s (PEU) from hydroxy-telechelic PLAs and hydroxy-telechelic PLA and PCL prepolymers, respectively. Homo or multiblock PEUs were obtained by using purified and dried telechelic PLA prepolymer or PLA and PCL prepolymers with the presence of chain extender (hexamethylene diisocyanate) and catalyst (neodymium tetrahydroborate or $SnOct_2$). They found that the properties of the PEUs are considerably affected by the diol units by considering their thermal, mechanical, and degradation properties. Especially, degradation rate of the PEUs were strongly dependent on the diol units. Random co-chain extension of a mixture of HO-PLLA-OH and HO-PCL-OH afforded multiblock PLLA-co-PCL in good yields. The increase in tensile modulus and elongation at break values of PCL-rich multiblock co-polymers with high molar mass were observed.

The identification of the copolymer structure is a requirement for the control of the copolymer properties such as mechanical, degradation, crystallization and solubility. The catalyst type and reaction temperature lead to the formation of different types of copolymers such as diblock, multi block or random due to the presence of side reactions known as transesterification reactions (Bero et al. 1993). Also, in the synthesis of homo and coPLA polymers, the type of catalyst or initiator is the main criteria determining

ring opening polymerization mechanism, transesterification reactions and polymerization kinetics. Ring opening polymerization of lactide is carried out in bulk at 110-180 ^{o}C or in solution at 25-80 ^{o}C (toluene, dichloromethane, 1,4-dioxane, tetrahydrofuran) according to cationic, anionic, or coordination-insertion mechanisms depending on the catalyst or initiator used (Aubrecht et al. 2002, Bourissou et al. 2005, Dechy-Cabaret et al. 2004, Jedlinski et al.1993, Krichelforf et al. 2000 b, Kricheldorf and Kreiser-Saunders 1990). Polymerization mechanism and transesterification reactions are explained in the next part due to their direct effects on chain microstructure and properties of any homopolymer or copolymer during polymerization.

2.2.2. Ring Opening Polymerization Mechanisms

The growing interest about PLA or PCL as biopolymers has also led to an increment of studies about mechanism governing polymerization of lactide and ε-caprolactone. The studies about the ring opening polymerization mechanisms of PLA and PCL have shown that the catalyst or initiator type directly affects the polymerization mechanism. The ring-opening polymerizations of lactide or ε-caprolactone monomers generally carried out *via* anionic, cationic, or coordination/insertion mechanism depending on the type of catalyst or initiator. In addition, a living or a non-living anionic, cationic or coordination/insertion ring opening polymerization is carried out by the effect of polymerization conditions such as temperature, initiator and solvent (Kamber et al. 2007, Kricheldorf et al 1988, Miola-Delaite et al.1999, Nomura et al 2000).

2.2.2.1. Anionic Ring Opening Polymerization

The anionic ring opening polymerizations of lactide or ε-caprolactone monomers are mostly initiated by alkali metal salts and alkoxides such as potassium methoxide, iron(II) alkoxides, lithium alkoxides, sodium and potassium alkoxides (Ito et al. 1977, Jedlinski et al. 1991, Kricheldorf and Kreiser-Saunders 1990, Kricheldorf and Boetcher 1993b, Mcguinnes et al. 2003). Also, the other anionic initiators based on

alkaline and alkaline earth metal phenoxides or carboxylates such as potassium phenoxide, potassium benzoate, butyl lithium, zinc stearate show catalytic activity at higher temperatures compared to the alkali metal alkoxides due to the weaker base (Kricheldorf 2001, Mazarro et al. 2009). On the other hand, high basicity of the metal alkoxides leads to side reactions in anionic ring opening polymerizations of lactones (Kricheldorf and Kreiser-Saunders 1990, O'Keefe et al. 2001)

Anionic ring opening polymerizations of lactide and ε-caprolactone require nucleophilic attack of the negatively charged anion of initiator on the carbonyl carbon atom with acyl-oxygen scission. The initiation and subsequent chain-growth steps of anionic polymerization of lactide by using a metal alkoxide (M-OR) as catalyst are shown in Figure 2.18. Anionic polymerization of lactide is carried out by the nucleophilic reaction of the anion with the carbonyl carbon of the monomer and the subsequent acyl-oxygen cleavage. In the chain growth step, propagation occurs by an alkoxide end group. More nucleophilic alkoxide present in the anionic catalyst promote initiation and propagation of the polymer chain (Mcguinnes et al. 2003, Garlotta 2001).

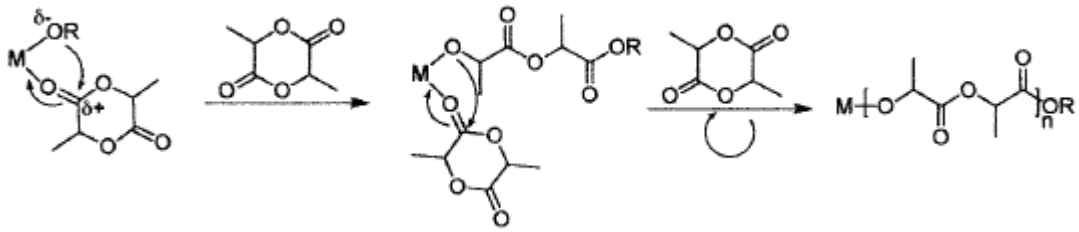


Figure 2.18. Anionic ring opening polymerization of lactide
(Source:Mcguinnes et al., 2003)

Kricheldorf and Kreiser-Saunders (1990) indicated that anionic polymerization of lactide significantly depends on basicity of initiator. Hovewer, the use of initiator or alkoxide group as strong bases leads to the partial racemization of lactide due to the deprotonation of lactide. Racemization proceeds as an unavoidable side reaction of anionic polymerization. Low and moderate molecular weight polymers are obtained in anionic polymerization due to the side reactions. The side reactions strictly depend on type of anionic initiator and solvent type. Kricheldorf and Lee (1995) and Luximon et al. (2001) investigated the effect of reaction conditions such as solvent type (dioxane, toluene, THF), temperature and monomer initiator ratio on the anionic polymerization of L or D,L-lactide initiated by dibutylmagnesium (Bu_2Mg) and lithium

diisopropylamide, respectively. Although the anionic polymerization of D,L-lactide initiated with lithium diisopropylamide in dioxane solution at 25 ^{o}C completed in few minutes, transesterification reactions were observed. They also observed no polymerization of D,L-lactide in THF solution with lithium diisopropylamide. In addition, the anionic polymerization of D,L or L-lactide initiated with Bu_2Mg in toluene and crown ether solution at 0 ^{o}C completed in four days and high molecular weights of PLLA ($3x10^5$) and PDLA (10^5) were obtained without transesterification reactions.

The atomic metal electronegativity of the catalysts is one of the influential parameters with respect to the catalytic activity for anionic mechanism. Mazarro et al. (2009) investigated the catalytic activities of different catalysts in the bulk copolymerization of D,L-lactide and glycolide by considering electronegativity values. Potassium (I) 2-ethylhexanoate (KOct), strontium (II) 2-ethylhexanoate ($SrOct_2$), lithium (I) 2-ethylhexanoate (LiOct), calcium (II) 2-ethylhexanoate ($CaOct_2$) were used as different alkaline and alkaline earth metal catalysts. The catalytic activity of catalysts as KOct>$SrOct_2$>LiOct>$CaOct_2$ was ranked by considering conversion data as shown in Figure 2.19. The electronegativity values of K, Sr, Li and Ca are 0.82, 0.95, 0.98, and 1, respectively. The catalytic activity results fit exactly to the electronegativity values in the same manner due to the dissociation of metal having low electronegativity value from the carboxylate group easily.

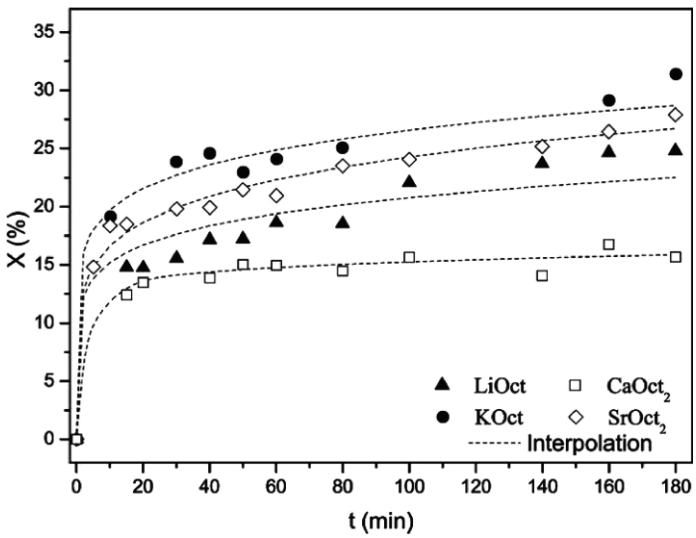
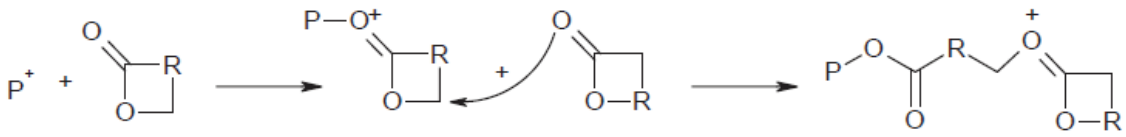


Figure 2.19.Total mass conversion as a function of reaction time for the bulk polymerization of D,L-lactide and glycolide using different catalysts. Molar ratio (L:G) in the initial monomer mixture: 80/20; molar ratio monomer to catalyst: 500, 130 ^{o}C (Source: Mazarro et al. 2009).

2.2.2.2 Cationic Ring Opening Polymerization

The cationic ring opening polymerizations are initiated by using alkylating agents, acylating agents, Lewis acids and protics acids. The mostly used initiators in the cationic polymerization of lactide and ε-caprolactone cyclic monomers are trifluoromethanesulfonic acid (triflic acid) and methyl trifluoromethanesulfonic acid (methyl triflate) (Bourissou et al. 2005, Kamber et al. 2007, Kricheldorf and Dunsing 1986, Kricheldorf et al 1986). The initiation and subsequent chain-growth steps of cationic polymerization of lactone are shown in Figure 2.20. The cationic ring opening polymerization requires the formation of a positively charged cationic species attached to the carbonyl oxygen of the cyclic monomer in the initiation step. The propagation mechanism begins with the positively charged cyclic monomer ring being cleaved at the alkyl-oxygen bond through S_N2–type reaction. The cationic chain growth proceeds by the cleavage of alkyl-oxygen bond rather than the acyl-oxygen bond. Low molecular weight PCL or PLA polymers have been obtained by using cationic initiators (Garlotta 2001, Kamber et al. 2007, Stridsberg et al. 2002).



initiation propagation

Figure 2.20. Cationic ring opening polymerization of lactone
(Source: Stridsberg et al. 2002).

2.2.2.3. Coordination-Insertion Ring Opening Polymerization

The coordination-insertion ring opening polymerizations also referred as pseudoanionic polymerization of lactide or ε-caprolactone monomers are initiated mostly by metal alkoxides similar to anionic ring opening polymerization. However, the covalent metal alkoxides containing free p-, d-, or f- orbitals such as calcium, aluminium, magnesium, tin, titanium, ytrium, zinc or zirconium promote the polymerizations of lactide or ε-caprolactone through the coordination-insertion mechanism, but not anionic mechanism (Albertsson and Varma 2003, Cayuela et al. 2006, Chamberlain et al. 2001, Dubois et al. 1991, Kricheldof et al. 1998 and 2000b,

Miola-Delaite et al. 1999, O'Keefe et al. 2001, Stevels et al. 1996, Zhong et al. 2001) Coordination-insertion ring opening polymerization of lactone is shown in Figure 2.21. As seen in the figure, metal alkoxide (M-OR) coordinates the carbonyl of the lactone monomer by following the cleavage of the acyl-oxygen bond of the monomer and insertion of the monomer into the metal-oxygen bond. The coordination provides enhancement of the electrophilicity of the CO-group and nucleophilicity of OR-group for the insertion of the lactone into the metal O-bond. (Jerome and Lecomte 2008, Stridsberg et al.2002)

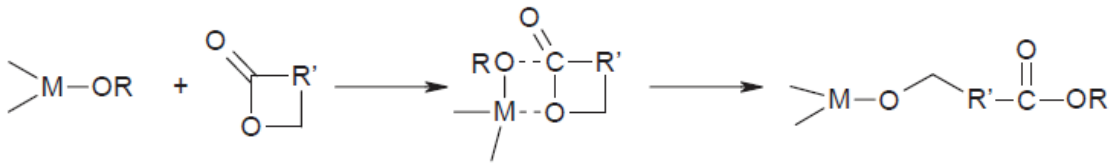


Figure 2.21. Coordination-insertion ring opening polymerization of lactone (Source: Stridsberg et al. 2002).

The use of co-initiators also affects the molecular weight and structure of the polylactones. Zhang et al. (1994) studied the effect of hydroxyl and carboxylic acid substances on lactide polymerization in the presence of stannous octoate. Stannous alkoxide, a reaction product between stannous octoate and alcohol, was proposed as the substance initiating the polymerization through coordinative insertion of lactide. Alcohol could affect the polymerization through reactions leading to initiator formation, chain transfer, and transesterification. Carboxylic acids affect the polymerization though a deactivation reaction. Figure 2.22 shows the effects of hydroxyl and carboxylic acid substances on the molecular weight of PLLA. As seen in the figure, alcohol increases PLLA production rate while carboxylic acid decreases it. The high alcohol concentration leads to the decrease of molecular weight of PLLA. However, the final molecular weight of PLLA is not sensitive to the carboxylic acid concentration.

The use of coordination-insertion initiators provides a decrease in the side reactions also called as transesterification reactions compared to anionic or cationic initiators. For that reason, the higher molecular weight polymers are synthesized and the control of the molecular weight during synthesis is possible by change of monomer/initiator ratio.

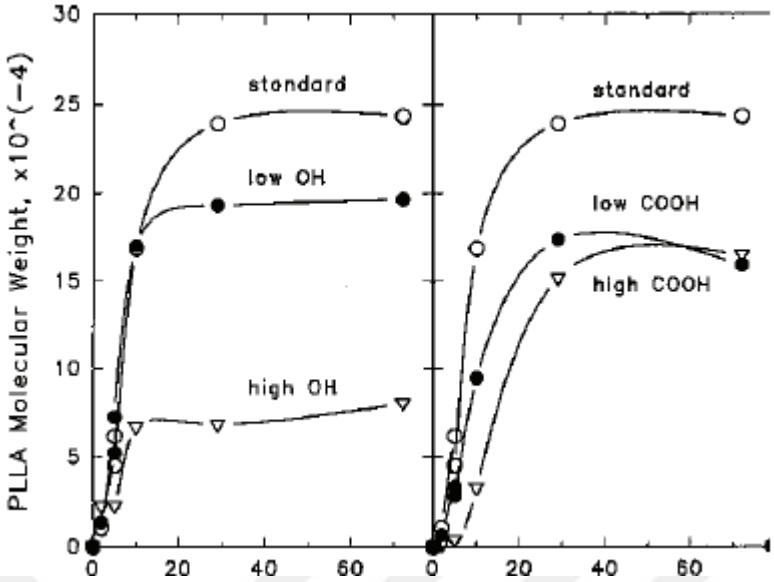


Figure 2.22. The effects of hydroxyl and carboxylic acid on the molecular weight of PLLA (Source: Zhang et al. 1994).

2.2.3. Transesterification Reactions during Polymerization

The presence of transesterification reactions in the ring opening polymerization of lactide based homo and copolymers is one of the important criteria that affects the molecular weight, molecular weight distribution of synthesized polymer and structure of copolymers. Transesterification reactions lead to the redistribution of polymer chains and hindering of block structure during polymerization. These reactions called as intramolecular or intermolecular transesterification reactions occur due to the effects of type and concentration of catalyst or initiator, reaction temperature or reaction time (Bero and Kasperczyk 1996, Bero et al. 1993, 1999, Contreras and Davilla 2006, Kasperczyk and Bero 1993, Kricheldorf et al. 1988). Bero and Kasperczyk (1996) also indicated the influence of monomer structure and catalyst type on the transesterification reactions in copolymerization with ε-caprolactone and L,L-lactide or racemic D,L-lactide. They concluded that the use of racemic D,L-lactide instead of L,L-lactide as monomer type in the copolymerization provides the enhancement of transesterification processes and also randomization of the copolymer chain related with higher flexibility and atactic nature of the backbone. Many studies about the LA or CL polymerization by

using different type of initiators in the literature indicate the direct effect of metal ion and ligand of initiator on side reactions. Alkali and alkaline metal alkyl and alkoxides lead to the fast living polymerization of lactones and also at the same time formation of macrocyles and depolymerization reactions occur due to the intramolecular and intermolecular transesterification reactions as an undesired effect of these initiators (Agarwal et al. 2000, Ito et al. 1979, Penczek et al. 1999). The higher contribution of transesterification is found in the case of $ZnEt_2$ used instead of $Al(acac)_3$ or $AlEt_3$ as initiator in the copolymerization of lactide and ε-caprolactone (Bero and Kasperczyk 1996). The reactivity of different metal alkoxides for transesterification reactions is ordered as $Bu_2Sn(OR)_2$ > $Bu_3Sn(OR)$ > $Ti(OR)_4$ > $Zn(OR)_2$ > $Al(OR)_3$ (Kricheldorf et al. 1988).

The mechanisms of intramolecular and intermolecular transesterification reactions during polymerization are shown in Figure 2.23. Intramolecular transesterification or also known as back-biting reactions lead to the formation of cyclic oligomers as a result of polymer degradation. Intermolecular transesterification affects the sequences of different polymeric segments and prevents the formation of a block structure in the polymer. These reactions lead to the decrease in molecular weight and increase in polydispersity index of the polymer (Lipik et al. 2010). Montaudo et al. (1996) showed the formation of cyclic lactides and odd-membered low molecular weight polylactide oligomers in the ring opening polymerization of lactide with an aluminium alkoxide initiator by making MALDI-TOF mass spectroscopy analyses. The cyclic lactides and odd-membered low molecular weight polylactide oligomers are formed due to the random cleavage of the polylactide chain during intramolecular and intermolecular transesterification reactions, respectively.

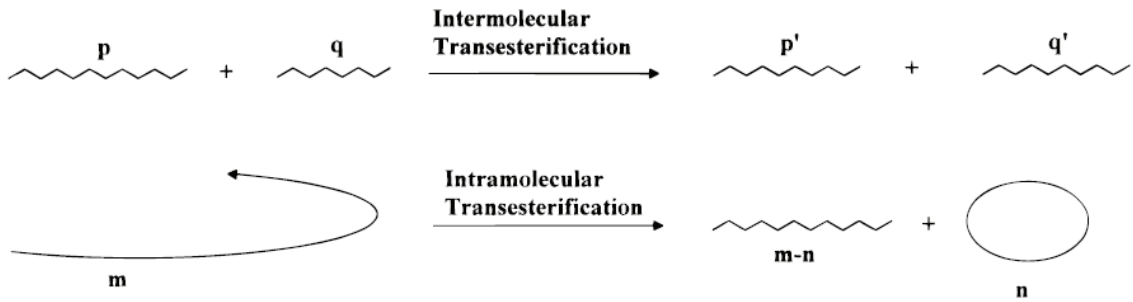


Figure 2.23. Intermolecular and intramolecular transesterification reactions (Source: Montaudo et al. 1996).

The transesterification reactions depend on the monomer addition sequence due to the effect of end groups or end active center and also reactivity difference between the monomers. The presence of transesterification reactions during copolymerization affects the sequence length and crystalline structure. Transesterification reactions lead to the formation of random copolymers with amorphous structure. Especially in the copolymerization of lactide and ε-caprolactone, the influence of transesterification reactions on the structure of the copolymers was reported by considering monomer addition sequence in the studies. Diblock copolymers are obtained when caprolactone is polymerized first. Random or multiblock copolymers are generally obtained when LA is polymerized first due to the transesterification reactions (Contreras and Davilla 2006, Florczak et al. 2007, In't Veld et al. 1997)

Lipik et al. (2010) investigated the effects of transesterification and degradation on properties and structure of polycaprolactone and polylactide copolymers synthesized by using different combinations of the order of monomer addition. The transesterification was observed for the synthesis of PLA or PCL-co-PLA at the first step in the case of the addition of CL or a mixture of LA and CL in the second step. The presence of transesterification in these cases can be explained from the production of low crystalline or totally amorphous P(LA-co-CL) block or random copolymers with high values of polydispersity indexes and low values of Young's Modulus such as 1.61 and 0.85 MPa, respectively. They concluded that PLA segments are more vulnerable to transesterification compared to PCL by considering NMR, MALDI-TOF and DSC results due to the presence of decarboxylation reactions.

Kasperczyk and Bero (1993, 1996) determined the two modes of transesterification in the copolymerization of L,L-lactide and ε-caprolactone. They also proposed a possible route of formation of the sequences via transesterification in the copolymerization of L,L-lactide and ε-caprolactone by using two different type of initiators containing Al or Zn of Figure 2.24. In the first mode of transesterification, a cleavage of the polylactide block occurs between lactidyl units (LL). The second mode of transesterification also required the cleavage of lactidyl units. In addition, the difference in the second mode of transesterification is the formation of CLC, CCLC or CLLLC chain sequences, where C and L denote the caproyl and lactly group, respectively.

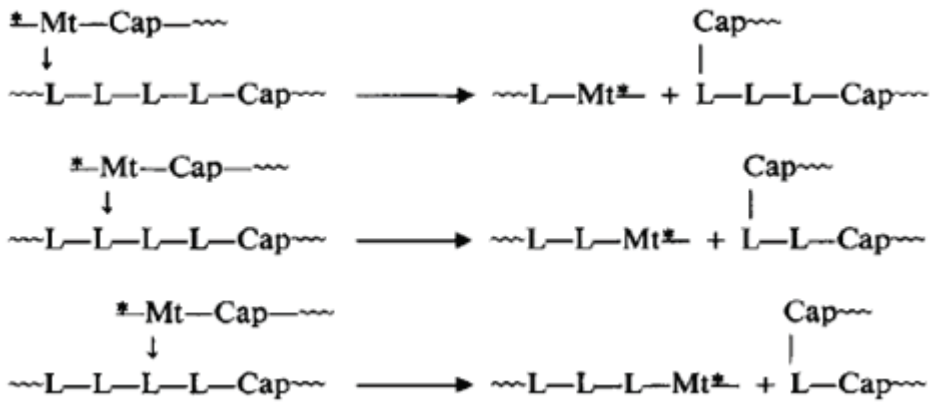


Figure 2.24.First and second modes of transesterification reactions. Mt denotes Al or Zn atom in the initiator molecule (Source: Kasperczyk and Bero 1996).

The chain microstructure of any copolymer such as average block length is determined by evaluation of the composition by means of ^{1}H-NMR and ^{13}C-NMR spectrum. The average length of the blocks is determined on the basis of attribution of lines in ^{1}H-NMR or ^{13}C-NMR. ^{13}C-NMR spectroscopy is highly sensitive tool for the evaluation of the monomer sequence in any copolymer (Kasperczyk and Bero 1993, Kricheldorf et al. 1985, Vanhoorne et al. 1992). Distribution of the repeat unit sequence in a copolymer structure obtained from the number average sequence lengths of the building blocks (l_i), the Bernoullian random number average sequence lengths $(l_i)_{random}$ and the degree of randomness (R). Kasperczyk and Bero (1993) determined the experimental number average sequence lengths of lactidyl blocks (l_{LL}^{e}) and caproyl blocks (l_{C}^{e}) by considering the type of transesterification reaction using Equations (2.2 and 2.3). In the case of first mode of transesterification,

$$l_{LL}^{e} = \frac{[LLLLLL]+[LLLLC]+[CLLLL]+[CLLC]}{[CLLC]+\frac{1}{2}([CLLLL]+[LLLLC])} \qquad \text{Equation 2.2}$$

$$l_{C}^{e} = \frac{[LLCLL]+[CCLL]+[LLCC]+[CCC]}{[LLCLL]+\frac{1}{2}([CCLL]+[LLCC])} \qquad \text{Equation 2.3}$$

where [seq.] indicate the contents of appropriate sequences in the copolymer chain represented by the intensities of corresponding lines in the ^{13}C NMR spectrum.

In the case of second mode of transesterification reaction, the recommended number average lengths of lactidyl and caproyl blocks are written as the following Equations (2.4 and 2.5) due to the formation of additional sequences such as CLC, CCLC or CLLLC sequences.

$$l_{LL}^{e} = \frac{1}{2}\frac{([LLL]+[LLC]+[CLL]+[CLC])}{[CLC]+\frac{1}{2}([CLL]+[LLC])} \qquad \text{Equation 2.4}$$

$$l_{C}^{e} = \frac{[LCL]+[CCL]+[LCC]+[CCC]}{[LCL]+\frac{1}{2}([CCL]+[LCC])} \qquad \text{Equation 2.5}$$

The coefficient of second mode of transesterification coefficient (T_{II}) is calculated from the following equation.

$$T_{II}=[CLC]/[CLC]_R \qquad \text{Equation 2.6}$$

where [CLC] is the experimental concentration of CLC sequence in the copolymer chain determined from the ^{13}C NMR, and $[CLC]_R$ is the theoretical concentration for completely random chains calculated via Bernoullian statistics by using Equation 2.7

$$[CLC]_R=k'^2/(k'+1)^3 \qquad \text{Equation 2.7}$$

Denoting the ratio of [CL]/[LL] as k', where [CL] stands for the concentration of caproyl groups and [LL] for lactidyl groups in the copolymer chain.

The coefficient (R) known as the degree of randomness (R) of the copolymer chain is an important parameter in the classification of the copolymer structure. The value of R ranges from 0 and 1 for block and completely random copolymer, respectively. For the copolymerization of caprolactone and lactide, it can be calculated as the ratio of the average length of randomly distributed lactidyl (l_{LL}^{R}) or caproyl (l_{C}^{R}) blocks to the experimental average length of the lactidyl or caproyl blocks, respectively.

$$R = \frac{l_{LL}^{R}}{l_{LL}^{e}} = \frac{l_{C}^{R}}{l_{C}^{e}}$$ Equation 2.8

l_{LL}^{R} and l_{C}^{R} are calculated from the following equations by considering complete transesterification via the first and second mode (Kasperczyk and Bero 1993).

$$l_{LL}^{R} = (k'+1)/2k'$$ Equation 2.9

$$l_{C}^{R} = k'+1$$ Equation 2.10

The number average sequence lengths of lactide and caprolactone, the Bernoullian random number average sequence lengths and the degree of randomness are calculated from the ^{1}H-NMR spectrum by using following equations (Fernandez et al. 2012; Herbert, 1993);

$$l_{LA} = \frac{2(LA)}{(LA-CL)}$$ Equation 2.11

$$l_{C} = \frac{2(CL)}{(LA-CL)}$$ Equation 2.12

$$l_{LA}^{R} = \frac{1}{(CL)}$$ Equation 2.13

$$l_{C}^{R} = \frac{1}{(LA)}$$ Equation 2.14

$$R = \frac{(LA-CL)}{2(LA)(CL)}$$ Equation 2.15

where (LA) and (CL) are the comonomer molar fractions obtained from the integration of the lactide methane signals and the ε-caprolactone methylene signals, and (LA-CL) is the average dyad relative molar fraction (Fernandez et al. 2012 a,b).

The chain structure of any copolymer such as repeat unit sequence directly affects the thermal, degradation and mechanical properties of the copolymers. For that

reason, the determination of number average sequence lengths and randomness character is a requirement in the characterization of any copolymer. The copolymers with higher randomness character have shorter number average sequence lengths. The reaction temperature also directly affects the formation of transesterification reactions. Fernandez et al (2012a) synthesized PLCL copolymers of different compositions at various temperatures (120, 130, 140, 150 ^{o}C) using $SnOct_2$ catalyst and variable LA:CL feed ratio (90:10, 80:20, 75:25, 70:30). They investigated the effects of chain microstructures on the crystallinity and mechanical properties of poly(L-lactide-co-ε-caprolactone) copolymers. They reported that the highest yields and molecular weight were obtained at the optimum temperature of 140 ^{o}C. In addition, the average sequence length of lactidyl (l_{LA}) in the PLCL(70:30) copolymer with 36 % crystallinity at 140 ^{o}C is obtained as 6.5, it reached to 16.7 in the case of PLCL (90:10) copolymer with 26.8 % crystallinity at the same reaction temperature due to the increase of lactidyl content. The randomness character of PLCL (90:10) is shifted from 0.37 to 0.69 and the average sequence length of lactidyl (l_{LA}) in the PLCL(90:10) decreased from 32.79 to 12.66 with the increase of temperature from 120 ^{o}C to 150 ^{o}C due to the increase of randomness character as related to the transesterification reactions. Fernandez et al (2012 b) also synthesized random PLCL copolymer having randomness character and number average sequence lengths of LA and CL as 0.92, 3.45 and 1.54, respectively. The average sequence lengths of LA and Cl in three PLCL copolymers around (70:30) are in the range of 6.5-3.45 and 3.29-1.54, respectively. The randomness characters of these copolymers were found as 0.47, 0.59 and 0.92, respectively. The second DSC scans of all three PLCL copolymers indicate fully amorphous structure. These results deduce that the crystallization ability significantly depends on randomness character and sequence lengths of the blocks in the copolymers.

2.2.4. Reaction Kinetics and Thermodynamics of Ring-Opening Polymerization

Reaction kinetics and thermodynamics data of any polymerization give information about the ease and possibility of polymerization by using different types of monomers, catalyts and /or initiators. To satisfy the ring-opening polymerization of L-

LA or ε-CL as cycling monomers, both reaction kinetics and thermodynamic data should be favored to open the ring of the monomer. The standard Gibbs free energy (ΔG^o_p) of polymerization determined by Equation 2.16 is one of the important criteria determining the spontaneous ($\Delta G^o_p<0$) or non-spontaneous ($\Delta G^o_p>0$) possibility of ring-opening polymerization.

$$\Delta G^o_p = \Delta H^o_p - T\Delta S^o_p \qquad \text{Equation 2.16}$$

ΔH^o_p and ΔS^o_p represent the standard polymerization enthalpy and the standard polymerization entropy at constant pressure, respectively. ΔG^o_p values indicating the thermodynamic polymerizability can not be taken as a direct measure of a monomer's reactivity. The rate constant by considering reaction kinetics of polymerization and as well as ΔH^o_p are required to make a comment about the reaction and the ring strain of the cycling monomer (Duda and Kowalski 2009). Ring strain is a thermodynamic property caused by either forcing the bonds between ring atoms into angular distortion or by steric interaction of subtituents on the ring atoms (Chanda 2000). The driving force for the ring-opening polymerization of any cyclic monomers is the release of ring strain (Chanda 2000 and Duda and Kowalski 2009).

The ring-opening polymerization consisting of initiation and propagation steps as shown in Figure 2.25 should be taken into account for the investigation of ring-opening polymerization kinetics. The formation of an ionic reactive centre occurs with the opening of the ring in initiation step. In the propagation step, monomer adds to a chain reaction for the chain growth.

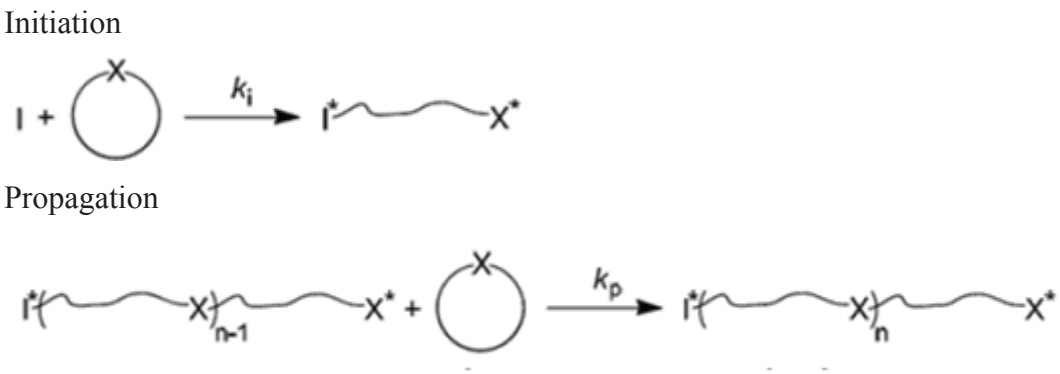


Figure 2.25. Ring-opening polymerization of cyclic monomers

The general rate law for polymerizations is given by Equation 2.17.

$$-\frac{d[M]}{dt} = k[M]^m [I]^n \qquad \text{Equation 2.17}$$

Where k, [M], and [I] denote reaction rate, monomer and initiator (or catalyst) concentrations, respectively. Polymerization kinetics directly depends on the type of catalyst, monomer, initiator and solvent, and as well as reaction temperature. In most of the studies related with the polymerizations of lactide and ε-caprolactone, first order reactions with respect to monomer and initiator (or catalyst) were determined (m=n=1). (Alcazar et al 2003, Chen et al. 2012, Contreras et al.2013, Dubois et al. 1991,1996, Ikpo et al. 2012, Pepels et al. 2013). In addition, different reaction orders were also determined in some of the studies (Chen et al 2005, Dubois et al. 1996, Hsiao and Lin 2013, Katiyar and Nanavati 2010, Wu et al 2005). Kinetic studies of Wu et al (2005) defined the first order and second order dependency on lactide monomer by using zinc complex and magnesium complex as catalyst, respectively. Hsiao and Lin (2013) studied the ring-opening polymerization of L-LA catalyzed by calcium complexes with the presence of a variety of alcohols such as benzyl alcohol (BnOH) or ethylene glycol (EG). Kinetic studies showed a first-order dependency on [LA] and a second-order dependeny on [BnOH].

The semi-logarithmic plot of –ln([M]/[M_0]) versus reaction time gives information about the apparent reaction rate (k_{app}), induction period and order of propagation reaction with respect to monomer concentration. [M_0] and [M] denote the monomer concentrations at initial and time t, respectively. The kinetic equations describing the apparent rate constant for first order according to the monomer are determined in the Equations (2.18 and 2.19).

$$-\frac{d[M]}{dt} = k_{app}[M] \qquad \text{Equation 2.18}$$

$$k_{app} = \frac{-\ln([M]/[M_0])}{t} \qquad \text{Equation 2.19}$$

Kinetic studies about the polymerization of L-LA or ε-CL show that the k_{app} values directly depend on the concentrations of initiator and co-initiator. Stridsberg et al (2000) defined different k_{app} values for the polymerization of L-lactide initiated by tin alkoxide with variable concentrations. The k_{app} values calculated from the Figure 2.26, range from 0.39 min^{-1} at [M]/[I]=400, 2.4 min^{-1} at [M]/[I]=100 up to 6.8 min^{-1} at [M]/[I]=25. Hao et al (2012) defined different k_{app} values for the polymerization of ε-CL initiated by zinc undecylenate catalyst (ZU) and benzyl alcohol (BnOH) The calculated k_{app} values are 0.72 h^{-1} and 0.13 h^{-1} at molar ratios of [CL]:[ZU]:[BnOH]as 50:1:1 and 200:1:1, respectively.

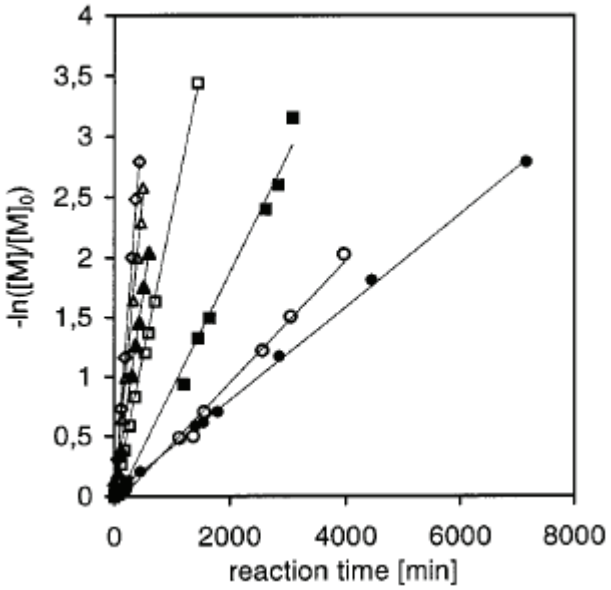


Figure 2.26. Plots of –ln([M]/[M_0]) versus reaction time for the polymerization of L-lactide initiated by the tin alkoxide system at different initiator concentrations (◊) 25, (Δ)35, (▲) 50, (□) 100, (■) 250, (o) 360, and (•) 400. (Source: Stridsberg et al. 2000)

Plots of k_{app} versus [I] gives information about the absolute rate constant (k_{abs}) and the order of reaction with respect to the initiator. k_{abs} can be written as Equation 2.20.

$$k_{abs} = \left[\frac{-\ln([M]/[M_0])}{t}\right]/[I] = \frac{k_{app}}{[I]^x[CoI]^y} \qquad \text{Equation 2.20}$$

x and y depict the order of initiator (I) and coinitiator (CoI). Chen et al (2013) defined the order of I and CoI in the polymerization of L-lactide initiated by $SnOct_2$ and benzyl

alcohol as -0.52 and 0.8, respectively. Chen et al (2013) defined also the order of I and CoI in the polymerization of ε-CL as -0.46 and 0.53, respectively.Reaction rates were found as 28.36 and 0.34 for L-LA and ε-CL, respectively.

Arrhenius Equation as given in Equation 2.21 is applied for the determination of the temperature dependence of reaction rates.

$$k=A\exp(-E_a/RT) \qquad \text{Equation 2.21}$$

A, E_a, R, and T denote the Arrhenius coefficient, activation energy, molar gas constant, and absolute temperature.

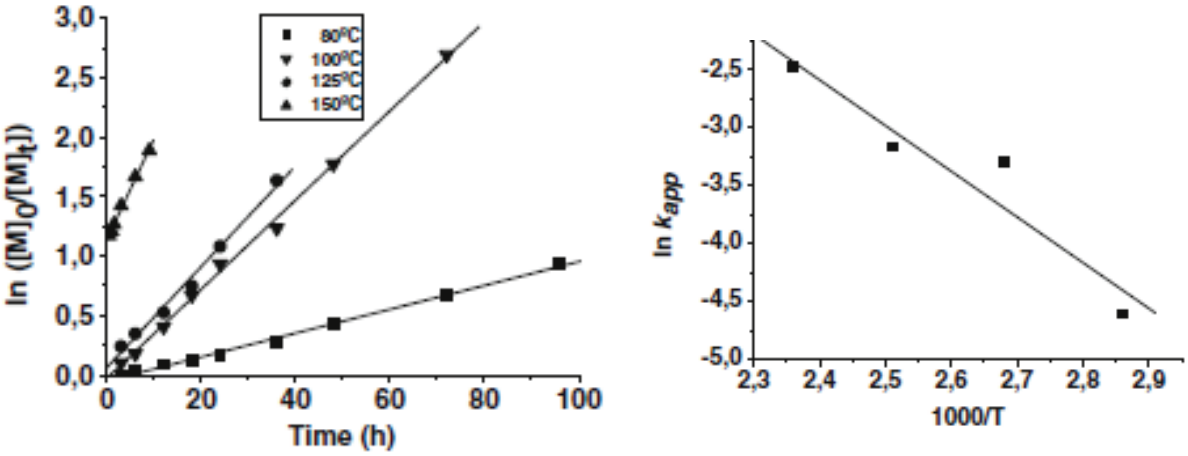
Contreras et al. (2013) investigated the effect of temperature on the bulk polymerization of ε-CL initiated by samarium acetate. Figure 2.27 shows straight-line relationships between ln([M_0]/[M]) versus reaction time and ln k_{app} versus the reciprocal of absolute temperature. The calculated k_{app} values from the figure (a) are 0.01h^{-1}, 0.037 h^{-1}, 0.041 h^{-1} and 0.085 h^{-1} at 80, 100, 125 and 150 ^oC, respectively. The higher k_{app} values were obtained by increasing temperature. The activation energy from the Arhenius plot was found as 34.25 kJ/mol. Ikpo et al. (2012) studied the effect of benzyl alcohol on the reaction rates and activation energies in the ring-opening polymerization of ε-CL by lithium piperazinyl-aminephenolate complexes. They found that the presence of benzyl alcohol leads to the increase in reaction rates and decrease in activation energies for ε-CL polymerization. Reaction rates increased from 0.036 to 0.273 L/(mol.min) and activation energies decreased from 56.8 kJ/mol to 48.8 kJ/mol with the addition of benzyl alcohol.

Thermodynamic parameters of ring-opening polymerization can be obtained by considering polymerization and depolymerization equilibria as given as

$$M + I \xrightarrow{k_i} P^*_m \qquad \text{Equation 2.22}$$

$$P^*_m + M \underset{k_d}{\overset{k_p}{\rightleftharpoons}} P^*_{m+1} \qquad \text{Equation 2.23}$$

where P^*_m, P^*_{m+1}, k_i, k_p, and k_d represent the growing polymer chain, initiation, polymerization and depolymerization reaction rate constants, respectively.



(a) (b)

Figure 2.27. (a) Plots of ln([M]/[M_0]) versus reaction time for the polymerization of ε-CL initated by samarium acetate at different temperature (b) The Arhenius plot (Source: Contreras et al. 2013)

The equilibrium monomer concentration ([M]$_e$) and the standard free energy change of the polymerization can be calculated by the following equations;

$$[M]_e = \frac{1}{K_{eq}} = \frac{k_d}{k_p} \qquad \text{Equation 2.24}$$

$$\Delta G^o = -RT \ln K = RT \ln [M]_e \qquad \text{Equation 2.25}$$

Standard enthalpy change of polymerization (ΔH_p^o) and standard entropy change of poymerization (ΔS_p^o) are calculated from Equation 2.26. Equilibrium monomer concentration depends on reaction temperature. Duda and Penczek (1990) determined the equilibrium monomer concentrations in L-LA polymerization as 0.15 mol/L at 80 °C and 0.058 mol/L at 133 °C. ΔH_p^o, ΔS_p^o, [M]$_e$ and ceiling temperature of L-LA and ε-CL polymerization are tabulated in Table 2.3.

$$\ln \frac{1}{[M]_e} = \frac{-\Delta H_p^o}{RT} + \frac{\Delta S_p^o}{R} \qquad \text{Equation 2.26}$$

Table 2.3 Standard thermodynamic parameters of ring-opening polymerization of L-LA and ε-CL.

Monomer	ΔH_p^o (kJ/mol)	ΔS_p^o (J/molK)	$[M]_{eq}$ (mol/L)	Tc (^oC)
L-LA	-22.9[a] -29.1[a]	-25[a] -40.7[a]	$1.2x10^{-2}$ [b]	284 in dioxane 640 in bulk
ε-CL	-28.8[b] -14[c]	-53.9[b] -6[c]	$5.1x10^{-2}$ [b] $7x10^{-3}$[c]	2060[c]

[a] values from Duda and Penczek 1990

[b] values from Duda and Kowalski 2009

[c] values from Save et al. 2002

The Eyring equation as given in Equation 2.27 is used for the determination of the activation parameters such as entropy of activation ($\Delta S^{\neq}$) and enthalpy of activation ($\Delta H^{\neq}$) (Chisholm et al. 2007 and Darensburg et al 2008).

$$\ln\frac{k}{T} = -\frac{\Delta H^{\neq}}{R} + \ln\frac{k_B}{h} + \frac{\Delta S^{\neq}}{R} \qquad \text{Equation 2.27}$$

T, k_B, h and R denote absolute temperature, the Boltzmann constant, Planck's constant and gas constant, respectively. The plot of ln(k/T) versus 1/T gives a straight line having slope as $-\Delta H^{\#}$/R and intercept as $\ln(k_B/h) + \Delta S^{\neq}/R$. Darensburg et al.(2008) determined the enthalpy, entropy and Gibbs free energy of activation parameters for ring-opening polymerization of L-LA as 73.5 kJ/mol, -42.5 J/mol.K and 86.1 kJ/mol. Dubois et al. (1996) also calculated $\Delta S^{\neq}$ by considering E_a from the following equation.

$$\frac{\Delta S^{\neq}}{4.576} = \log k - 10.573 - \log T + \frac{E_a}{4.576T} \qquad \text{Equation 2.28}$$

They determined the Ea, $\Delta H^{\#}$, $\Delta S^{\neq}$and $\Delta G^{\neq}$ for the ring opening polymerization of ε-CL initiated by aluminium trialkoxides as 10.3 kcal/mol, 9.7 kcal/mol, -26.7 cal/mol.K and 16.5 kcal/mol, respectively.

2.3. Biocompatibilty of Biodegradable Polymers

Biocompatibility is defined as the ability of a material to perform with an appropriate host response in a specific application where host response is the reaction of a living system to the presence of a material (Williams,1987). Basically, biocompatibility means the acceptance of an implant by surrounding tissues and by the body as a whole. The implant should be compatible with tissues in terms of mechanical, chemical, surface, and pharmacological properties. Body fluids can act as a solvent for impurities entrapped within a polymer matrix. On the other hand, compounds issued from polymerization and processing stages, namely monomers, oligomers, catalysts, initiators, solvents, etc., can generate particular morphological characteristics such as crystallinity or porosity, and cause toxicity or undesired physical aging due to slow release or slow uptake of low molecular weight compounds according to phase partition (Vert, 2000). For the prevention and reduction of risks, biodegradable polymers should posses the prerequisites for medical applications.

2.3.1. Prerequisites for Biomedical Applications

Due to the close contact of biomaterials with biological systems, the interaction and biocompatibility of synthetic polymers are of prime concern. The required properties of biodegradable polymers are similar to other biomaterials, i.e. biocompatibility, sterilizability, adequate mechanical and physical properties, and manufacturability. The biodegradable polymers used in direct contact with human tissue are not allowed to be toxic, immunogenic and not to inhibit the normal function in vivo studies.

Also, degradation products of biodegradable polymers should be biocompatible with human tissue. Biodegradable polymers consist of hydrolytically unstable linkages in the backbone. The most common chemical functional groups of this kind are esters, anhydrides, orthoesters, and amides. Depending on the chemical structure of the polymer backbone, degradation can occur by either surface or bulk erosion (Domb et al., 1997, Heller et al., 2000). PLA and PCL are aliphatic polyesters that contain flexible ester bonds. The degradation of PLA, PCL homopolymers and copolymers first involves non-enzymatic hydrolysis of ester linkages, autocatalyzed by the generation of

carboxylic acid end groups, followed by the loss of mass. (MacDonald, 1996, Gan et al., 1999, Chen et al., 2000, Ikada and Tsuji 2000, Li et al., 2001). In addition, degradation time of PCL is longer than PLA due to the presence of more hydrophobic $–CH_2$ groups in its repeating unit (Abedalwafa et al. 2013). Biocompatible degradation products of PLA or PCL such as lactic acid or 6-hydroxycaproic acid and oligomers are eliminated from body by normal metabolic pathway. These biocompatible degradation products are broken down into water and and carbon dioxide via the citric acid cycle (Nair and Laurencin, 2007). $SnOct_2$ is the mostly used catalyst in the polymerization of PLA or PCL. In addition, Nakayama et al. (1996) and Yamada et al. (2008) defined the toxic effect of $SnOct_2$. Tsuji et al. (2010) indicated the neurotoxic effect of dibutyltin. They also indicated that the toxicity of PLLA used as synthetic artifical dura mater after brain surgery increases with the increase in tin concentration. The brain tissue is directly exposed to the tin catalyst leading to the cytotoxic effect on brain during biodegradation of low molecular weight of PLLA (5000 Da), poly(glycolic acid-co-ε-caprolactone) (6400 Da) and poly(L-lactic acid-co-glycolic acid-co-ε-caprolactone) (6400 Da). Therefore the non-toxic catalyst in synthesis of biodegradable polymers for medical applications should be considered.

In the evaluation of any polymer for bioapplications, it is necessary to investigate their biocompatibility properties by considering toxicity tests. Cytotoxicity testing is the first step in the evaluation of biocompatibility. Animal tests and clinical tests are carried out in further steps. Cytotoxicity refers to toxic effect at the cellular level due to DNA damage, permeabilization of the cellular membrane, apoptosis, and other causes (Xian 2009). Cell viability and cytotoxicity tests are Neutral Red uptake (NR), Kenacid R-binding method (KB), Lactic acid Dehydrogenase assay (LDH) and 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay (MTT) (Fotakis and Timbrell 2005, Idris et al. 2010, Serrano et al. 2004). The neutral red assay is based on the accumulation of the neutral red dye in the lysosomes of viable, uninjured cells. The widely used method for the testing of cytotoxicity is MTT assay. LDH assay is also very sensitive method for the determination of cytotoxicity. Although MTT assay depends on only mithochondrial activity of cell, LDH assay is based on the measurement of lactate dehydrogenase activity in the extracellular medium (Fotakis and Timbrell 2005). The biocompatible properties indicating the cell affinity of the biopolymers are investigated by evaluation of cell adhesion and proliferation

experiments. The introduction about MTT assay and cell affinity of biodegradable polymers is given in the following parts.

2.3.1.1. MTT Assay

MTT is the widely used biochemical test in the determination of cytotoxicity. This test is based on the determination of the number of in vitro viable cells. MTT assay is a rapid calorimetric method based on the mitochondrial succinate dehydrogenase conversion of tetrazolium to MTT formazan (Ciapetti et al. 1993, Mei et al. 2005). The addition of MTT to metabolically active cells leads to the formation of water-insoluble and dark purple MTT formazan. The amount of MTT formazan produced is directly proportional to the number of proliferating cells present in culture. Dead cells or metabolically inactive cells do not react with MTT. In spectrophotometry, MTT formazan soluted with dimethylsulfoxide (DMSO) gives maximum absorption at 570 nm (Xian 2009).

The cytotoxicity of PLA/PCL homopolymers and copolymers were carried out recently in the literature. The cytotoxicity tests of PLA/PCL homopolymers and copolymers revealed that none of the polymers were toxic (.Jung et al. 2013, Idris et al.2010, Serrano et al 2004). In addition, unwanted severe responses such as inflammation had been observed in the long term uses of implants made of PLA/ PCL homopolymers or copolymers in body. These adverse effects had been explained by the formation of acidic degradation products and mild foreign-body reactions (Bergsma et al.1995, Böstman and Pihlajamäki 2000, Ekholm et al. 1999, Kum et al. 2013, Kwak et al. 2008). The decrease of pH due to the formation of hydroxy acids during degradation of biocompatible PLA/PCL homopolymers and copolymers can lead to the inflammation or injury. In addition, although these polymers were synthesized by $Sn(Oc)_2$, the toxic effects of tin based materials had not been mentioned for the reason of inflammation, acute brain injury and foreign body reactions. The toxic effect of $Sn(Oc)_2$ and tin based materials had been proved in vitro and in vivo studies (Ahmed and Tsuchiya, 2006, Arakawa 2000, Chmielnicka et al. 1993, Cooney and Wuertz, 1989, Tsuji et al. 2010, Yamada et al. 2008). Yamada et al. (2008) investigated the effects of $Sn(Oc)_2$ on cell viability in vitro according to MTT assay and on neurotransmission behavior in the rat. Figure 2.28 shows the effect of $Sn(Oc)_2$ on

astrocytes. Astrocytes were cultured with $Sn(Oc)_2$ for 7 days. As shown in the figure, they showed that the cytotoxicty of $Sn(Oc)_2$ to astrocytes in vitro and reduction of the mitochondrial activity upto 16% of the control group were obtained when treatment of normal human astrcytes with 10 ppm.

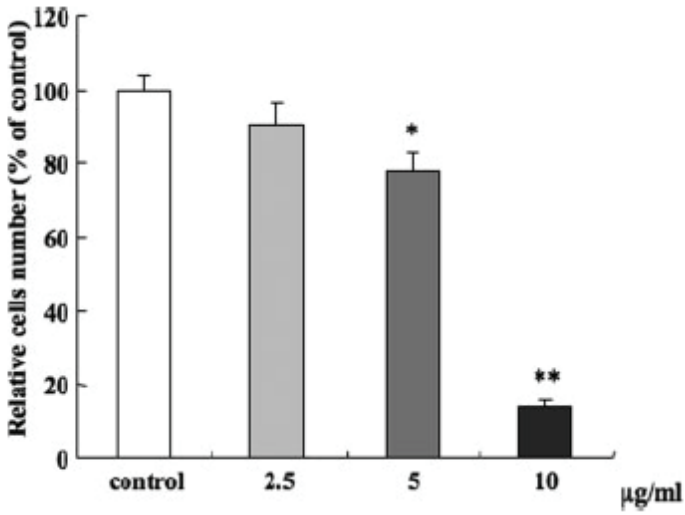


Figure 2.28. The effect of $SnOct_2$ on astrocytes
(Source:Yamada et al. 2008)

Limited number of studies is available about the residual catalyst in biodegradable polymers such as PLA or PCL. Schwach et al. (2002) studied the influence of polymerization conditions on the hydrolytic degradation of poly(DL-lactide) polymerized in the presence of $SnOct_2$ or zinc metal. The residual catalyst amount during aging of the polymers synthesized by $SnOct_2$ and zinc metal were monitored and shown in Figure 2.29. As seen in the figure, residual tin concentration in lab scale production of PDLA is increased from 306 to 795 ppm during hydrolytic degradation of PDLA synthesized by $SnOct_2$. The residual zinc concentration found as 40 ppm remained unchanged throughout the degradation of PDLA synthesized by zinc metal. This study indicated that the amounth of residual catalyst changes according to catalyst type during degradation of PDLA and total removal of residual catalyst in polymer is not possible.

The residual amount of initiators or catalysts in biodegradable polymer synthesis depends on the initiator type. Schappacher et al. (2010) compared in vitro cytotoxicity toward human osteoprogenito cells of PCLs synthesized from various metallic initiators.

The amounts of residual metal present in the PCLs synthesized by $Al(OiPr)_3$, borohydrate $La(BH_4)_3(THF)_3$, $La(OiPr)_3$, $SnOct_2$, $Ti(OiPr)_4$ or $Zn[O(CH_2)_3NHBoc]_2$ were reported as 765, 165, 395, 3370, 815 and 350 ppm, respectively. The lowest residual metal contents (3.1-7.2 %) were obtained with lanthanium and zinc based initiators whereas the highest ones (72.4-99.6%) were obtained with titanium, tin and aluminium derived PCLs. The highest residual amount was found as 3370 ppm in PCL synthesized by $SnOct_2$ at [M]/[I] : 220. In the previous studies about the PLA and PCL synthesis, Schwach et al. (2002) and Schappacher et al. (2010) indicated the presence of highest residual metal concentration in PLA or PCL synthesized by $SnOct_2$. Compared to the other studies about the tin toxicity (Floera and Büsselberg 2005, Tsuji et al. 2010, Yamada et al. 2008), the residual amounts of tin as 800 and 3370 ppm found in the studies of Schwach et al.(2002) and Schappacher et al. (2010) are very high concentrations that will lead to toxic effects in the utilization of this polymers for biomedical applications. However, Schappacher et al. (2010) evaluated in vitro properties of PCL film extractions from a series of cytotoxicity tests involving MTT and neutral red assays upon exposure to human osteoprogenitor cells. Although the presence of huge amount of residual tin and other metals in PCLs and observation of toxic effects of some PCL extracts were reported as shown in Figure 2.30, they defined the absence of toxic influence of all PCLs according to MTT and neutral red assays. Since MTT and neutral red assays give information about the short term cytotoxicity results. It will be only possible to see toxic effect of metallic residues by increasing the extraction time of PCL films or carrying out long term degradation studies of PCL films.

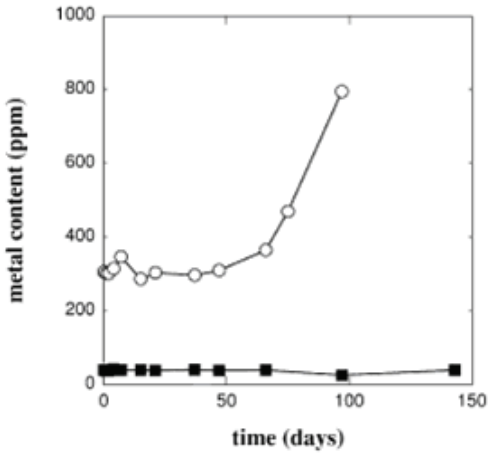


Figure 2.29. Variation of the residual metal content during the degradation of poly(D,L-lactide) (Source: Schwach et al. 2002).

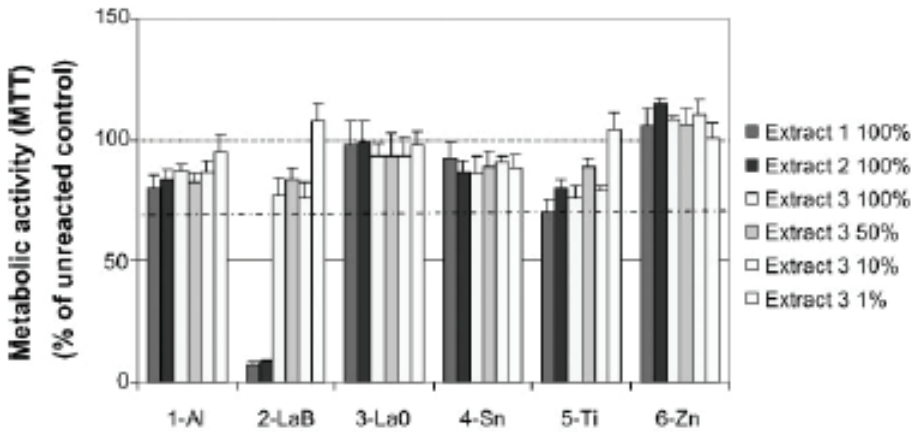


Figure 2.30. Effect of incubation and catalysts on MTT cytotoxicity results of PCLs extracts (Source: Schappacher et al. 2010)

2.3.1.2. Cell Affinity and Medical Applications of PLA/PCL Homopolymers and Copolymers

Cell-material interactions play pivotal role in the classification of the biodegradable polymers for biomedical applications. Although the biopolymers used in bone, skin or cartilage should be capable of promoting cell adhesion property, the biopolymers used in intraocular lenses, blood contacting devices such as hearth valves, catheters for hemodialysis or vesicles for therapeutic drugs should be capable of preventing cell adhesion (Bacakova et al. 2004, Pamula et al. 2008). Cell affinity of biopolymers is related with their surface properties such as hydrophilicity, surface energy, surface charge, chemical composition, and morphology. The synthesis of biopolymers should be designed by considering their biomedical applications.

PLA and PCL based biocompatible homopolymers and copolymers are mostly used in various medical applications due to their hydrolytic degradation properties in the human body. Some of their medical applications are scaffolds such as cartilage repair, drug delivery, implants, orthopedic devices (screws, pins) and injectable cell carriers for knee repair, wound dressing, temporary prosthetic devices and vascular graft (Abedalwafa et al. 2013, He et al. 2009, Liu et al. 2011, Mei et al. 2005, Middleton and Tipton 2000, Wildemann et al. 2005, Zhang et al 1993). Jung et al (2008) investigated the cartilage regenerations with three dimensional scaffolds prepared from PLA, poly(lactide-co-glycolide) (PLGA) and poly(lactide-co-caprolactone) (PLCL). They

indicated the significant enhancement of cartilage regeneration with elastic PLCL scaffolds comparing to PLA and PLGA scaffolds. Ye et al. (1998) developed a tubular PLLA/PCL microporous stent for delivering gene transfer vectors to the arterial wall. Yu et al. (2012) described a shape memory stent of poly(ε-caprolactone-co-DL-lactide) copolymer to displace the conventional metallic stent for potential treatment of esophageal stenosis. They deduced the shape recovery process in vivo by implantation of the PCLA stent into a dog's esophagus as shown in Figure 2.31. They suggested that the complications of esophageal treatment has been decreased by using biodegradable tubular shaped PCLA copolymer instead of current used metallic stent made of Ni-Ti based shape memory alloy.

High crystallinty property and acidic degradation products restrict the medical applications of PLLA and PCL homopolymers due to its long time degradation, poor cell affinity and local inflammation in vivo (Nakagawa et al. 2006, Yanagida et al. 2009). Vogth et al. (2004) reported the usability of a paclitaxel-eluting coronary PDLA stent having double helical geometry even if it was local inflammotory response. Plasma treatment and copolymerization methods are used to improve the cell compatibility of PLLA (Nakagawa et al. 2006, Yang et al. 2002). In this chapter, the introduction about the cell affinity properties of PLA, PCL homopolymers and copolymers are given. Copolymerization of L-LA with different types of monomers such as ε-caprolactone, glycolide, polyethylene glycol, poly(etylene oxide) or trimethylene carbonate (TMC) have been carried out extensively (Fernandez et al. 2014, Ji et al. 2010, Nagahama et al.2006, Jung et al. 2013, Wan et al. 2003, Wang et al.2012).

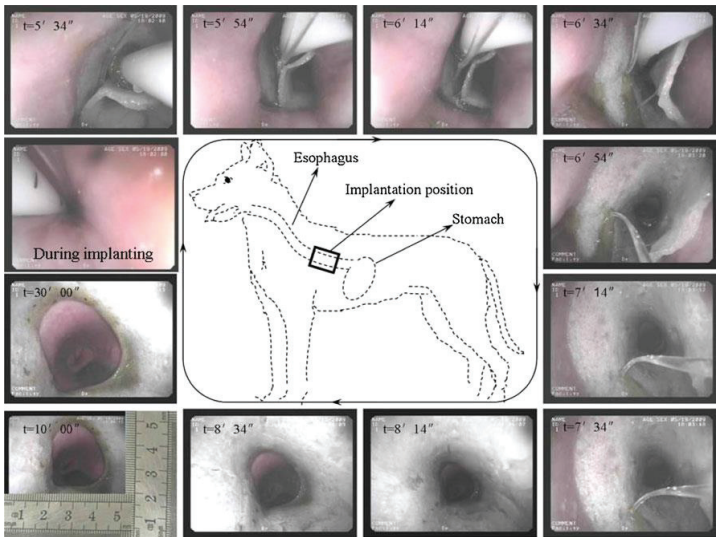


Figure 2.31. The endoscopic photos showing the shape memory recovery of PCLA stent implanted in the dog's esophagus (Source:Yu et al. 2012)

The cell affinity strictly depends on the hydrophilic structure. Wan et al. (2003) investigated the cell affinity properties of high molecular weight poly(L-lactide)-poly(ethylene glycol) multiblock copolymers (multi-PLE) by using mouse NIH 3T3 fibroblasts according to MTT assay. They found that the multi–PLE copolymer scaffolds with appropriate hydrophilicity were more favorable to mass transport and cell proliferation and cell affinity compared to that of PLLA. Cell proliferation was improved by increasing the hydrophilicity of the scaffold due to the incorporation of hydrophilic PEG to hydrophobic PLA.

The cell adhesive and cell affinity of PLA/PCL homopolymers and copolymers depend on their crystallinity, porosity, streoregularity properties and also film preparation technique such as solvent casting, electrospinning, etc. Increase in cell adhesion is observed by increase of crytallinity, porosity, stereoregularity, hydrophilicity and 3D morphology by the preparation of electrospun fabric (Finne-Wistrand et. al. 2008, Nagahama et al. 2007, Sarasua et al. 2011, Serrano et al. 2004, Wan et al. 2003).

Cell adhesive properties of any copolymer also depend on the copolymer composition and branching structure. Limited number of studies are available related to the effects of branching and copolymer composition on biocompatibility studies of PLA and PCL based biopolymers. Miyasako et al. (2007) and Wang et al. (2012) studied the biocompatibility of crosslinked poly[(ε-caprolactone)-co-lactide] copolymers and multibranched pentablock bearing PEG, hyperbranched polyglycidol (HPG) and PLA copolymers, respectively. Miyasako et al. (2007) prepared the crosslinked polymer from the branched macromonomer consisting different CL/LA compositions (90/10, 80/20 and 70/30) with constant pentaerythritol and $SnOct_2$. Although they investigated the HeLa cells adhesion on these crosslinked copolymers having different composition, they did not investigated the branching effect on cell adhesion. Figure 2.32 indicates the microscobic views of HeLa cells adhered to CL/LA membrane cultured for 48 h. As seen in the microscobic views, the Hela cells adhered well and extended on the CL/LA:70/30 surface similar to the polystyrene dish (TCPS). Figure 2.33 shows the cell growth on the CL-LA membranes and TCPS. As seen in the figure, the high adhered cell numbers were obtained for CL/LA: 70/30 surfaces. They suggest that the Hela cells adhesion and growth on CL/LA:70/30 is comparable to that of commercially available TCPS.

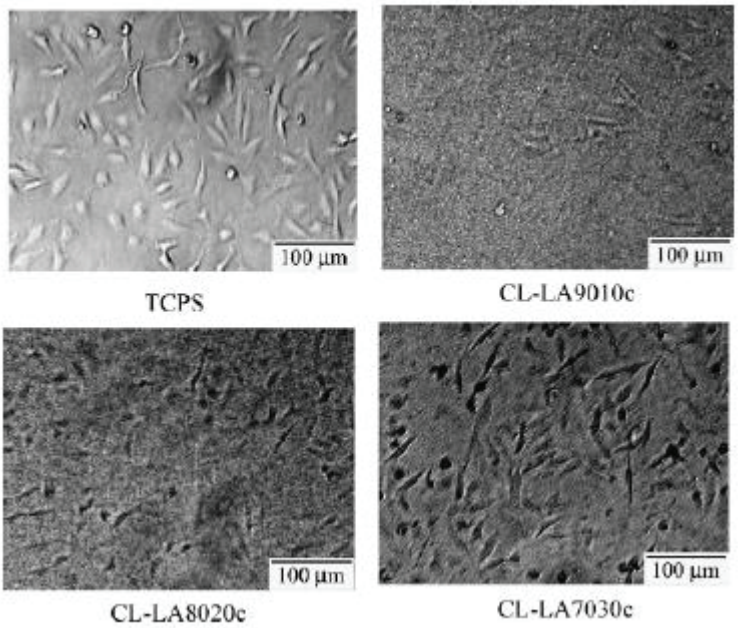


Figure 2.32. Optical microscope pictures of HeLa cells adhered to CL-LA membrane cultured for 48 h. (Source: Miyasako et al. 2007).

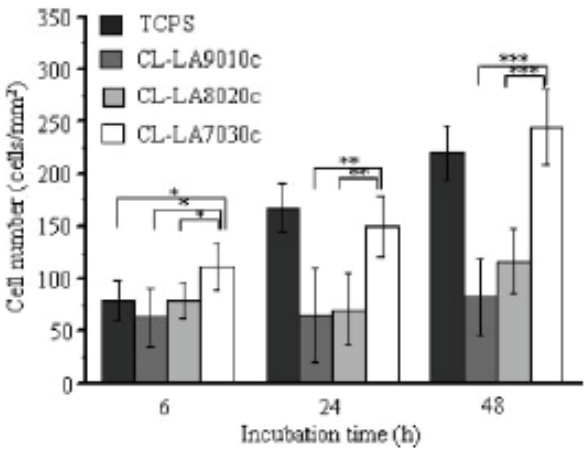


Figure 2.33.Cell growth on the CL-LA membranes and TCPS
(Source: Miyasako et al. 2007)

Wang et al. (2012) studied the biocompatibility of pentablock and multibranched bearing PEG, hyperbranched polyglycidol (HPG) and PLA copolymers for controlled drug release. The molecular weight range of the copolymers measured by dynamic light scattering is between 4360 and 15300 Da. The cytotoxic properties of the micelles at various concentrations prepared from the copolymers having different lengths of PLA were determined according to MTT assay. Human embryonic kidney 293T and

colorectal carcinoma HCT-116 cells were used in MTT assay. Figure 2.34 indicates the cytotoxicity of the nanosized micelles of the PLA-b-HPG-b-PEG-HPG-b-PLA copolymers for 293T and HCT-116 cells. As seen in the figure, the viability of both types of cell was higher than 90 % in a concentration range of 1 to 50 μg/mL. The samples 2a, 2b, 2c, 2d, and 2e denote the amount of PLA in the copolymers given as 18, 23, 50, 68, and 78 wt%, respectively. The lowest cell viability values for both types of cells were obtained in the 50 μg/mL micelle concentration prepared by the copolymer consisting lowest amount of PLA given as 18 wt%. They reported that no acute and cytotoxicity of the polymers against normal cells were obtained and good affinities of the copolymers with anticancer drug doxorubicin were also observed.

Tissue regeneration directly depends on the chemical composition, physical structure and biologically functional moieties of the scaffolds. Electrospinning method is mostly preffered in the production of nanofibrous scaffolds due to the similarity of the electrospun nanofibers to native extracellular matrix. (Bhutto et al. 2016, Cui et al 2009, Kim et al 2009, and Kwon and Matsua 2005). Bhutto et al. (2016) developed the PLCL 3D multichannel nerve conduit with aligned electrospun nanofibers for Schwann cell proliferation. They performed MTT assay to investigate the effect of multichannel stucture of nerve conduit on cell viability as shown in Figure 2.35. They reported that 19-channel conduits showed higher cell viability compared to nine-channel conduits as shown in the figure. The nerve conduit with a high number of channels provides high surface area for cell attachment compared to that with a low number of channels.

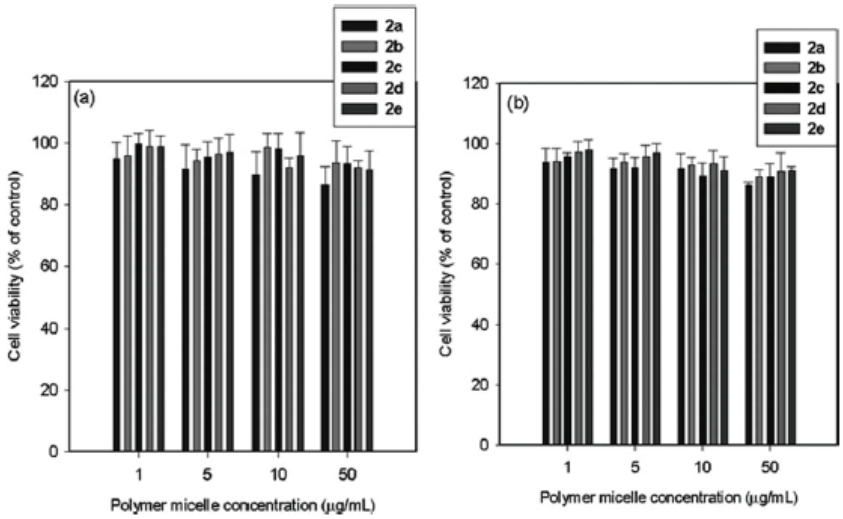


Figure 2.34. Cytotoxicity of the nanosized micelles of the PLA-b-HPG-b-PEG-HPG-b-PLA copolymers (a) 293T and (b) HCT-116 cells (Source: Wang et al. 2012).

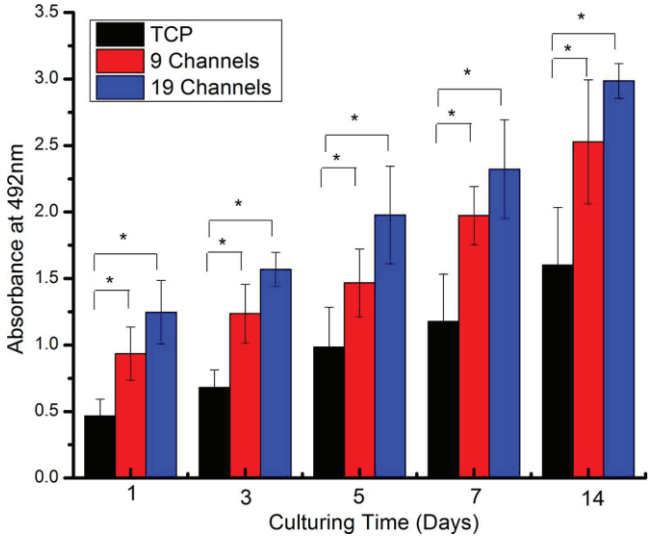


Figure 2.35. MTT results of 3D PLCL multichannel nerve conduits

(Source: Bhuto et al. 2016)

CHAPTER 3

THE EXPERIMENTAL STUDY

3.1. Materials

In this study, the required materials for synthesis of homo/co-PLAs and PCLs such as monomers, catalysts, solvents, initiators and co-initiators are given in detail. Purification of the monomers is required to achieve a controlled polymerization. To minimize the effect of monomer purity on the polymerization, the purification of L-Lactide (LA) and ε-caprolactone monomers (CL) supplied from Boehringer Ingelheim and Sigma Aldrich were carried out, respectively. Some of the impurities available in L-lactide monomer are L- or D-lactic acid, D or meso lactide, oligomers of L-lactide and water. Purification of L-Lactide monomer was carried out by applying recrystallization process with dried toluene for three times and dried at room temperature under vacuum for 48 h before use. Purification procedure of ε-caprolactone monomer is different than that of L-lactide monomer. ε-caprolactone monomer (CL) was dried over powdered calcium hydride for 24 h at room temperature and was subsequently distilled at reduced pressure prior to use. Tin(II) 2-ethylhexanoate as known as stannous octoate ($SnOct_2$ 95 %), creatinine (99 wt%) and bismuth (III) acetate as catalysts and initiators were supplied from Aldrich Co. Ethylene glycol (99 %), pentaerythritol, dipentaerythritol and myo-inositol (Myo) as co-initiators were supplied from Aldrich Co. $SnOct_2$ and ethylene glycol were purified by distillation under reduced pressure. After the purification of ε-caprolactone monomer and ethylene glycol are stored over activated 4 ^{o}A molecular sieves. Bismuth (III) acetate (Bi(III)Ac), creatinine, pentaerythritol, dipentaerythritol, myo-inositol (Myo) dichloromethane, hexane, methanol, acetone were used without further purification. Toluene (Lab-Scan, 99.8%) was dried over Na wire before use. Dichlorodimethyl silane (Merck, ≥98 %) and triethylamine (Acros organics, 99%) were used for silanization of glassware.

The materials used for in vitro cytotoxicity tests such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (>97.5 %) and Roswell Park Memorial Institute-1640 (RPMI-1640) with L glutamine were supplied from Sigma. Heat

inactivated fetal bovine serum (FBS), trypsin and gentamycin sulphate were supplied from Biological Industries (Israel). Glutaraldehyde solution (25 %) and ethanol (>99.5%) supplied from Merck were used for fixation and dehydration of cells, respectively.

3.2. Methods

Experimental methods can be summarized in:

- Synthesis of OH functional PLLA polymers by using Bi(III)Ac and creatinine as catalysts
- Synthesis of OH functional PCL polymers by using creatinine as catalyst
- Synthesis of poly(L-lactide-co-ε-caprolactone) P(LL-co-CL) copolymers by using Bi(III)Ac and creatinine as catalysts
- Characterization of homo/co PLLAs and PCLs by NMR, DSC, FTIR, and SEC
- In vitro cytotoxicity tests of homo/co PLLAs and PCLs synthesized by using Bi(III)Ac and creatinine catalysts

3.2.1. Synthesis of OH Functional PLA Polymers

OH functional PLAs were synthesized at a variety of conditions using different types and concentrations of catalyst and co-initiators at different temperatures. For the synthesis of di, tetra, and hexa -OH functional PLLA, ethylene glycol, pentaerythritol and myo-inositol were used as co-initiators, respectively. Bismuth (III) acetate (Bi(III)Ac) and creatinine were used as biocompatible catalysts. L-lactide/Bi(III)Ac mole ratios were taken as 200, 500 and 1000. 500 and 50 were used for L-lactide/creatinine mole ratios. L-lactide/Co-initiator mole ratios were taken as 20, 100 and 1000.

The ring-opening polymerization of L-lactide was carried out by bulk polymerization process in a 25ml round bottom flask containing teflon coated magnetic bar and a three-way opening equipped with a butyl rubber septum to collect sample during polymerization under nitrogen or argon atmosphere. Lactide, catalyst and

initiator were transferred to the flask under nitrogen atmosphere in a glove box (MBraun MB 150B-G-I, Germany). The flask was sealed with the three way opening and kept in an oil bath at 120 and 140 ^{o}C. The reaction product synthesized by using Bi(III)Ac was purified by dissolving in dichloromethane and precipitation in a mixture of cold hexane and methanol (v/v = 95/5). The creatinine initiated polymers were isolated by dissolving in acetone and precipitated in a mixture of cold water-methanol. After drying of the polymers under vacuum, the OH functional PLA polymers were stored in dry conditions.

3.2.2. Synthesis of OH Functional PCL Polymers

The di, tetra and hexa OH functional poly (ε-caprolactone) (PCL) polymers were synthesized at 140 ^{o}C by using different types and concentrations of co-initiators. The ε-caprolactone monomer/catalyst ratios were kept constant as 500 and 50 for Bi(III)Ac and creatinine, respectively. CL/CoI mole ratios were taken as 20, 100 and 1000. The reaction product synthesized by using Bi(III)Ac was purified by dissolving in dichloromethane and precipitation in a mixture of cold hexane and methanol (v/v = 95/5). In addition, crude polymers synthesized by using creatinine were purified by dissolving in acetone, and then precipitated in cold water and methanol mixture. The polymers were dried under vacuum and stored in dry conditions until use.

3.2.3. Synthesis of Poly(L-lactide-co-ε-caprolactone) P(LL-co-CL) Copolymers

P(LL-co-CL) copolymers were synthesized by successive two step ring opening polymerization of L-lactide and ε-caprolactone at 140 ^{o}C. Firstly, di, tetra and hexa OH functional PCL prepolymers were synthesized by using ethylene glycol, pentaerythritol, dipentaerythritol and myo-inositol as co-initiators, respectively. The ε-caprolactone monomer/catalyst ratios were kept constant as 500 and 50 for Bi(III)Ac and creatinine, respectively. CL/CoI ratios were taken as 20 and 100. The [CL]/[LA] molar feed ratio in the copolymers was kept at 50/50. Then, 10 mmol lactide was added under argon atmosphere whenever the conversion level CL reached up to 93%. The copolymers

prepared with Bi(III)Ac catalyst were isolated by dissolving in dichloromethane and precipitation in a mixture of cold hexane-methanol. Acetone and cold water were used in the isolation of the copolymers prepared by creatinine catalyst. The copolymers were dried at room temperature under vacuum before characterization test.

3.2.4. Characterization of Homo/co PLAs and PCLs

3.2.4.1. Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR analyses were performed with Bruker Advance DPX-400, Varian Mercury plus-AS400 or Varian VNMRJ 400 nuclear magnetic resonance spectrometers operating at 400 MHz to determine the structure, monomer conversion and number average molecular weight of the polymers. Deuterated chloroform ($CDCl_3$) was used for dissolving of the samples. Nondeuterated chloroform was used as an internal standard (δ=7.26 ppm).

Conversion tests were performed by ^{1}H NMR analyses of withdrawing samples from the reaction mixture during polymerization. Monomer conversion values are calculated by integrated the specified proton of a polymer versus the sum of the integration of the same specified protons of monomer and the polymer. For PLLA, monomer conversion was determined by integrating the methine proton peak of LA monomer at 5 ppm (-COCH(CH_3)-O-, δH = 5 ppm) and the methine proton peak of PLA polymer at 5.2 ppm (-CO-CH(CH_3)-O-, δH^m= 5.2 ppm). For PCL, monomer conversion was determined by integrating the triplet methylene peak of CL monomer at 4.20 ppm and the triplet methylene peak of PCL polymer at 4.00 ppm.

$M_{n,NMR}$ was calculated according to following equation using peak intensity for methine protons of L-lactide units at chain terminals (HO-CH(CH_3)CO-, δH^c = 4.36 ppm) and inside the main chain of the polymer (-CO-CH(CH_3)-O-, δH^m= 5.2 ppm) as given in Equation 3.1.

$$M_{n,NMR} = M_{initiator} + \frac{\delta H^m}{\delta H^c} x72xArmnumber \qquad \text{Equation 3.1}$$

$M_{n,NMR}$ of PCL was calculated using peak intensity for methylene protons of CL monomer at chain terminals (δH^c = 3.6 ppm) and inside the main chain of the polymer (δH^m= 4.0 ppm) as given in Equation 3.2

$$M_{n,NMR} = M_{initiator} + \frac{\delta H^m}{\delta H^c} x114xArmnumber \qquad \text{Equation 3.2}$$

The chain microstructure of any copolymer such as average block length is determined by evaluation of the composition by means of ^{1}H NMR and ^{13}C NMR spectrum. The average length of the blocks is determined on the basis of attribution of lines in ^{1}H NMR or ^{13}C NMR as given in Chapter 2.

3.2.4.2 Size Exclusion Chromatography (SEC)

SEC was used to determine the molecular weights and polydispersity index values of polymer synthesized at different time periods during polymerization. Chloroform and THF SEC's were used for polymers synthesized by Bi(III)Ac and creatinine, respectively.

For SEC analyses of polymers synthesized by Bi(III)Ac, the instrument comprised of a Waters 717 plus autosampler unit, PL-ELS 1000 evaporative light scattering detector (Polymer Labs., UK) and a Waters model 510 solvent pump equipped with three PLgel 10 μm mixed B-columns, 300x7.5 mm from Polymer Labs. Polystyrene standards in the range of 4000-900000 g/mol range were used for calibration. Chloroform/methanol (95/5 v/v%) was used as an eluent with a flow rate of 1 ml/min. The injection volumes of the samples and polystyrene standards were as 50 and 35 μL, respectively. Millenium version 3.20 software program was used for data transfer.

SEC analyses of polymers synthesized by creatinine were performed using a Viscotek TDA model 301 equipped with two GMH_{HR}-M columns with TSK-el (mixed bed, MW resolving range: 300-100000) from TOSOH Biosep, a VE 5200 GPC autosampler, a VE 1121 GPC solvent pump, and a VE 5710 GPC degasser. Flow rate of THF as a mobile phase was 1 ml/min. Universal and conventional calibration methods

were created using narrow linear PS standards. OmniSEC version 4.0 software was used to process the data.

3.2.4.3. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC-1 Star system, Metler Toledo) and TA Instruments Q10 were used to determine the glass transition, cold crystallization, melting temperatures and enthalpies of cold crystallization and the melting of the polymers under non-isothermal crystallization condition. DSC analyses of homo and OH functional PLAs were conducted under a nitrogen atmosphere with a flow rate of 80 ml/min. 5 ±0.1 mg of the polymer was heated from 25 to 200 ^{o}C at a heating rate of 10 ^{o}C/min. In the second step, the samples were cooled down from 200 to -20 ^{o}C at a rate of 10 ^{o}C/min cooling rate. After melt-quenching application, the samples were heated again from -20 to 200 ^{o}C at a rate of 10°C/min. In the DSC analyses of caprolactone based homo/copolymers, cooling temperature was set to -80/-40 ^{o}C instead of -20 ^{o}C.

The degree of crystallinity (χ) values of the samples were calculated by the ratio of the measured melting enthalpy (ΔH_m J/g) of the sample to the value of enthalpy of 100 % crystalline polymer (ΔH_o=93.6 J/g for PLLA (Fischer et al. 1973) and ΔH_o=139.5 J/g for PCL (Pitt et al. 1981).

$$\chi = \frac{\Delta H_m}{\Delta H_o} * 100 \qquad \text{Equation 3.3}$$

3.2.5 In Vitro Cytotoxicity Tests and Cell Adhesion

Mouse NIH 3T3 fibroblasts used in MTT tests were gently gifted by Prof Dr. Neşe Atabey (Department of Medical Biology and Genetics, Dokuz Eylül University). The cells were cultured using RPMI-1640 supplemented with 10 % fetal bovine serum (FBS) and 1% gentamycin sulphate (50 µg/ml) in 5 % CO_2 humidified incubator at 37 ^{o}C. RPMI 1640 contains L-glutamine buffered with 25mM N-(2-hydroxyethyl)piperazine-N$^{'}$-2-ethanesulfonic acid (HEPES). The cell cultures were passaged by trypsinization after formation of 80-85 % cell confluence.

3.2.5.1. In Vitro MTT Cytotoxicity Tests of Catalysts

MTT tests of three different types of catalysts ($SnOct_2$, $BiAc_3$ and creatinine) were carried out in order to determine their cytotoxic effect and half maximal inhibitory concentrations (IC50). The mouse fibroblast 3T3 cells were cultured for 24 h at 37 ^oC in a humidified atmosphere with 5% CO_2 to achieve a cell monolayer in 96-well plates at $1x10^4$ cells. 100μl cell suspension was used in 96 well-plate. The all catalysts were used after autoclaving and sterile filtration in cyto-toxicity tests. Serial dilutions were made for preparation of catalyst concentrations as 500, 100, 50, 25, 10, 1, and 0.1 μg/ml. After the end of each incubation period (24 h, 48 h and 72 h), MTT stock solution was added into each well at 1:10 ratio and incubated for four hours Controls without cells were carried out in order to subtract the colorant retained by the substrate. After incubation, the plates were centrifuged at 1800 rpm for 10 minutes at room temperature to prevent removal of formazan crystals. Supernatant was removed before the DMSO addition. DMSO was used to dissolve blue formazan crystals for spectrophotometric quantification at a wavelength of 540 nm by using an automated plate reader. Each catalyst was assayed three times in triplicate. IC50 values of the catalysts were calculated by non-linear regression analysis of these separate triplicate experiments by "GraphPad Prism 5" software.

3.2.5.2. In Vitro MTT Cytotoxicity Tests of Biodegradable Polymers

MTT cytotoxicity tests of PLA, PCL homopolymers and P(LL-co-CL) copolymers synthesized with four different initiators having M/I:100 by using creatinine and Bi(III)Ac catalysts were carried out. The polymeric films (1cmx1cm) were prepared by using hot press. The mouse fibroblast 3T3 cells were cultured for 24 h at 37 ^oC in a humidified atmosphere with 5% CO_2 to achieve a cell monolayer in 24-well plates at $1x10^5$ cells concentration. The polymeric films (1.25 cmx1.25 cm) were sterilized under UV light for 2 h. After the sterilization of the polymeric films were inserted to the well plate for incubation 24 h, 48 h and 72 h at 37 ^oC with 5% CO_2. RPMI 1640 supplemented with 10 % fetal bovine serum (FBS) and 50 μg/ml gentamycin sulphate were used for culture medium. 600 μl cell suspension was used. After the end of each incubation period (24 h, 48 h and 72 h), MTT stock solution was added into each well at

1:10 ratio and incubated for four hours. Viable cells convert MTT to soluble formazan crystals during incubation. Controls without cells were carried out in order to subtract the colorant retained by the substrate. The amount of formed formazan depends on the viable cell number. After incubation, the plates were centrifuged at 1800 rpm for 10 minutes at room temperature to prevent removal of formazan crystals. Supernatant was removed before the DMSO addition. DMSO was used to dissolve blue formazan crystals for spectrophotometric quantification at a wavelength of 540 nm by using an automated plate reader. Cytotoxicity data obtained from experiments were performed in three times in triplicate. The results were expressed as percentages of the mean absorbance (optical density) compared with that in the control cells. The mean optical density of the control indicating the normal growth medium was set to represent 100 % viability.

3.2.5.3. Cell Adhesion and Growth on Biodegradable Polymers

Cell attachment and cell morphology on PLLA/PCL homopolymers and P(LL-co-CL) copolymers synthesized with four different co-initiators having M/CoI:100 by using creatinine and Bi(III)Ac catalysts for one day were studied. The mouse fibroblast 3T3 cells were cultured for 24 h at 37 ^{o}C in a humidified atmosphere with 5% CO_2 to achieve a cell monolayer in 24-well plates at $1x10^5$ cells concentration. The polymeric films (1.25cmx1.25cm) were sterilized under UV light for 2 h. After the sterilization, the polymeric films were inserted to the well plate for incubation 24 h. 600µl cell suspension was used in 24 well-plate. After being cultured for 24 h, the samples were taken from the culture plate and washed with phosphate buffer (PBS) for two times, then fixed with 2.5 % glutaraldehyde in PBS and stored for one night at 4 ^{o}C. After the washing of polymeric film samples with PBS for three times, the samples were dehydrated with graded ethanol series (50, 60, 70 and 90 %) for 10 minutes and two times respectively. They also subjected to pure ethanol for 30 minutes before drying of the samples under crtical drying conditions. Glutaraldehyde and ethanol were used as crosslinking fixative and coagulating fixative agents, respectively. The samples were sputter-coated with a thin layer of gold. Cell attachment and cell morphology of the films were observed by Scanning Electron Microscopy (Quanta 250 SEM).

CHAPTER 4

RESULTS AND DISCUSSIONS

The tailoring of molecular structure of PLLAs and PCLs by means of biosafe catalysts and co-initiators has attracted a great importance in biomedical applications. Enhancement of degradation and physical properties of PLLAs and PCLs can be optimized by incorporation of different co-initiators.

The synthesis of linear and branched hydroxy telechelic PLLAs and PCLs is possible by using different types of co-initiators. Also, these hydroxy telechelic PLLAs and PCLs can be used as intermediate polymers for further synthesis of various kinds of poly(ester-urethane)s. In this study, it was aimed to compare linear and star-shaped PLLAs and PCLs synthesized by using Bi(III) acetate and creatinine as biocompatible catalysts. Ethylene glycol, pentaerythritol and myo-inositol as co-initiators were used to synthesize linear and branched PLLAs and PCLs at different architectures. Especially, myo-inositol as a non-toxic co-initiator is selected due to the availability in the body and plant. Also, the thermal properties and molecular weights of linear and star-shaped PLLAs and PCLs were tailored by varying the type and amount of co-initiators.

Experimental results are given as four parts in this chapter. The results of synthesis of OH functional PLLA by using Bi(III)Ac and creatinine and the synthesis of OH functional PCL by using creatinine are given in first and second part, respectively. The third part includes the results of diblock, tetrablock and hexablock LA/CL copolymers by using creatinine. The MTT test results of $SnOct_2$, Bi(III)Ac and creatinine catalysts as well as the polymers synthesized, and , cell adhesion results of the selected synthesized PLLA and PCL homopolymers and copolymers are given in the fourth part in this chapter.

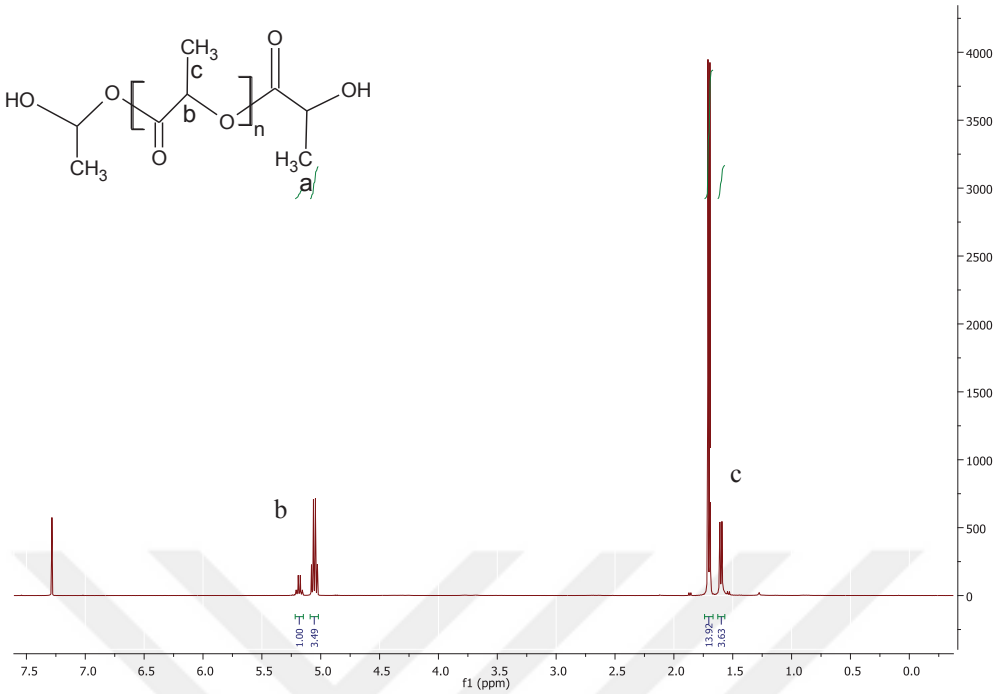
4.1. Synthesis of Homo and OH Functional PLLA Polymers by using Bi(III)Ac and Creatinine as Catalysts

The effects of catalyst and co-initiator type, the ratios of monomer/catalyst (M/C) and monomer/co-initiator (M/CoI) and temperature on polymerization of lactide were investigated.

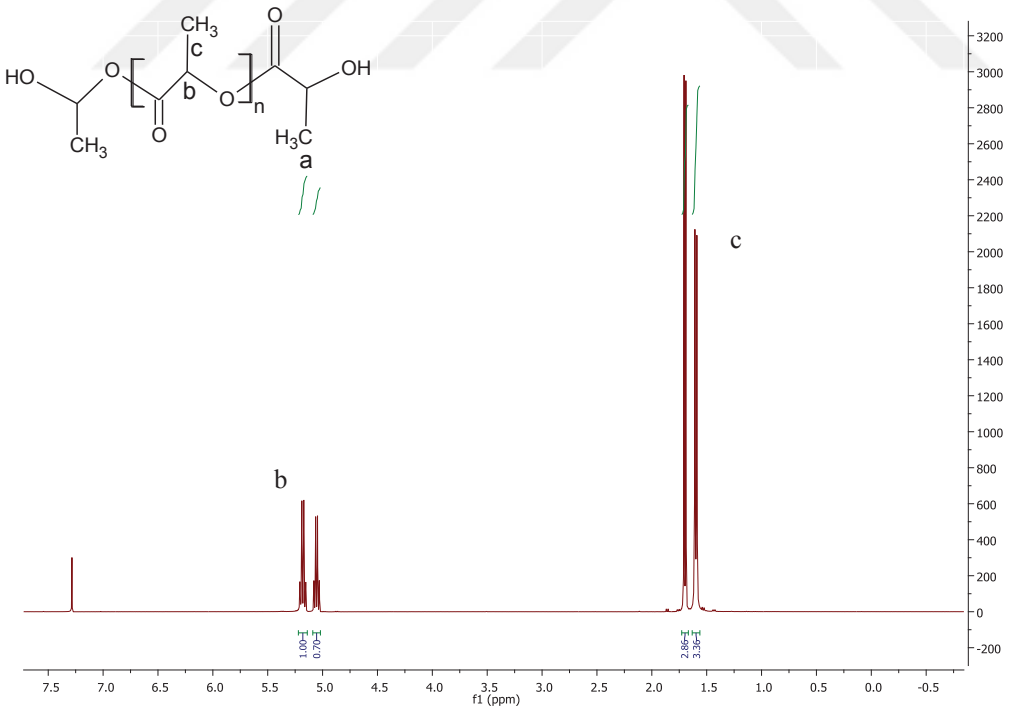
4.1.1. Catalyst type and temperature effects on synthesis of PLLA

The catalysts have been used together with alcohols for initiation in many polyester based polymerization systems. For the evaluation of the three types of catalysts in ring opening polymerization of lactide, Bi(III)Ac, $SnOct_2$ and creatinine catalysts have been used without any initiator. Monomer conversion values for ring opening polymerization reactions of lactide by using three different catalysts were calculated from ^{1}H NMR analyses. LA monomer conversion was determined by integrating the methine proton peak of LA monomer at 5 ppm (-COCH(CH_3)-O-, δH = 5 ppm) and the methine proton peak of PLLA polymer at 5.2 ppm (-CO-CH(CH_3)-O-, δH^m= 5.2 ppm). Figure 4.1.a and Figure 4.1.b show ^{1}H NMR spectra of crude PLLA polymers synthesized by Bi(III)Ac catalyst for 60 min at 120 and 140 ^oC, respectively. Lactide monomer/Bi(III)Ac catalyst [M/C] ratio was hold constant as 500 for this polymerization step at two different reaction temperatures. The conversion values of the synthesized PLLA polymers for 60 min at 120 and 140 ^oC were calculated as 22 and 59 %, respectively.

Figure 4.2.a and Figure 4.2.b show ^{1}H NMR spectra of crude PLLA polymers synthesized by $SnOct_2$ and creatinine catalysts for 45 minutes at 120 ^oC and 15 hours at 140 ^oC, respectively. Lactide monomer/catalyst ratios for $SnOct_2$ and creatinine catalysts were taken as 500 and 50, respectively. The conversion value of the crude PLLA polymer synthesized by $SnOct_2$ catalyst for 45 min at 120 ^oC was found as 55 %. The lower conversion value as 38 % was obtained for the crude PLLA polymer synthesized by creatinine catalyst at the end of 15 hours.

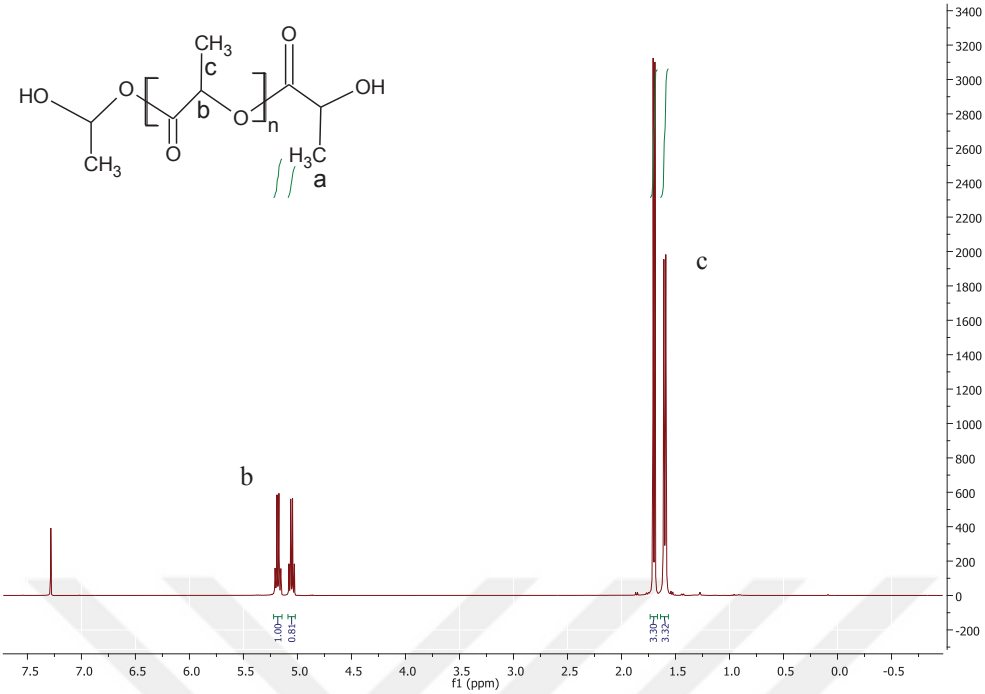


(a)

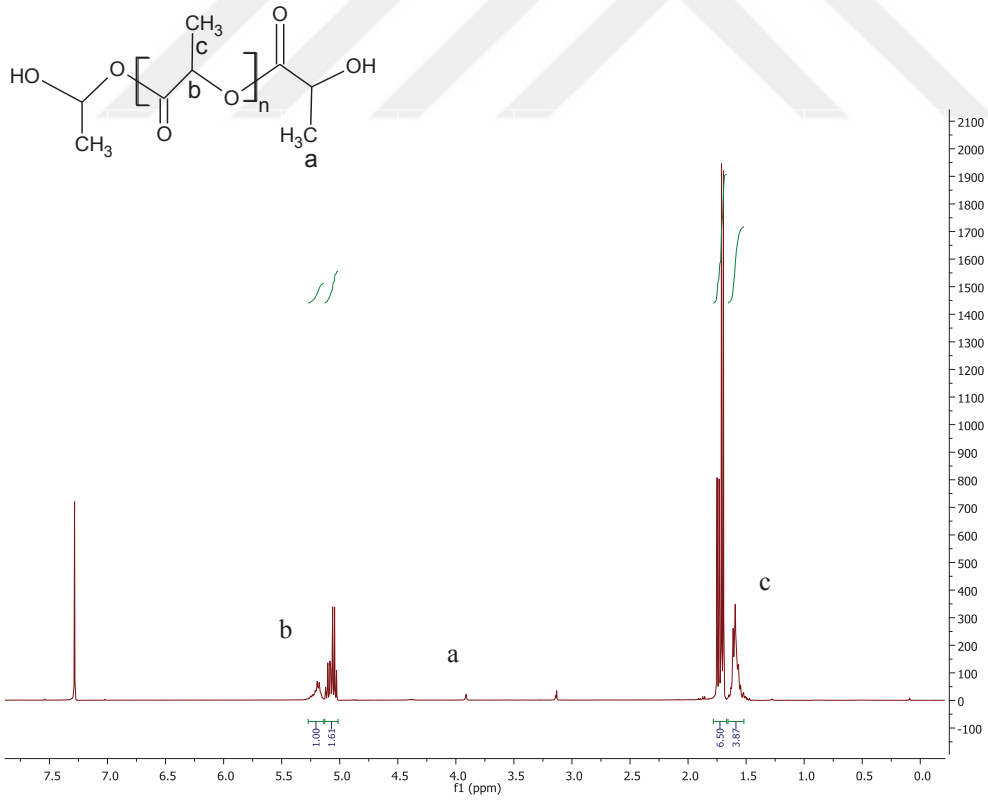


(b)

Figure 4.1. ^{1}H NMR spectra of crude PLLA polymer synthesized for 60 min by Bi(III)Ac catalyst (a) at 120 ^{o}C and (b) 140 ^{o}C.



(a)



(b)

Figure 4.2. ^{1}H NMR spectra of crude PLLA polymers synthesized by (a) $SnOct_2$ for 45 minutes at 120 ^{o}C , (b) creatinine for 15 hours at 140 ^{o}C.

Figure 4.3 and Figure 4.4 show the time-conversion curves for lactide polymerization in bulk by using three different catalysts; Bi(III)Ac, $SnOct_2$ or creatinine according to amount of monomer/catalyst ratio (M/C) and temperature. Since the amount of catalyst may affect the reactivity and molecular weight, polymers were synthesized at two different temperatures and M/Bi(III)Ac ratios. Figure 4.3 illustrates time temperature curves for lactide polymerization with Bi(III)Ac catalyst at M/C ratio of 500 at 120 and 140 ^{o}C. and compares the results obtained with $SnOct_2$ catalyst. As seen from the Figure, Bi(III)Ac is less active than $SnOct_2$., but all reactions have high conversion values. In the case of monomer/catalyst ratio being 500 (M/C:500), it can be said that the catalyst efficiency of $SnOct_2$ is slightly higher than Bi(III)Ac systems at 120 and 140 ^{o}C. The difference in reaction rates at two different temperatures is obviously clear. As seen in the figure, a higher polymerization temperature results in a faster reaction. The achivement of higher conversion values with increase of temperature for a specified reaction time is related with the reaction rate. It can be said that the reaction rates directly depend on temperature (Contreras et al 2013). The effects of temperature on reaction rates are investigated in polymerization kinetics part in more detail.

Figure 4.4 shows time conversion data of polylactide polymerization with creatinine catalyst for two different M/C ratios of 50 (50:1) and 500 (500:1) at 120 and 140 ^{o}C and compares with $SnOct_2$ for M/C ratio of 10000 at 120 ^{o}C. It can be seen that significant differences were observed between the conversion values of the polylactides synthesized. A lower M/C ratio and a higher temperature give higher conversion values at the same temperature or same M/C ratio, respectively. Effect of water impurity on the polymerization rate of lactide was also studied. As seen in the figure, conversion values were increased significantly in the presence of water in the synthesis of lactide by creatinine catalyst at 120 ^{o}C. The increase in the conversion values in the presence of water indicates the initiator effect of water for the ring opening polymerization of lactide. Thus, drying of solvents and glassware and purification of L-lactide monomer are extremely important to carry out the controlled polymerization of lactide. The use of high amount of creatinine as a catalyst is required for synthesis of poly(L-lactide). At a conversion of 95%, although the lactide polymerization initiated by M/$SnOct_2$:500 at 120 ^{o}C was found as 30 times higher than the polymerization initiated by M/$SnOct_2$:10000 at 120 ^{o}C, the polymers obtained at M/$SnOct_2$:500 are not biosafe due to the cytotoxicity for biomedical applications. Therefore, it is better to compare our

data with safe M/$SnOct_2$ ratio of 10000. The lactide polymerization initiated by M/$SnOct_2$:500 at 120 ^oC was found as higher 1.25, 1.75 and 0.4 times than the systems initiated by M/Bi(III)Ac:500 at 140 ^oC, M/Bi(III)Ac:500 at 120 ^oC, and M/Creatinine:50 at 140 ^oC, respectively. The high conversion values were obtained by using $SnOct_2$ catalyst in LA polymerization. However, the toxic effects of residual tin amount in biopolymers synthesized by $SnOct_2$ catalyst had been reported (de Mattos et al. 2000, Silva et al. 2002, Tsuji et al. 2010, Yamada et al. 2008). Also, the impossibility of complete removal of tin from the synthesized biopolymers limits their medical applications (Fernandez et al. 2013, Schwach et al. 1997 and 2002, Stjerndahl et al. 2007 and 2008, Tanzi et al. 1994). Stjerndahl et al. 2007 and 2008 studied about the minimization of residual tin content in polymerization of lactide and caprolactone. They indicated that monomer/$SnOct_2$ ratio of 10000 or higher is required for achievement of polymer consisting acceptable tin content for biomedical applications. The FDA (Food and Drug Administration) has set a limit of 20 ppm of residual tin in commercially used medical polymers, and it is essential not to exceed that limit.

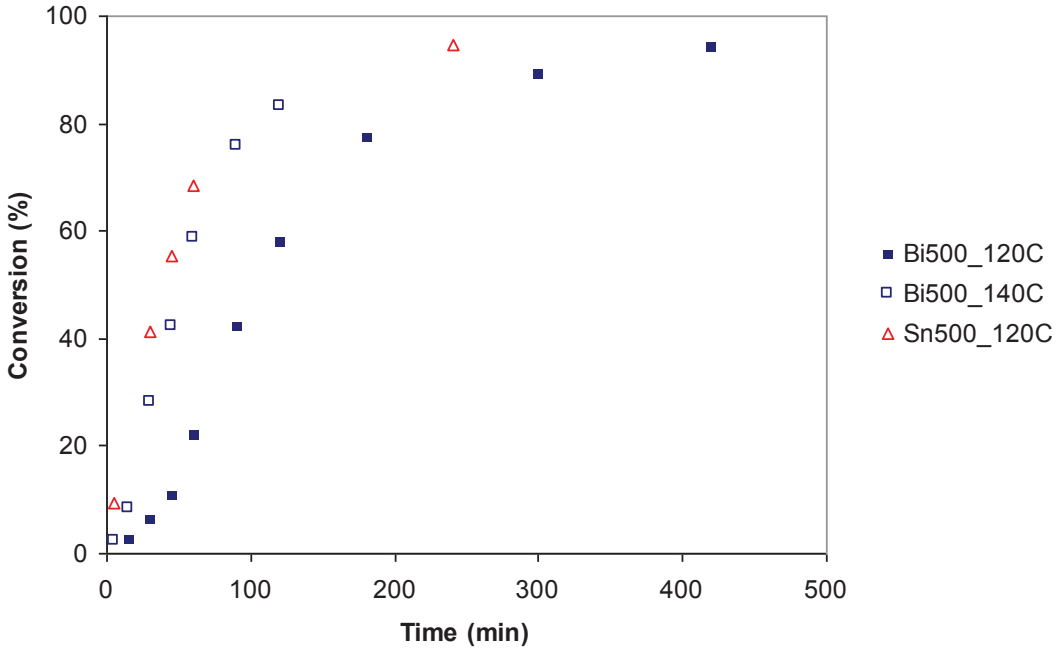


Figure 4.3. Time-conversion curves for bulk polymerization of lactide initiated by Bi(III)Ac, or $SnOct_2$ without co-initiators at 120 -140 ^oC.

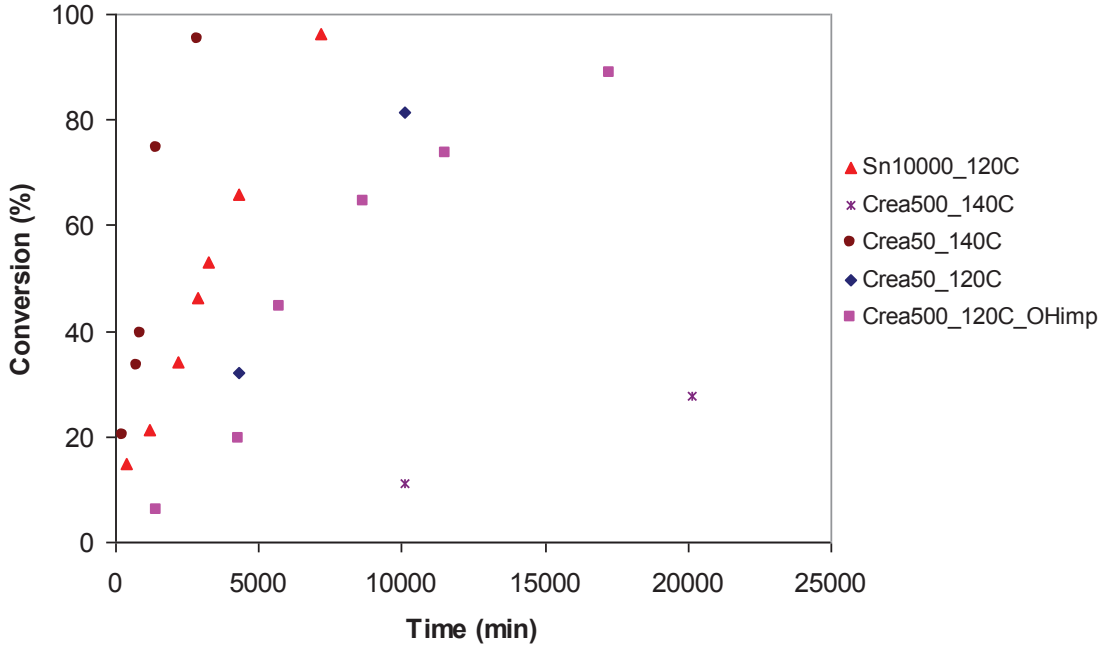


Figure 4.4.Time-conversion curves for bulk polymerization of lactide initiated by creatinine at different M/I ratios and 120-140 ^{o}C and $SnOct_2$ (M/I:10000) at 140 ^{o}C without co-initiators.

Properties of homopolymers synthesized by Bi(III)Ac without co-initiators at three different M/Bi(III)Ac ratios at 120 and 140 ^{o}C are given in Table 4.1. The results in the Table gives number average molecular weight determined by both SEC and NMR as well as the thermal properties of T_g and T_m and crystallinity values. The results revealed that Bi(III)Ac initiated the L-lactide polymerization without addition of co-initiators. The monomer conversion values were determined by integrating the methine proton peak of LA monomer at 5 ppm (-COCH(CH_3)-O-, δH = 5 ppm) and the methine proton peak of PLA polymer at 5.2 ppm (-CO-CH(CH_3)-O-, δH^m= 5.2 ppm) from ^{H}NMR The conversion values were found as higher than 96.8 %. The M_n values from ^{H}NMR were calculated by considering peak intensities for methine protons of L-lactide units at chain terminals (HO-CH(CH_3)CO-, δH^c = 4.36 ppm) and the main chain of the polymer (-CO-CH(CH_3)-O-, δH^m= 5.2 ppm). The M_n values obtained from ^{H}NMR were found as between 44900 and 97200 Da. The M_n values of two samples synthesized by M/I ratios 500 and 1000 at 140 ^{o}C had not been calculated due to the unvisible of end group from ^{H}NMR. The M_n values measured from SEC were obtained between 37300 and 105900 Da. The decrease in M_n values were observed with increase in temperature and decrease with M/I ratio.

It is known that the drawback of the faster reaction is a lower M_n and a higher PDI. At constant M/I ratio, as temperature increases, rate of polymerization increases and M_n values obtained both SEC and NMR decreased. The reduction in M_n values is observed with increase in temperature and catalyst amount. When the M/I ratio is increased at the constant temperature, it was observed that M_n values increased (SEC), however the increase is not linear fashion as initial monomer/initiator ratio(Mo/Io) increase. The reason for these results is that the system is not totally free from OH-co-initiating impurities or water. This uncontrolled reaction leading to inter and intra molecular transesterification reactions is also identified by the relative high PDI values found as in the range of 1.34 and 1.49.

Thermal properties of the homopolymers synthesized by Bi(III)Ac without co-initiators were determined by DSC and melting temperatures of synthesized PLLA polymers by Bi(III)Ac catalyst were found to be between 170 and 176 ^{o}C. The glass transition temperatures were obtained between 51 and 59 ^{o}C. The degree of crystallinity (χ) values of the PLLAs were calculated by the ratio of the measured melting enthalpy (ΔH_m J/g) to the value of enthalpy of 100 % crystalline PLLA polymer given as ΔH_o=93.6 J/g in the literature (Fischer et al. 1973). The crystallinity values of the synthesized PLLA polymers by Bi(III)Ac catalyst were obtained between 57 and 66 %. The increase in crystallinity values was observed by increase in M/I ratio and decrease in temperature. The increase in crystallinity values, glass transition and melting temperatures were observed with increase in molecular weight of PLLAs. The lowest conversion, melting point, molecular weight, melting enthalpy.and crystallinity values were obtained in the case of M/I:200 and 140 ^{o}C. In the case of M/I ratio:1000 and 120 ^{o}C., the highest crystallinity value and glass transition temperature were determined as 66 % and 59 ^{o}C.

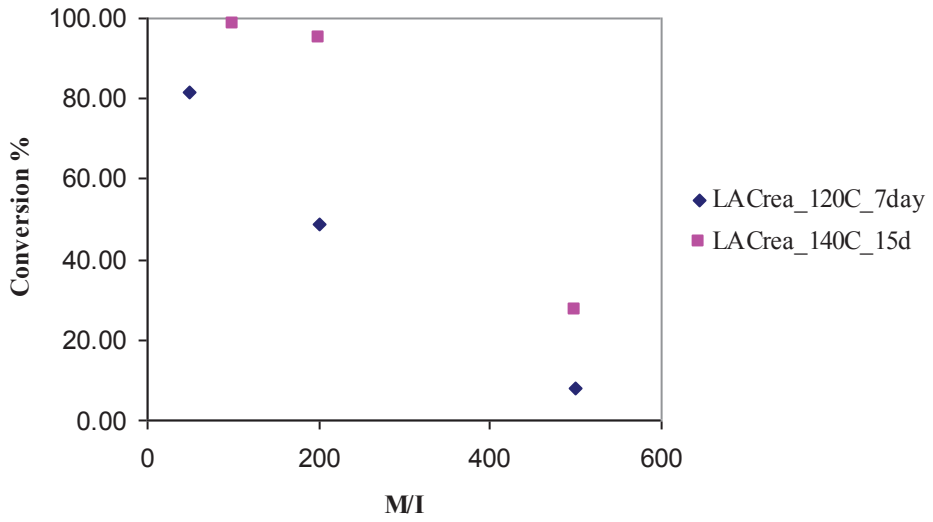
The high values in molecular weights, glass transition and melting temperatures of PLLAs are obtained with increase of M/I ratio and decrease of temperature. These results show that the Bi(III) acetate initiator can be used succesfuly in bulk polymerization without any co-initiators. However, the systems may work better using a co-initiator under controlled reaction and therefore, we will continue to examine these systems by using ethylene glycol, myo-inositol and pentaerythritol as co-initiators in bulk polymerization.

Table 4.1. Polymerizations of L-lactide initiated by Bi(III)Ac without co-initiators

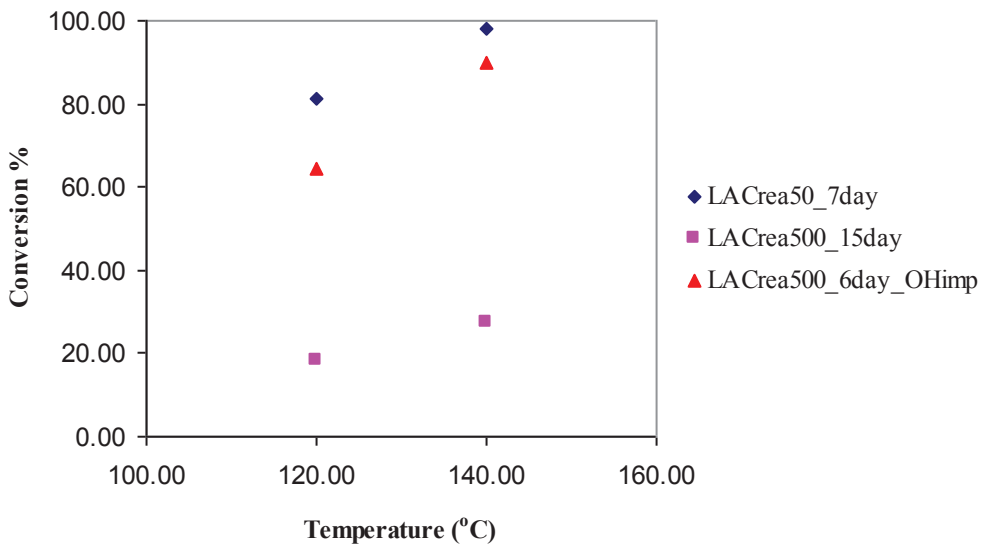
Mo/I	Rxn Temp (^oC)	Conv NMR (%)	M_n SEC (Da)	PDI	M_n HNMR (Da)	T_g (^oC)	T_m (^oC)	Crys (%)
200	120	97.7	64300	1.45	55100	56.4	175	58.3
200	140	96.8	37300	1.49	44900	51.6	170	57.4
500	120	99.6	93000	1.34	51200	51.4	174	60.1
500	140	99.8	87400	1.52	*	55.5	176	61.0
1000	120	99.6	105900	1.38	97200	59	176	66.4
1000	140	99.7	96200	1.40	*	56.4	177	62.6

**The end groups were not visible in the NMR spectrum*

Figure 4.5 a and 4.5.b show the effect of M/I and temperature on the bulk polymerization of L-lactide initiated by creatinine catalyst. Four different M/I ratios (50, 100, 200 and 500) were used to find the optimum M/I ratio for L-lactide polymerizations initiated by creatinine catalyst at 120 and 140 ^oC. The long polymerization times are required to reach the high conversion values in bulk polymerization of LA by creatinine catalyst without co-initiator. The high conversion values found as 98.2, and 95.2 % in the case of M/I: 50 and M/I:200 for creatinine catalyst were obtained at the end of seven and fifteen days at 140 ^oC, respectively. In the case of M/I: 500, the conversion value were found as 27.6 % at the end of fifteen days at 140 ^oC. For the conditions of M/I:100, the conversion value was determined as 82 % at 120 ^oC. As seen in Figure 4.5.a and 4.5.b, the conversion values increase with decrease of M/I ratios and increase of polymerization temperature. Figure 4.5.b also indicates the effect of water impurities on the bulk polymerization of LA initiated by creatinine. In the case of M/I: 500 and the presence of OH impurities, the conversion values at 120 and 140 ^oC were obtained as 64.5 and 90 % at the end of six days, respectively. Therefore, it was concluded that the presence of OH impurities significantly increased the conversion values.



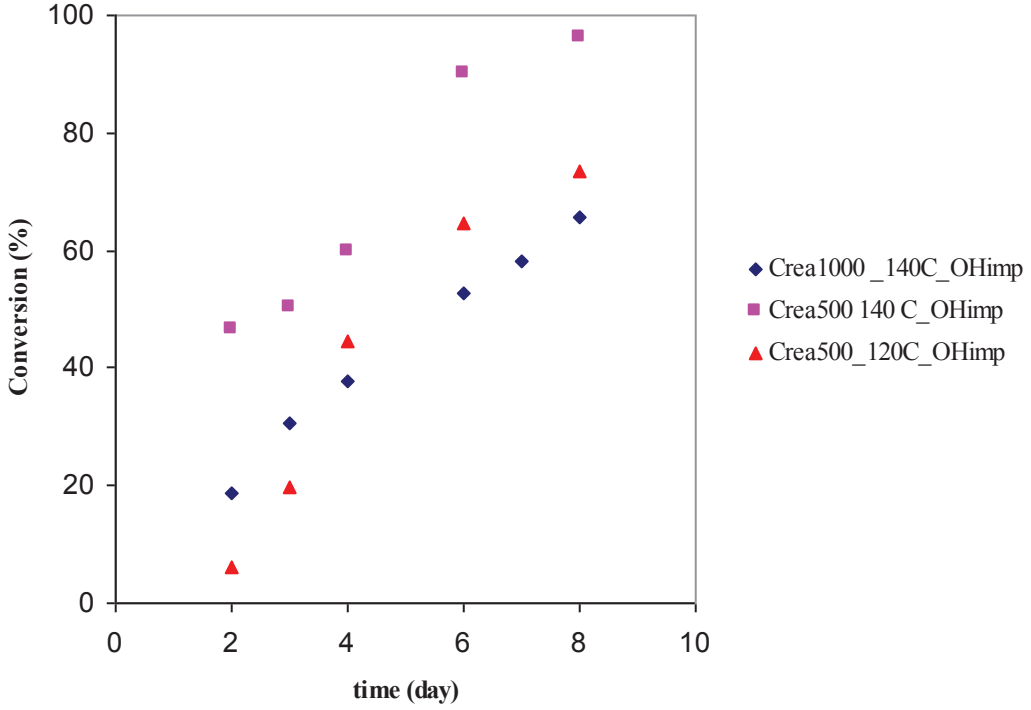
(a)



(b)

Figure 4.5. Effect of a) M/I and b) temperature on the conversion curves for bulk polymerization of lactide initiated by creatinine.

Figure 4.6 indicates the effect of water (OH) impurities in the polymerization of lactide initiated by creatinine catalyst at different M/I ratios and temperature. The increase in the conversion values with the presence of water indicates the initiator effect of water for the ring opening polymerization of lactide. The presence of water impurities leads to significant decrease in polymerization time of LA polymerization initiated by creatinine catalyst.



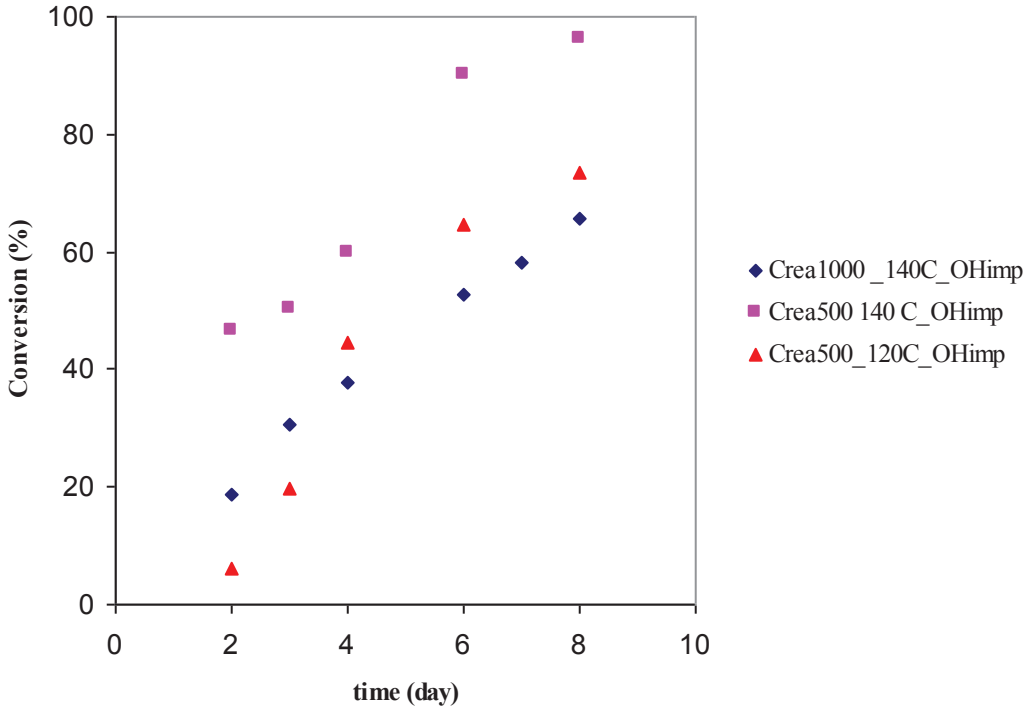


Figure 4.6. Effect of OH impurities and temperature on the conversion curves for bulk polymerization of lactide initiated by creatinine.

It can be said that the increase in the amount of creatinine and polymerization temperature leads to significant increase in the conversion values and the decrease in reaction time. The M/I ratio and polymerization temperature as 50 and 140 ^{o}C seem to

be reasonable (or optimum values) for the polymerizations synthesized by creatinine catalyst without co-initiator by considering reaction time.

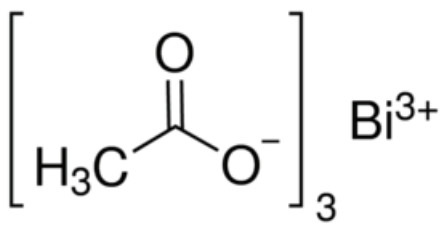
The properties of homo PLLAs synthesized by creatinine catalyst without co-initiator was given in Table 4.2. At constant M/I ratio: 50, polymers were synthesized at two different temperatures. As shown in Table 4.2, the increase in conversion values from 81.4 to 98.2 % were tabulated with increase of polymerization reaction temperature from 120 to 140 ^{o}C at the end of seven days.

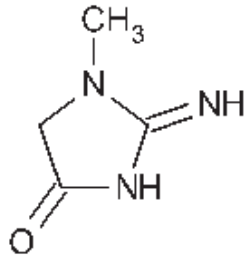
The M_n values obtained from SEC and ^{H}NMR were found to be between 4240 and 11600 Da. The low M_n values of this synthesized polymers initiated by creatinine catalyst without co-initiator were obtained and compared to that of LA polymerization initiated by Bi(III)Ac. The presence of water in polymerization as OH impurity led to the significant decrease in polymerization time from seven to two days. The polydispersity values were obtained in the range of 1.75-1.88. The glass transition temperatures were found to be between 33 and 45 ^{o}C. Melting peaks were not observed for PLAs synthesized by creatinine catalyst. Amorphous PLAs with low molecular weight and high PDI values were obtained by using creatinine as a catalyst.

Table 4.2.Bulk polymerization of L-lactide initiated by creatinine without co-initiator.

Mo/I	Rxn time (days)	Rxn temp (^{o}C)	Conv NMR %	M_n SEC (Da)	PDI	M_n ^{1}H NMR (Da)	T_g (^{O}C)
50	7	120	81.4	8500	1.88	4240	33
50	7	140	98.2	9400	1.87	11600	45
50	2	140	95.2	9350	1.75	10800	43

In this ring opening polymerization of PLLA with creatinine catalyst, the mechanism obeys coordination-insertion mechanism based on the presence of OH functional impurities such as trace amount of water effect as a co-initiator.The structures of Bi(III)Ac and creatinine catalysts were shown in Figure 4.7. Kricheldorf et al. 2004b also revealed that L-lactide polymerizations initiated by Bi(III)Ac as a catalyst and tetraethylene glycol, 1,1,1-tris(hydroxymethyl)propane or pentaerythritol as co-initiators in chlorobenzene are based on coordination-insertion mechanism.





(a) (b)

Figure 4.7. Structures of Bi(III)Ac and creatinine catalysts.

4.1.2. Effect of Co-Initiator type on synthesis of PLLA

The effects of different types of co-initiators on synthesis of PLLA by using Bi(III)Ac and creatinine catalysts have been investigated by considering ^{1}H NMR, ^{13}C NMR, SEC and DSC results. The synthesis of linear and star shaped PLLA polymers is possible by incorporation of branch structure by using different type of co-initiators. Figure 4.8 indicates the ring opening polymerizations of L-lactides by using Bi(III)Ac and creatinine catalysts and ethylene glycol, pentaerythritol and myo-inositol as co-initiators to form di, tetra and hexa hydroxyl functional PLLAs, respectively.

4.1.2.1. Effect of Co-Initiator type on synthesis of PLLA by using Bi(III)Ac catalyst

4.1.2.1.1. Effect of Ethylene Glycol as Co-Initiator on synthesis of PLLA by using Bi(III)Ac

Figure 4.9 shows the ^{1}H NMR structure of PLLA synthesized by Bi(III)Ac as an initiator and ethylene glycol as a co-initiator in the case of M/I: 500, M/CoI:20 at 120 ^{o}C. The monomer conversions were determined from ^{1}H NMR spectrum of the crude reaction mixture by taking the ratio of the peak intensity of methine proton of monomer (δH = 5.0 ppm, -COCH(CH_3)-O-) to that of polymer(δH= 5.2 ppm, -CO-CH(CH_3)-O-). Figure 4.10 indicates the ^{13}C NMR spectrum of PLLA synthesized by Bi(III)Ac and ethylene glycol at the same polymerization conditions as in Figure 4.9. In the ^{13}C NMR spectrum, -CH, -CH_3, groups in the repeating and terminal units, -CO and the CH_2

group coming from ethylene glycol were observed at 68.9, 16.6, 66.6, 20.4, 169.80 and 62.64 ppm, respectively. Although methylene protons of ethylene glycol were not detectable in the ^{1}H NMR spectrum, methylene carbon of ethylene glycol was recognized in ^{13}C NMR spectrum.

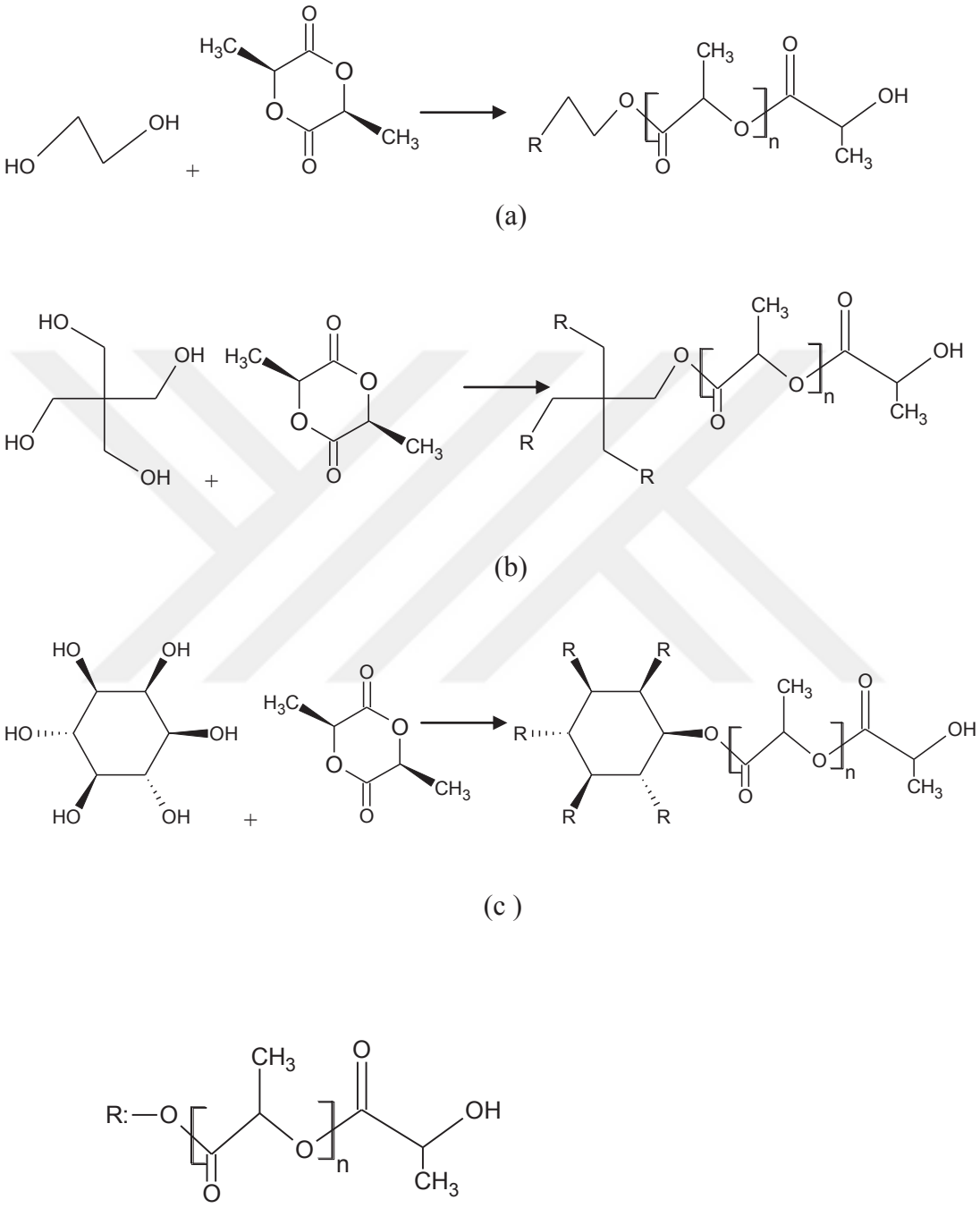


Figure 4.8. Ring opening polymerizations of L-lactides by using (a) ethylene glycol, (b) pentaerythritol and (c) myo-inositol as co-initiators.(R denotes PLLA units).

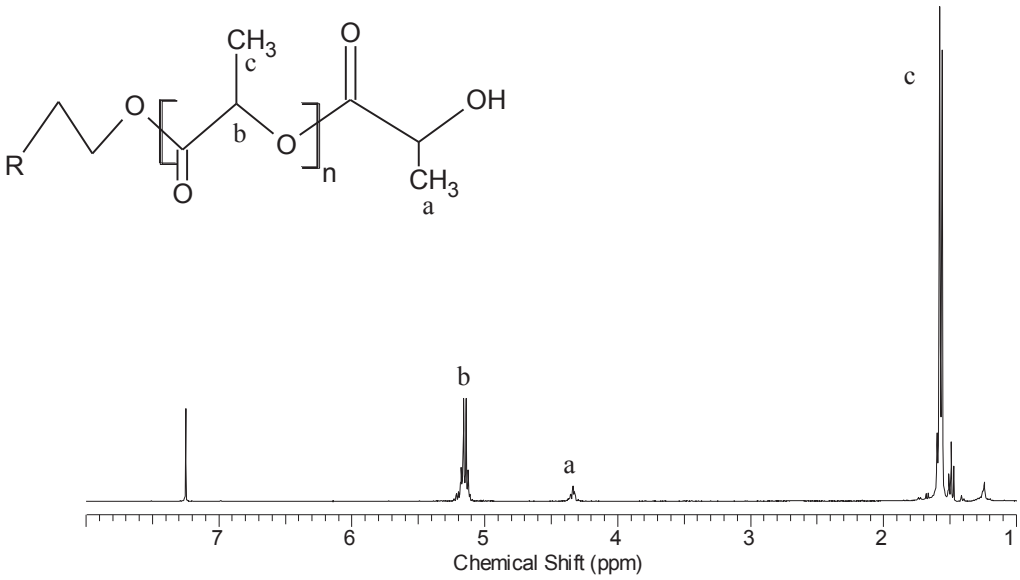


Figure 4.9. ^{1}H NMR spectrum of PLLA synthesized by Bi(III)Ac and ethylene glycol in the case of M/I: 500, M/CoI:20 at 120 ^{o}C.

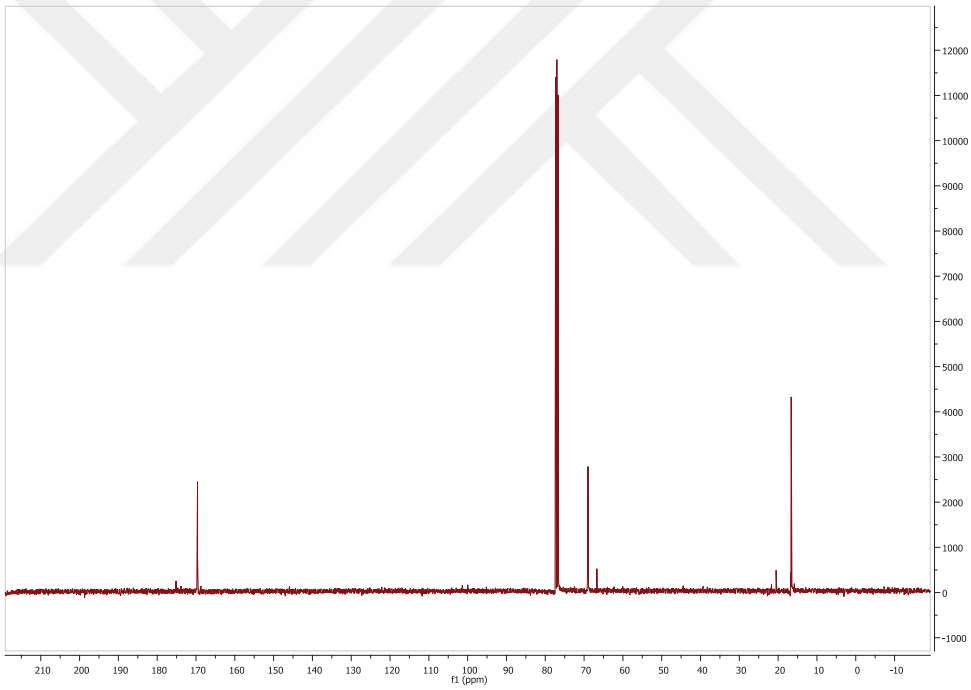


Figure 4.10. ^{13}C NMR spectrum of PLLA synthesized by Bi(III)Ac and ethylene glycol in the case of M/I: 500, M/CoI:20 at 120 ^{o}C

Figure 4.11 presents the time-conversion curves for bulk polymerization of lactide initiated by Bi(III)Ac (M/I: 500) and ethylene glycol (M/CoI:20, 100, and 1000) as an initiator and co-initiator, respectively. The longest polymerization time was obtained in the polymerization of L-LA in the absence of EG at 120 ^{o}C. The addition of EG makes the reaction faster, a lower Mo/CoI ratio results in a higher reaction rate. It is

also obvious as in the case above that a higher temperature gives a faster reaction rate, independent on the Mo/Io ratio. The achivement of higher conversion values with increase of EG amount and temperature for a specified reaction time is related with the reaction rate. It can be said that the increasing of EG amount and temperature leads to the increase of reaction rates. The effects of EG amount and temperature on reaction rates are also investigated in polymerization kinetics part. Bi500EG20 at 140 ^{o}C reach 100 % conversion within 120 min. At a conversion of 90%, the lactide polymerization initiated by M/Bi(III)Ac:500 and M/EG:20 at 140 ^{o}C was found as higher 9 and 21 times than the systems initiated by M/Bi(III)Ac:500 without co-initiator at 140 ^{o}C and 120 ^{o}C, respectively. The decrease of the polymerization time by using EG as co-initiator is attributed the activator effect of EG in the polymerization of L-LA. This case indicates that the catalytic activity of Bi(III)Ac in rop of L-LA is increased with the presence of EG. Our results are also in good agreement with the literature results. In the literature, variable coinitiators such as alcohols, dodecanol, glycerol were used in the polymerization of cyclic esters to decrease the polymerization time (Ikpo et al. 2012, Yu et al. 2009)

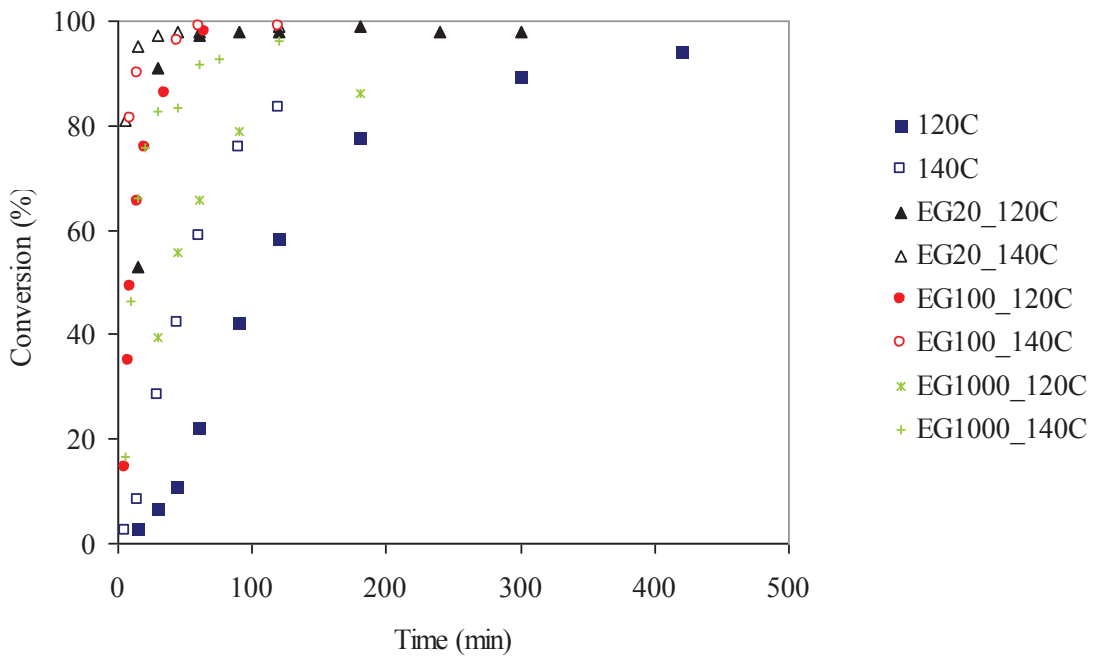


Figure 4.11. Time-conversion curves for bulk polymerization of lactide initiated by Bi(III)Ac with ethylene glycol as a co-initiator.

The results of bulk polymerization of L-lactide initiated by Bi(III)Ac and ethylene glycol with different M/I and M/CoI ratios at 120 and 140 ^{o}C are given in Table 4.3 and Table 4.4, respectively. The molecular weights of the synthesized -OH functional PLAs with ethylene glycol were much lower compared to the system obtained without co-initiators. The same trend regarding the temperatures can though be seen at 140 ^{o}C results in somewhat lower molecular weights. The molecular weight and also arm length of the polymers is increasing with decreasing the amount of ethylene glycol. The observation of decrease in molecular weight with the increase of CoI amount is in good aggrement with the literature (Yu et al. 2009). This indicates the controlling effect of CoI on polymerization of L-LA. The molecular weights obtained from ^{1}H NMR of the functional PLAs with ethylene glycol synthesized at 120 ^{o}C were found in the range of 1100 and 25100 Da for different M/I and M/CoI ratios. The lower molecular weights obtained from ^{1}H NMR of the functional PLAs with ethylene glycol synthesized at 140 ^{o}C were obtained in the range of 850 and 16240 Da for different M/I and M/CoI ratios. In the case of M/I:1000 and 120 ^{o}C, molecular weights obtained from ^{1}H NMR of functional PLAs with ethylene glycol for M/CoI ratios : 20,100,and 1000, were tabulated as 1102, 6260 and 25100 Da, respectively. In the case of M/I:1000 and 140 ^{o}C, molecular weights obtained from ^{1}H NMR of functional PLAs with ethylene glycol for M/CoI ratios : 20, 100, and 1000 were tabulated as 950, 5710 and 16240 Da, respectively.

Glass transition and melting temperatures of PLAs are decreased by the decrease in M/CoI ratios (Tables 4.3 and 4.4). In the case of M/I:500, at 120 ^{o}C; glass transition temperatures of functional PLAs with ethylene glycol for M/CoI ratios :1000, 100,and 20 were obtained as 48, 38.2 and 32.6 ^{o}C, respectively. In the case of M/I:1000 and 120 ^{o}C, glass transition temperatures of functional PLAs were found to be higher as seen in Table 4.3. However, in the conditions of M/CoI:20 at 140 ^{o}C given in Table 4.4, all glass transition temperatures as found as 31.7, 33 and 25 ^{o}C, were below the body temperature for EG functional PLAs synthesized at all M/I ratios :200, 500 and 1000 respectively. The glass transition temperatures found as below the body temperature can be used as shape memory polymers. In the case of M/I:500 at 120 ^{o}C, melting temperatures of functional PLAs with ethylene glycol for M/CoI ratios :1000, 100,and 20 were tabulated in Table 4.3 as 171, 156 and 115 ^{o}C., respectively. The reduction in glass and melting temperatures is attributed to the disruption of crystal formation and related on the decrease in chain length. The crystallinity values of the EG functional

PLAs synthesized at 120 °C were found as between 23.2 and 58.4 %. The range of crystallinity values of these polymers synthesized at 140 °C were recorded in the range of 9.7 and 57.6 %. The decrease in crystallinity values of functional PLAs were observed with decrease in M/CoI ratios for all M/I ratios. As seen in the Tables 4.3 and 4.4, two melting temperatures were obtained. The presence of multiple melting peaks and the decrease in enthalpy values can be explained by the dispersion of polymer chains and hindering of chain ordering leading to decrease in crystallinity values.

Table 4.3. Effect of M/I and Mo/CoI ratios on bulk polymerization of L-lactide by Bi(III)Ac and ethylene glycol at 120 °C.

Mo/I	Mo/ CoI	Conv NMR %	M_n SEC (Da)	PDI	M_n ^{1}HNMR (Da)	T_g (°C)	T_m (°C)	Crys (%)
200	20	99.17	6700	1.15	1150	24	128.2/ 110.1	30
200	100	99.7	39300	1.10	6300	35.6	161.1/ 156.6	53.5
200	1000	99.7	66800	1.33	10240	37.4	169.9/ 161.4	58
500	20	99.6	6800	1.12	1100	32.6	130.2/ 114.9	31
500	100	100	34400	1.08	6500	38.2	162.9/ 155.9	49
500	1000	99.7	89200	1.13	14800	48	170.8	52
1000	20	99.3	6600	1.10	1102	42	129/ 114.9	23
1000	100	99.5	33500	1.09	6260	41.6	162.9/ 156.1	50
1000	1000	88.9	143600	1.33	25100	51	173	53

Table 4.4. Effect of M/I and Mo/CoI ratios on bulk polymerization of L-lactide by Bi(III)Ac and ethylene glycol at 140 ^{o}C

Mo/I	Mo/ CoI	Conv NMR %	M_n SEC (Da)	PDI	M_n ^{1}HNMR (Da)	T_g (^{o}C)	T_m (^{o}C)	Crys (%)
200	20	99.5	5050	1.2	850	31.7	118 / 106	22
200	100	99.7	26200	1.23	5120	35.1	131/ 114	34.5
200	1000	99.8	45000	1.39	13540	48.3	165	38.6
500	20	98.7	5800	1.18	980	33	124/ 108.	22
500	100	99.7	28700	1.15	6170	38.3	162/ 153	50.3
500	1000	99.8	78600	1.21	15110	48.1	169	57.6
1000	20	98.9	5650	1.16	950	25	136/ 124	9.7
1000	100	99.6	29200	1.18	5710	42.2	162/ 154	51
1000	1000	99.6	70100	1.65	16240	50.8	172/ 155	51.5

It is clear that the M/CoI ratio and not the M/I ratio is decisive for the degrees of polymerization. Kricheldorf et al. 2004 a and 2004 b have also reported dependence of co-initiator amount on molecular weight of the polymers. The increase in M_n values obtained from SEC and ^{1}H NMR were observed with the decrease of co-initiator amount from M/CoI: 20 to 1000 due to the decrease of number of active units during polymerization.

Srivastava et al. 2007 have earlier performed similar experiments for PLLA polymerization under enzymatic polymerization conditions by using lipase PS (Pseudomonos fluorescence) as a biocatalyst and these results gave a much lower M_n value and a much longer time to reach 100 % conversion. The lower amount of Bi(III)Ac was required in the same polymerization systems compared to enzymes as a catalyst.

4.1.2.1.2. Effect of Myo-Inositol as Co-Initator on synthesis of PLLA by using Bi(III)Ac

The conversion curves of poly(L-lactides) initiated by Bi(III)Ac catalyst with myo-inositol as a co-initiator were presented in Figure 4.12. The increase in the amount of myo-inositol as a co-initiator leads to the decrease in reaction rate of poly(L-lactide) in contrast to EG addition. This observation is not expected in ring-opening polymerization of L-LA with the presence of CoI. Myo-inositol does not indicate a significant effect as a co-initiator in bulk polymerization of L-lactide due to the poor solubility in the Bi(III)Ac and lactide melt. Also, the reaction rate is getting faster by increasing M/CoI and temperature. As in the case with EG, all reactions reached 100 % conversion, and also the use of EG as a co-initiator provides a significant increase in rate of L-lactide polymerization by Bi(III)Ac, however , the reactivity is opposite of that of EG system. One difference is that in the case of Myo-inositol, the curves are much closer to each other, the differences between the Mo/CoI ratios are not large.

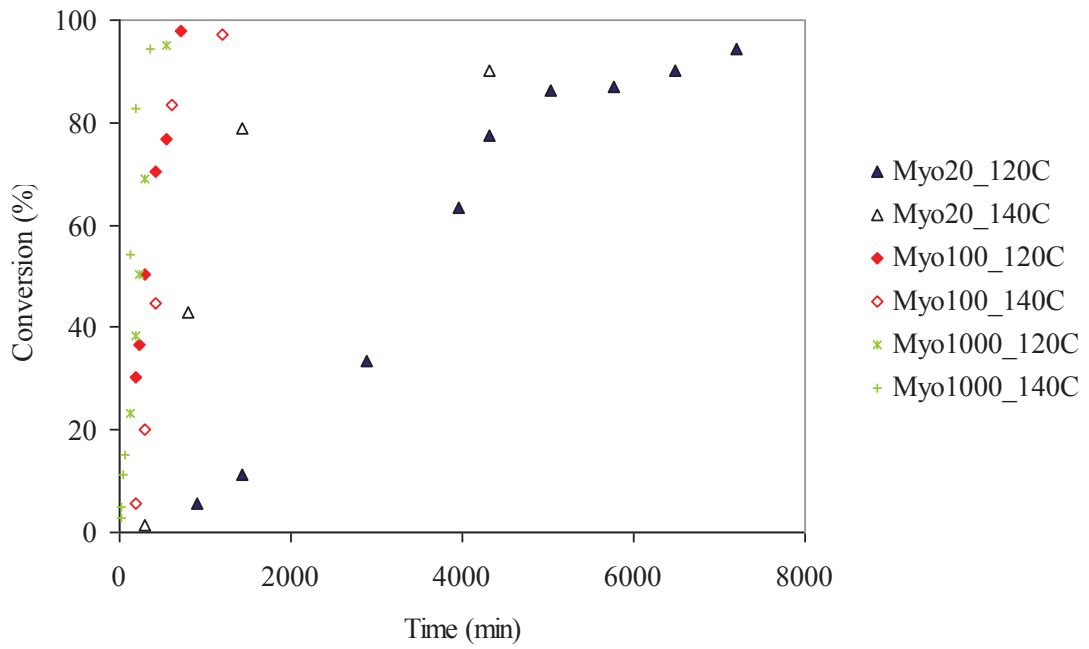


Figure 4.12. Time-conversion curves for bulk polymerization of lactide initiated by Bi(III)Ac with myo-inositol as a co-initiator.

The polymerizations results of L-lactide initiated by Bi(III)Ac and myo-inositol at 120 and 140 ^{o}C are given in Table 4.5 and Table 4.6, respectively. At constant M/I ratio, the decrease in M_n values were obtained with a decrease in M/CoI

ratios. The molecular weights calculated from ^{1}H NMR except in the case of M/I: 1000 were found as higher than theoretical M_n values. The higher M_n values indicated that all myo-inositol was not reacted as a co-initiator. Amorphous PLAs were obtained in the case of M/Myo: 20 at 120 ^oC. The molecular weights calculated from ^{1}H NMR were found as higher than M_{ntheo} values except in the case of M/Myo=1000. This indicates that the possibility of propagation rate is higher than chain transfer between active species and unreacted -OH groups. Another possibility is also reduction of accessibility of the unreacted -OH groups due to steric hindrance of the previously formed armed PLAs. As seen in the Table, the conversion values at M/I: 500 and 1000 are decreased from 94.3 to 77 % , respectively at the end of five and six days at constant M/CoI.

Molecular weights obtained at 140 ^oC are decreased with increasing temperature. Conversion values are increased with an increase in temperature. The molecular weights and melting temperatures of polymers initiated with Bi(III)Ac and myo-inositol at 140 ^oC were found to be higher than the polymers synthesized with enzymes at the same conditions by Numata et al.(2007).

Table 4.5.Effect of M/I and Mo/CoI ratios on bulk polymerization of L-lactide by Bi(III)Ac and myo-inositol at 120 ^oC

Mo/I	Mo/CoI	Conv NMR (%)	M_n (SEC) (Da)	PDI	M_n HNMR (Da)	T_g (^oC)	T_m (^oC)	Crys (%)
500	100	96.5	56200	1.42	32650	35.2	170.5	45.3
500	1000	95.9	88480	1.55	132960	38.3	176	54.2
500 5days	20	94.3	4300	1.59	3260	31.3		
1000 6days	20	77.5	3420	1.48	2880	28.9		

Table 4.6.Effect of M/I and Mo/CoI ratios on bulk polymerization of L-lactide by Bi(III)Ac and myo-inositol at 140 ^{o}C

Mo/I	Mo/CoI	Conv NMR (%)	M_n SEC (Da)	PDI	M_n ^{1}HNMR (Da)	T_g (^{o}C)	T_m (^{o}C)	Crys (%)
500	20	93.45	2880	1.08	3550	38.1	163.6/ 146.9	5.7
500	100	99.8	20000	1.09	23800	51.2	148.2/ 137.5	26.5
500	1000	97.97	63000	1.39	53420	45.5	171.9	52.4

4.1.2.1.3. Effect of Pentaerythritol as Co-Initator on synthesis of PLLA by using Bi(III)Ac

Figure 4.13 and Figure 4.14 indicate ^{1}H NMR and ^{13}C NMR spectra of PLLA synthesized by Bi(III)Ac and pentaerythritol in the case of M/I: 500, M/CoI:20 at 120 ^{o}C, respectively. As seen in the figures, -CH, -CH_3 in the repeating unit and the end groups were seen. CH_2-O-CO signal of the pentaerythritol was also seen in the ^{1}H NMR spectrum at 4.14 ppm and also ^{13}C NMR spectrum at 62.6 ppm.

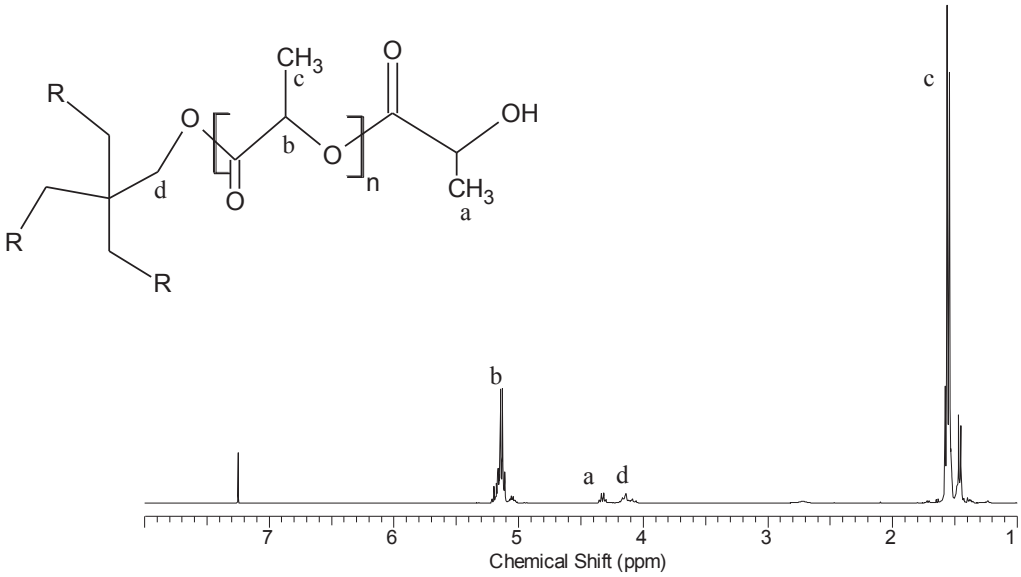


Figure 4.13. ^{1}H NMR spectrum of PLLA synthesized by Bi(III)Ac and pentaerythritol in the case of M/I: 500, M/CoI:20 at 120 ^{o}C.

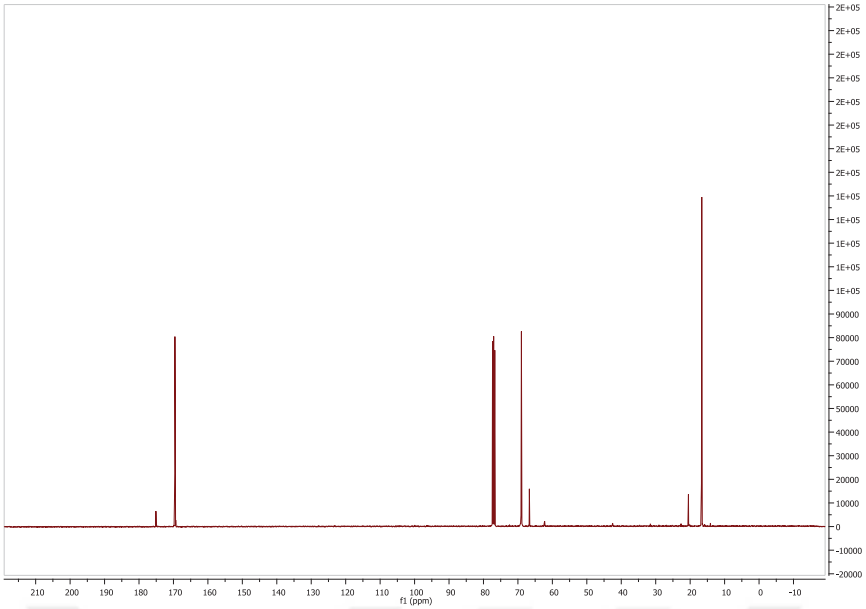


Figure 4.14. ^{13}C NMR spectrum of PLLA synthesized by Bi(III)Ac and pentaerythritol in the case of M/I: 500, M/CoI:20 at 120 ^{o}C.

Table 4.7 indicates the results of bulk polymerization L-lactide by Bi(III)Ac and pentaerythritol at 120 ^{o}C. As seen in the table, the decrease in molecular weight, the increase in glass transition and melting temperature were found with increase of M/CoI ratio. The observation of decrease in glass transition and melting temperatures with the increase of branching number due to the use of different type of co-initiators is in good aggrement with the literature (Wang and Dong 2006b)

Table 4.7. Effect of Mo/CoI ratio on bulk polymerization of L-lactide by Bi(III)Ac and pentaerythritol at 120 ^{o}C.

Mo/I	Mo/CoI	Conv NMR (%)	M_n SEC (Da)	PDI	M_n ^{1}HNMR (Da)	T_g (^{o}C)	T_m (^{o}C)	Crys (%)
500	20	90.6	4200	1.26	3950	33.7		
500	100	98.5	28400	1.32	33300	46.1	58.4/ 147.6	39
500	1000	96.5	62300	1.24	110450	57.3	174.7	43.8

Comparison of the effects of different co-initiators on the initiation of L-lactide with Bi(III)Ac at 120 and 140 °C are shown in Figure 4.15. Evaluation of biosafe initiatior with several co-initiators were investigated and compared with their reactivies. Although ethylene glycol and pentaerythritol lead to a significant increase in conversion values of L-lactide, the use of myo-inositol has a adverse effect that leads to the decrease in conversion values. Reaction time is much shorter to reach 100% conversion for ethylene glycol and pentaerythritol than that of myo-inositol. The lower conversion values in all cases were obtained in reduction of temperature. The highest co-initiator effect in polymerizations with Bi(III)Ac was found in the use of ethylene glycol as a co-initiator at 140 °C. As seen Tables 4.1-4.7, the higher molecular weights of the polymers consisting the same amount of co-initiator were obtained in the case of myo-inositol. The synthesis of high molecular weight PLA polymers having low PDI values by using Bi(III)Ac as a catalyst indicates the controlled ring opening polymerization of L-LA due to supressing effect of Bi(III)Ac on side reactions.

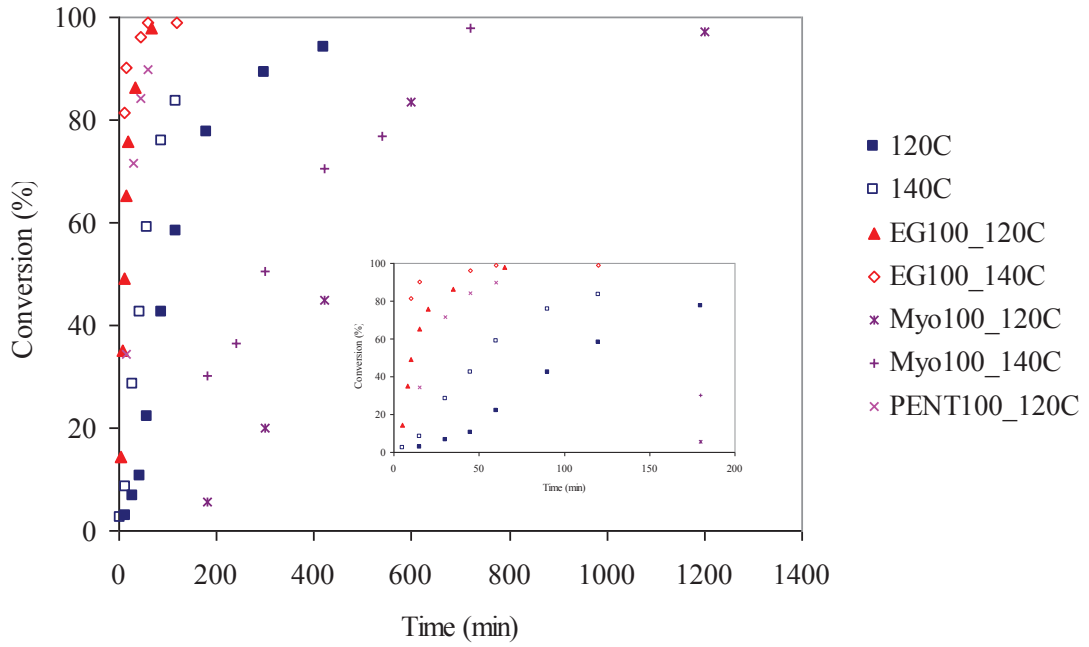


Figure 4.15. Conversion-time curves of Bi(III)Ac initiated L-lactide with and without different co-initiators at 120 and 140 °C.

4.1.2.2. Effect of Co-Initiator type on synthesis of PLLA by using creatinine catalyst

4.1.2.2.1. Effect of Ethylene Glycol as Co-Initiator on synthesis of PLLA by using creatinine

As another biosafe catalyst, creatinine was used for the polymerization of PLLA. The increase in the amount of creatinine catalyst used from M/I: 500 to:50 and increase in polymerization temperature from 120 to 140 ^{o}C were led to significant increase in the conversion values and the decrease in reaction time of bulk polymerization of lactide initiated by creatinine catalyst without co-initiator. In the case of M/I: 500 and 50 for lactide polymerization initiated with creatinine catalyst without co-initiator, the conversion values were found as 27.6 % at the end of fifteen days and 98.2,% at the end of seven days at 140 ^{o}C. At constant M/I:50 ratio, the conversion values increased from 81.4 to 98.2 % with increase in temperature from 120 to 140 ^{o}C at the end of seven days.

Effects of the ratios of monomer/initiator (M/I) and monomer/co-initiator (M/CoI) on the polymerizations of L-lactide initiated by creatinine and ethylene glycol were given in the Table 4.8. The molecular weight of the polymers decreases with the use of ethylene glycol. Significant differences in molecular weight and reaction time were observed as temperature and the amounts of co-initiator changes due to the effects of temperature and co-initiator on reaction rate. The decrease in molecular weight with the increase of CoI amount was observed due to the increasing number of living chains.

T_g, molecular weights and PDI values did not change with M/I ratio. However, significant change in the values of T_g and molecular weight were observed by the effect of M/CoI ratio. All T_g values for the condition of M/CoI:20 were obtained below the body temperature in the range of 22.4 and 44.4 ^{o}C. The melting peaks were not observed from the DSC analyses of these synthesized PLA polymers by using creatinine catalyst. These results indicate that creatinine catalyst leads to the formation of amorphous PLA polymers.

Although crystalline EG functional PLAs were synthesized by Bi(III)Ac catalyst, amorphous EG functional PLAs were obtained with creatinine catalyst. This difference in crystal structure of synthesized EG functional PLAs with two different

catalysts can be explained by the capability of Bi(III)Ac catalyst on stereo-controlled polymerization of LA. The molecular weights of EG functional PLAs synthesized by Bi(III)Ac catalyst were found as higher than that of the EG functional PLAs synthesized by creatinine catalyst. In the case of M/I: 200 and 120 ^{o}C, the molecular weights obtained from ^{1}H NMR of EG functional PLAs synthesized by creatinine were 1200, 3500 and 6800 Da for M/CoI: 20, 100 and 1000, respectively. In the same polymerization conditions (M/I: 200 and 120 ^{o}C), the molecular weights obtained from ^{H}NMR of EG functional PLAs synthesized by Bi(III)Ac were 1150, 6300 and 10240 Da for M/CoI: 20, 100 and 1000, respectively. The polymerization times for respective EG functional PLAs synthesized by creatinine catalyst were significantly different. Seven days are required to reach high conversion values when M/I: 200 and 120 ^{o}C. Also, the range of respective PDI values of these polymers synthesized by Bi(III)Ac and creatinine were found as 1.15 -1.33 and 1.76-1.82, respectively. The lower PDI values obtained from Bi(III)Ac catalyst indicate that Bi(III)Ac catalyst provides more controlled polymerization comparing to creatinine catalyst. For two types of the catalysts, the decrease in molecular weight and decrease in glass transition temperature were observed with the decrease in M/CoI due to the increase in living chains during polymerization.

Table 4.8. Effect of Mo/CoI ratios on bulk polymerization of L-lactide initiated by creatinine and ethylene glycol

Mo/I	Mo/ CoI	Rxn Temp (^{o}C)	Rxn time days	Conv NMR (%)	M_n SEC (Da)	PDI	M_n ^{1}HNMR (Da)	T_g (^{o}C)
200	20	120	7	98.2	3200	1.79	1200	22.4
200	100	120	7	97.1	4800	1.76	3500	38.7
200	1000	120	15	86.2	7200	1.82	6800	44.4
50	20	140	2	99	2500	1.23	1030	23.4
50	100	140	2	98.8	5350	1.87	3140	41.4
50	1000	140	2	97.2	8000	1.76	8250	43.3

4.1.2.2.2. Effect of Pentaerythritol as Co-Initiator on synthesis of PLLA by using Creatinine

The results of bulk polymerization of L-lactide by using creatinine and pentaerythritol were given in Table 4.9. As seen in the Table, the use of pentaerythritol as a co-inititor also provides the decrease in T_g and molecular weights of the polymers due to the formation of branch structure. The molecular weights obtained from ^{H}NMR of the functional PLAs with pentaerythritol synthesized at 140 ^{o}C were obtained as 3030, 12600 and 32800 Da for different M/CoI ratios 20,100 and 1000, respectively. The PDI values of these polymers were found between 1.44 and 1.52. The T_g values with increasing amount of pentaerythritol used were obtained ad 42.9, 36.8 to 33.6 ^{o}C for M/CoI: 1000, 100 and 20, respectively. The PDI values were obtained in the range of 1.44 and 1.67. The molecular weight of functional PLA with pentaerythritol (M/CoI:1000, M/I:50 at 140 C) were found as higher than that of the PLA synthesized by creatinine catalyst without co-initiator (M/I:50 at 140 C) and lower than the same respective PLA synthesized by Bi(III)Ac catalyst (M/CoI:1000, M/I:500 at 120 C). Although amorphous pentaerythritol functional PLAs were synthesized with creatinine catalyst, crystalline pentaerythritol functional PLAs were obtained with Bi(III)Ac catalyst. The decrease in molecular weights and glass transition temperatures of pentaerythritol functional PLAs were observed with decrease in M/I ratio for Bi(III)Ac and creatinine catalysts.

Table 4.9. Effect of Mo/CoI ratios on bulk polymerization of L-lactide initiated by creatinine and pentaerythritol at 140 ^{o}C for 2 days.

Mo/I	Mo/CoI	Conv NMR (%)	Mn SEC (Da)	PDI	M_n ^{1}H NMR (Da)	T_g (^{o}C)
50	20	90.4	2760	1.44	3030	33.6
50	100	89.4	7820	1.67	12600	36.8
50	1000	95.5	16100	1.52	32800	42.9

4.1.2.2.3. Effect of Myo-inositol as Co-Initiator on synthesis of PLLA by using Creatinine

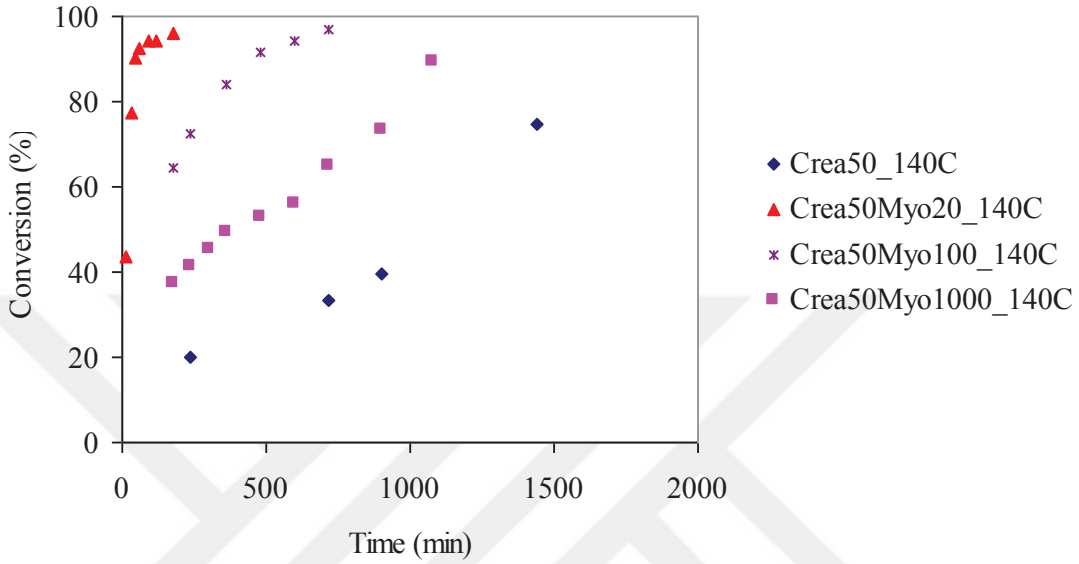
The effect of myo-inositol on the polymerization of L-lactide with creatinine was seen in Table 4.10. Highest PDI value was obtained in the case of M/CoI=100. The decrease in T_g and molecular weights of the polymers were observed by the increase in the amount of my-inositol as a co-initiator. As seen in the Table, lowest conversion value in the condition of M/CoI:1000 was found as 74% for functional PLA with myo-inositol at the end of two days polymerization reaction. The molecular weights obtained from ^{H}NMR of the functional PLAs with myo-inositol synthesized at 140 ^{o}C were obtained as 5000, 16400 and 22700 Da for different M/CoI ratios 20,100 and 1000, respectively. The acceptable PDI values were obtained in the range of 1.21 and 1.35. The increase in T_g values of functional PLAs with myo-inositol were obtained as 34.8, 39.9 and 42.4 ^{o}C for increase of M/CoI ratios:20, 100 and 1000, respectively. The T_g value below body temperature were also observed in the case of M/CoI:20 like the other our previous results of functional PLAs initiated with different co-initators as EG or PENT and different catalysts as Bi(III)Ac and creatinine..

Table 4.10. Effect of Mo/CoI ratios on bulk polymerization of L-lactide initiated by creatinine and myo-inositol at 140 ^{o}C for 2 days.

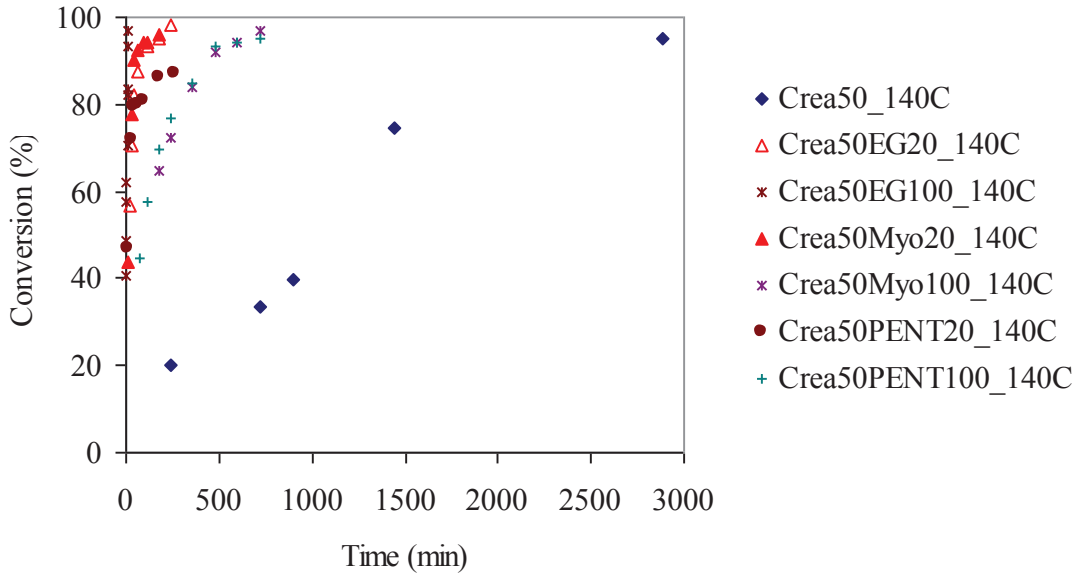
Mo/I	Mo/CoI	Rxn Temp (^{o}C)	Conv NMR (%)	M_n SEC (Da)	PDI	M_n ^{1}HNMR (Da)	Tg (^{o}C)
50	20	140	94.2	3200	1.35	5000	34.8
50	100	140	98	10050	1.43	16400	39.9
50	1000	140	79.4	27000	1.21	22700	42.4

Figure 4.16 shows the conversion-time curves of creatinine initiated L-lactide with and without different co-initiators at 140 ^{o}C. Figure 4.16.a shows the effects of different CoI ratios of myo-inositol on conversion of lactide polymerization initiated with creatinine catalyst. It is clearly seen that the decrease in M/I ratio of myo-inositol

leds to the increase in the conversion due to the increase of number of living chains during polymerization. As seen in Figure 4.16 a and Figure 4.16 b, different types of co-initiators were significantly decreased the polymerization reaction time required for high conversion values. The decrease in M/CoI leads to the increase in reaction rate of polymerization.



(a)



(b)

Figure 4.16. Conversion-time curves of creatinine initiated L-lactide with and without (a) myo-inositol (b) different co-initiators at 140 ^{o}C.

The changes in molecular weights, glass transition temperatures and PDI values indicate the presence of controlled polymerization of lactide by using different co-initiators and catalysts. The high molecular weight PLAs synthesized by Bi(III)Ac and creatinine catalysts are obtained at the high [M]/[I] ratios for all cases as seen in Tables 4.1-4.10. This can be explained by the presence of low initiator concentration at high [M]/[I] ratio. Low initiator concentration leads to the less initiation sites during polymerization. Less initiating sites are lead to the enhancement of growing chains resulting to the synthesis of high molecular weight PLAs. Also, the longer reaction time is required to reach high conversion at the high [M]/[I] ratios. On the contrary, the low molecular weight PLAs are obtained at the low [M]/[I] ratios due to the presence of more initiator sites. Furhermore, the shorter reaction time is required to reach high conversion at the low [M]/[I] ratios. The effects of initiator and catalyst on polymerization of PLA also investigated in polymerization kinetics part.

4.1.3. Polymerization Kinetics of PLLA

Different ranges of homo PLAs were synthesized by using different types of catalysts at the same polymerization conditions due to the difference in catalyst activity of Bi(III)Ac or creatinine. The kinetic factors affecting the ring-opening polymerization of L-LA by using Bi(III)Ac or creatinine were investigated. Figure 4.17 shows semilogarithmic plots of $\ln([LA]_o/[LA]_t)$ versus time for $SnOct_2$, Bi(III)Ac and creatinine catalysts. The linear plots of $\ln([LA]_o/[LA]_t)$ versus time indicated that the polymerization rates are first order dependency on lactide monomer by using different types and amount of initiators. The apparent constant rates of polymerization were determined from the slope of the plot of $\ln([LA]_o/[LA]_t)$ versus time. The apparent rate constants (k_{app}) for LA polymerization initiated by $SnOct_2$ (M/C:10000), Bi(III)Ac (M/C: 500) at 120 ^oC and creatinine (M/C:50) at 140 ^oC were calculated as $2x10^{-4}$, $7.8x10^{-3}$ and $9x10^{-4}$ min^{-1}, respectively. The linearity of the plot also indicates constant concentration of active centers throughout the polymerization (Hao et al. 2012)

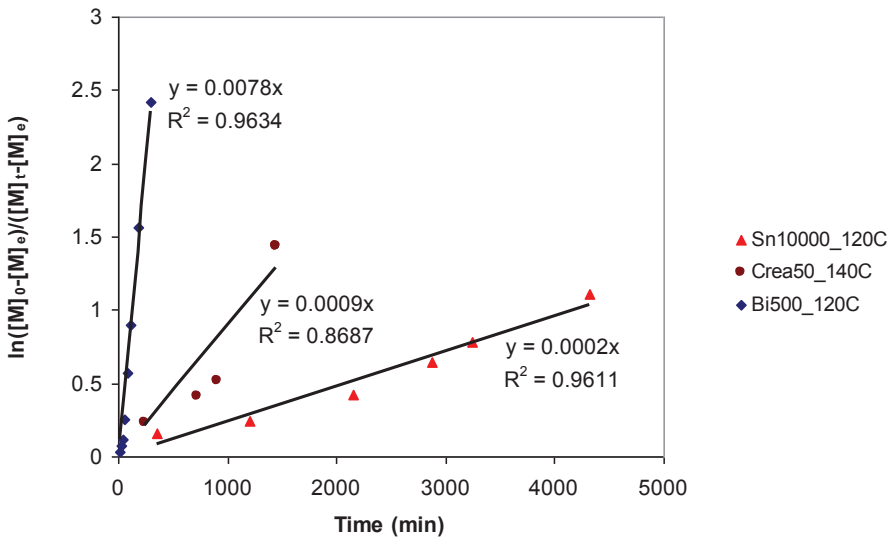


Figure 4.17. Semilogarithmic plots of ln ($[LA]_o/[LA]_t$) versus time for PLLA synthesis initated by $SnOct_2$, Bi(III)Ac and creatinine catalysts.

The k_{app} values for PLLA synthesis initiated by Bi(III)Ac and ethylene glycol were shown in Figure 4.18. As seen in the figure, the k_{app} values are decreased with increase of EG co-initiator amount and decrease in reaction temperature. The k_{app} values for the polymerization of L-LA by using Bi(III)Ac catalyst was tabulated in Table 4.11. As shown in the figure and table, the k_{app} values directly depend on reaction temperature, type and concentrations of initiator and co-initiator. The k_{app} values for PLLA polymerization reaction inititated by Bi(III)Ac at 120 and 140 ^oC were found as 0.0078 and 0.0136 min^{-1}, respectively. The k_{app} values for PLLA polymerization reaction initiated by creatinine catalyst are shown in Figure 4.19 and Table 4.12. The higher k_{app} values in the polymerizations initiated with Bi(III)Ac were defined comparing to the polymerizations initiated with creatinine. These high k_{app} values indicated that the polymerizations initiated with Bi(III) Ac catalyst are faster than the polymerizations initiated with creatinine. The high conversion values in the polymerization of L-LA were achieved by using Bi(III)Ac instead of creatinine as a catalyst at the reasonable polymerization time due to the high rate constants. By comparing the k_{app} values, it can be concluded that the catalytic activity of Bi(III)Ac is higher than that of creatinine during polymerization of LA. Also, the increase of kapp values were observed with the increase of co-initiator amount such as EG and PENT. This increase in k_{app} values can be explained by the effect of co-initiator as active centers (Chen et al. 2013). The increase of CoI amount leads to the improvement in the

ring opening polymerization of L-LA by the effect of increasing the concentration of active centers. This increase in k_{app} values with CoI amount also confirms the suggested coordination-insertion mechanism for the ring-opening polymerization of L-LA. The increase in reaction rate with the increase of CoI amount indicates the controlling effect of CoI on polymerization. The increase in reaction rates with the presence of co-initiators shows that co-initiators lead to the enhancement of polymerization by lowering the activation energy required for ring-opening polymerization of L-LA. This relationship between CoI and reaction rate is in good aggrement with the literature findings (Chen et al. 2013, Ikpo et al. 2012, Yu et al. 2009).

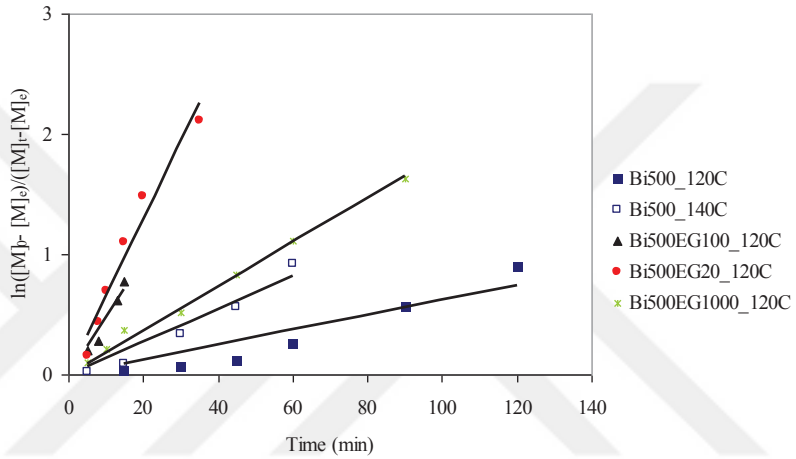


Figure 4.18. Semilogarithmic plots of $\ln([LA]_o/[LA]_t)$ versus time for PLLA synthesis initiated by Bi(III)Ac and ethylene glycol.

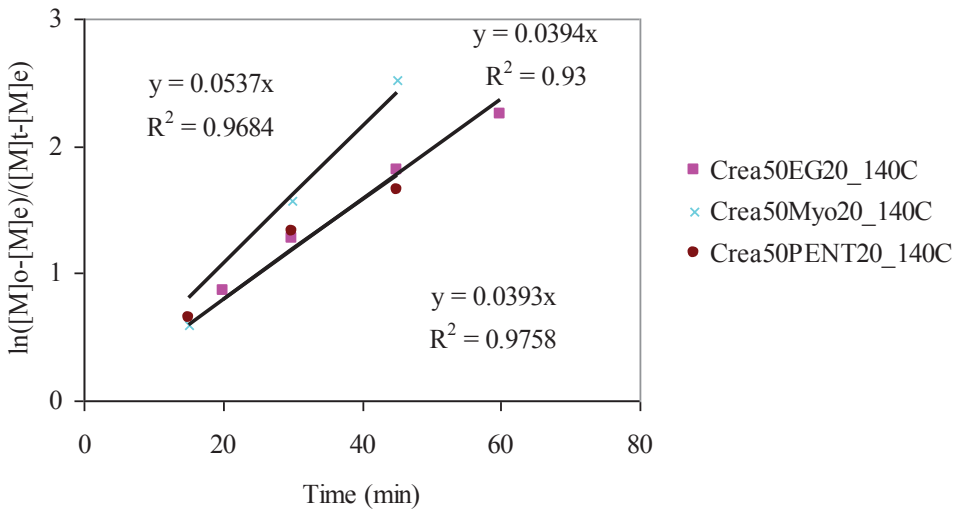


Figure 4.19. Semilogarithmic plots of $\ln([LA]_o/[LA]_t)$ versus time for PLLA synthesis initated by creatinine and co-initiators.

Table 4.11. Apparent rate constants (k_{app}) for for PLLA synthesis initiated by Bi(III)Ac catalyst.

Coinitiator type	Reaction Temp (°C)	M/CoI	k_{app} (min^{-1})
-	140	-	0.0136
EG		20	0.3898
EG		100	0.1706
EG		1000	0.0664
-	120	-	0.0078
EG		20	0.0735
EG		100	0.0645
EG		1000	0.0183
PENT		20	0.0436
PENT		100	0.0395
PENT		1000	0.0209
Myo		20	0.0053
Myo		100	0.0027
Myo		1000	0.0003

Table 4.12 Apparent rate constants for PLLA synthesis initiated by creatinine catalyst at 140 °C

Coinitiator type	M/CoI	k_{app} (min^{-1})
-	-	0.0009
EG	20	0.0393
EG	100	0.0041
EG	1000	0.001
PENT	20	0.0394
PENT	100	0.0061
PENT	1000	0.0014
Myo	20	0.0537
Myo	100	0.0055
Myo	1000	0.0016

4.2. Synthesis of OH functional PCL polymers by using creatinine as catalyst

OH functional polycaprolactone (PCL) were synthesized by using ethylene glycol, pentaerythritol, dipentaerythritol and myo-inositol as initiators at different molar ratios of ε- caprolactone to initiator. The molar ratio of ε-caprolactone and initiators (M/I=20, 100 and 1000) was varied to obtain ε-caprolactone prepolymers with different

molecular weights. Also, it was found that the structure of PCL polymers was dependent on the initiator type as shown in Figure 4.20. Linear PCL polymers were obtained by using ethylene glycol as initiator. Pentaerythritol was used for synthesis of tetra functional PCL polymer. Hexa functional PCL polymers were obtained by the use of dipentaerythritol and myo-inositol.

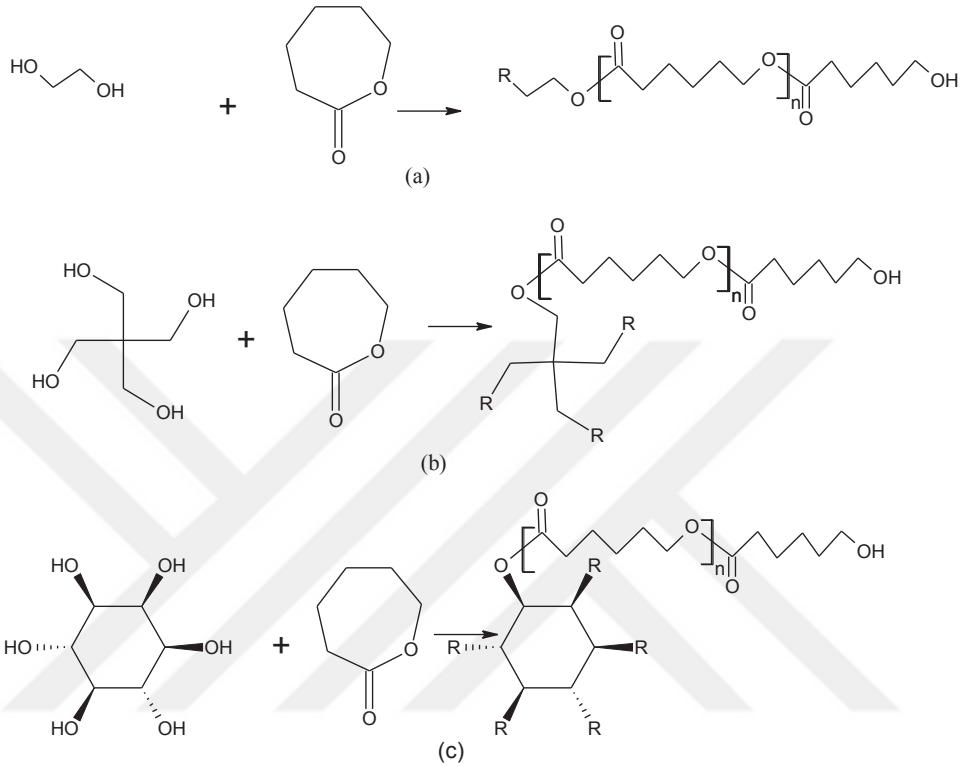
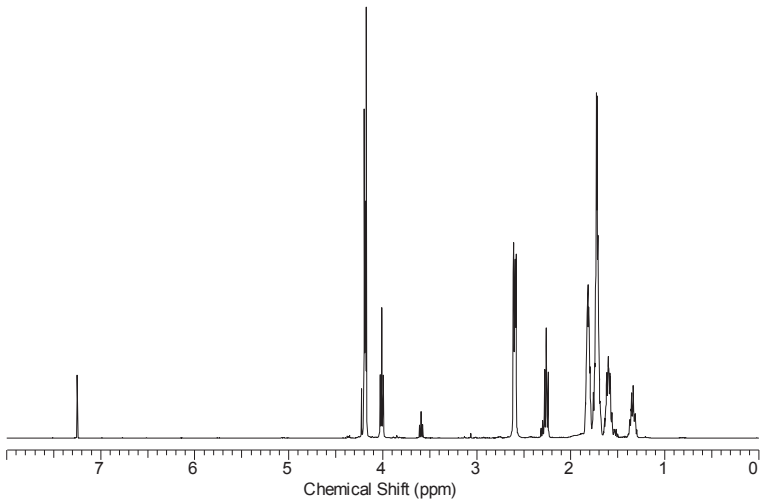
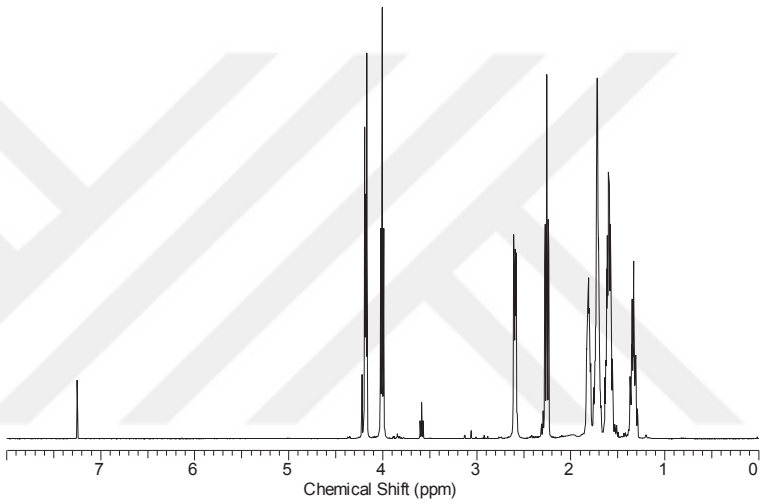


Figure 4.20.Ring opening polymerizations of ε-caprolactone by using (a) ethylene glycol, (b) pentaerythritol and (c) myo-inositol as initiators (R denotes PCL)

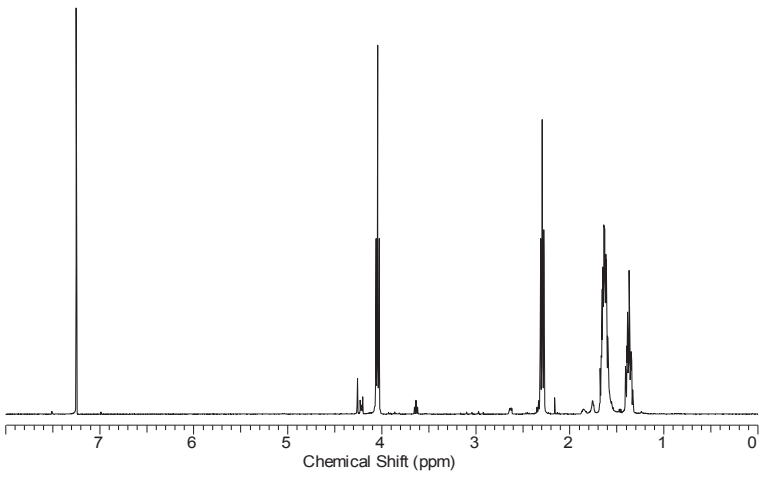
Samples were withdrawn from the reaction mixture and analyzed by ^{1}H NMR to determine the mechanism and conversion of reaction mixture. Figures 4.21- 4.23 show the ^{1}H NMR spectra of crude reaction mixture during polymerization of linear, four arm and six arm PCL prepolymers for M/I=100, respectively. As shown in the Figures, the peak intensity at 4.18 ppm of oxymethylene proton (t, 2H,-CH_2-O-) of CL monomer especially decreased with increase in polymerization time like the other CL monomer peaks observed at 2.61 ppm (t, 2H,-CH_2-CH_2-COO-) and 1.81 ppm (m, 4H,-CH_2-CH_2-COO-). On the other hand, the peak intensity at 4.01 ppm of oxymethylene proton (t, 2H,-CH_2 -O -) of PCL prepolymer increased with increasing polymerization time like the other PCL peaks observed at 2.27 ppm (t, 2H ,-CH_2-CH_2-COO-), 1.61 ppm (m, 4H, -CH_2-CH_2-COO-) and 1.34 ppm (m, 2H, -CH_2-CH_2-CH_2-CH_2-CH_2). The peak at 3.65 ppm denotes the end group of PCL prepolymer (t, 2H,-CH_2-OH).



(a)

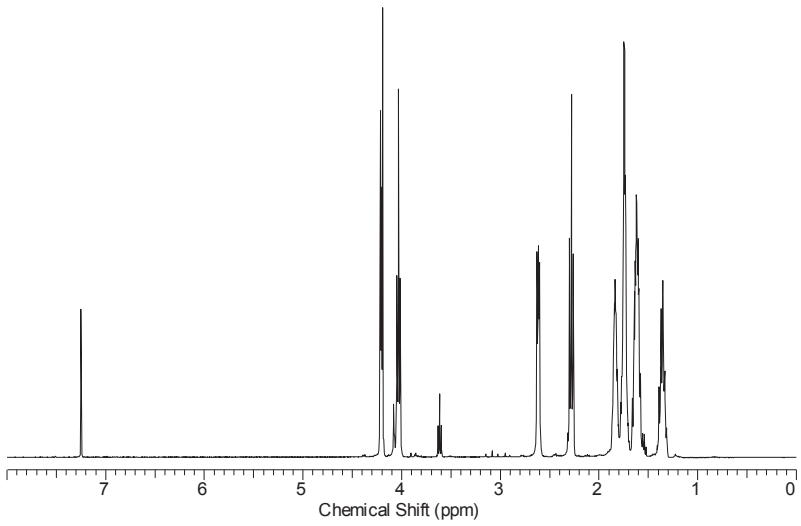


(b)

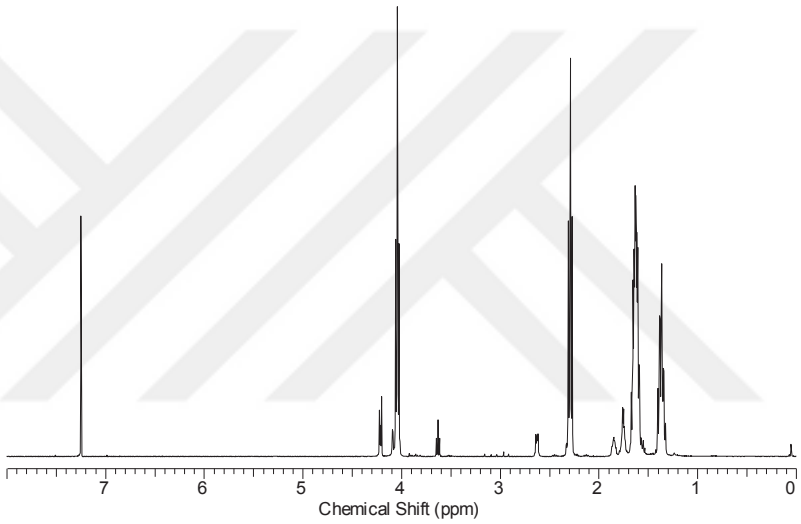


(c)

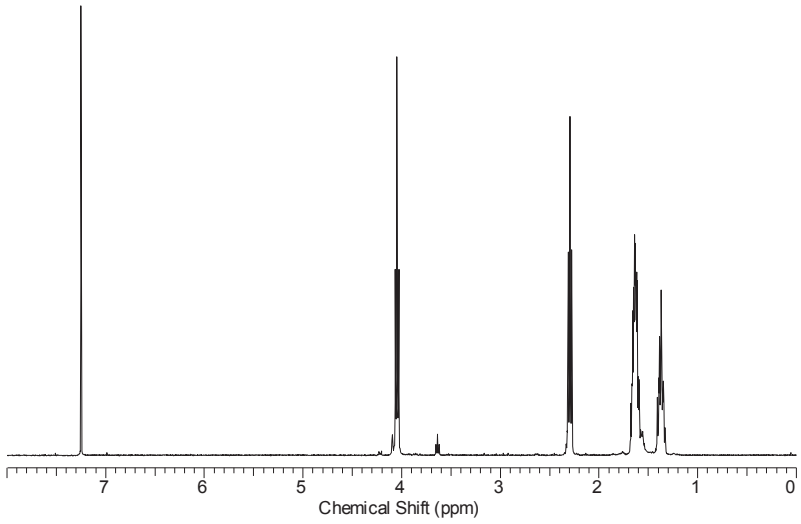
Figure 4.21. ^{1}H NMR spectra of crude reaction mixture during polymerization of diblock PCL prepolymers by using ethylene glycol as initiator (M/I=100) (a) 3 days, (b) 6 days and (c) 10 days.



(a)

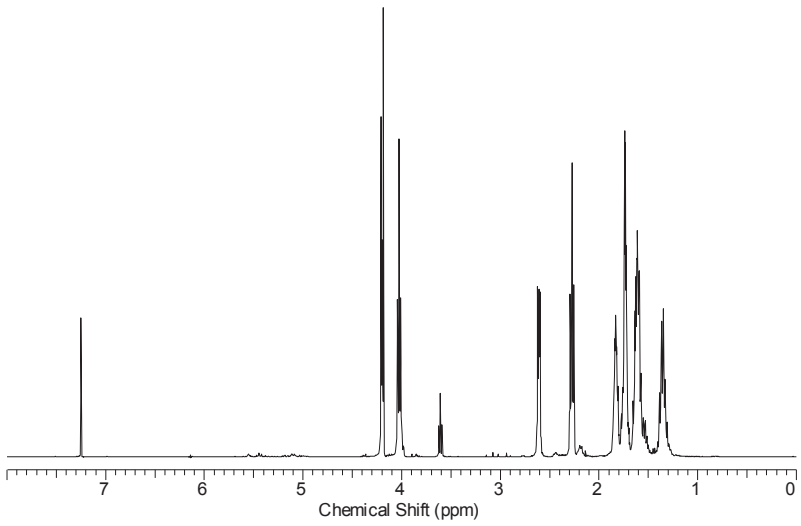


(b)

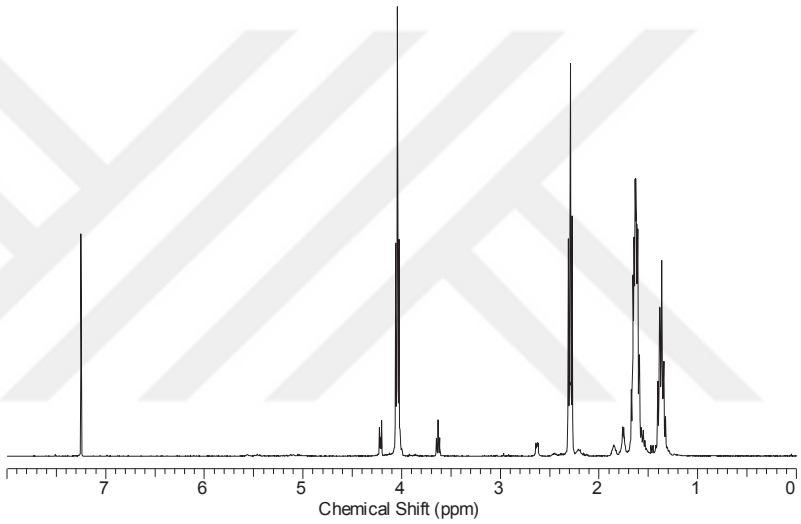


(c)

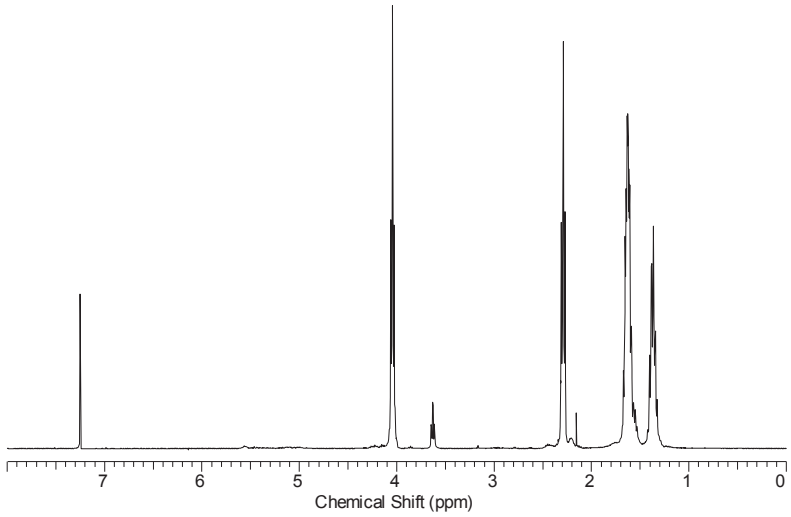
Figure 4.22. ^{1}H NMR spectra of crude reaction mixture during polymerization of tetrablock PCL prepolymers by using pentaerythritol as initiator (M/I=100) (a) 3 days, (b) 6 days and (c) 8 days.



(a)



(b)



(c)

Figure 4.23. ^{1}H NMR spectra of crude reaction mixture during polymerization of hexablock PCL prepolymers by using myo-inositol as initiator (M/I=100) (a) 3 days, (b) 6 days and (c) 8 days.

The polymerization of ε-caprolactone was monitored by ^{1}H-NMR following the resonance signal of oxymethylene protons (-O-CH_2-) of CL monomer at 4.17 ppm and PCL prepolymer at 4.01 ppm by calculating the conversions. Figures 4.24- 4.26 indicate the effects of ethylene glycol, pentaerythritol and myo-inositol as initiators on the conversions of PCL polymerization, respectively. As seen in the Figures, decrease in conversion values were obtained with the increase in the M/I ratio for all type of initiators. The use of initiator led to the decrease in reaction time due to the increase in the reaction rate of PCL polymerization due to the increase in number of living chains.

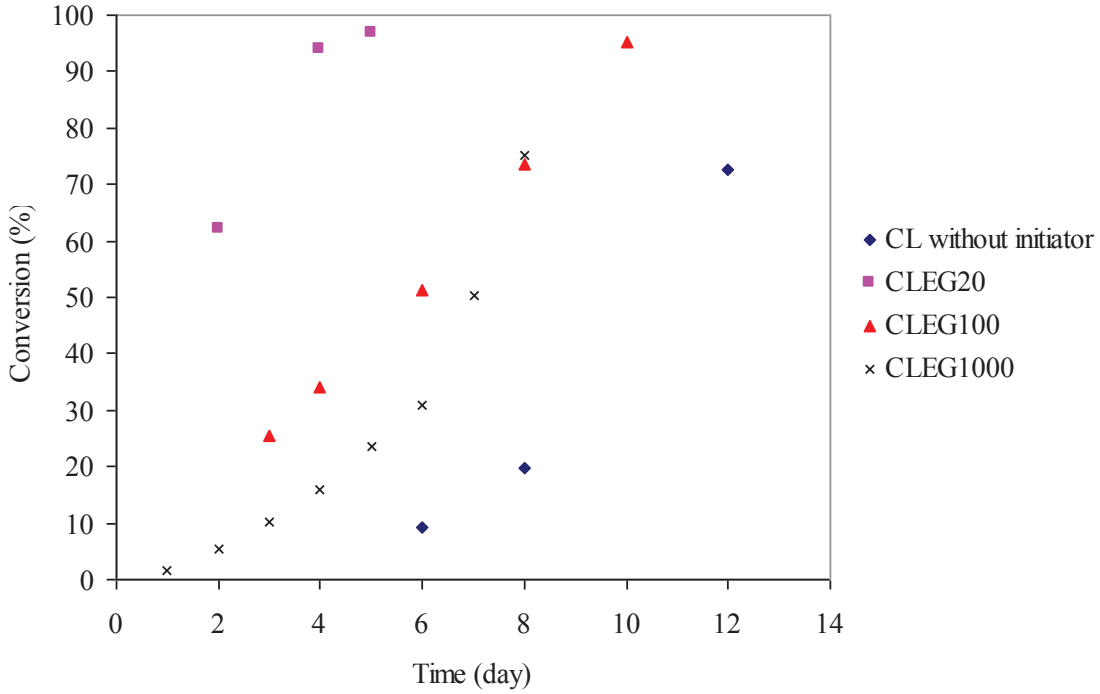


Figure 4.24. The effect of ethylene glycol on conversion of di hydroxy functional PCL

Figure 4.27 shows the comparison of the co-initiators on the effects of PCL polymerization for M/CoI:20. Ethylene glycol, pentaerythritol and myo-inositol lead to significant increase in conversion values of ε-caprolactone polymerization. The OH groups found in the structure of the co-initiators react with the monomer unit. The number of OH groups in ethylene glycol, pentaerythritol and myo-inositol is two, four and six, respectively. The highest conversion values of ε-caprolactone monomer were reached fastly in the case of myo-inositol having the highest number of hydroxly(-OH) group comparing to the other co-initiators. This result verified the relation between the number of active chains and the number of OH groups during polymerization.

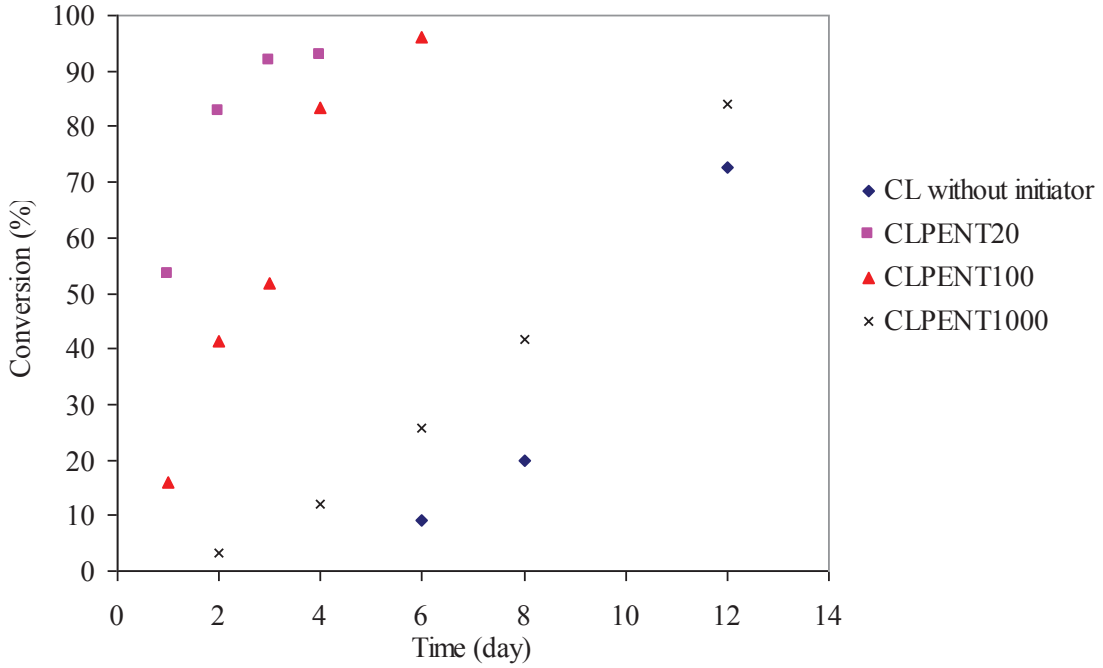


Figure 4.25. The effect of pentaerythritol on conversion of tetra hydroxy functional PCL

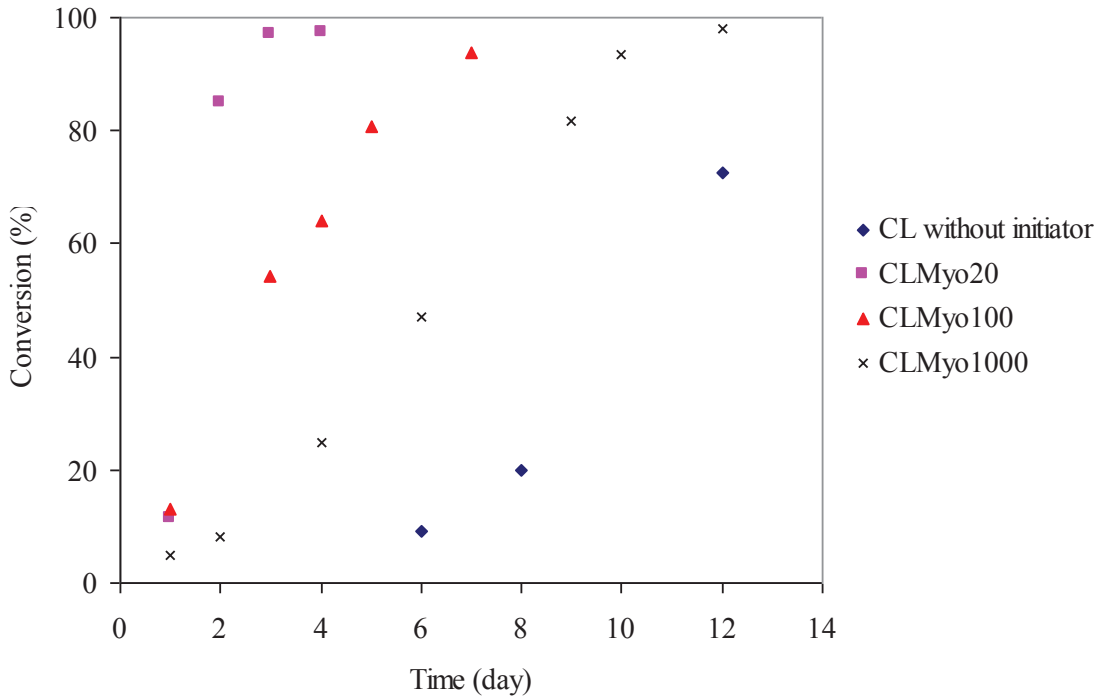


Figure 4.26. The effect of myo-inositol on conversion of hexa hydroxy functional PCL

Figures 4.28- 4.30 indicate the ^{1}H NMR spectra of di, tetra, and hexa hydroxy functional PCL synthesized from ethylene glycol, pentaaerythritol and myo-inositol in the conditions of M/I=20 for 4 days at 140 ^{o}C. The major peaks of PCL at 4.01, 2.27,

1.61, 1.34 and 3.65 ppm were observed in each case. Methylene protons of ethylene glycol and pentaerythritol were determined at 4.22 and 4.06 ppm, respectively. However, the methine proton of inositol was not recognized in ^{1}H NMR.

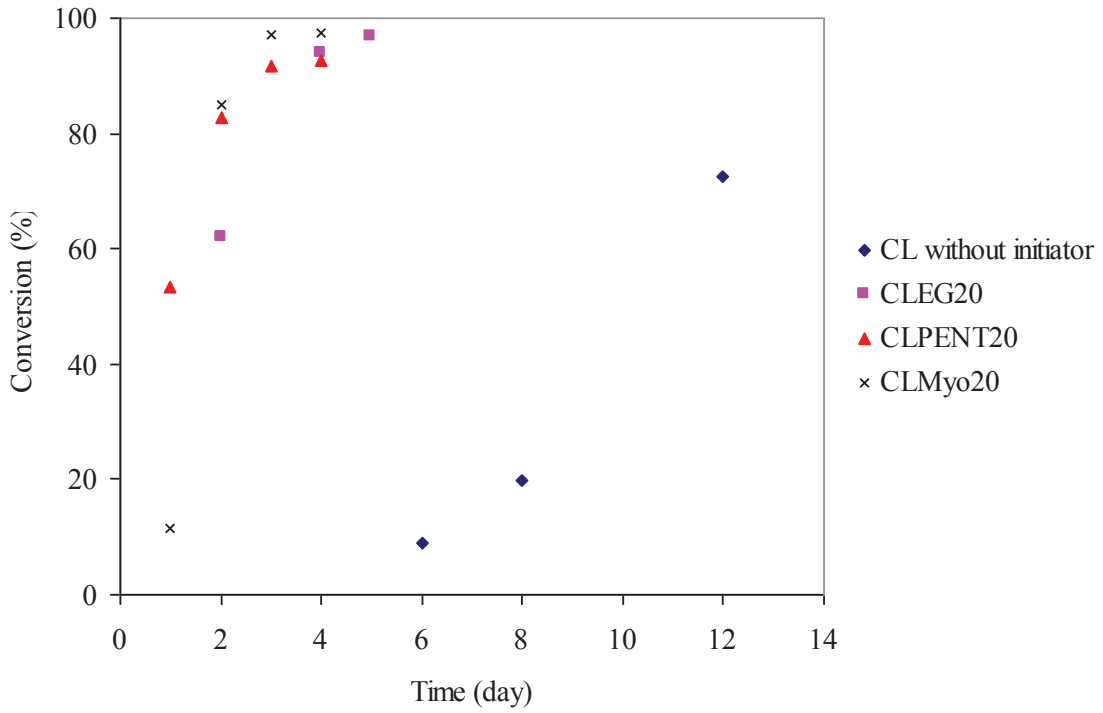


Figure 4.27. Comparison of co-initiators in PCL polymerization.

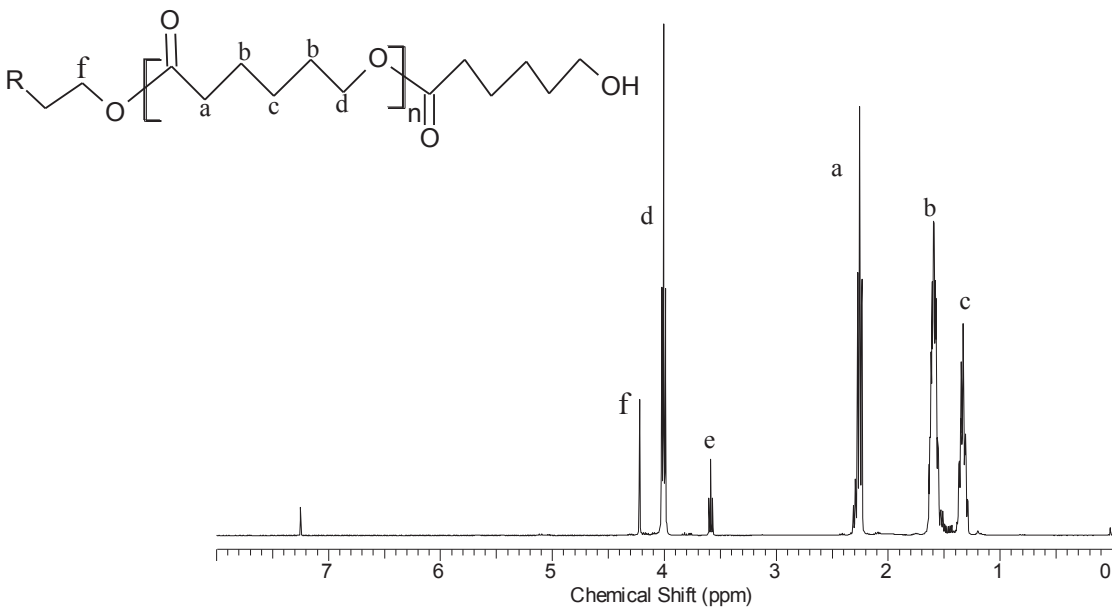


Figure 4.28. ^{1}H NMR spectrum of di hydroxy functional PCL synthesized from ethylene glycol for M/I=20

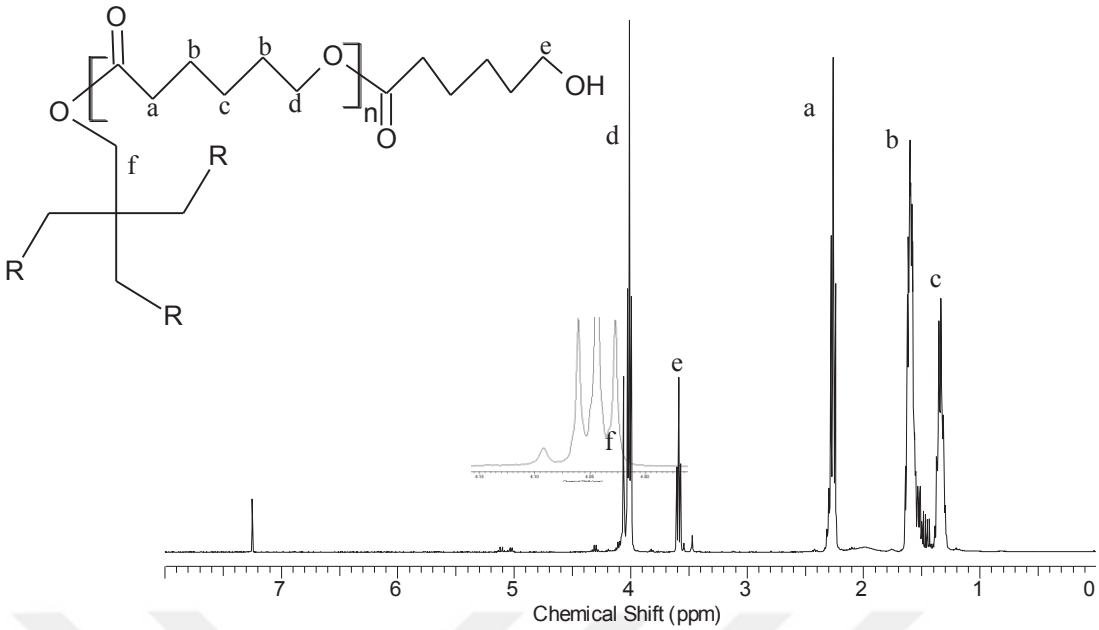


Figure 4.29. ^{1}H NMR spectrum of tetra hydroxy functional PCL synthesized from pentaerythritol for M/I=20.

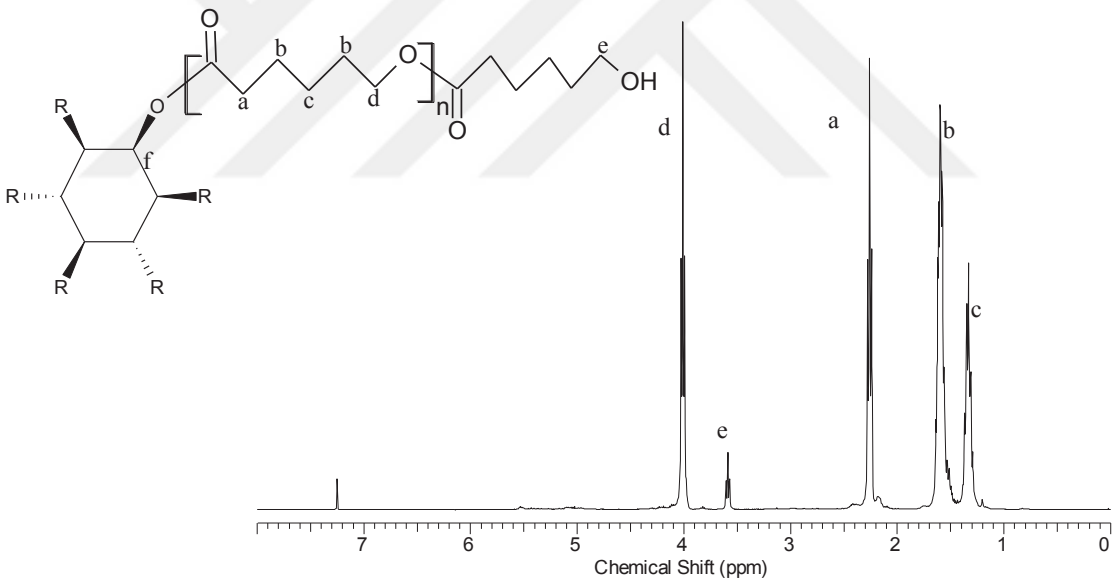


Figure 4.30. ^{1}H NMR spectrum of hexa hydroxy functional PCL synthesized from myo-inositol for M/I=20.

^{13}C NMR spectra of di, tetra and hexa hydroxy functional PCL were also presented in Figures 4.31, 4.32 and 4.33, respectively. As shown in these figures, the major carbonyl peaks of PCL were observed at 173.5, 64.04, 33.9, 25.42, and 24.45 ppm. The methine carbon of myo-inositol and methylene carbon of ethylene glycol and pentaerythritol were detected also ^{13}C NMR around 62.4 ppm.

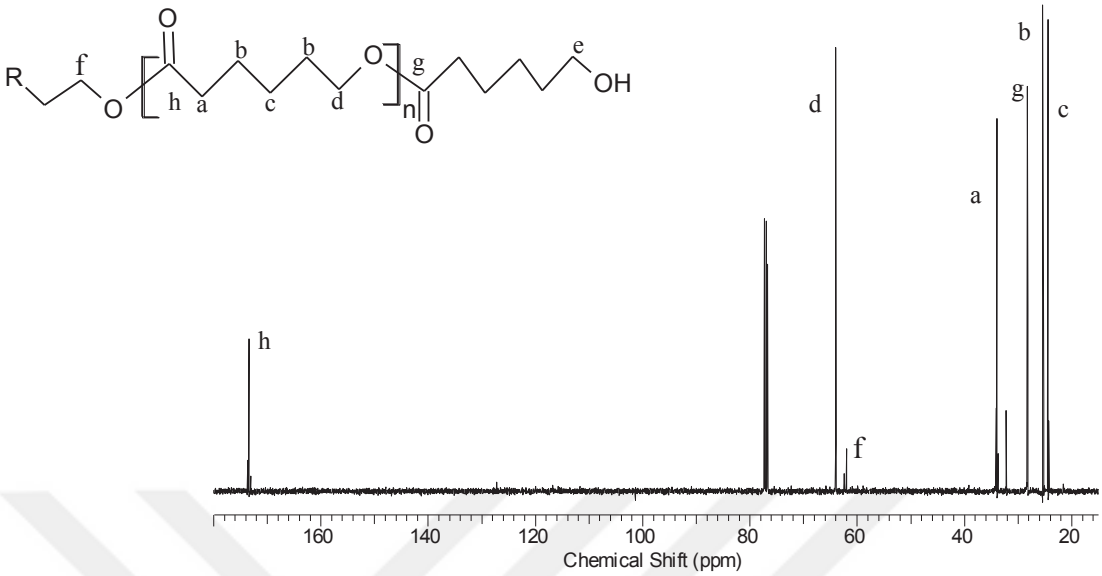


Figure 4.31. ^{13}C-NMR spectrum of di hydroxy functional PCL for M/I=20.

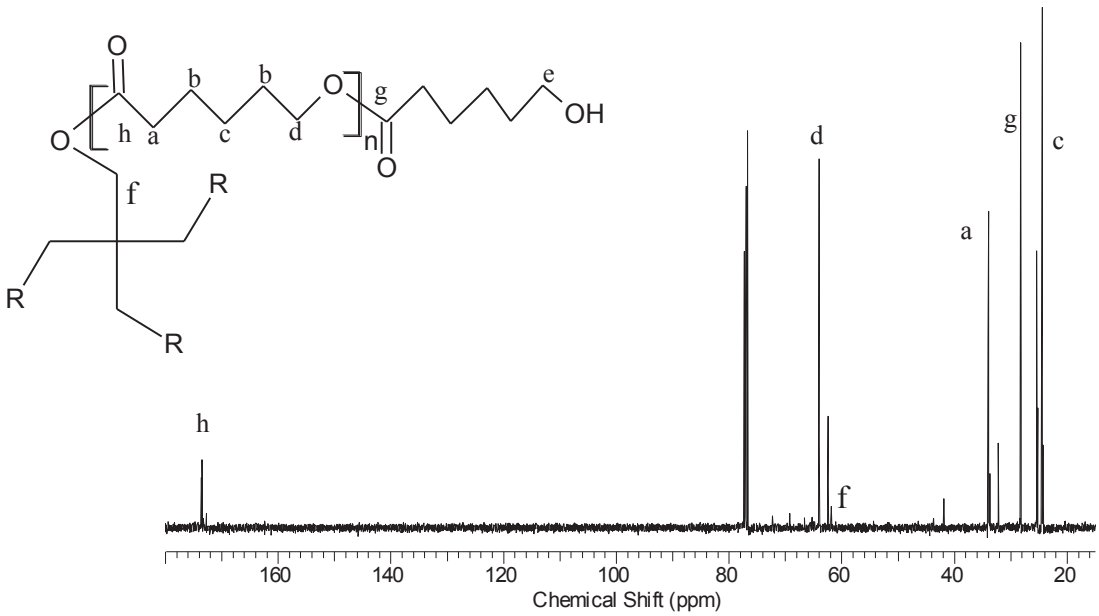


Figure 4.32. ^{13}C NMR spectrum of tetra hydroxy functional PCL for M/I=20.

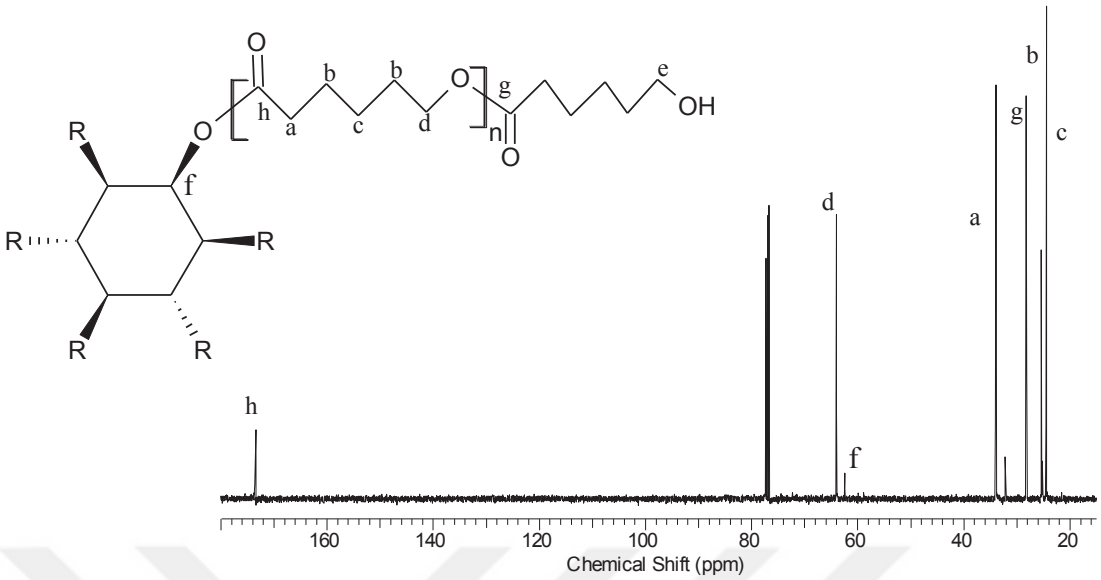


Figure 4.33. ^{13}C NMR spectrum of hexa hydroxy functional PCL for M/I=20.

Polymerizations of ε-caprolactone with initiators are summarized in Table 4.13. The conversion values were obtained by considering the resonance signals of oxymethylene protons (-O-CH_2-) of CL monomer at 4.17 ppm and PCL prepolymer at 4.01 ppm from ^1H NMR. The conversion values of ε-caprolactone monomer were determined between 73 and 99 %. The number average molecular weights of PCL-OHs and degrees of polymerization were calculated via ^1H NMR from the intensity ratios of the oxymethylene proton (-O-CH_2-) signals at 4.01 ppm in a repeating unit and the methylene proton (–CH_2-0H) signals at 3.64 in the end group of PCL prepolymer. The peak of the protons of the terminal methylene at 3.64 ppm indicates the termination of PCL with hydroxyl groups. The similar M_n values of PCL polymers were obtained from ^1H NMR and SEC . The M_n values were obtained between 2300 and 21400 Da. The decrease in M_n values were observed with the increase in the co-initator amount. The PDI values were determined between 1.2 and 2.37. The glass transition temperatures were found between -72 and -76 ^oC. Crystalline PCL polymers by using creatinine catalyst were sythesized in contrary to amourphous PLA polymers synthesized by creatinine catalyst. The melting temperatures were determined between 16.4 and 53 ^oC. Crystallinity values were obtained between 34 and 54.5 %. The decrease in melting temperature, molecular weight and crystallinity values were observed with the increase in co-initiator amount.

Table 4.13 Properties of ε-caprolactone polymers synthesized with co-initiators and creatinine catalyst.

CoI type	Mo/ CoI	Conv NMR (%)	M_n ^{1}HNMR (Da)	M_n SEC (Da)	PDI	T_g (^oC)	T_m (^oC)	Crys (%)
EG	20	99	2300	2550	1.16	-73.8	46.2	44.4
EG	100	95.2	5080	7800	1.44	-73.5	52.7	52
EG	1000	72.7	8270	5350	1.9	-73.6	53	54.5
PENT	20	92.7	2570	3150	1.32	-72.6	35.5/ 42.2	36.1
PENT	100	96.2	13360	8900	1.3	-72	46.6/ 50.4	44
PENT	1000	84	14000	13400	2.1	-74.2	52.7	46
Myo	20	97.5	7020	5240	1.4	-76.3	16.4/ 25.4	34
Myo	100	93.9	10098	11250	1.93	-73	43.4	39.4
Myo	1000	98	21400	16250	2.37	-74	53	47.5

Figure 4.34 indicates the FTIR spectra of homo, di, tetra and hexa block PCL polymers. Main absorption bands of the synthesized PCL polymers were defined as 2944 (ν_{as} CH_2), 2866 (ν_s CH_2), 1721 (νC=O), 1239 (ν_{as} COC) and 1171 (ν_s COC). As seen in the figure, FTIR spectrums of homo and hydroxyl functional PCL polymers are the same. Any peak in FTIR spectra of homo and hydroxyl functional PCL was recognized related to the presence of ethylene glycol, pentaerythritol and myo-inositol as initiators.

Samples were withdrawn from the reaction mixture and analyzed by Size Exclusion Chromatography (SEC) for determination of the change in number average molecular weight (M_n) and molecular weight distribution (M_w/M_n). Figure 4.35 indicates the M_n determined by SEC as a function of monomer conversion during CL polymerization by using ethylene glycol, pentaerythritol and myo-inositol as co-initiators (M/I=20). In the case of M/I=20, linear relationship between M_n and conversion were observed for CL polymerization with different initiators. Linear relationship is related to the low frequency of transesterification reactions. Linearity also indicates that the propagation and termination reactions occur in a controlled manner. Highest M_n values were obtained for the hexa hydroxyl functional PCL polymers initiated by myo-inositol.

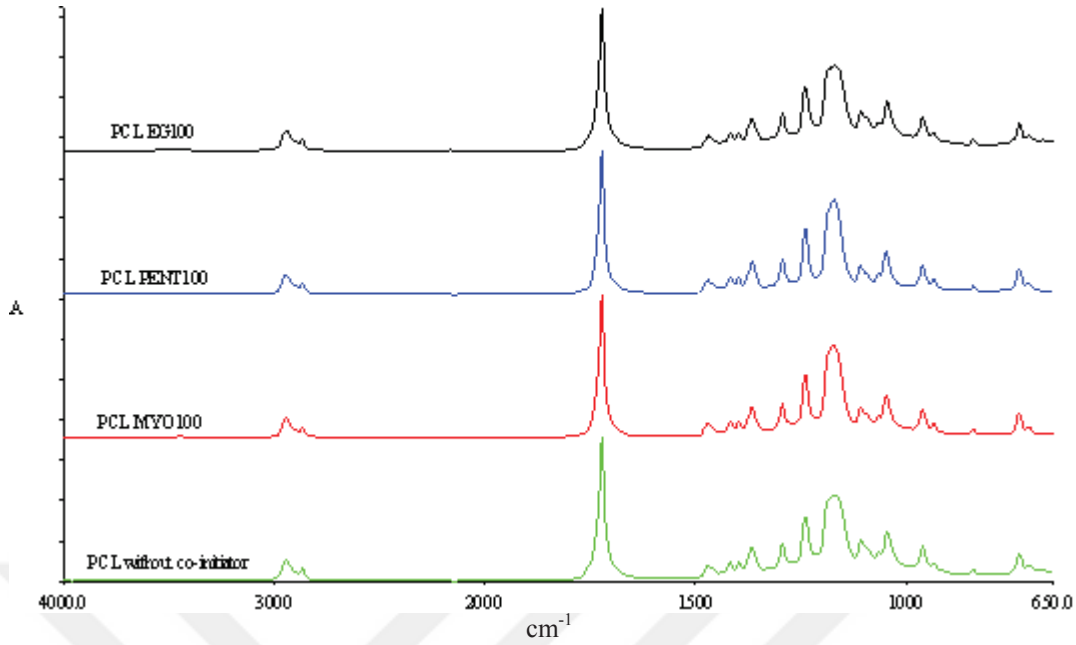


Figure 4.34 FTIR spectra of homo and di, tetra and hexa block PCL prepolymers synthesized by using creatinine.

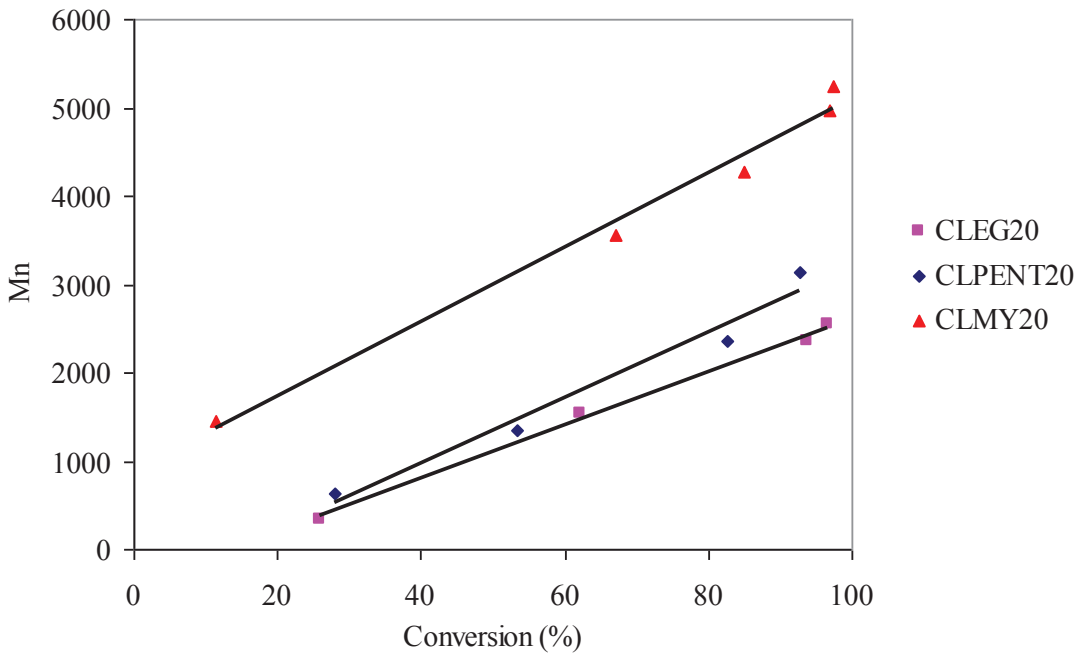


Figure 4.35. The influence of monomer conversion on number average molecular weight (M_n) determined by SEC for M/I=20.

Figure 4.36 shows the influence of monomer conversion on polydispersity (PDI) during CL polymerization with different initiators. As seen in the plots of PDI versus conversion, the functional PCL polymers indicate narrow molecular weight distribution

except PCL initiated by myo-inositol after conversion of 98%. This can be explained by the presence of backbiting reactions after conversion of 98 % in the polymerization of hexa hydroxyl functional PCL.

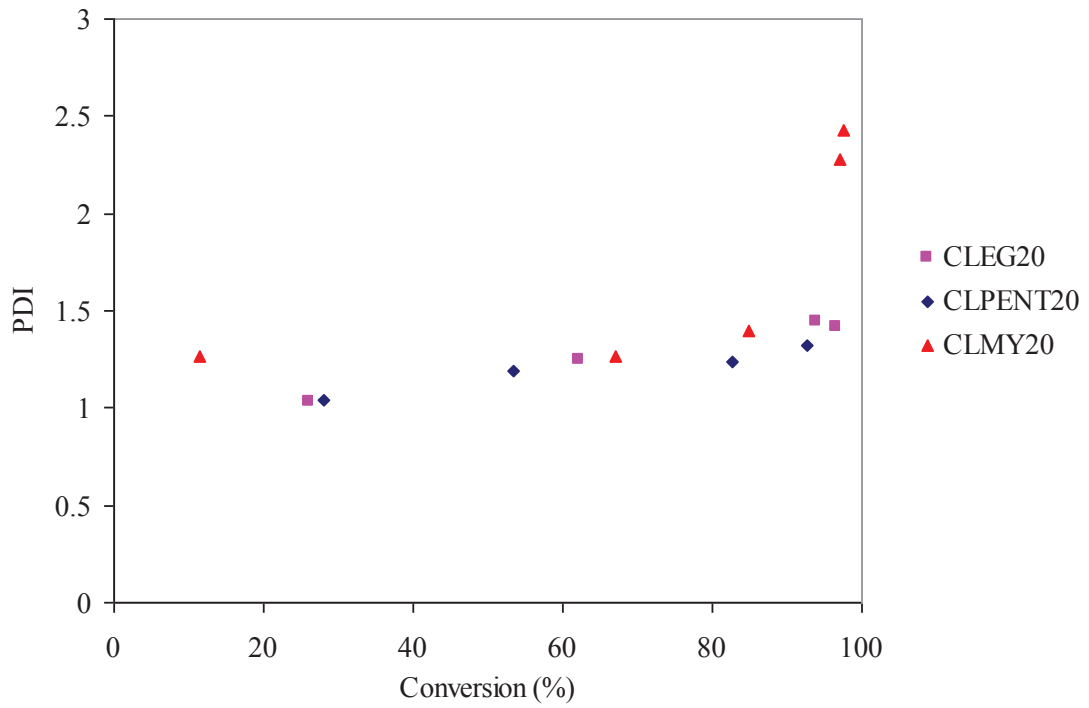


Figure 4.36. The influence of monomer conversion on PDI for M/I=20.

Figure 4.37 and Figure 4.38 show the influence of monomer conversion on number average molecular weight (M_n) and PDI determined by SEC at the conditions of M/I=1000, respectively. As seen in the figure, linear relationship between M_n and conversion was also obtained in the functional PCL polymerization by using initiators at the rate of M/I=1000. In the case of M/I=1000, the polymerization takes approximately 15 days at 140 ^{o}C. In the case of M/I=1000, oxygen can be transferred to the reaction flask during the withdrawal of the sample from the reaction mixture during the long polymerization time at high polymerization temperature. This led to decrease in molecular weight and increase in PDI value.

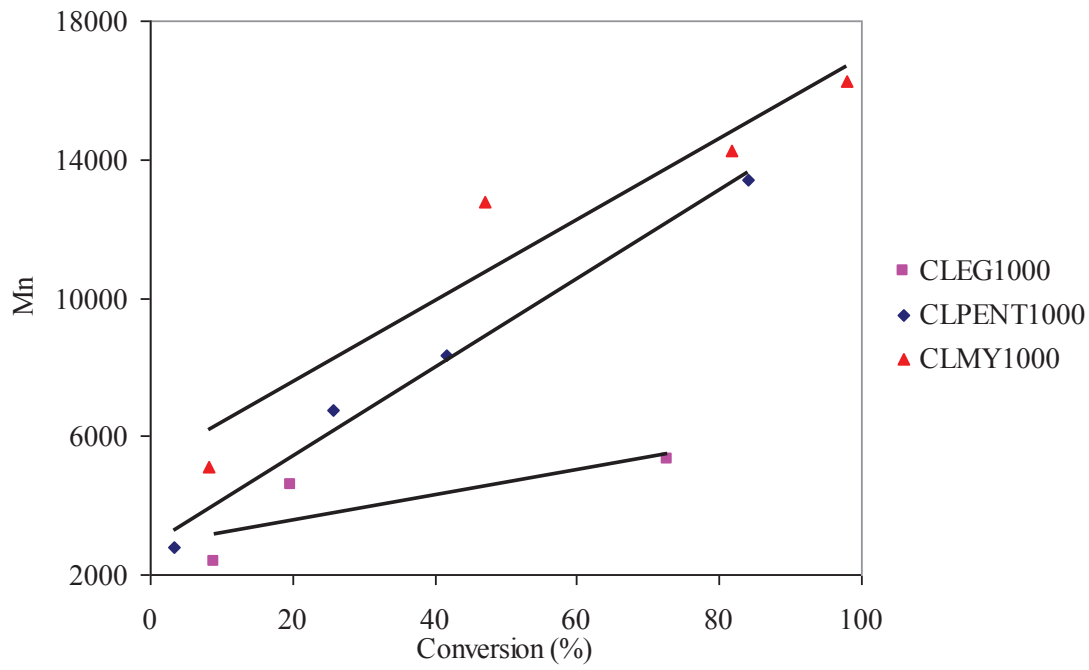


Figure 4.37. The influence of monomer conversion on number average molecular weight (M_n) determined by SEC for M/I=1000.

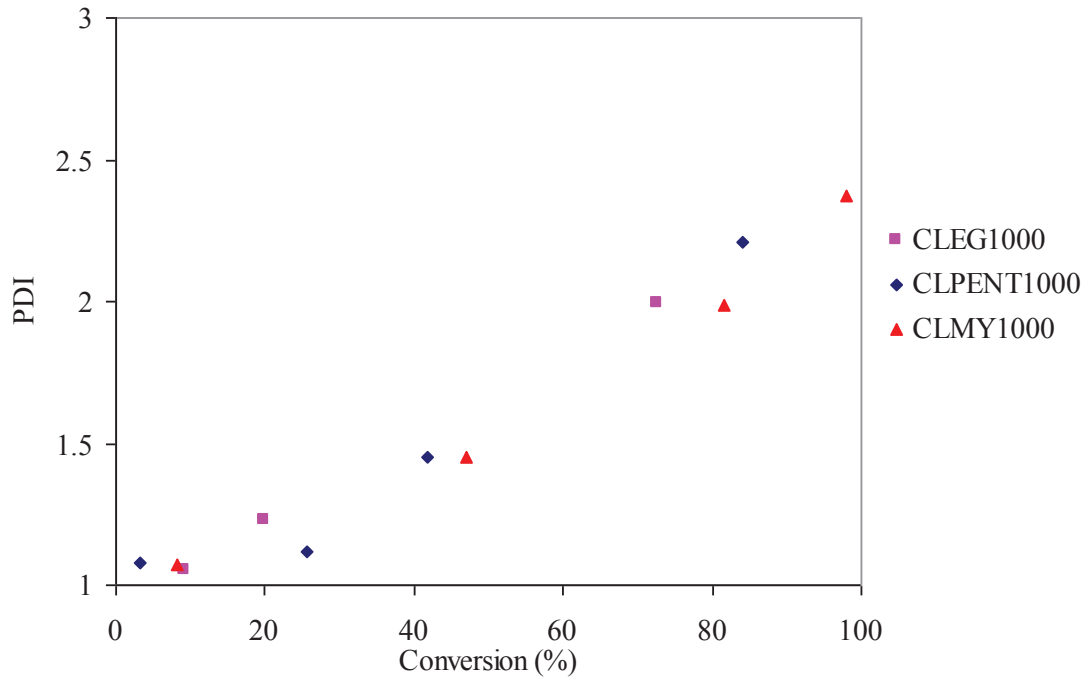


Figure 4.38. The influence of monomer conversion on PDI for M/I=1000.

Figure 4.39 indicates the SEC chromatograms of tetra hydroxy functional PCL prepolymers as a function of reaction time. Unimodal and narrow molecular weight distributions were observed during polymerization of tetra hydroxy functional PCL like

the other PCL prepolymers except PCL prepolymers synthesized by using initiator at a rate of M/I=1000. As seen in the SEC chromatograms, the number average molecular weights of the tetra functional PCL increase with time. M_n values of the polymer were obtained 1800, 4045, 8840 and 8900 Da for 1, 2, 4 and 6 days, respectively. PDI values of the polymer were obtained as 1.15, 1.16, 1.12 and 1.3, respectively.

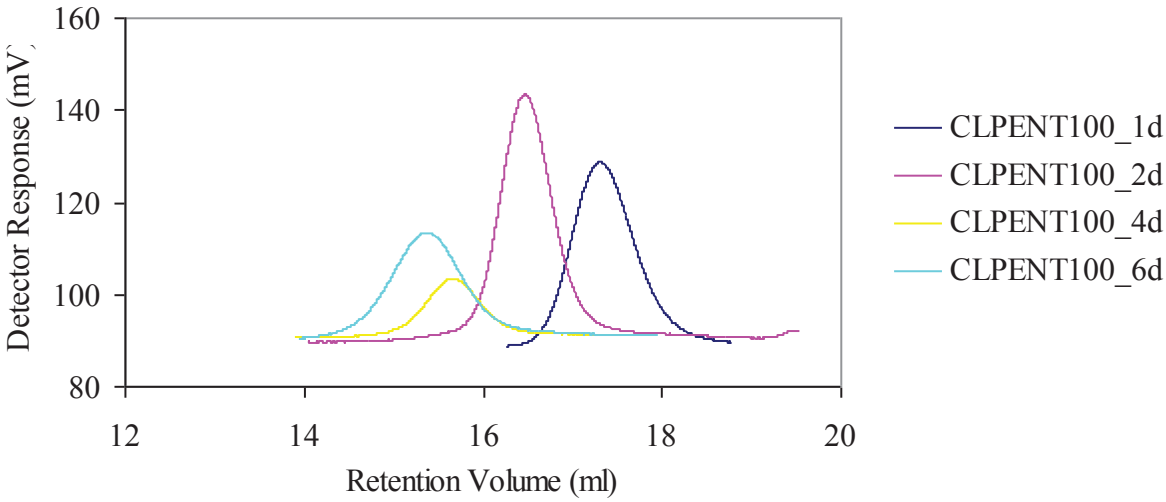


Figure 4.39. SEC chromatograms of tetrahydroxy functional PCL prepolymers as a function of reaction time.

Figure 4.40 illustrates the SEC chromatograms of dihydroxy functional PCL prepolymers as a function of M/I ratio. As seen in the figure, unimodal molecular weight distributions were obtained in the polymers synthesized by using ethylene glycol at a rate of 20 and 100. These polymers also have narrow molecular weight distributions. Hovewer, the SEC chromatogram of PCL prepolymer synthesized with ethylene glycol at a rate of 1000 did not show the unimodal distribution. This bimodal distribution result indicated the uncompleted polymerization reaction. This uncompleted polymerization result was also confirmed with low conversion value found as 77 % obtained from ^{1}H NMR results.

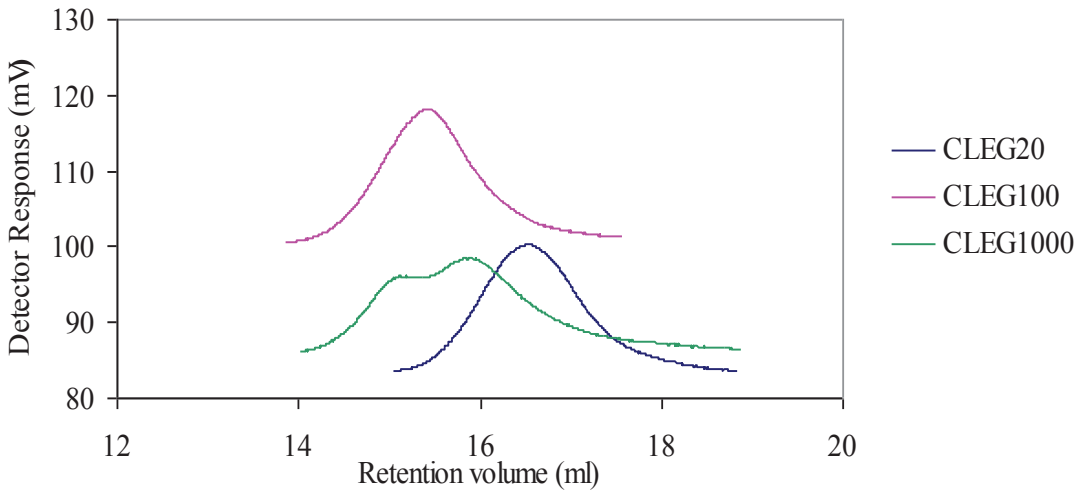


Figure 4.40. SEC chromatograms of dihydroxy functional PCL prepolymers as a function of M/I ratio.

Thermal properties of the hydroxyl functional PCL polymers are given in Table 4.13 and Figure 4.41. Glass transition temperatures of the polymers are in the range of -70.6 and -74.2 ^{o} C. The melting temperatures are found between 16.4 -53.0 ^{o}C. Glass transition and melting temperatures of PCLs are decreasing by the decrease of M/I ratios. The reduction in glass and melting temperatures is attributed to the disruption of crystal formation and related on the decrease in chain length. The presence of multiple melting peaks and the decrease in enthalpy values can be explained by the dispersion of polymer chains and hindering of chain ordering leading to decrease in crystallinity values.

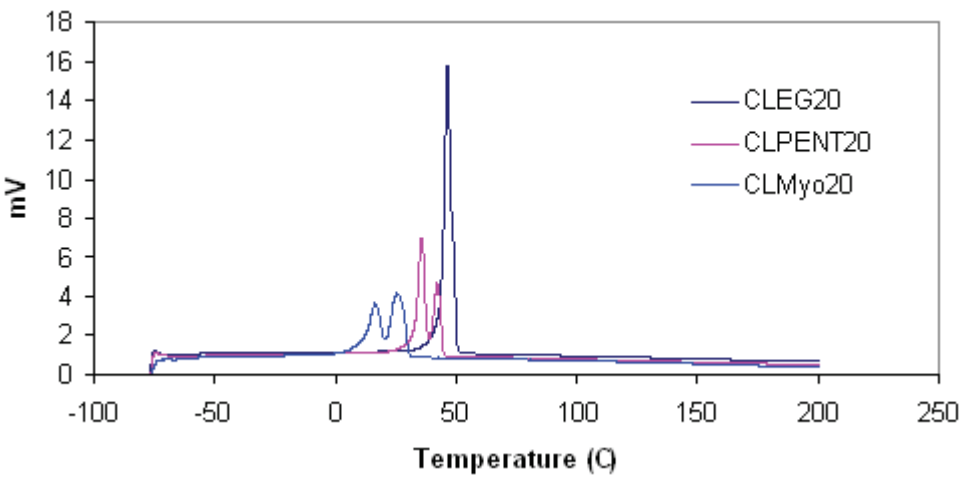


Figure 4.41. DSC thermograms of di, tetra and hexa functional PCL for M/I=20 after melt quenching

4.3. Synthesis of diblock, tetrablock and hexablock LA/CL copolymers by using creatinine

Copolymers of PLLA are the most innovative materials being actively investigated for their use in biomedical materials due to their biodegradability, biocompatibility and product of renewable resources. In this section, the copolymers of ε-caprolactone and L-lactide were synthesized by the sequential one-pot copolymerization process to study the effects of the process on possibility of block copolymer formation and the transesterification reactions during ring opening polymerization.

The copolymers were prepared by synthesizing poly(ε-caprolactone) prepolymer as the first block, and subsequently copolymerizing this prepolymer with L-lactide according to sequential copolymerization method. The sequential polymerization method were used due to two reasons, one is the differences in reactivity of CL and LA homopolymers and the other is high sensitivity of LA to transesterification. In this study as given in the previous chapters, the reactivity of LA was found as higher than that of CL similar to other studies of Bero et al. 1993, Karsperczyk et al. 1993 and Vanhooorne et al. 1992. Lipik et al. (2010) investigated the effects of order of monomer addition on transesterification during copolymerization of lactide and caprolactone. They observed transesterification for the synthesis of PLA or PCL-co-PLA at the first step. They concluded that PLA segments are more vulnerable to transesterification compared to PCL. From that point of view, PCL prepolymers were produced in the first step to decrease the transesterification sensitivity of lactide in this study.

In the sequential copolymerization, two, four and six arm OH functional Poly(ε-caprolactone) prepolymers were obtained by using creatinine as catalyst (monomer/catalyst molar ratio M/C = 50) and three different initiators such as ethylene glycol, pentaerythritol, and myo-inositol, respectively. L-lactide was added to the during the polymerization reaction of PCL prepolymer when the conversion of PCL prepolymer was higher than 95%. Figure 4.42 shows the copolymerization scheme of L-lactide with PCL prepolymer.

The polymerization of ε-caprolactone was monitored by ^{1}H-NMR following the resonance signal of oxymethylene protons (-O-CH_2-) of CL monomer at 4.17 ppm and PCL prepolymer at 4.01 ppm by calculating the conversions. The conversions of

polymerization reactions for di, tetra and hexablock PCL prepolymers in the case of M/I=100 reached to higher than 95 % for 10, 8 and 7 days, respectively. The conversion of diblock PCL polymer in the case of M/I=20 was found as 93.83 after 4 days. The conversion values of polymerization reactions for four and six arm PCL prepolymers in the case of M/I=20 were higher than 97 % for 4 and 3 days, respectively. In all cases, polymerization reactions of hexablock polymers were completed earlier than the others due to the reactivity of myo-inositol. LA monomer was added in different time periods during the polymerization of each PCL prepolymers due to the variable conversions. After the addition of LA, all polymerization reactions were completed one day later. The difference in reaction time can be explained by the difference in rates of polymerization of L-lactide and ε-caprolactone monomers due to the difference in Lewis basicities of L-lactide and ε-caprolactone.

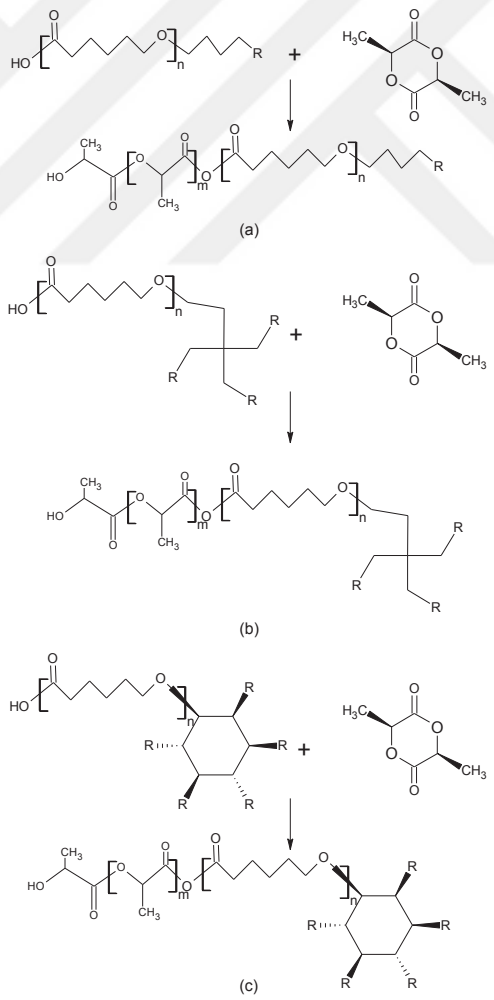


Figure 4.42. Copolymerizations of L-lactide with (a) diblock PCL prepolymer (b)tetrablock PCL prepolymer and (c) hexablock PCL prepolymer (R denotes PCL and PLLA units).

^{1}H NMR spectra of di, tetra and hexa hydroxy functional PCL-PLLA copolymer with assignments were shown in Figures 4.43- 4.45, respectively. The peak at 5.13 ppm corresponds to the methine proton of PLLA (q, 1H, -CO-CH(CH_3)-O-). The peak at 1.54 ppm is attributed to metyhlene proton of PLLA (d, 3H, -CH_3).

As seen in the figures, major peaks of PCL were also observed at 4.02, 2.27 and 1.34 ppm. The signals of –CH_2OH, methylene protons of the hydroxyl end groups of the PCL prepolymers at 3.64 ppm disappeared, and new signals of –CH(CH_3)OH, methyne proton at 4.33 ppm of the hydroxyl end of the copolymer coming from PLA were observed. These indicated that the PCL reactive chains have succeeded the polymerization of the second monomer L-LA by using sequential polymerization method. Also, it can be concluded that PCL prepolymers affect as macroinitiators in the copolymerization of LA due to the reaction of hydroxyl group in PCL prepolymer with LA monomer in the second step reaction.

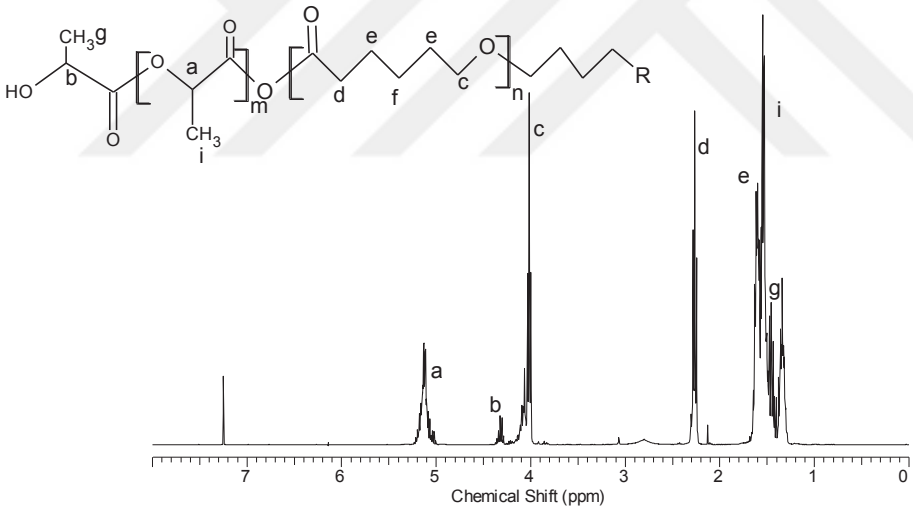


Figure 4.43. ^{1}H NMR spectrum of di-hydroxy functional PCL-PLLA copolymer.

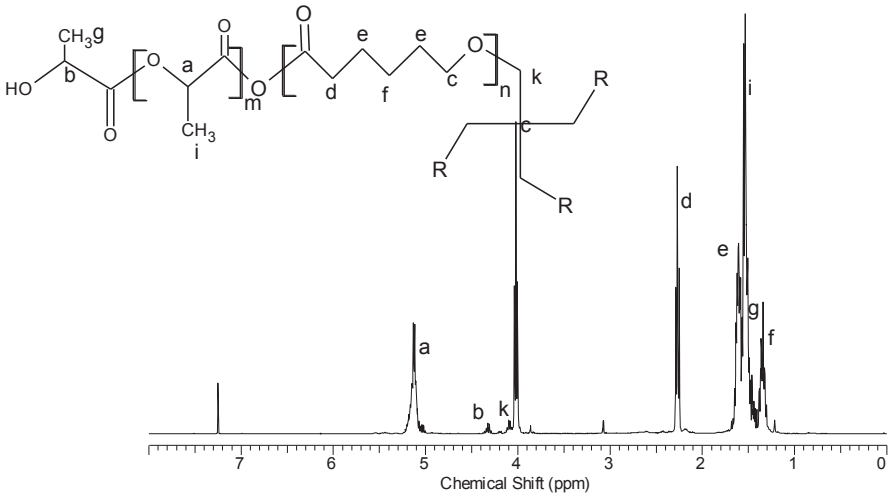


Figure 4.44. ^{1}H NMR spectrum of tetra-hydroxy functional PCL-PLLA copolymer.

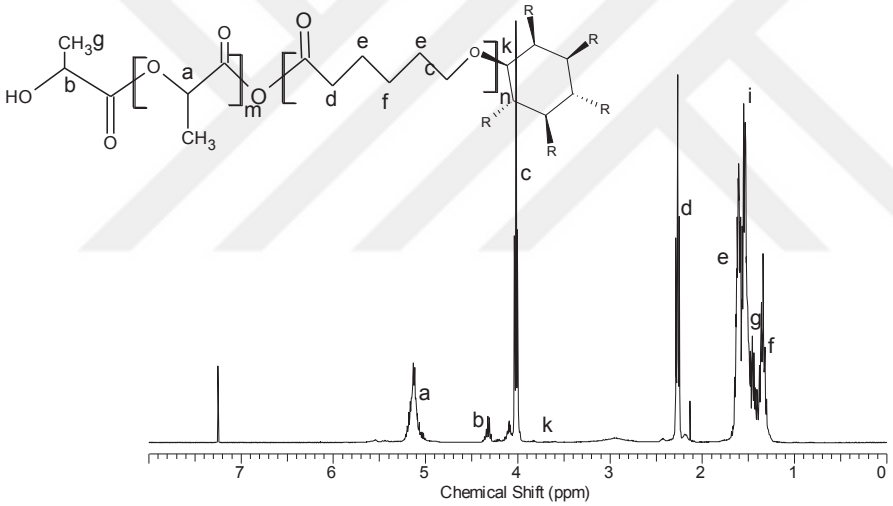


Figure 4.45. ^{1}H NMR spectrum of hexa-hydroxy functional PCL-PLLA copolymer.

The number average molecular weight $M_{n,NMR}$ of the copolymers were calculated from the sum of the $M_{n,NMR}$ values of the caprolactone prepolymers and the number average molecular weight of the PLA part of the copolymers. The number average molecular weights of crude PCL prepolymer were calculated from the intensity ratios of the oxymethylene proton (-O-CH_2-) signals at 4.05 ppm and the methylene proton signals (–CH_2-0H) at 3.64 ppm in the end group of PCL prepolymer. The number average molecular weights of PLA part was determined from the intensity ratios of the OCH methine proton signals at 5.13 ppm and the HOCH terminal methine

proton signals at 4.33 ppm. As seen in the Table 4.14, the molecular weight of the prepolymers increased with the copolymerization with L-lactide. The increase in molecular weight depends on the M/I ratio and the conversion of L-lactide.

Mole fraction of PCL in the copolymer was determined from the intensities of methylene protons in the caprolactone prepolymer observed at 4.02 ppm ($-CH_2CH_2OCO-$) and methine proton of lactide polymer observed at 5.13 ppm ($-CO-CH(CH_3)-O-$) by the area ratio of the peak at 4.02 ppm to the area of the peak at 5.13 ppm.

The average polymerization degree of L-LA is also determined by the integration ratio of methine protons of the PLLA block at 4.35 ppm and the methylene protons of PCL at 2.30 ppm.

Table 4.14 Copolymerization of ε-caprolactone and L-lactide

CoI type	Mo/ CoI	CL Conv HNMR (%)	LA Conv HNMR (%)	^{1}HNMR CL/LA	M_n ^{1}HNMR PCL (Da)	M_n ^{1}HNMR (CL-LA) (Da)	M_n SEC (Da)	PDI	T_g (^oC)
EG	20	93.5	98	0.67	1550	4570	4500	1.26	32.4
EG	100	96.2	46.7	1.19	4620	6940	10445	1.48	30.4
PENT	20	97.1	97.2	0.87	2110	3050	6200	1.13	28.9
PENT	100	98	84	1.00	11080	13090	21510	1.08	25.8
Myo	20	98	93.5	1.18	6100	9780	9340	1.23	24.8
Myo	100	95.2	94.5	1.00	9410	16300	23020	1.09	22.5

Chain microstructure and randomizing effects of transesterification reactions were studied by means of ^{13}C NMR since this technique is very sensitive to monomer sequencing and is therefore a powerful tool for determining the average sequence length for each type of monomer unit. In particular, the carbonyl carbon signals between 175 and 165 ppm are the most sensitive to the sequence distribution of the CC and LL units. Figure 4.46 and Figure 4.47 show the expanded carbonyl carbon regions of the

^{13}C NMR spectrum of hexa-hydroxy functional PCL-PLLA copolymer at different M/I ratio as 20 and 100, respectively. The peak at 173.46 and 169.5 ppm corresponds to the CL-CL (CC) and LA-LA (LL) sequence, respectively. The other peaks observed at 173.37, 173.43, 172.78 and 169.99 ppm correspond to LLCC, CLCC, LLCLL and CLLL. In the case of M/I=100, the presence of the CLCC and LLCLL peaks indicate the presence of transesterification. LL unit undergoes bond cleavage leading to the formation of anomalous sequences of CLC. Thus, transesterification plays an important role in the redistribution of monomer sequences, thereby influencing the microstructure. These odd-number L sequences cannot be formed by the opening of LA rings alone during the growth reaction of the copolymer chains. In the ^{13}C-NMR analyses, the peak at 171 ppm corresponds to CLC sequence was not detected for all samples. This triad is used generally to indicate the occurence of transesterification reactions (Pappalordo et al, 2009).

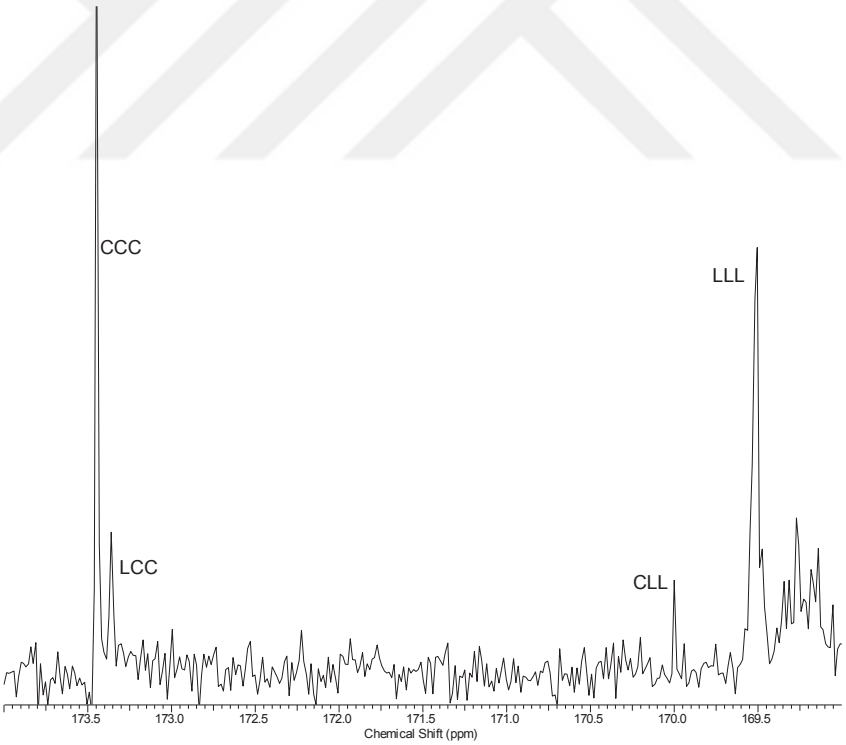
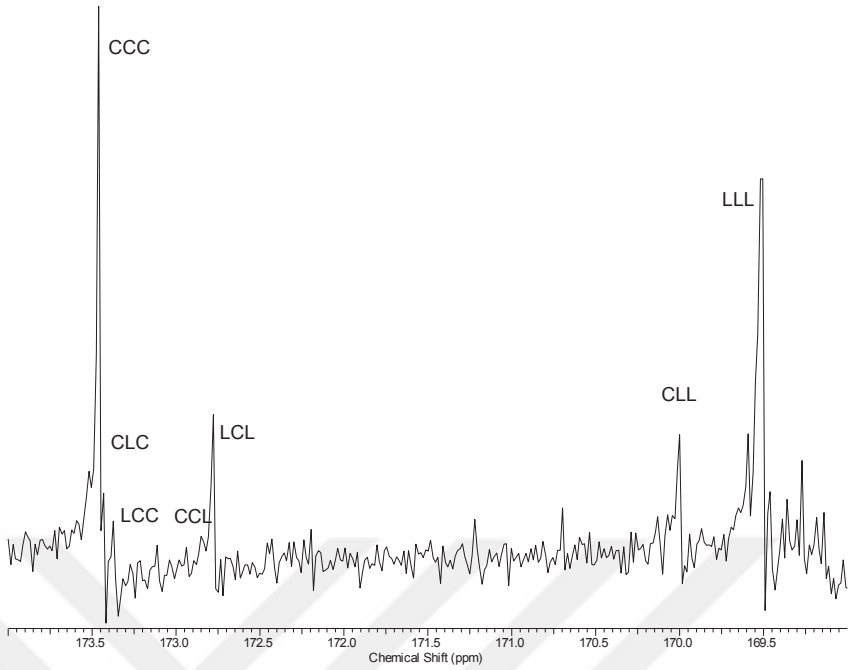


Figure 4.46. Expanded carbonyl carbon regions of the ^{13}C NMR spectrum of hexa-hydroxy functional PCL-PLLA copolymer for M/I=20.



(b)

Figure 4.47. Expanded carbonyl carbon regions of the ^{13}C NMR spectrum of hexa-hydroxy functional PCL-PLLA copolymer for M/I=100.

Figures 4.48-4.50 show the GPC chromatograms of PCL-PLA block copolymers. As seen in the figures, the GPC chromatograms of all of the copolymers synthesized by successive addition of monomer according to sequential polymerization method resulted in unimodal curves. If the PCL prepolymer did not initiate the polymerization of L-lactide, there should be two peaks on the GPC curve. This indicated that the PCL prepolymer initiate the polymerization of L-lactide in the copolymerization. Also, there was no evidence to suggesting any copolymer products consist of only poly (L-lactide) (PLA) or PCL homopolymeric fractions. The results showed that PCL with derived hydroxyl end-groups is an active macroinitiator for LA polymerization leading ultimately to homogeneous copolymer products. This finding is in good agreement with the results obtained by In't Veld et al. As seen in Table 4.15, the copolymers had narrow molecular weight distributions ranging from 1.08 to 1.26. The high PDI value was only obtained for the diblock copolymers synthesized by using EG as initiator at a rate of M/I=100 due to the low conversion of L-LA.

Figure 4.48 indicates an increase in the molecular weight of polymer with time. As seen in the figure, the addition of LA leads to the significant increase in the

molecular weight of the polymer. It can be said that the reactivity of LA is higher than caprolactone due to reaching the high conversion value in less reaction time for one day.

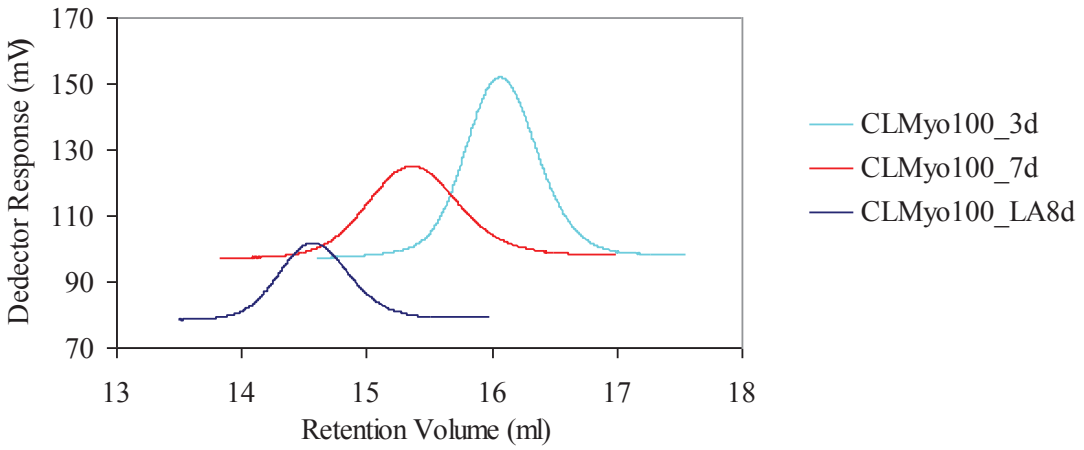


Figure 4.48. GPC chromatograms of hexablock PCL-PLA polymers during the polymerization.

Figures 4.49 and 4.50 illustrate the GPC chromotagrams of di, tetra and hexa block PCL-PLA copolymers synthesized by using M/I=20 and 100, respectively. As seen in the chromatograms, hexa block PCL-PLA copolymer synthesized at M/I=100 has the highest molecular weight as compared to retention volumes. Also, it can be concluded that the molecular weight increases with an increase in number of branches and M/I ratio on account of increase in number of living chains.

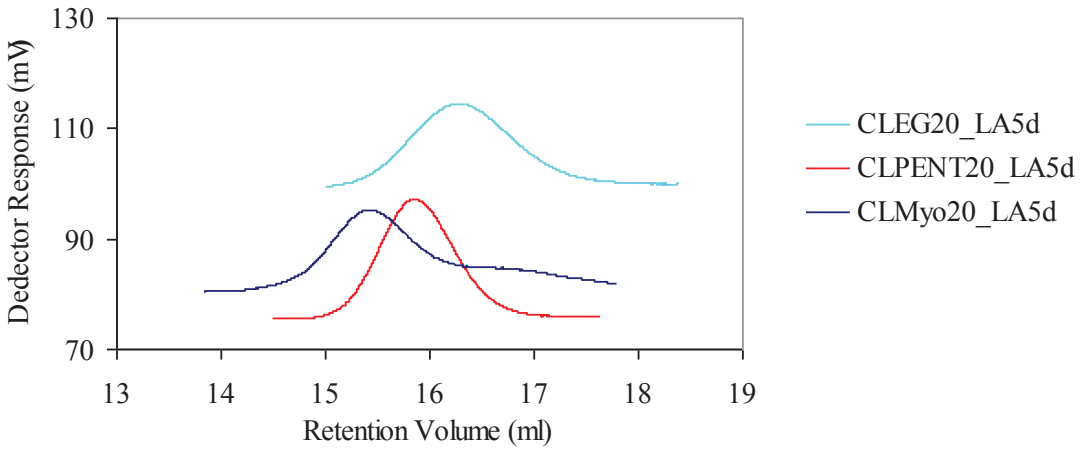


Figure 4.49. GPC chromotagrams of di, tetra and hexa block PCL-PLA copolymers synthesized by using M/I=20.

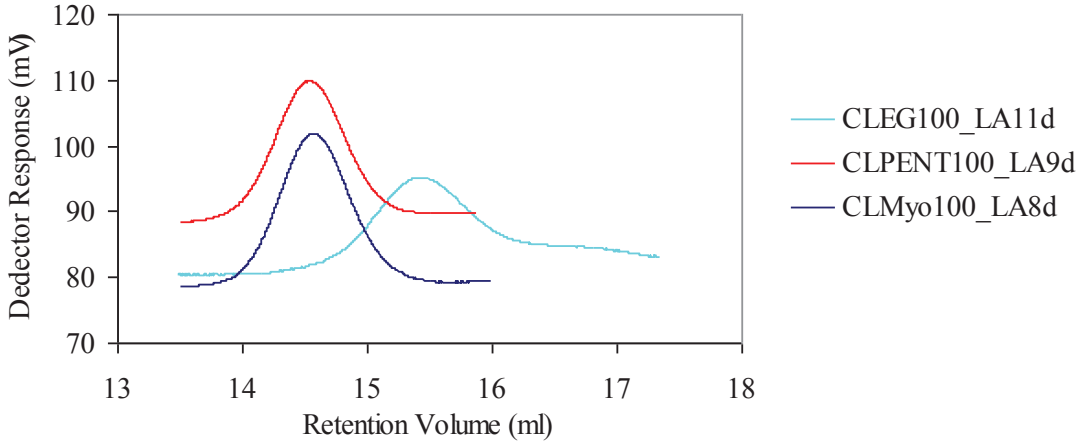


Figure 4.50. GPC chromotagrams of di, tetra and hexa block PCL-PLA copolymers synthesized by using M/I=100.

As seen in all of the GPC chromatograms (Figures 4.45-4.47), the monomodal profiles of molecular weight distribution of branched PCL-PLA copolymers demonstrate the evidence of controlled synthesis of branched PCL-PLA copolymers without formation of cyclic oligomers.

The chemical structure of polymers was also investigated by Fourier Transform Infrared Spectroscopy (FTIR). Figure 4.51 shows the FTIR spectra of di, tetra and hexa hydroxy functional PCL-PLA copolymers. The major peaks of PCL and PLA units were observed on the FTIR spectra of the copolymers. As seen in the Figure 4.51, additional peaks at 1750 (νC=O), 1267 (ν_{as} COC + CH), 1085 ((ν_sCOC) and 1029 ($\nu_{as}CH_3$) cm^{-1} assigned to PLA unit were detected according to the FTIR spectra of homo and di, tetra and hexa block PCL prepolymers shown in Figure 4.44. Especially, the main difference between the IR spectrum of functional PCL prepolymers and functional PCL-PLA copolymers was the only carbonyl absorption band region ((νC=O). In the PCL-PLA copolymers, two peaks at 1750 and 1728 cm^{-1} were defined as the carbonyl absorption bands of PLA and PCL units, respectively. The presence of these peaks is proved that FTIR analysis confirmed the formation of PCL-PLA block copolymers by using sequential addition polymerization method.

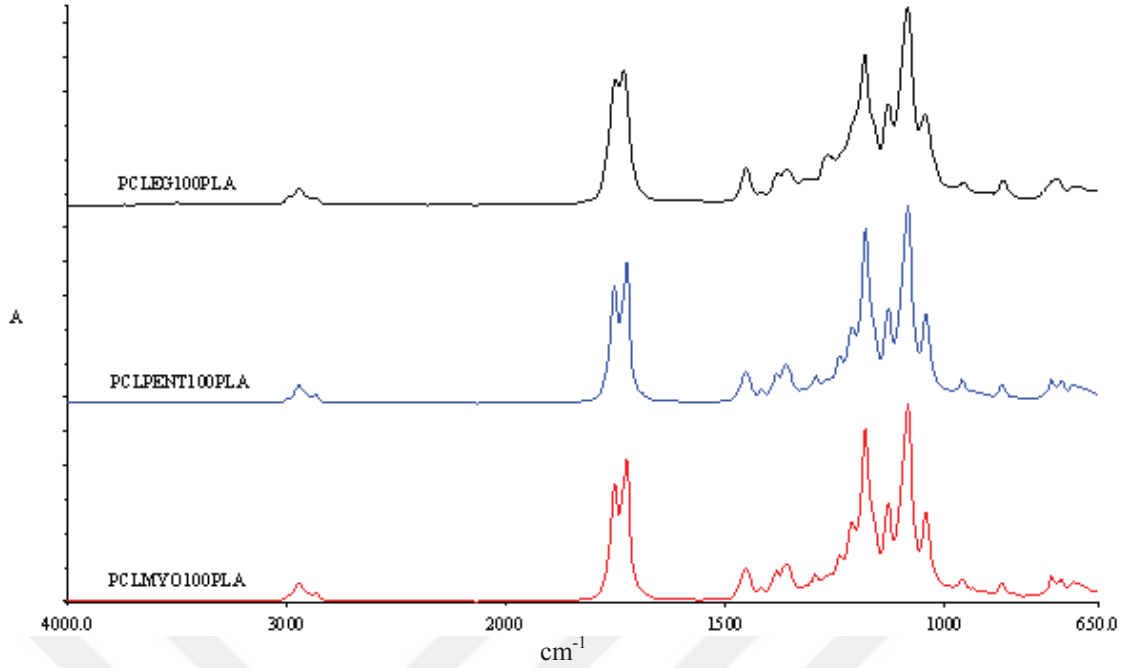


Figure 4.51.FTIR spectra of di, tetra and hexa hydroxy functional PCL-PLA copolymers synthesized by using creatinine.

As seen in Table 4.14, no melting peak in PCL-PLA copolymers was observed Single glass transition temperatures are significant in that there is no phase separation between prepolymer block and PLLA block. Melting peaks were not observed due to the shorter monomer sequence lenght. The formation of random copolymers was attributed to the occurrence of transesterification reactions. These side reactions were caused by the ε-caprolactone derived hydroxyl end groups generated during the copolymerization of ε-caprolactone with prepolymers of L-lactide.

The introduction of CL segment in copolymerization provides a decrease in the crystallinity of the PLLA homopolymer due to the low T_g value of PCL segment having flexible chain. The homopolymers and copolymers having higher initiator content possessed lower crystallinity when compared with those of lower initiator content (EG, PENT). The PLLA chains have higher ester group content compared with PCL chain. For that reason, the incorporation of the lactide segment to the PCL improved the degradation properties of the polymer.

4.4. In vitro cytotoxicity and cell adhesion of biodegradable PLA and PCL homo and copolymers

Biocompatibility properties of PLA/PCL homopolymers and copolymers were investigated by carrying out in vitro cytotoxicity tests of the used catalysts ($SnOct_2$, Bi(III)Ac and creatinine), and cell adhesion tests of PLA/PCL homopolymers and copolymers.

4.4.1.Cytotoxicity test of $SnOct_2$, Bi(III)Ac and creatinine catalysts

Cytotoxicity of the $SnOct_2$, Bi(III)Ac and creatinine catalysts were tested according to MTT test protocol. MTT test gives information about the viability of cells. The cytotoxicity of the three types of catalysts have been tested by checking the dose inhibiting 50% of cell growth (IC50) of 3T3 mouse fibroblast cells. Variable catalyst concentration in the range of 500, 100, 50, 25, 10, 1, 0.1 μg/ml were used for the determination of IC50 values. IC50 values obtained from MTT tests are tabulated in the Table 4.15. IC50 values for Sn $(Oct)_2$ catalytst were found as 34, 24, 20 μg/ml for 1, 2 and 3 days, respectively. IC50 values for Bi(III)Ac catalyts were found as 132, 98 and 40 μg/ml for 1, 2 and 3 days, respectively. However, creatinine at all dosages does not show any cell inhibiting effect. Therefore, creatinine catalyst can be used safely as a biocatalyst for the synthesis of low molecular weight amorphous PLA and PCL homopolymers and copolymers in biomedical applications. Since the higher amount of Bi(III)Ac catalyst are required to reach IC50 values compared to that of $SnOct_2$ catalyst, cytotoxicity of Bi(III)Ac catalyst is much better than that of $SnOct_2$. The physical form and solubility of catalysts can led to the determination of different IC50 values in cyto-toxicity tests. Although the presence of $SnOct_2$ and creatinine in liquid form was directly used in cyto-toxicity test due to the solubility of these catalysts in PBS, the unsoluble Bi(III)Ac catalyst was not used directly in tests. Mazzaoro (2012) also defined the IC50 values of $SnOct_2$ catalysts as 26.1 or 125.9 ppm for 3T3 mouse fibroblast cells and human endothelial cells, respectively.The standard recipe in the industry uses a concentration of $SnOct_2$ between 140 and 281 ppm. It can be concluded by considering our IC50 values with literature that the industrial PLA synthesized by

$SnOct_2$ is not biocompatible for long term bioapplications. Recent studies published by Tsunoda et al. 2012 and Tuji et al (2010) also reported toxic effects of tin based catalysts.

Table 4.15. IC50 values of catalysts

Catalyst type	**IC50 1 day** (μg/ml)	**IC50 2 days** (μg/ml)	**IC50 3 days** (μg/ml)
$SnOct_2$	34	24	20
Bi(III)Ac	132	98	40
Creatinine	-	-	-

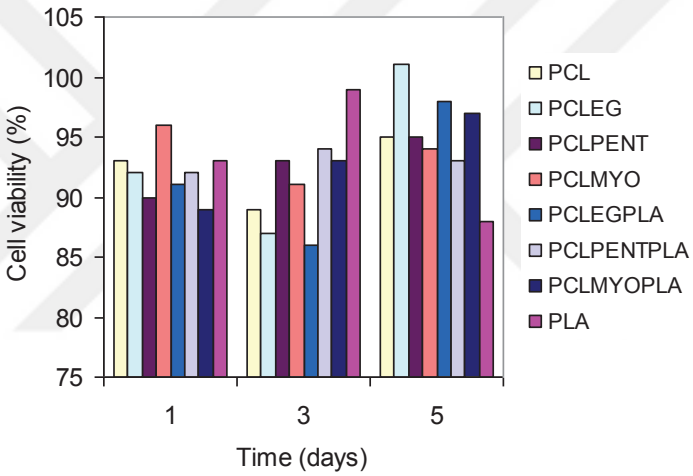
MTT cytotoxicity results indicate that creatinine is absolutely non-toxic, any adverse effect on cell viability was not observed for creatinine catalyst. Therefore, PLA or PCL based homopolymers and copolymers synthesized by creatinine catalyst can be directly used in medical and pharmaceutical application. Tin and bismuth are not available in physiological medium of the body contrary to creatinine. The toxicity of bismuth based catalyst (Bi(III)Ac) was found lower than tin based catalyst ($SnOct_2$). The residual creatinine catalyst does not lead to the toxic effect compared to the widely used tin based catalyst.

4.4.2. Cell Adhesion and Growth on Biodegradable Polymers

The cell viability and adhesion ability of PLLA/PCL homopolymers and P(LL-co-CL) copolymers synthesized with creatinine and Bi(III)Ac catalysts for fibroblast 3T3 cells were evaluated. Figure 4.52.a and b indicate the cell viability results for one three and five days of PLLA/PCL homopolymers and P(LL-co-CL) copolymers synthesized with three different co-initiators having M/CoI:100 by using creatinine and Bi(III)Ac catalysts, respectively.The cell viability results were found higher than 86 %. No cytotoxic properties were observed all of the polymer samples synthesized with Bi(III)Ac and creatinine catalysts.

Figures 4.53 and 4.54 show SEM micrographs of 3T3 fibroblast cell adhesion for 24 hr on PCL, PCL-EG100, PCLPENT100, PCL MYO100, PCL-EG100PLA,

PCLPENT100PLA, PCLPENT100PLA and as well as PLA homopolymers and copolymers synthesized by Bi(III)Ac catalyst and creatinine catalyst, respectively. As shown in the figures, the lowest fibroblast cell adhesion were observed on non functional PLLA and PCL homopolymers synthesized by both catalysts. The moderate cell adhesion abilities were observed on PCL/PLA copolymers. However, as seen in the SEM micrographs, cell adhesion on functional PLAs with EG, PENT and Myo surfaces are significantly much higher than the other nonfunctional PLA/PCL homopolymers and copolymer surfaces. It can be concluded that the number of hydroxyl groups on polymer structure directly effects on cell adhesion capability of polymeric surfaces. Therefore, functional PLA/PCL homo or copolymers could be a good candidate for biomedical applications.



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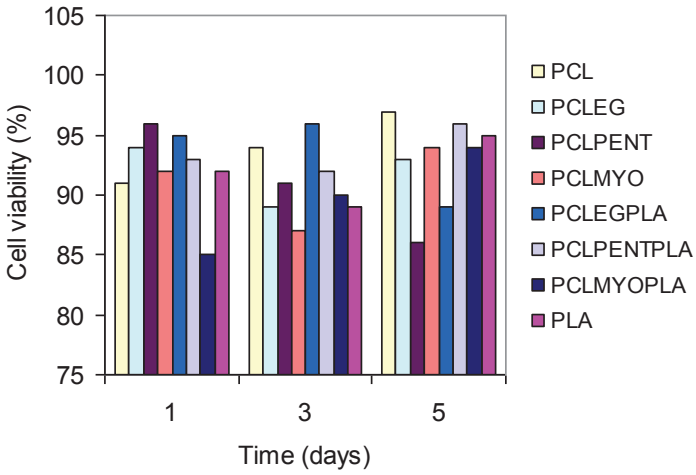
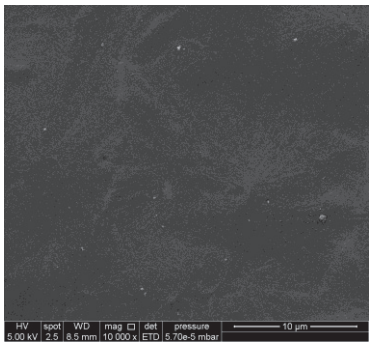


(b)

Figure 4.52.The cell viability results of PLLA/PCL homopolymers and P(LL-co-CL) copolymers synthesized with three different co-initiators having M/CoI:100 by using (a) creatinine, (b) Bi(III)Ac catalysts.



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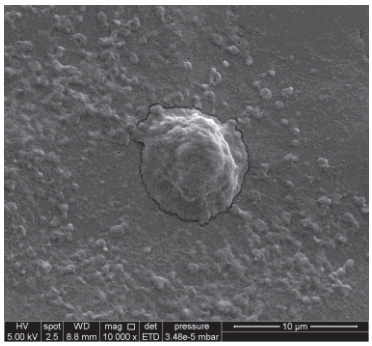

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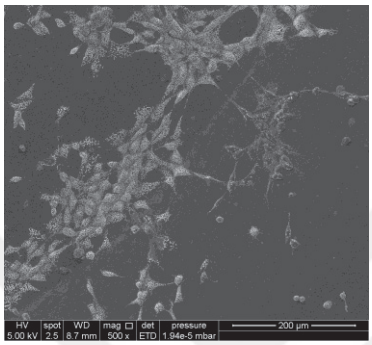

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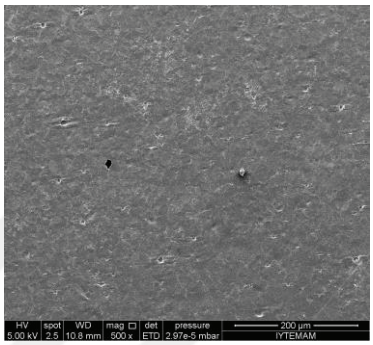

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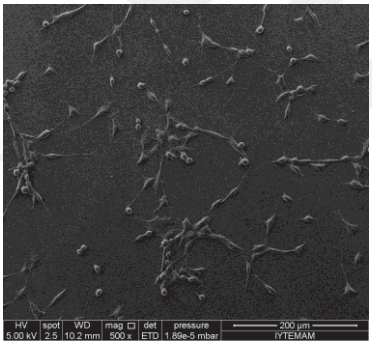

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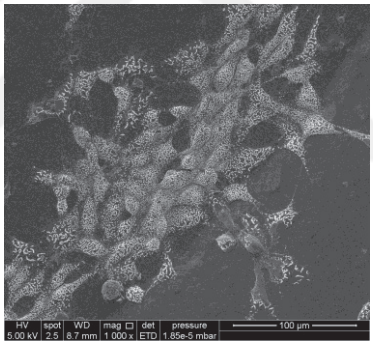
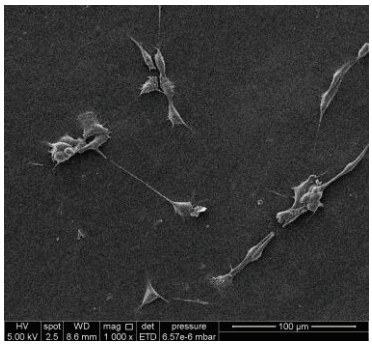


(f)



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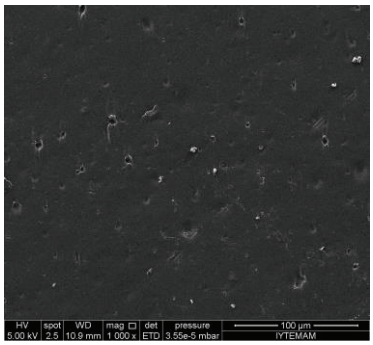
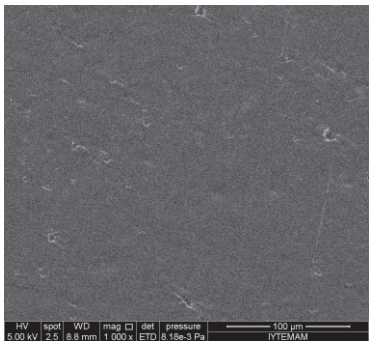


(h)

Figure 4.53. SEM micrographs of 3T3 fibroblast cell adhesion on (a)PCL (b) PCL-EG100, (c) PCLPENT100, (d) PCL MYO100 ,(e) PCL-EG100PLA, (f) PCLPENT100PLA, (g) PCLPENT100PLA and (h) PLA homopolymers and copolymers synthesized by Bi(III)Ac catalyst



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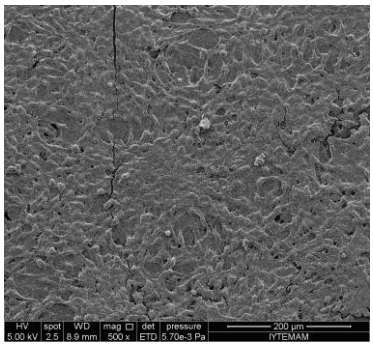

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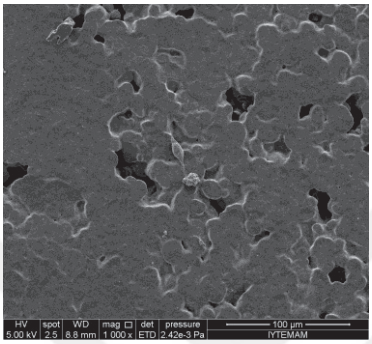

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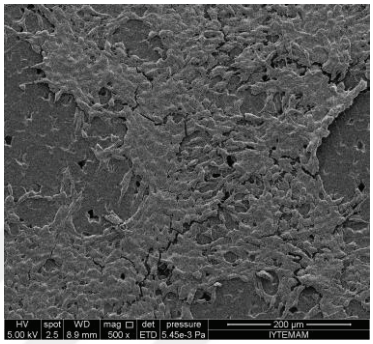
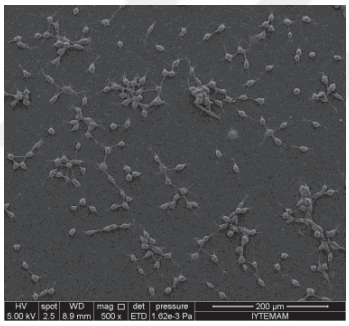
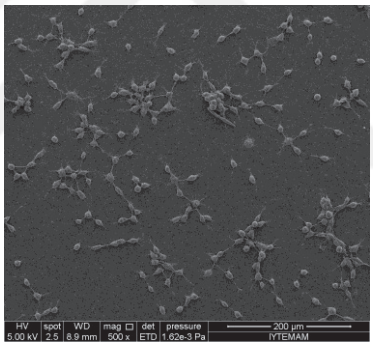


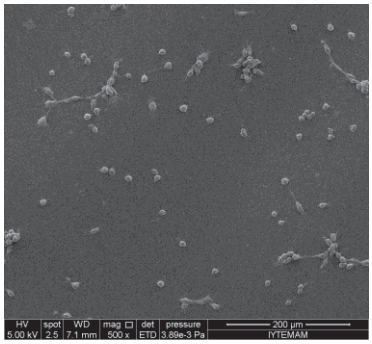
(d)



(e)



(f)



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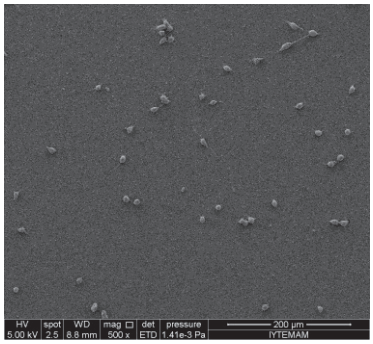

(h)

Figure 4.54. SEM micrographs of 3T3 fibroblast cell adhesion on (a)PCL (b) PCL-EG100, (c) PCLPENT100, (d) PCL MYO100 ,(e) PCL-EG100PLA, (f) PCLPENT100PLA, (g) PCLPENT100PLA and (h) PLA homopolymers and copolymers synthesized by creatinine catalyst

CHAPTER 5

CONCLUSIONS

In this study, linear and star shaped PLLAs were synthesized by using biosafe catalysts such as bismuth(III)Ac and creatinine to be used confidently for bio-applications such as tissue and drug delivery systems. The effects of catalyst type, monomer/initiator and monomer/co-initiator ratios and temperature on final properties of PLLAs were investigated. The effect of catalyst type on polymer properties was observed by differences in crystalline structure and stereoregularities. Although the use of Bi(III)Ac leads to the synthesis of semicrystalline PLLAs, amorphous PLLAs were synthesized by using creatinine catalyst due to the difference in stereochemical preference of the catalysts. This difference in crystal structure indicates the capability of Bi(III)Ac catalyst on stereo-controlled polymerization of LA and CL. The high molecular weight polymers having low PDI values were obtained by using in a limited reaction time. However, low molecular weight polymers having high PDI values were obtained by using high amount of creatinine in a long reaction time. The results showed that the molecular weight of the polymers synthesized depends significantly on catalyst type but not significantly catalyst amount. The number average molecular weights of PLA homopolymers were obtained in the range of 850 to 97000 Da. It was found that initiators used in the synthesis of branched polymers significantly affect directly the molecular weight of the final polymer together with structural and thermal changes. The decrease in glass transition temperatures and molecular weights of PLLAs synthesized by bismuth(III)Ac and creatinine were observed with an increase in amount of co-initiators due to the decrease in chain length and disruption of crystal formation. The glass transition temperatures of PLA homopolymers were obtained between 59 and 24 ^{o}C. The molecular weights of the PLLAs also increased with an increase in the number of functional groups in the structure. The decrease in glass transition temperature and crystallinity of the homopolymers and copolymers lead to the enhancement of degradation of the polymers due to the increase of chain flexibility. The utilization of di, tetra or hexa -OH functional homo and coPLLAs having low glass transition temperature could be used for bioapplications demanding fast degradation rate.

Synthesis of linear and star shaped homopolymers is possible by using appropriate catalyst and initiator. Initiators should have one or more hydroxyl end group to initiate the ring opening polymerization of lactide resulting in a growing polymer chain of lactide units at the sides of the initiator. The structure of the polymer is dependent on the number of the hydroxyl groups in the initiator. In this study, linear PLLA and PCL were synthesized by using ethylene glycol. Tetra and hexa functional PLLAs and PCLs were synthesized by using pentaerythritol and myo-inositol, respectively. Also, the use of initiator led to the decrease in reaction time due to the increase in the reaction rate of polymerization.

Alternatively, synthesis of linear and star shaped block copolymers is possible by sequential melt ring-opening polymerization of the corresponding monomers using catalyst and initiators. Firstly, ε-caprolactone was polymerized using creatinine as catalyst and different initiators to synthesis prepolymers having different number of hydroxyl end group. After the addition of lactide monomer, the hydroxyl end group of the prepolymer initiates the copolymerization of the lactide. Using this technique linear and star-shaped block copolymers of L-lactide and ε-caprolactone had been synthesized. The number average molecular weights of PCL homopolymer and copolymers were obtained as 2300 to 21400 Da. The glass transition temperatures of PCL homopolymers were significantly increased from -76 to 22.5 ^{o}C with copolymerization of LA.

Synthesis and characterization of di, tetra, and hexa OH functional PLLA, PCL and P(LA-co-CL) were carried out by using creatinine catalyst. Although di, tetra and hexa functional PLLAs and block and random P(LA-co-CL) polymers have amorphous structure, di, tetra and hexa functional PCLs have crystalline structure. The reactivity of LA was found as higher than ε-caprolactone monomer. In the case of copolymerization of lactide with *ε*- caprolactone, the reactivity of lactide was found considerably higher than that of *ε*-caprolactone. Formation of random copolymers was attributed to the occurrence of transesterification reactions.

The cytotoxic properties of $SnOct_2$, Bi(III)Ac and creatinine catalysts were analyzed according to MTT test procedure. MTT test results deduced the no cytotoxic effect of creatinine usage and lower cytotoxic effect of Bi(III)Ac in bioapplications instead of $SnOct_2$.

In the continuation of this study, the compatibility of synthesized homo or copolymers having variable molecular weight, glass transition temperature and

crysttallinity value for different medical applications such as drug delivery or implant can be tested by considering their required properties especially protein adsorption and degradation properties. Also, in the continuation of this study, the use of microwave reactor in the synthesis of PLLA homo polymers and copolymers with non-toxic creatinine catalyst is recommended for the reduction of polymerization time and the production of crystalline polymers.

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