Novel methods to synthesize aliphatic polyesters of vivid architectures

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विशुद्धं परं सचिविदानन्दरूपम्
गुणाधारमाधारहीं वरेणयम् ।
महान्तं विभान्तं गुहान्तं गुणान्तं
सुखान्तं स्वयं धाम रामं प्रपद्ये ॥
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ABSTRACT

Cross-linked films of ε-caprolactone (CL) and 1,5-dioxepan-2-one (DXO) having various mole fractions of monomers and different cross-link densities were prepared using 2,2’-bis-(ε-caprolactone-4-yl) propane (BCP) as cross-linking agent and Sn(Oct)$_2$ as catalyst. Reaction parameters were examined to optimize the film-forming conditions. Networks obtained were elastomeric materials, easy to cast and remove from the mould. Effect of CL content and cross-link density on the final properties of the polymer network was evaluated. Thermal, mechanical and surface properties of the films were controlled by monomer feed composition and cross-link density. The films have potential to be used for tissue engineering applications as shown by preliminary cell growth studies. To avoid organometallic catalysts in the synthesis of poly(1,5-dioxepan-2-one) (PDXO), the enzyme-catalyzed ring-opening polymerization (ROP) of DXO was performed with lipase-CA (derived from Candida antarctica) as a biocatalyst. A linear relationship between number-average molecular weight ($M_n$) and monomer conversion was observed, which suggested that the product molecular weight can be controlled by the stoichiometry of the reactants. The monomer consumption followed a first-order rate law with respect to monomer and no chain termination occurred. Effect of reaction water content, enzyme concentration and polymerization temperature on monomer conversion and polymer properties was studied. An initial activation by heating the enzyme was sufficient to start the polymerization as monomer conversion occurred at room temperature afterwards. Terminal-functionalized polyesters and tri-block polyesters were synthesized by lipase-CA catalyzed ROP of DXO and CL in the presence of an appropriate alcohol as initiator. Alcohol bearing unsaturation introduced a double bond at the chain end of the polyester, which is a useful pathway to synthesize comb polymers. Dihydroxyl compounds were used as macro-initiators to form tri-block polyesters. The enzyme-catalyzed polymerization of lactones has been shown to be a useful method to synthesize metal-free polyesters.

Keywords: polyesters, 1,5-dioxepan-2-one, ε-caprolactone, 2,2’-Bis-(ε-caprolactone-4-yl)propane, Sn(Oct)$_2$, enzyme, lipase-CA, ring-opening polymerization, architecture, networks, tissue engineering
SAMMANFATTNING

Tvärbundna filmer av ε-kaprolakton (CL) och 1,5-dioxepan-2-on (DXO) har framställts med 2,2'-bis-(-ε-kaprolakton-4-yl)propan (BCP) som tvärbindare och Sn(Oct)$_2$ som katalysator. Monomersammansättningen och tvärbindningstättheten har varierats och reaktionsparametrarna undersöks för att optimera filmbildnings parametrarna. De bildade nätverken var elastiska, lätta att gjuta och lätta att avlägsna ur sina former. CL-haltens och tvärbindningstätthetens inverkan på de slutliga egenskaperna hos nätverken har undersökt. De termiska-, mekaniska- och ytegenskaper hos filmerna kan kontrolleras via monomersammansättningen samt via tvärbindningstätthen. Preliminära celltillväxtsstudier visar att materialen har potential för användning inom vävnadsersättning. För att undvika användandet organometalliska katalysatorer i syntesen av poly(1,5-dioxepan-2-on) (PDXO) har enzymet lipas CA (från Candida antarctica) använts som "biokatalysator" för ringöppningspolymerisationen (ROP) av DXO. Ett lineärt förhållande mellan molekylvikten och monomeromsättning observerades, vilket visar att produktens molekylvikt kan kontrolleras via reaktanternas stöckiometri. Reaktionen var av första ordningen med avseende på monomerens stöckiometri och ingen terminering förekom. Inverkan av vattenhalten, enzymkoncentrationen och polymerisationstemperaturen på monomeromsättningen och polymerens egenskaper har undersökt. En initial aktivering genom uppvärmning av enzymet var tillräcklig för att påbörja polymerisationen. Åndfunktionalisera- och triblockpolyestrar syntetiserades med lipas CA-katalysert ROP av DXO och CL med en alkohol som initiatör. En dubbelbindning i polyesterns kedjéända har introducerats genom användandet av en alkohol med en omättnad som initiatör. Dubblebindningen utgör sedan en användbar reaktionsväg för att syntetisera kamformade polymerer. Dihydroxyföreningar har använts som makroinitiatorer för syntetisering av triblockpolyestrar. Sammanfattningsvis, enzymkatalysert polymerisation av laktoner har påvisats vara en användbar metod för syntetiseringen av metallfria polyestrar.

Nyckelord: polyestrar, 1,5-dioxepan-2-on, ε-kaprolakton, 2,2'-bis-(-ε-kaprolakton-4-yl)propan, Sn(Oct)$_2$, enzym, lipas CA, ringöppningspolymerisation, arkitektur, nätverk, vävnadsrekonstruktion
LIST OF PAPERS

This thesis is a summary of the following papers:

- “Potential tissue implants from the networks based on 1,5-dioxepan-2-one and \( \varepsilon \)-caprolactone”, Natalia Andronova, Rajiv K. Srivastava, Ann-Christine Albertsson, *Polymer*, **2005**, 46, 6746-6755


- “Enzyme catalyzed ring-opening polymerization of 7-member ring lactones leading to terminal-functionalized and tri-block polyesters”, Rajiv K. Srivastava, Ann-Christine Albertsson, accepted in *Macromolecules*

and following review:

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ABBREVIATIONS

AFM  Atomic Force Microscopy
BCP  2,2’-Bis-(ε-caprolactone -4-yl) propane
CL  ε-Caprolactone
DS  Degree of Swelling
DSC  Differential Scanning Calorimetry
DXO  1,5-Dioxepan-2-one
FTIR  Fourier Transform Infra-red Spectroscopy
mCPBA  meta-Chloroperoxybenzoic acid
N435  Novozyme 435, Lipase-CA (Candida antarctica)
NMR  Nuclear Magnetic Resonance
PCL  Poly(ε-caprolactone)
PCL-diol  Dihydroxy Terminated Poly(ε-caprolactone)
PDXO  Poly(1,5-dioxepan-2-one)
PEG  Poly(ethylene glycol)
ROP  Ring Opening Polymerization
SEC  Size Exclusion Chromatography
TGA  Thermogravimetric Analysis

SYMBOLS

DP  Degree of Polymerization
ΔH_f  Heat of Fusion
ΔH_0^f  Heat of Fusion of 100% crystalline polymer
[I]  Initiator concentration (moles)
 k_{app}  Apparent Rate Constant
[M]  Monomer concentration (moles)
[M]_0  Initial Monomer concentration (moles)
M_n  Number Average Molecular Weight
M_w  Weight Average Molecular Weight
T_c  Crystallization Temperature
T_g  Glass Transition Temperature
T_m  Melting Point
W  Final Weight
W_0  Initial Weight
W_c  Crystallinity
1. PURPOSE OF THE STUDY

The ability to precisely control the synthesis of advanced macromolecular structures has been an important objective in the polymer research during the last decade. The development of macromolecules with strictly defined structures and properties, aimed specifically at biomedical applications, lead to complex and advanced architectures and diversification of biodegradable polymers. Among them aliphatic polyesters and poly(ether-esters) have a leading position as compared to the others, because of their hydrolytic degradation to non-toxic hydroxy-carboxylic acids which can be metabolized in the human body and their synthesis with controlled molecular architecture, stereochemistry and molecular weight distribution.

Extensive research has been done to improve and develop different polymerization methods to synthesize aliphatic polyesters and out of them ring-opening polymerization of lactones and lactides is one of the most versatile and studied method. Several possible combinations of initiators and catalysts have been evaluated to achieve the desired polymer architecture and properties. The main objective of the present work has been to synthesize aliphatic polyesters of different architectures suitable for biomedical applications. The specific purposes of the thesis includes

- To develop cross-linked networks based on 1,5-dioxepan-2-one (DXO) and ε-caprolactone (CL) for biomedical applications using traditional organometallic catalyst.
- To evaluate the enzyme-catalyzed ring-opening polymerization method as an alternative route to synthesize aliphatic polyesters based on DXO and CL. The method will eliminate the contamination of synthesized polymers with metallic residues from organometallic catalysts used until now and bring a “green chemistry” appeal with it.
- To synthesize functionalized and block aliphatic polyesters via enzyme-catalyzed ring opening polymerization of DXO and CL.
2. INTRODUCTION

2.1 Background

Aliphatic polyesters prepared by ring-opening polymerization (ROP) of lactones and lactides, are versatile polymers having good mechanical properties, hydrolyzability and biocompatibility. These attributes make them a leading candidate in biomedical and pharmaceutical industries as a resorbable implant material and a vehicle for controlled drug delivery. Earlier studies on aliphatic homopolyesters and copolyesters in the 1960s were aimed at developing materials for surgical implants and tissue repair. American Cyanamid Co. first developed resorbable sutures from polyglycolide in 1962 under the trade name of Dexon. A copolyester with composition poly[L-lactide (8%)-co-glycolide (92%)] became available a few years later as a biodegradable suture material under the name Vicryl. Since then aliphatic polyesters have been considered in a variety of medical applications such as prosthetics, artificial skin, dental implants, vascular grafts, pins, bone screws, stents and plates for temporary internal fracture fixation. The use of erodible polymer vehicles for controlled drug delivery was also explored in early 1970s. Systems based on polylactides were investigated for the long term delivery of antimalarial drugs and contraceptives etc. A commercially successful drug delivery system is Lupron Depot, which is intended for endometriosis and prostate cancer therapy.

Research effort has been extended in the past few years to refine the technique of ROP of lactones and lactides so that aliphatic polyesters with controlled architecture and tailor made properties could be prepared. Several review articles describing the mechanism of such polymerization, types of monomers, initiators and catalysts have been published. The main focus of such studies was to enhance the properties of these polymers to meet the stringent requirements of biomedical and pharmaceutical industries. Instead of linear polyesters, more complex architectures such as stars, brushes, cyclics, hyperbranched and cross-linked materials have been synthesized for improving the mechanical properties, hydrophilicity and degradability of aliphatic polyesters and extending the potential application areas of these polymers.
2.2 Ring-opening polymerization of lactones and lactides

One method to synthesize aliphatic polyesters is polycondensation of hydroxy-carboxylic acids. This is the least expensive method, which however, usually gives poor yields and low molecular weights. Furthermore, it is very hard to achieve polymers with well-defined architectures, end groups and composition by polycondensation. The most common method for synthesizing aliphatic polyesters is ROP of lactones or lactides. Carothers et.al. 36 developed this method in 1930s. Many different initiators and catalysts have been developed since then. By ROP it is possible to control properties such as molecular weight, molecular weight distribution and architecture of the polymer. The method also provides the possibility to achieve desired end groups and copolymerization of various monomers, depending upon the initiator or catalyst system.

The polymerization of lactones is generally carried out in bulk or in solution (THF, dioxane, toluene etc.), emulsion 37 or dispersion 38. Many organometallic compounds such as metal-oxides, carboxylates and alkoxides are effective catalysts for the controlled ROP of lactones. Scheme 2.1 presents the reaction pathway for the ROP of lactones. Each macromolecule formed generally contains one chain end terminated with a functional group originating from the termination reaction and the other end-capped with a functional group from the initiator. By altering the catalyst or initiator and the termination reaction, the nature of the functional groups can be varied which plays an important role in both the thermal and hydrolytic stability of the resulting polyester 39-41. Functional groups accessible to post polymerization reactions can also be introduced into the polymer structure in this way.

\[
\begin{align*}
\text{ROP of lactones} \\
\begin{array}{c}
\text{M} - \text{O} - \text{R'} \\
\text{R = (CH}_2\text{n)}
\end{array}
\end{align*}
\]

Depending on the initiator, the polymerization proceeds according to three different reaction mechanisms, i.e. cationic, anionic or coordination-insertion mechanism 17,20,21.

2.2.1 Cationic ROP

4-, 6- and 7-membered ring lactones form polyesters when reacted with cationic catalysts 42-44. The cationic ROP involves the formation of a positively charged species which is subsequently attacked by the monomer. The attack results in ring-opening of the positively charged species through an S_N2 type process (Scheme 2.2). The cationic polymerization is difficult to control and often only low molecular weight polymers are formed.
2.2.2 **Anionic ROP**

Anionic ROP of lactones takes place by the nucleophilic attack of a negatively charged initiator on the carbonyl carbon or on the carbon atom adjacent to the acyl oxygen of the monomer, resulting in a linear polyester (Scheme 2.3). The propagating species is negatively charged and counter-balanced with a positive ion. Depending on the nature of the ionic propagating chain end and the solvent, the reacting complex can vary from completely ionic to almost covalent.

Jedlinski and coworkers developed living anionic ROP method of 4- and 5- membered ring lactones leading to well defined polymers and copolymers of high molecular weight. The anionic ring opening of 4-membered rings (β-lactones) occurs through alkyl- or acyl-oxygen cleavage giving a carboxylate or alkoxide respectively. Larger lactones, such as ε-caprolactone, react only by an attack of the anion on the carbonyl carbon atom with acyl-oxygen scission and the formation of an alkoxide as the growing species.

2.2.3 **Coordination-insertion ROP**

The pseudo anionic ROP is often referred to as coordination-insertion ROP, since the propagation proceeds by the coordination of the monomer to the active species and then insertion of the monomer into the metal-oxygen bond by rearrangement of the electrons.
Scheme 2.4 shows a coordination-insertion mechanism. The growing chain remains attached to the metal through an alkoxide bond during the propagation. The reaction is terminated by hydrolysis forming a hydroxyl end-group. Macromers having end groups for post polymerization reactions can be produced using functional alkoxy-substituted initiators.

\[ M-OR' + O\overline{\bigcirc}R \rightarrow R'O\overline{\bigcirc}M\overline{\bigcirc}OR \rightarrow M\overline{\bigcirc}ORR' \]

\( R = (\text{CH}_2)_n \)

Scheme 2.4: Coordination-insertion ROP of lactones

The coordination-insertion type of polymerization has been thoroughly investigated since it yields well-defined polyesters through living polymerization.

### 2.3 Monomers

The most widely used lactones or lactides in ROP includes D- and L-lactide (LA), glycolide (GA) and \( \varepsilon \)-caprolactone (CL) (Scheme 2.5) due to their suitable hydrolyzability and biocompatibility as polymers. In the human body these polymers degrade through hydrolysis into their corresponding hydroxy acids. The hydroxy acids can be metabolized, which makes the polymers suitable as resorbable implant materials. Their application ranges from sutures to bone fixation. However, the homopolymers are all semi-crystalline and therefore offer only a limited range in tensile properties. Consequently, extensive work has been performed on the monomer 1,5-dioxepan-2-one (DXO). It is valuable complementary monomer, giving a completely amorphous homopolymer. The elastic properties of resorbable materials based on semi-crystalline polymers are improved by copolymerization with DXO.

\[ \text{(a)} \quad \text{(b)} \quad \text{(c)} \quad \text{(d)} \]

Scheme 2.5: Monomers (a) glycolide (b) D- or L-lactide (c) \( \varepsilon \)-caprolactone (d) 1,5-dioxepan-2-one
2.4 Organometallic compounds as catalysts for ROP of lactones and lactides

A large variety of organometallic compounds, i.e. metal-oxides, carboxylates and alkoxides have been studied in order to achieve effective polyester synthesis via ROP of lactones or lactides. Many reactions catalyzed by metal complexes are highly specific, and by careful selection of metals and ligands the reactions can be generated to form a desired polymer structure. The covalent metal oxides with free p or d orbital react as coordination catalysts and not as cationic or anionic catalysts. Several organometallic catalysts based on Zn, Al, Sn and Ge have been explored to carry out the ROP of lactones and lactides. Out of them catalysts based on Sn and Al, in particular Sn(Oct)$_2$ and Al(iPr)$_3$ (Scheme 2.6), have been researched extensively.

![Scheme 2.6: Structures of (a) Sn(Oct)$_2$ and (b) Al(iPr)$_3$](image)

2.4.1 Sn(Oct)$_2$

Sn(Oct)$_2$, commonly referred to as stannous octoate is a frequently used catalyst in the ROP of lactones and lactides. The mechanism of Sn(Oct)$_2$ catalyzed polymerization has been widely discussed and several proposals have been made. The Sn(Oct)$_2$ is not thought to be the actual initiator since the molecular weight does not depend on the monomer-to-Sn(Oct)$_2$ molar ratio. The mechanism is a coordination-insertion mechanism where a hydroxyl functional group is thought to coordinate to Sn(Oct)$_2$, forming the initiating tin-complex.

Investigations of the coordination-insertion mechanism have resulted in two slightly different reaction pathways. In the first proposal the co-initiating alcohol functionality and the monomer both are coordinated to the tin-complex during propagation (Scheme 2.7). In the second proposal the tin-complex is converted into a tin-alkoxide before complexing and ring-opening of the monomer (Scheme 2.8).
Introduction

Scheme 2.7: Sn(Oct)$_2$ catalyzed ROP; complexation of monomer and alcohol prior to ROP

Scheme 2.8: Sn(Oct)$_2$ catalyzed ROP; formation of tin-alkoxide before ROP

2.5 Enzyme-catalyzed polymerization

A vast number of enzymes catalyze metabolic reactions via biosynthetic pathways in living cells. Natural polymers such as polysaccharides, proteins, polyesters etc are synthesized in nature by enzymes. By conservative estimates over $10^{12}$ tons of cellulose, starch and related biomaterials are generated on our planet each year due to natural processes. The annual production of synthetic polymers derived from petroleum feedstock is four to five magnitudes smaller than these natural polymers $^{62}$. The need for developing environmentally friendly processes and products has culminated in alternative routes to generate synthetic polymers and in-vitro enzyme catalysis is one of the most promising options. More than 100 years ago (1894), Emil Fisher proposed a “key and lock” theory as to the specific substrate selectivity by the enzyme, which is presently understood as molecular recognition of the substrate by the enzyme through supramolecular interactions. If the enzymatic reaction takes place in-vivo, it is always involved to recognize the substrate by the enzyme. This is also true for the enzymatic reactions in-vitro. However, the substrate-enzyme relationship is not as strict as the key and lock relationship, as enzymes are dynamic and sometimes very generous in recognizing even unnatural substrates in-vitro $^{63}$. This situation allows enzymes to catalyze the synthesis of not only some natural polymers but also a variety of synthetic polymers.

There has been an exponential increase in research in the area of in-vitro enzyme catalyzed polymerizations, since many families of enzymes can be utilized for transformation of not only their natural substrates but a wide range of unnatural
compounds, yielding a variety of useful materials. All the enzymes are generally classified into six groups and typical polymers produced \textit{in-vitro} with catalysis by respective enzymes are given in table 2.1 \textsuperscript{63}.

Table 2.1: Families of enzymes

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Typical polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidoreductases</td>
<td>Polyphenols, polyanilines, vinyl polymers</td>
</tr>
<tr>
<td>Transferases</td>
<td>Polysaccharides, cyclic oligosaccharides, polyesters</td>
</tr>
<tr>
<td>Hydrolases</td>
<td>Polysaccharides, polyesters, polycarbonates, poly(aminoc acid)s</td>
</tr>
<tr>
<td>Lyases</td>
<td>None</td>
</tr>
<tr>
<td>Isomerases</td>
<td>None</td>
</tr>
<tr>
<td>Ligases</td>
<td>None</td>
</tr>
</tbody>
</table>

2.5.1 \textit{Hydrolases}

Hydrolases are enzymes which catalyze a bond cleavage reaction by hydrolysis. It is generally accepted that an enzymatic reaction is virtually reversible, and hence, the equilibrium can be controlled by appropriately selecting the reaction conditions. On the basis of this view, many hydrolases have been employed as catalysts for the reverse reaction of hydrolysis, leading to polymer production by a bond forming reaction.

2.5.2 \textit{Lipase}

Lipase, a member of the hydrolase family, is an enzyme which catalyzes the hydrolysis of fatty acid esters normally in an aqueous environment in living systems. On the other hand, some lipases are stable in organic solvents and can be used as catalyst for esterifications and trans-esterifications \textsuperscript{64-68}. This specific catalysis enabled production of useful polyesters and polycarbonates by various polymerization modes. Lipases catalyze the ROP of lactones (small to large rings), cyclic diesters (lactides) and cyclic carbonates to produce polyesters or polycarbonates. Enzyme catalyzed synthesis of these polymers is being actively pursued in several laboratories with an aim to get high molecular mass polymers and a summary of lipase catalyzed ROP of different lactones, lactides and cyclic carbonates is presented in table 2.2.
Table 2.2: Lipase catalyzed ring-opening polymerization

<table>
<thead>
<tr>
<th>Monomer</th>
<th>Enzyme</th>
<th>Temp (°C)</th>
<th>Time (h)</th>
<th>Medium</th>
<th>$M_n$ (g/mol)</th>
<th>$M_w$ (g/mol)</th>
<th>Con (%)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 1,3-DXO</td>
<td>PPL</td>
<td>60-120</td>
<td>24-96</td>
<td>Bulk</td>
<td>169000</td>
<td>99</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>100</td>
<td>24</td>
<td>Bulk</td>
<td>25400</td>
<td>97</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LIM</td>
<td>100</td>
<td>24</td>
<td>Bulk</td>
<td>11200</td>
<td>94</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>2 1,3DXO'</td>
<td>AK</td>
<td>80</td>
<td>72</td>
<td>Bulk</td>
<td>6100</td>
<td>97</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>3 MBC</td>
<td>AK</td>
<td>80</td>
<td>72</td>
<td>Bulk</td>
<td>9000</td>
<td>82</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>4 1,4-DXO</td>
<td>N435</td>
<td>60</td>
<td>15</td>
<td>Bulk</td>
<td>41000</td>
<td>69</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>5 IPMD</td>
<td>PPL</td>
<td>100,130</td>
<td>72</td>
<td>Bulk</td>
<td>11500</td>
<td>90</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PS</td>
<td>100</td>
<td>72, 168</td>
<td>Bulk</td>
<td>17500</td>
<td>74</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>6 MDs</td>
<td>PPL</td>
<td>110-130</td>
<td>72, 144</td>
<td>Bulk</td>
<td>6900-15200</td>
<td>0-92</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>7 PL</td>
<td>PPL</td>
<td>60</td>
<td>430</td>
<td>n-hexane</td>
<td>2323</td>
<td></td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>8 BL</td>
<td>PPL</td>
<td>RT</td>
<td>500</td>
<td>Bulk</td>
<td>1045</td>
<td></td>
<td>76</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PPL</td>
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<td>24</td>
<td>Bulk</td>
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**Enzymes**: N435 or CA: Novozyme-435 (Candida Antartica); AK or PF: Pseudomonas flourescens; PS: Pseudomonas species; i-PS: immobilized PS; PPL: Porcine pancreatic lipase; i-PPL: immobilized PPL; AYS or CR: Candida rugosa; PC: Pseudomonas cepacia; CC: Candida cylindracea; A or AN: Aspergillus niger; PR: Penicillium roueorti; RD: Rhizopus delemer; MM: Mucor mehei; SPS: surfactant coated PS; PA: Pseudomonas aeruginosa; **Monomer**: 1,3-DXO: 1,3-dioxane-2-one; 1,3-DXO*: substituted 1,3-DXO; MBC: 5-methyl-5-benzyloxycarbonyl-1,3-dioxane-2-one; 1,4-DXO: 1,4-dioxane-2-one; IPMD: 3(S)-isopropylmorpholine-2,5-dione; MDs: Morpholine-2,5-dione derivatives; PL: β-propiolactone; BL: β-butyrolactone; TMC: trimethylene carbonate; MTMC: 1-methyl trimethylene carbonate; CL: ε-caprolactone; OL: 0-octanolide; DL: δ-decalactone; UDL: undecanolide; DDL: 12-dodecanolide; PDL: ω-pentadecalactone; MMLs: α-methylene macrolides; MVL: α-methylene-δ-valerolactone **Solvents***: toluene, acrylonitrile, dioxane, tetrahydro furan, chloroform, butyl ether, isopropyl ether **Con**: Conversion

#### 2.5.3 Advantages of enzyme-catalyzed polymerization

Enzyme catalysis has provided a new synthetic strategy for useful polymers. It may greatly contribute to global sustainability by using non-petrochemical renewable resources as starting substrates for functional polymeric materials. The organometallic catalysts used for ROP of lactones or lactides are based on derivatives of heavy metals (Zn, Al, Sn or Ge) \(^{56}\), which are toxic in nature. This is of concern in biomedical applications of these polymers as it is difficult to remove the metallic impurities, which may become concentrated within matrix remnants after degradation of the polymer \(^{99}\). Enzymes are non-toxic natural catalysts and, therefore a better candidate for ROP. The enzymatic polymerization, therefore, can be regarded as environmentally friendly synthetic process of polymeric materials, providing a good example to achieve “green polymer chemistry”. The benefits of enzyme-catalyzed polymerization are:

- Enzyme catalyzed reaction proceed under mild reaction conditions i.e. temperature, pressure and pH etc. with high enantio- and regio-selectivity.
- Enzymes can be used in bulk, organic media and at various interfaces.
- Enzymes are derived from renewable resources. They are recyclable eco-friendly non-toxic materials. Therefore the need for their complete removal from the polymers is not that stringent.
- Polymers with well-defined structures may be formed by enzyme-catalyzed process.
Lipases do not require the exclusion of water and air when used as catalysts for polyester synthesis. This is in contrast to traditional chemical initiators where strict precautions are to be taken to exclude air or water from the system.

Small (4-7 membered) cyclic lactones have ring strains and are easily polymerized by organometallic initiators. However polymerization of large ring lactones (macrolides) is slow and only low molecular weight products are obtained. For example traditional chemical catalysts have poor activity for the polymerization of $\omega$-pentadecalactone, (PDL) whereas lipase catalyzed routes to polyPDL have great promise. The unstrained $\gamma$-butyrolactone could not be polymerized using aluminoxane initiators but it could be polymerized using enzymes.

2.6 Macromolecular architecture

The macromolecular design of a polymer regulates its physico-chemical properties. The ability to design and control the macromolecular structure is of utmost importance when new materials are developed. More advanced structures such as stars, combs, brushes, ladders, cyclics, hyperbranched and cross-linked networks (Scheme 2.9) have been synthesized to meet vast demands from different targeted applications of such polymers. As an alternative to linear polymers, such advanced structures, specially cross-linked systems, offer several advantages over linear systems. A more consistent loss of mass over time is observed with cross-linked systems, which can avoid a sudden decrease in strength before the total loss of mass occurs. A broader spectrum of mechanical properties and degradation patterns can also be achieved with cross-linked systems to fulfill the end application requirements. These can be synthesized by copolymerizing the monomer with a multi-functional cross-linking agent or by reacting a low molecular weight prepolymer with a cross-linking agent.

Scheme 2.9: Macromolecular architecture; (a) star (b) comb (c) ladder (d) cyclic (e) brush (f) hyperbranched (g) cross-linked
3. EXPERIMENTAL

3.1 Materials

Tetrahydro-4H-pyrane-4-one (Maybridge Chemical, UK), m-chloroperbenzoic acid (Acros, Belgium), 2,2′-bis-(4-hydroxycyclohexyl) propane, chromium oxide (Aldrich, Germany), acetic acid (Fluka, Sweden), dichloromethane, MgSO₄, NaHSO₃, NaHCO₃, diethyl ether, isopropanol, toluene and acetone (Labora, Sweden) were used as received. ε-Caprolactone (Aldrich, Germany) was dried and distilled over CaH₂ at reduced pressure prior to use. Poly(caprolactone-4-yl) propane (PCL-diol, mol.wt.1250 and 2000 g/mol, Aldrich, Germany), poly(ethylene glycol) (PEG, mol.wt. 1000 and 2000 g/mol, Aldrich, Germany) were used as received. Novozyme 435 (activity approx. 10,000 PLU/g according to the supplier) was donated by Novozyme Inc. The enzyme, Novozyme 435, was dried over P₂O₅ (Merck, Germany) at 0.1 mm Hg for 42 hours whenever required before use. Stannous octoate (Aldrich, Germany) was distilled under reduced pressure. BCP and DXO were synthesized in the lab as described in the following sections.

3.2 Synthesis of 1, 5-dioxepan-2-one

1,5-dioxepan-2-one (DXO) was synthesized from tetrahydro-4H-pyrane-4-one through Bayer-Villiger oxidation according to the method reported by Mathisen et. al. The 1,5-dioxepan-2-one obtained was purified by recrystallization from diethyl ether and two subsequent distillations under reduced pressure. Finally the monomer was dried over CaH₂ overnight and distilled under reduced pressure. ¹H-NMR δ(ppm): 4.25 (t, 2H, -CH₂-OCO-), 3.85 (t, 2H, -CH₂-CH₂-OCO-), 3.78 (t, 2H, -CH₂-CH₂-COO-), 2.85 (t, 2H, -CH₂-COO-)

3.3 Biodegradable networks

3.3.1 Synthesis of 2,2′-bis-(ε-caprolactone-4-yl) propane (BCP)

BCP was synthesized according to scheme 3.1 by a modified approach to the procedure given elsewhere. An isomeric mixture of 2,2′-bis-(4-hydroxycyclohexyl) propane (30g) was dissolved in 240 ml of acetic acid in a RB flask kept at 10°C in a water-ice
bath. 37.2 g of CrO$_3$ was dissolved in a mixture of 200 ml of acetic acid and 40 ml of water, and added drop-wise to the flask over a period of 1 hour under continuous stirring. After 30 minutes, 400 ml of isopropanol was added to the mixture and stirred overnight. The mixture was concentrated under reduced pressure and the resulting thick solution was precipitated in water. The precipitated white powder was recrystallized from toluene and the di-ketone, 2,2'-bis-(4-cyclohexanone) propane, was obtained after filtration and drying (yield = 76%). A Baeyer-Villiger oxidation was then performed on the di-ketone. 176.7 g of m-chloroperbenzoic acid (m-CPBA) was dissolved in 2000 ml of CH$_2$Cl$_2$ and, after removal of the aqueous phase; it was cooled to 10°C in a water-ice bath. 22 g of di-ketone was dissolved in 333 ml of CH$_2$Cl$_2$ and added drop-wise over a period of 1 hour to the m-CPBA solution with continuous stirring. The mixture was stirred overnight. The acid was neutralized by sodium bisulphate-sodium bicarbonate treatment and the resulting organic phase was concentrated to a white powder under reduced pressure. The white powder obtained was finally recrystallized from acetone and dried under vacuum (yield = 70%). $^1$H-NMR δ (ppm): 4.36 (R,R), 4.16 (S,R) (t, 2H, -CH$_2$OOC-), 2.74 (R,R), 2.58 (S,R) (t,2H, -CH$_2$COO-), 1.95 (q, 2H, -CH$_2$-C-OOC-), 1.59 (q, 2H, -CH$_2$-CH$_2$-COO-) 1.39 (m, 1H, -CH$_2$-CH-CH$_2$-), 0.80 (3H, CH$_3$-)

Scheme 3.1: Synthesis of BCP

### 3.3.2 Preparation of cross-linked films

Networks were produced by ring-opening polymerization of DXO or CL or their mixture using BCP as cross-linking agent in the presence of stannous octoate (Sn(Oct)$_2$) as a catalyst. Monomer(s), BCP and Sn(Oct)$_2$ were weighed in a flask and the mixture was dissolved in a small amount of chloroform and spread over a pre-silanized petri-dish. The chloroform was evaporated under a nitrogen atmosphere and the resultant well-spread mixture was kept in an oven for cross-linking at 140°C for an initial 1.5 hours and finally at 180°C for 30 min. Both homo- and co-polymers containing different mole fractions of CL and DXO and cross-linking agent were prepared. The theoretical cross-linked density $\rho$ (%) was calculated according to the equation (3.1):

\[
\rho(\%) = \frac{M_r \times X}{M_c} \times 100
\]
\[ \rho = \frac{2n}{2n + m} \times 100 \]  
Equation (3.1)

Where \( n \) is the mole fraction of cross-linking agent and \( m \) is the mole fraction of monomer(s).

### 3.4 Enzyme-catalyzed polymerization of DXO and CL

#### 3.4.1 Bulk polymerization of DXO with Lipase-CA

The polymerization was carried out in bulk in a 10 ml round-bottom flask containing a Teflon-coated magnetic bar and a three-way opening equipped with a butyl rubber septum to enable samples to be taken out during the polymerization under nitrogen flushing. DXO and Lipase-CA (5.0 wt\% of monomer) were transferred to the flask under a nitrogen atmosphere in a glove-box (MBraun MB 150B-G-I, Germany). The flask was then sealed with the three-way opening and kept in an oil bath at 60°C under continuous stirring for 4 hours. Samples for \(^1\)H-NMR, size exclusion chromatography (SEC) and differential scanning calorimetry (DSC) were withdrawn with the help of a flamed syringe under nitrogen flushing, at regular intervals. Each sample was divided into five parts. One part for \(^1\)H-NMR analysis was dissolved in CDCl\(_3\) and then separated from the enzyme by filtration using a membrane filter of pore size 0.45 \( \mu \)m. The second part for SEC analysis was dissolved in dimethylformamide (DMF) and separated from the enzyme by filtration through a 0.45 \( \mu \)m membrane filter. The third part was dissolved in the minimum necessary amount of chloroform and filtered through a 0.45 \( \mu \)m membrane filter to separate the enzyme. The filtrate was vacuum dried before analysis by DSC. The remaining two parts were left for 24 hours at room temperature under air and then analyzed by \(^1\)H-NMR and SEC as described above. The residual portion of the reaction mixture was dissolved in a small amount of chloroform, the enzyme was removed by filtration and the filtrate was dried under vacuum to obtain the final polymer. A control experiment (without enzyme) was also carried out to determine the thermal polymerization of DXO under similar reaction conditions.

#### 3.4.2 Bulk polymerization of CL with Lipase-CA

In order to compare the behavior of DXO with another seven-member ring lactone, \( \varepsilon \)-caprolactone (CL) was polymerized with Lipase-CA (5.0 wt \% of monomer) at 60°C for 4 hours. The polymerization was carried out under identical conditions as mentioned above for DXO and samples were analyzed by \(^1\)H-NMR and SEC.

#### 3.4.3 Effect of reaction water content

The water content during the polymerization was varied from 100 to 400 ppm in the standard formulation of DXO and Lipase-CA (5.0 wt% of monomer) by the addition of demineralized water with the help of a syringe under nitrogen atmosphere. All polymerizations were performed at 60°C for 4 hours. Samples were analyzed immediately by \(^1\)H-NMR, SEC and DSC by dissolving them in CDCl\(_3\), DMF and CHCl\(_3\).
respectively, followed by filtration through 0.45 μm membrane filter to remove the enzyme. The DSC sample was vacuum dried prior to analysis. All polymerizations were terminated by dissolving the residual reaction mixture in chloroform and removing the enzyme by filtration. Filtrates were dried under vacuum to obtain the final polymers.

3.4.4 Effect of enzyme concentration

The Lipase-CA concentration was varied from 0.5 to 2.0, 5.0, 7.0 and 10.0 wt% with respect to monomer, and polymerizations were performed at 60°C for 4 hours following the same procedure as described above. Samples were tested by 1H-NMR, SEC and DSC immediately as mentioned above. The final polymer samples were obtained by dissolving the residual reaction mixture in chloroform, removing the enzyme by filtration and drying the filtrates under vacuum.

3.4.5 Effect of temperature

In the standard formulation of DXO and Lipase CA (5.0 wt% of monomer) the polymerizations were performed at different temperatures (40, 60, 80, 100 and 120°C) for 4 hours. Samples were analyzed and final polymers were obtained following the same procedures as described above.

3.5 Terminal-functionalized and tri-block polyesters

3.5.1 Polymerization of DXO with Lipase-CA using alcohol as initiator

All the glassware was silanized, flame-dried and stored under a nitrogen atmosphere at 0 ppm moisture in a glove-box. In a typical polymerization, the lactone, enzyme (5.0 wt% of monomer) and alcohol were weighed in a round-bottom flask inside the glove-box. The flask was sealed and immersed in an oil bath at 60°C for 2 hours under continuous stirring. After 2 hours, when the viscosity of the reaction mixture became very high and the magnetic bar stopped stirring, the flask was removed from the oil-bath and a sample of the reaction mixture was taken for 1H-NMR analysis to determine the percentage monomer conversion. The polymer was obtained by dissolving the reaction mixture in a small amount of chloroform followed by precipitation in excess cold hexane. The polymer was dried under vacuum before analysis. Different series of polymers with different degrees of polymerization were synthesized with each initiator.

3.5.2 Polymerization of CL with Lipase-CA using alcohol as initiator

A procedure similar to that described above for DXO was used to polymerize CL with different alcohols as initiators, except that the polymerization was performed for 4 hours at 60°C. The percentage monomer conversion was determined by 1H-NMR analysis of the crude reaction mixture. The polymer was obtained by dissolving the reaction mixture in a small amount of chloroform followed by precipitation in excess cold hexane. The polymer was dried under vacuum before characterization.
4. CHARACTERIZATION & METHODS

4.1 Nuclear Magnetic resonance (NMR)
Monomer conversion and degree of polymerization were determined by $^1$H-NMR (400 MHz Bruker Avance) using CDCl$_3$ as solvent and tetramethyldisilane as internal standard.

4.2 Size Exclusion Chromatography (SEC)
The molecular weight of polymers was determined by size exclusion chromatography (SEC). Dimethylformamide (DMF) was used as eluent at a flow rate of 1.0 ml/min. The injection volume was 50µL. A Waters 717 plus auto sampler and a Waters model M-6000A solvent pump equipped with a PL-EMD 960 light scattering evaporative detector, two PL gel 10-mm mixed B columns (300 x 7.5 mm) from Polymer Laboratories and one Ultrahydrogel linear column (300 x 7.8mm) from Waters, connected to an IBM-compatible PC was used. Narrow molecular weight polystyrene standards were used for calibration. Millenium version 3.20 software was used to process the data.

4.3 Thermal Analysis
The thermal properties of the polymers were determined by differential scanning calorimetry (DSC) using a Mettler-Toledo DSC 820 module under a nitrogen atmosphere (nitrogen flow rate 80 ml/min) with a sample mass of 5 ± 1 mg and a heating rate of 5°C/min. The samples were subjected to a heating-cooling-heating cycle from -70°C to 100°C and the analysis was performed on the second heating plot. The relative crystallinity of the PCL segment of different samples was calculated according to the equation (4.1).

$$w_c = \frac{\Delta H_c}{\Delta H_{f}} \times 100$$  
Equation (4.1)
where \( w_c \) is the crystallinity, \( \Delta H_f \) is the heat of fusion of the sample, and \( \Delta H_f^0 \) is the heat of fusion of 100% crystalline PCL. The value of \( \Delta H_f^0 \) used for the calculations was 139.5 J/g \(^{101}\).

Thermal stability was evaluated by thermo-gravimetric analysis (TGA) under a nitrogen atmosphere (nitrogen flow rate 50ml/min) with a sample mass of 10 ± 1 mg and a heating rate of 10°C/min.

### 4.4 FTIR

FTIR measurements were performed on a Perkin-Elmer 2000X FTIR spectrometer, equipped with a golden gate single reflection ATR unit with a diamond crystal. The spectra were taken as an average of 30 scans at a resolution of 4 cm\(^{-1}\).

### 4.5 Optical microscopy

A polarized optical microscope (Leitz Ortholux POL-BK II) was used to examine the morphology of the samples.

### 4.6 Tensile testing

Tensile testing was performed on an Instron 5566 equipped with pneumatic grips and controlled by a Dell 466/ME personal computer. The tensile measurements were made with a crosshead speed of 50 mm/min and an initial grip separation of 32 mm. The samples had dimensions of 80x5 mm and a thickness of approximately 0.5 mm. The average thickness of each sample was calculated from five independent measurements with a Mitutoyo micrometer. The samples were preconditioned for 48 h at 50 ± 5% RH and 23 ± 1°C. Five different samples from the same film were tested for each network. All tests were carried out in accordance with ASTM D882-95A.

### 4.7 Hydrophilicity evaluation

The static contact angles were measured on the air side of the cross-linked films with a Ramé Hart goniometer using the sessil drop technique. Deionized water was used (Millipore, resistivity: 18.4 MΩ cm). The static contact angle was calculated from the mean value of at least 8 contact angle measurements at four different positions on the surface.

### 4.8 Swelling

The degree of swelling of the network was determined gravimetrically. A piece of network film was weighed and kept in a sealed beaker containing chloroform. At regular intervals, the film was taken out; the excess solvent was removed from the
surface with the help of a tissue paper. The film was then weighed and returned to the medium. This procedure was continued until a constant weight was attained. The equilibrium degree of swelling (DS) was calculated according to equation (4.2).

\[ DS = \left(\frac{W - W_0}{W_0}\right) \times 100 \]  

Equation (4.2)

where \( W_0 \) is the initial weight of the dry sample and \( W \) is the final weight of the swollen sample.

### 4.9 Atomic Force Microscopy (AFM)

The surface topographies of the cross-linked films were analyzed with an atomic force microscope (AFM), CSM Instruments Nano indentor with combined Atomic Force Microscope. The analysis was performed in dynamic contact mode in air using a Pointprobe plus probe with a nominal spring constant of \( \sim 46.5 \text{ N} \cdot \text{m}^{-1} \) and a resonance frequency of 181-200 kHz. The length of the cantilever was 223 nm. Image analysis was performed in CSM Instruments ImagePlus v. 3.1.10.
5. Networks

Biomedical applications such as tissue implants, artificial cartilages and skin require strong, elastic and tough polymers, which can withstand the specific requirement of these uses. Poly (1,5-dioxepan-2-one) (PDXO) is an amorphous, sticky material with low impact strength, while poly (ε-caprolactone) (PCL) is a semi-crystalline and tough polymer. When 1,5-dioxepan-2-one (DXO) and ε-caprolactone (CL) are cross-linked during their ring-opening polymerization using 2,2’-bis-(ε-caprolactone-4-yl) propane (BCP) as the cross-linking agent, a strong elastomeric polymer network is formed, where tetra-functional cross-links are incorporated into the growing polymer chains (scheme 5.1).

![Scheme 5.1: Network formation of DXO and CL using BCP](image)

The experimental procedure for such a cross-linking reaction was developed after thorough scouting runs on reaction conditions, particularly the cross-linking
Results and Discussion

Cross-linking could be performed under relatively milder conditions than previously observed with DXO and BCP alone\(^3\), and this avoided the thermal oxidation of the material during cross-linking, which was indicated by the generation of colorless films. The molar ratio of co-monomers was varied from 0.2 to 1.0 with respect to CL. For the films having CL molar fraction at 0.2, the theoretical cross-link density was changed from 10 to 40%. The details of composition with the designations of the cross-linked films are given in Table 5.1. It was observed that BCP dissolved in caprolactone and this meant that it was not necessary to use chloroform as solvent. Cross-linking of PCL and PDXO with BCP under the described conditions led to the formation of smooth, homogeneous, colorless and elastic films which could easily be removed from the mould (Figure 5.1). Introduction of CL gave more resilient films with improved tear properties. Films with a high DXO content were transparent and flexible and they became slightly opaque and tough when the CL content was increased to 0.8-mole fraction.

<table>
<thead>
<tr>
<th>Polymer designation</th>
<th>Theoretical X-link density (%)</th>
<th>CL</th>
<th>DXO</th>
<th>BCP</th>
<th>Sn(Oct)(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>10</td>
<td>56</td>
<td>222</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>P2</td>
<td>10</td>
<td>111</td>
<td>167</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>P3</td>
<td>10</td>
<td>166</td>
<td>111</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>P4</td>
<td>10</td>
<td>222</td>
<td>56</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>P5</td>
<td>10</td>
<td>279</td>
<td>0</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>P6</td>
<td>20</td>
<td>54</td>
<td>217</td>
<td>34</td>
<td>1</td>
</tr>
<tr>
<td>P7</td>
<td>30</td>
<td>54</td>
<td>218</td>
<td>58</td>
<td>1</td>
</tr>
<tr>
<td>P8</td>
<td>40</td>
<td>55</td>
<td>220</td>
<td>92</td>
<td>1</td>
</tr>
</tbody>
</table>

5.1 \(^1\)H-NMR

The consumption of monomer and incorporation of polymer into cross-linked network was monitored by \(^1\)H-NMR by following the intensity of the resonance signals of oxymethylene protons of monomer (CL or DXO) and polymer (PCL or PDXO) as a function of time. For example, in figure 5.2, the intensity of the peak at 4.16 ppm due to oxymethylene protons (-O-CH\(_2\)-) of CL monomer decreased with time and finally disappeared after 2 hours, indicating that the monomer was completely polymerized. The resonance signals due to the oxymethylene protons of polymer (PCL) appeared at 3.99 ppm. The intensity of these signals relative to the oxymethylene protons of the
monomer increased with increasing polymerization time. Once the polymer was completely cross-linked (after 2 hours), no free polymer was available to be dissolved in CDCl$_3$ and analyzed by NMR and therefore no proton signal due to PCL was observed.

Figure 5.1: Easy to remove, flexible, elastic and transparent film of cross-linked DXO-CL

![Figure 5.1](image1.png)

![Figure 5.2](image2.png)

Figure 5.2: $^1$H-NMR spectra of reaction mixture during cross-linking of CL with BCP: (A) 0 hours; (B) 30min; (C) 1 hour; (D) 1.5 hours; (E) 2 hours; (F) 2.5 hours
A similar trend was observed with DXO, as shown in figure 5.3. The peaks due to DXO monomer disappeared with time indicating complete monomer conversion. The intensity of peaks due to PDXO first increased and then decreased with time as more and more of polymer was getting incorporated into the cross-linked network and therefore it was not soluble in CDCl$_3$ to be analyzed by the NMR.

Figure 5.3: $^1$H-NMR spectra of reaction mixture during cross-linking of DXO with BCP: (A) 30 min; (B) 1 hour; (C) 1.5 hour; (D) 2 hours; (E) 2.5 hours

Figure 5.4 shows the $^1$H-NMR results when CL and DXO were cross-linked together in a 20:80 molar ratio. A similar trend to that of the individual monomer cases was observed. Peaks due to both monomers disappeared with time indicating almost complete monomer conversion. The intensity of the peaks due to poly(ɛ-caprolactone-co-1,5-dioxepan-2-one) [poly(CL-co-DXO)] first increased and then decreased due to non-availability of free polymer for analysis by NMR as the cross-linking reaction progressed. The proton resonance signals appeared at the same positions as were observed for the monomers (CL and DXO) and cross-linked homopolymers (PCL and PDXO).
5.2 Thermal Analysis

The DSC results showed a single glass transition temperature ($T_g$) for all the cross-linked copolymers, which was somewhere between the $T_g$ of the respective homopolymers of PDXO (-39°C) and PCL (-60°C), indicating the formation of relatively random copolymers. This is in agreement with the results obtained from the bulk polymerization of CL and DXO, which formed ideal copolymers due to the similar reactivity ratios of the two co-monomers. The molecular architecture including molar mass, degree of cross-linking and chain branching also affects the $T_g$. Cross-links reduce the available free volume and the $T_g$ is thus expected to increase with increasing cross-linking density, and an increase in $T_g$ from −39.6°C to −33.6°C was observed when the cross-link density was increased from 10 to 40%. The approximate crystallinity of the copolymers was calculated with respect to 100% crystalline PCL.
Results and Discussion

DSC results and $T_g$ values predicted by the FOX equation (equation 5.1) are summarized in table 5.2.

$$\frac{1}{T_g} = \frac{W_{CL}}{T_{g,CL}} + \frac{W_{DXO}}{T_{g,DXO}}$$

Equation (5.1)

where $W_{CL}$ and $W_{DXO}$ are the weight fractions of CL and DXO and $T_g$, $T_{g,CL}$, $T_{g,DXO}$ are the glass-transition temperatures of network, PCL and PDXO, respectively.

Table 5.2: Thermal properties of cross-linked CL-DXO-BCP films

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$T_g$ (°C)</th>
<th>$w_c$ (%)</th>
<th>$T_g$ (°C) (FOX)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>-41.2</td>
<td>0.0</td>
<td>-39.2</td>
</tr>
<tr>
<td>P2</td>
<td>-46.8</td>
<td>0.0</td>
<td>-43.0</td>
</tr>
<tr>
<td>P3</td>
<td>-52.3</td>
<td>0.0</td>
<td>-47.6</td>
</tr>
<tr>
<td>P4</td>
<td>-56.9</td>
<td>1.0</td>
<td>-53.5</td>
</tr>
<tr>
<td>P5</td>
<td>-62.5</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td>P6</td>
<td>-39.6</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>P7</td>
<td>-34.7</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>P8</td>
<td>-33.5</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

A representative thermo-gravimetric analysis trace of cross-linked copolymers of CL and DXO is shown in figure 5.5. The characteristic decomposition temperatures and percentage weight losses depend on the backbone structure of the copolymers.

Figure 5.5: Thermogravimetric trace of cross-linked CL:DXO with BCP (sample P2): TGA (—), DTG (---)
As can be seen in figure 5.5, the largest weight loss occurred in the temperature range of 250°C to 350°C. Within this temperature range, the temperature of the maximum rate of weight loss $T_{\text{max}}$ and the percentage weight loss at $T_{\text{max}}$ were determined from the differential thermo-gravimetric (DTG) traces. They are plotted against CL content in poly(CL-co-DXO) copolymers having 10% cross-link density in figure 5.6. A linear relationship between the $T_{\text{max}}$ or the percentage weight loss and the CL content was observed, which can be utilized to determine the copolymer composition from thermo-gravimetric traces. A linear relationship was also observed when $T_{\text{max}}$ and the percentage weight loss were plotted against cross-link density of the networks, as shown in figure 5.7. An increase in CL content led to more stable polymers, as shown by the increase in $T_{\text{max}}$ and decrease in percentage weight loss in figure 5.6. This was due to the introduction of crystalline domains, which accounted for the increased thermal stability of films with a high CL content.

![Graph 5.6](image1)

Figure 5.6: Effect of CL content on decomposition temperature, $T_{\text{max}}$ (■) and percentage weight loss (◊) of networks of CL and DXO having 10% theoretical cross-link density

![Graph 5.7](image2)

Figure 5.7: Effect of cross-link density on decomposition temperature, $T_{\text{max}}$ (■) and percentage weight loss (◊) loss of networks of CL and DXO (molar ratio 20:80)
5.3 Tensile properties

In the copolymers of CL and DXO cross-linked with BCP, the modulus increased gradually with increasing CL content. On the other hand, a decrease in elongation at break was observed (Figure 5.8). These findings were attributed to the semi-crystalline nature of the PCL segments present in the network. When only PCL cross-linked with BCP was tested, a modulus of 0.1 GPa and an elongation at break of 440 % were obtained. As expected, an increase in cross-link density led to an increase in the modulus and a decrease in the elongation at break, as shown in figure 5.9.

![Figure 5.8: Effect of CL content on tensile properties; Youngs modulus (■) and elongation at break (◊) in networks of CL-DXO having 10% theoretical cross-link density](image1)

![Figure 5.9: Effect of cross-link density on tensile properties; Youngs modulus (■) and elongation at break (◊) in cross of CL and DXO (molar ratio 20:80)](image2)
5.4 Hydrophilicity evaluation

It has been observed in previous study that higher the CL content, the more hydrophobic the surface will be. As shown in figure 5.10, a linear increase in contact angle was observed when the CL content was increased from 0.2 to 1.0 mole fraction. The hydrophilicity of the materials can thus be tailored by changing the CL content. A slight increase in the contact angle was observed with higher cross-link density (Figure 5.10). This was due to the presence of BCP, which increases the hydrophobicity of the network.

![Graph showing the relationship between CL content and contact angle.](image)

Figure 5.10: Effect of CL content (with 10% cross-link density) and cross-link density (with CL and DXO molar ratio of 20:80) on contact angle in cross-linked films of CL-DXO.

5.5 Swelling

With increase in CL content, a decrease in degree of swelling was observed, as shown in figure 5.11. This was again due to semi-crystalline nature of CL where the diffusivity of solvent molecules is less, leading to lower swelling values. The degree of swelling was expected to decrease with increasing cross-link density, since there was less space available for swelling of the network. As shown in figure 5.11, the expected trend was observed till the theoretical cross-link density was increased to 30%. Although a further increase in cross-link density led to an increase in the degree of swelling. This could be explained by the inhomogeneous cross-linking happened in this case due to very high amount of BCP. Since the amount of CL was very small in this film the solubility of BCP is greatly affected as it is more soluble in CL than DXO.

5.6 Morphology

PCL is a semi-crystalline polymer while PDXO is an ether-containing polyester which is totally amorphous. Results from thermal analysis showed a single T_g indicating that
the cross-linking of CL and DXO with BCP generates random copolymers. When the CL content was increased above a certain level, there was an expected tendency for PCL blocks to be formed that can crystallize in these cross-linked networks. Microcrystalline domains could be observed in photomicrographs of samples having a CL content of 80% or more. Below 80%, no significant features due to crystallinity could be seen. These crystalline domains were responsible for a gradual increase in the mechanical properties of networks with increase in CL content.

### 5.7 Surface topography

These cross-linked copolymers were developed as scaffolds for tissue engineering applications and it was therefore necessary to evaluate the surface properties of the networks. Surface topography is a crucial aspect, which is required to be at an optimal level for cell growth and proliferation, since cells adhere differently to different surfaces depending upon the topography. In order to have a better understanding of height variations on surface of the cross-linked network, atomic force microscopy measurements were made on series of cross-linked copolymers having cross-link density of 10% (P1 to P5). It was deduced from the surface analysis that roughness of the surface increased with higher CL content. This trend is shown in three-dimensional images of polymers P3, P4 and P5 in figure 5.12. Densely populated hills were observed in P5 and the surface became smoother from higher to lower CL content. On the other hand, the surface roughness was slightly increased in copolymer P1 as sparsely and unevenly distributed hills could be observed. This was due to the localized presence of cross-link points from BCP, which was possible as BCP has lower solubility in DXO and therefore tend to remain in the CL rich areas.
Figure 5.12: AFM topographical three-dimensional image of networks of CL-DXO having 10% cross-link density: (a) CL content 1.0 mol fraction (P5) (b) CL content 0.8 mol fraction (P4) (c) CL content 0.6 mol fraction (P3)
6. ENZYME-CATALYZED POLYMERIZATION OF DXO AND CL

Novozyme 435 is a Lipase from Candida antarctica produced by submerged fermentation of a genetically modified Aspergillus oryzae microorganism and absorbed on a microporous resin. It consists of bead-shaped particles with a diameter in the range of 0.3-0.9 mm. The bulk density of Novozyme 435 is approximately 0.43 g/cm$^3$ and it has a water content of 1-2 % w/w. Besides having a high polymerization activity for lactones, Novozyme 435 or lipase-CA also offers the merit of being a metal-free catalyst which can be easily removed from the final product by filtration and does not release any toxic metallic residues. Our interest in PDXO builds on the versatility of this polymer as a component in random and block copolymers and in cross-linked systems suitable for biomedical and pharmaceutical application. The bulk polymerization of DXO was performed at 60°C for 4 hours, under a nitrogen atmosphere using Lipase-CA (5.0 wt% with respect to monomer) as biocatalyst. The kinetic aspects of polymerization were checked under these conditions. DXO was a liquid at 60°C and the polymerization system could be described as a suspension of enzyme beads in DXO. An increase in viscosity was observed with time, as a typical characteristic of bulk polymerization. After 4 hours, samples could not be withdrawn for analysis due to very high viscosity of the reaction mixture and for this reason the polymerization time was set at 4 hours for all the experiments.

6.1 Bulk polymerization of DXO with Lipase-CA

The first set of experiments was conducted with 5.0 wt% of Lipase-CA (with respect to monomer) at 60°C for 4 hours. Samples were withdrawn at regular intervals for analysis by $^1$H-NMR, size exclusion chromatography (SEC) and differential scanning calorimetry (DSC). Monomer conversion was monitored by $^1$H-NMR, following the disappearance of the resonance signals of oxymethylene protons of DXO as a function of time. The intensity of proton resonance signals due to DXO monomer decreased with time, while the relative intensity of peaks due to PDXO increased with respect to monomer protons (Figure 6.1). The ratio of peaks due to polymer and monomer was used to calculate the percentage conversion of monomer. No thermal polymerization of DXO was observed in the control experiment (without enzyme) under these conditions.
Results and Discussion

Figure 6.1: $^1$H-NMR spectra depicting monomer conversion with respect to time ROP of DXO with Lipase-CA (5.0 wt% with respect to monomer) at $60^\circ$C for 4 hours

$^1$H-NMR results were used to construct a plot of monomer conversion versus time. 78% conversion was observed during the 4 hours of polymerization (Figure 6.2; series A). When a check on the monomer conversion for the samples left for 24 hours at room temperature in air was made, an interestingly high percentage conversion was obtained (Figure 6.2; series B) compared to samples analyzed immediately. For example, the sample taken out at 15 minutes showed a monomer conversion of 18% when analyzed immediately and when it was kept at room temperature for 24 hours in air, a monomer conversion of 54% was observed. Similarly, all the samples taken out at different time intervals showed an increase in monomer conversion when they were left at room temperature in air for 24 hours. As can be seen in the figure 6.2; series B, the final conversion increased from 78% to 92%.

The polymerization was performed in bulk and since the reaction became diffusion controlled as soon as the viscosity was high (after gel point), the rate of monomer conversion was determined from the slope of the best-fit curve during the initial 1 hour of polymerization (before gel point). For the samples analyzed immediately (Figure 6.2; series A), the rate of monomer conversion was 0.48% per minute. When a similar calculation of the monomer conversion was made for the samples left for 24 hours in air at room temperature (Figure 6.2; series B), the rate of monomer conversion was
0.43 % per minute. This showed that the enzyme was still active at room temperature and was able to catalyze the polymerization of DXO. An initial activation by heating the enzyme at a high temperature (60°C) was sufficient to start the polymerization.

![Graph showing conversion of DXO monomer](image)

Figure 6.2: Conversion of DXO monomer when polymerized with Lipase-CA (5.0 wt% with respect to monomer) at 60°C for 4 hours

A plot of \(-\ln ([M] / [M]_0)\) against polymerization time was made from the \(^1\)H-NMR results (Figure 6.3), where \([M]_0\) was the initial monomer concentration and \([M]\) was the monomer concentration at a given polymerization time \((t)\). From this plot, the polymerization kinetics of DXO initiated by 5.0 wt% of lipase-CA at 60°C can be said to follow a first order rate law with respect to monomer, indicating that the number of

![Graph showing -ln ([M] / [M]_0) vs. time](image)

Figure 6.3: Plot of \(-\ln ([M] / [M]_0)\) as a function of polymerization time for ROP of DXO catalyzed by Lipase-CA (5.0 wt% with respect to monomer) at 60°C for 4 hours
Results and Discussion

termination reactions was low and that the number of growing chains was almost constant. The apparent rate constant for polymerization of DXO with 5.0 wt% lipase-CA at 60°C was found to be \(7.1 \times 10^{-3}\) min\(^{-1}\) from the slope of figure 6.3, using the equation: 

\[ K_{app} = \frac{d(-ln[M]/[M]_0)}{dt}, \]

where \(K_{app}\) is the apparent rate constant.

The increase in number-average (M\(_n\)) as well as weight-average (M\(_w\)) molecular weight with time, as determined from SEC, is shown in figure 6.4. M\(_n\) of 38000 g/mol and M\(_w\) of 69000 g/mol were finally obtained. The linearity of the plot of M\(_n\) against percentage conversion of monomer (Figure 6.5) showed that the frequency of trans-esterification reactions during the polymerization was low.

![Figure 6.4: M\(_n\) and M\(_w\) as a function of time during ROP of DXO with Lipase-CA (5.0 wt% with respect to monomer) at 60°C for 4 hours](image)

![Figure 6.5: M\(_n\) as a function of monomer conversion during ROP of DXO with Lipase-CA (5.0 wt% with respect to monomer) at 60°C for 4 hours](image)
When the molecular weight of samples analyzed immediately was compared with that of samples analyzed after 24 hours, the values of the samples analyzed after 24 hours were found to be slightly lower (Figure 6.6). This was possible due to the reversible nature of enzymatic reactions, which led to enzyme-catalyzed hydrolysis of the polymer backbone when the samples were left at room temperature in air for 24 hours.

![Figure 6.6: M_n as a function of polymerization time for ROP of DXO with Lipase-CA (5.0 wt% with respect to monomer) at 60°C for 4 hours](image)

**6.2 Polymerization behavior of DXO and CL under Lipase-CA catalysis**

A comparison of polymerization behaviors of DXO and CL under lipase-CA catalysis was made, where both the monomers have a seven member ring structure. It was found that the lipase-catalyzed polymerization of CL is much slower than that of DXO. The increase in viscosity (gel point) in CL polymerization could only be observed after 3 hours. A time-conversion plot was made to determine the rate of monomer conversion for lipase-CA catalyzed polymerization of CL (Figure 6.7). The rate of monomer conversion (before gel point) in case of CL was 0.20 % per minute which is significantly lower than the rate of monomer conversion in DXO (0.48 % per minute) determined under identical polymerization conditions. The M_n and M_w of PCL synthesized in this way were 22000 g/mol and 31000 g/mol, respectively, as determined from SEC. These values were much smaller than the molecular weight values of PDXO discussed earlier. This difference in the behavior of these two lactones arises due to their specificity towards lipase catalysis. CL may have acted as a poor substrate for the lipase-catalyzed polymerization due to its weak binding at enzyme’s active surface. This behavior showed that even lactones with same ring-size can behave differently towards an enzyme. Thus, besides ring-size, the molecular structure of lactones also plays an important role in their recognition by lipase.
Results and Discussion

![Graph showing the relationship between time and conversion for CL monomer polymerized with Lipase-CA (5.0 wt% with respect to monomer) at 60°C for 4 hours. The equation Y = 9.487 + 0.202 X is given.](image1)

**Figure 6.7:** Time-conversion plot for CL monomer when polymerized with Lipase-CA (5.0 wt% with respect to monomer) at 60°C for 4 hours

## 6.3 Effect of reaction water content

Water bound to an enzymes surface plays an important role in maintaining the conformational flexibility of the enzyme\(^{103}\). The role of water and other molecules as chain initiator in the enzyme-catalyzed ROP of lactones has been thoroughly discussed\(^{82,91,92}\). It was found that water is an important factor that controls not only the rate of monomer conversion but also the polymer molecular weight\(^{94}\).

![Graph showing the effect of water content on monomer conversion and Mn for ROP of DXO carried out with Lipase-CA (5.0 wt% with respect to monomer) at 60°C for 4 hours.](image2)

**Figure 6.8:** Effect of water content on monomer conversion and Mn for ROP of DXO carried out with Lipase-CA (5.0 wt% with respect to monomer) at 60°C for 4 hours

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To study this effect of water on the lipase-CA catalyzed polymerization of DXO, the reaction water content was varied from 100 to 400 ppm (with respect to monomer). As can be seen in figure 6.8, an increase in monomer conversion and a gradual decrease in $M_n$ were observed when the water level was increased up to 300 ppm. This showed the role of water as an initiator. An increase in reaction water content led to a corresponding increase in the chain initiation, and this led to an increase in the number of propagating chain ends in the reaction system. However, beyond 300 ppm, the monomer conversion decreased and a sharp decrease in $M_n$ was also observed. This was due to a change in the role of water from initiator to a chain cleavage agent, where excess water led to product hydrolysis. The decrease in monomer conversion and $M_n$ may also be due to moving above an optimum water activity for the enzyme.

### 6.4 Effect of enzyme concentration

The effect of different loading amounts of Lipase-CA on the DXO conversion and PDXO molecular weight was examined. Both the monomer conversion and the polymer’s $M_n$ were found to increase with enzyme concentration, as shown in figure 6.9. PDXO having $M_a$ and $M_w$ of 56000 g/mol and 112000 g/mol respectively, could be synthesized with 10.0 wt% of enzyme and a monomer conversion of 97% was achieved. The increase in monomer conversion could be explained as being due to an enzyme-catalyzed ROP of DXO which has the characteristics of living polymerization. A decrease in $M_n$ was expected with increasing enzyme concentration, since the total number of propagating polymer chains increased as a result of higher enzyme loading. To its contrary an increase in $M_n$ with increasing enzyme concentration was observed.

![Figure 6.9: Effect of enzyme concentration on monomer conversion and $M_n$ for ROP of DXO carried out at 60°C for 4 hours](image)

The possible reason for this observation could be that the polymerization was performed only for a limited time (4 hours) until the viscosity of the reaction mixture
became high. This has prevented complete monomer conversion in experiments where the enzyme concentration was low (as confirmed by the $^1$H-NMR results) resulting in a lower molecular weight. According to Nishida et. al. an increase in molecular weight with increased enzyme concentration proves that the enzyme is actually acting as a polymerization catalyst and not as an initiator. Similar results of increase in molecular weight with increasing amount of enzyme have been found in literature with poly(1,4-dioxan-2-one) and ε-caprolactone.

6.5 Effect of temperature

The results of monomer conversion and $M_n$ as a function of polymerization temperature are presented in figure 6.10. Both, the monomer conversion as well as the $M_n$ showed a maximum at 80°C. Enzyme-catalyzed polymerization in bulk can encounter diffusion constrains. Viscosity of the bulk polymerization is greatly dependent on the temperature and affects the diffusion of the substrates in the reaction mixture. At lower temperatures the diffusion limitation may be present, leading to the formation of the polymer with lower molecular weight and monomer conversion. By increasing the reaction temperature in viscous reaction mixtures the mobility of monomer and polymer components is increased. Therefore higher monomer conversion and molecular weight was observed when temperature was increased from 40 to 80°C. A decrease in monomer conversion and molecular weight at polymerization temperature > 80°C could be the result of enzyme denaturation and deactivation at elevated temperatures.

![Figure 6.10: Effect of polymerization temperature on monomer conversion and $M_n$ for ROP of DXO carried out with Lipase-CA (5.0 wt% with respect to monomer) for 4 hours](image)

Figure 6.10: Effect of polymerization temperature on monomer conversion and $M_n$ for ROP of DXO carried out with Lipase-CA (5.0 wt% with respect to monomer) for 4 hours.
6.6 Relationship between monomer conversion, polymer molecular weight and $T_g$

DSC was used to determine the $T_g$ of all the polymer samples. For example, when DXO was polymerized with 5.0 wt% of Lipase-CA at 60°C for 4 hours, a gradual increase in $T_g$ was observed as the polymerization progressed, indicating a build-up in the molecular weight of the polymer. The DSC thermograms presented in figure 6.11 show this trend. The $T_g$ of the polymer increased from $-51^\circ C$ to $-44^\circ C$ with increasing polymerization time. A 3-D plot was constructed to show the general relationship of high monomer conversion–high $M_n$–high $T_g$ for different PDXO samples synthesized (Figure 6.12). The data from all the experiments were included in this plot.

![Figure 6.11: DSC thermograms depicting increase in $T_g$ of PDXO with polymerization time](image1)

![Figure 6.12: Relationships between monomer conversion, $M_n$ and $T_g$ for DXO ROP with Lipase-CA as catalyst](image2)
6.7 Mechanism of Lipase-CA catalyzed ring-opening polymerization of DXO

Lipase is an enzyme belonging to the family of Hydrolases and it catalyzes ester bond hydrolysis by means of a “catalytic triad”, composed of a nucleophilic serine residue activated by a hydrogen bond in relay with histidine and aspartate or glutamate \(^{67}\). It is this “catalytic triad” which is responsible for the ROP of lactones. The serine residue participates in the nucleophilic attack on a lactone to form an enzyme-activated monomer (EAM) complex. The initiation is a nucleophilic attack of water, which is believed to be present within the enzyme, onto the acyl carbon of the EAM to produce \(\omega\)-hydroxy carboxylic acid. During propagation, the nucleophilic attack by the terminal hydroxyl group of \(\omega\)-hydroxy carboxylic acid on EAM leads to the formation of polymer chain elongated by one more monomer unit \(^{83,91,105,106}\). A similar mechanism is proposed for the polymerization of DXO with Lipase CA (Scheme 6.1).

Initiation

![Scheme 6.1: Initiation](image)

Propagation

![Scheme 6.1: Propagation](image)
7. **TERMINAL-FUNCTIONALIZED & TRI-BLOCK POLYESTERS**

During the bulk polymerization of DXO with lipase-CA at 60°C, an increase in viscosity of the reaction mixture was observed in 1 hour. After 4 hours, the magnetic bar stopped stirring due to very high viscosity of the reaction mixture and no sample could be withdrawn for kinetic measurements. When DXO was bulk polymerized with lipase-CA at 60°C in the presence of different alcohols as initiators, an increase in viscosity was observed within 30 minutes in all the cases. The polymerization of DXO was therefore carried out only up to 2 hours, where the viscosity of the reaction mixture became very high and the magnetic bar stopped stirring. In the case of CL, the polymerizations were carried out up to 4 hours as the increase in viscosity was not observed until after 2 hours. This shows the difference in reactivity of DXO and CL towards lipase-catalyzed polymerization.

![Scheme 7.1: Enzymatic synthesis of end-functionalized or tri-block polyesters based on DXO using different alcohols as initiator; (a) PCL-diol forming the middle block of the tri-block copolymer (b) 4-pentene-2-ol introducing terminal unsaturation (c) PEG forming the middle block of the tri-block copolymer](image-url)

Scheme 7.1: Enzymatic synthesis of end-functionalized or tri-block polyesters based on DXO using different alcohols as initiator; (a) PCL-diol forming the middle block of the tri-block copolymer (b) 4-pentene-2-ol introducing terminal unsaturation (c) PEG forming the middle block of the tri-block copolymer
4-Pentene-2-ol was used as an initiator to synthesize PDXO or PCL macromers having a double bond at one chain end, which will be useful for the synthesis of comb-like polymers. Dihydroxyl-terminated macro-initiators such as PCL-diol or PEG were used in order to synthesize tri-block copolymers with DXO or CL. The hydroxyl group at the two ends of the macro-initiator chain initiated the polymerization generating a tri-block copolymer with the macro-initiator as the middle block. The reaction pathways for the synthesis of homo and copolymers of DXO or CL with these initiators are shown in schemes 7.1 and 7.2, respectively.

Scheme 7.2: Enzymatic synthesis of end-functionalized or tri-block polyesters based on CL using different alcohols as initiator; (a) PCL-diol acting as chain extender to form PCL homopolymer (b) 4-pentene-2-ol introducing terminal unsaturation (c) PEG forming the middle block of the tri-block copolymer

7.1 Use of 4-pentene-2-ol as initiator

4-Pentene-2-ol was used as an initiator to introduce terminal functionalization in PDXO or PCL. Polymers having different degrees of polymerization (DP) were synthesized by varying the molar ratio of monomer to initiator. Three different polymers of theoretical DP = 30, 55 and 100 were synthesized with DXO as shown in table 7.1. The percentage monomer conversion was determined from the $^1$H-NMR of the crude reaction mixture in all the cases and it was found to decrease with increasing molar feed ratio of monomer to initiator (table 7.1). This was because the polymerization was allowed to run for a limited period of time. With an increase in the monomer to initiator molar ratio the number of growing chains at any given time was less. Since the polymerization was performed in bulk, the viscosity increased with time so that the accessibility of monomer to the growing polymer chains was limited. Therefore high viscosity and fewer growing centers led to low monomer conversion.
Table 7.1: Lipase-CA catalyzed ROP of DXO in the presence of 4-pentene-2-ol as initiator

<table>
<thead>
<tr>
<th>No</th>
<th>M/In</th>
<th>Con (%)</th>
<th>DP</th>
<th>DP*</th>
<th>M_n*</th>
<th>M_w*</th>
<th>PDI</th>
<th>T_g</th>
</tr>
</thead>
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<tr>
<td>1</td>
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<td>92.6</td>
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<td>29</td>
<td>3400</td>
<td>7500</td>
<td>13000</td>
<td>1.7</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>78.7</td>
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<td>44</td>
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<td>9900</td>
<td>20000</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>63.0</td>
<td>63</td>
<td>63</td>
<td>7400</td>
<td>15300</td>
<td>33900</td>
<td>2.2</td>
</tr>
</tbody>
</table>

*monomer-to-initiator molar feed ratio. b conversion determined by 1H-NMR of crude reaction mixture. c DP calculated by feed composition and monomer conversion using the formula DP = (M/In) * (Conversion/100). d determined by 1H-NMR of precipitated polymer. e obtained by SEC analysis with polystyrene standards. f obtained by DSC analysis

The DP of different PDXO samples was determined from 1H-NMR by taking the ratio of the intensity of oxymethylene protons at 4.2 ppm to that of the terminal methylene protons at 3.6 ppm. As can be seen in the table 7.1, the DP increased with increasing molar feed ratio of monomer to initiator, which confirmed that the alcohol had acted as the initiator. The M_n of each sample was calculated from these DP values and they are also presented in table 7.1. Another way to calculate the DP of the polymers obtained was to take into account the monomer-to-initiator molar feed ratio (M/In) and the monomer conversion by using the equation (7.1). This is on the assumption that all the chains are initiated at the same time and equal numbers of monomer units are therefore attached to each growing polymer chain.

$$\text{DP} = \frac{\text{M}}{\text{In}} \times \left( \frac{\text{Conversion}}{100} \right)$$  \hspace{1cm} \text{Equation (7.1)}

The DP values calculated in these two ways were very close to each other, as can be seen in table 7.1 columns 4 and 5. This showed that all the chains were initiated at the same time and grew almost at the same rate consuming equal number of monomer units. This also confirmed the role of the added alcoholic moiety as an initiator.

An increase in molecular weight was observed in SEC chromatograms with an increase in the molar ratio of monomer to initiator. This further supports the role of added alcohol as initiator. The PDI values increased from 1.7 to 2.2 with increasing monomer-to-initiator molar feed ratio. This could be due to the fast polymerization kinetics of DXO, which led to a high viscosity of the reaction mixture in a short period of time. The longer chains generated at the high monomer-to-initiator ratio have a high probability of undergoing trans-esterification reactions under such conditions of polymerization. The increase in PDI was therefore due to diffusion controlled nature of the polymerization at higher conversion which resulted into occurrence of trans-esterification reactions and in-turn increase in $M_w$ without changing the $M_n$. 
The thermal properties of the polymers were determined from the DSC. The $T_g$ of PDXO samples increased with increasing molecular weight of the polymer, as shown in table 7.1.

Three different polymers of theoretical DP = 30, 65 and 130 were synthesized with CL and the results are presented in table 7.2. The monomer conversion decreased with increasing molar feed ratio of monomer to initiator and this could be explained on the basis of reasons similar to those discussed before for DXO. The incorporation of the pentene moiety was confirmed by the presence of respective proton signals in $^1$H-NMR. Figure 7.1 shows a representative $^1$H-NMR spectrum for PCL having an olefin moiety at one chain end.

Table 7.2: Lipase-CA catalyzed ROP of CL in the presence of 4-pentene-2-ol as initiator

<table>
<thead>
<tr>
<th>No</th>
<th>M/In$^a$</th>
<th>Con$^b$ (%)</th>
<th>DP$^c$</th>
<th>DP$^d$</th>
<th>$M_n$$^d$</th>
<th>$M_n$$^e$</th>
<th>$M_w$$^e$</th>
<th>PDI$^f$</th>
<th>$W_c$$^f$ (%)</th>
</tr>
</thead>
<tbody>
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<td>93.6</td>
<td>28</td>
<td>26</td>
<td>3000</td>
<td>6600</td>
<td>9500</td>
<td>1.4</td>
<td>57.6</td>
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<td>55</td>
<td>6400</td>
<td>12000</td>
<td>17500</td>
<td>1.5</td>
<td>51.8</td>
</tr>
<tr>
<td>3</td>
<td>130</td>
<td>66.7</td>
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<td>87</td>
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<td>22000</td>
<td>31500</td>
<td>1.4</td>
<td>47.8</td>
</tr>
</tbody>
</table>

$^a$ monomer-to-initiator molar feed ratio. $^b$ conversion determined by $^1$H-NMR of crude reaction mixture. $^c$ DP calculated by feed composition and monomer conversion using the formula DP = (M/In) * (Conversion/100). $^d$ determined by $^1$H-NMR of precipitated polymer. $^e$ obtained by SEC analysis with polystyrene standards. $^f$ obtained by DSC analysis

The ratio of oxymethylene protons at 4.0 ppm to terminal methylene protons at 3.6 ppm was used to calculate the DP of different 4-pentene-2-ol initiated PCL samples and the DP values were found to increase with an increase in monomer-to-initiator molar feed ratio. The $M_n$ values calculated from these DP numbers are presented in table 7.2. The DP for each sample was also calculated using equation (7.1) and the values calculated by both the methods were quite close to each other, as shown in table 7.2. This showed that all the chains were initiated at the same time and grew consistently under Lipase-CA catalyzed polymerization of CL in the presence of 4-pentene-2-ol as initiator.

The role of the added alcohol as initiator was also confirmed by the increase in molecular weight with increasing molar ratio of monomer to initiator observed in the SEC chromatograms. No significant change in PDI was observed with increase in monomer-to-initiator ratio indicating that transesterification reactions did not occur when the feed ratio was changed. All these values are presented in table 7.2.
No clear $T_g$ of PCL could be observed in the DSC thermograms as the heating began at -70°C which is very close to the expected $T_g$ of the polymer. The relative crystallinity of PCL homopolymers was calculated from the melting peak (heat of fusion) at about 60°C. A decrease in crystallinity was observed with increasing chain length of the PCL homopolymers, as shown in table 7.2. This could be due to a more regular packing of small chains generating more regular crystals. The short polymer chains crystallized in extended chains or in once or twice folded crystals with a very small proportion of the amorphous material. On the other hand, the long chains could not as effectively be accommodated into the crystals and have large proportion of statistical chains in the amorphous phase, resulting into reduced crystallinity.

The presence of carbon-carbon double bond due to 4-pentene-2-ol initiator in the PDXO or PCL homopolymers was also confirmed by FTIR. The spectra of all three PCL samples were compared with that of the commercial PCL in figure 7.2. The low intensity peak due to C=C stretching at ~1645 cm$^{-1}$ indicated the presence of unsaturation. The relative intensity of this peak decreased with increasing DP of the PCL chain.
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Results and Discussion

Figure 7.2: FTIR of PCLs having different degrees of polymerization (Nos. 1, 2 and 3 in table 7.2) synthesized by Lipase-CA catalyzed ROP of CL in the presence of 4-pentene-2-ol as initiator; peak at \(\sim 1645 \text{ cm}^{-1}\) confirmed the presence of unsaturation at the polymer chain end, intensity of peak decreased with increasing degree of polymerization of PCL.

7.2 Use of PCL-diol as macro-initiator

Dihydroxyl-terminated PCL-diols of different molecular weights (1250 and 2000 g/mol) were used to initiate the DXO or CL polymerization with lipase CA. With DXO, a triblock polymer poly (DXO-\text{b-CL-}b\text{-DXO}) was obtained. Six different polymers of DXO with PCL-diol were synthesized by changing the monomer-to-initiator molar feed ratio from 25 to 60 as shown in table 7.3. The percentage monomer conversion and DP were calculated from \(^1\text{H-NMR}\) analyses of the crude reaction mixture and the precipitated polymer, respectively. In both sets of triblock polymers synthesized by PCL1000 or PCL2000, the percentage monomer conversion decreased with increasing monomer-to-initiator ratio. This is due to similar reasons as discussed before in the case of 4-pentene-2-ol.

The ratio of the peak intensity of methylene protons at 2.6 ppm to that of the terminal methylene protons at 3.6 ppm was used to calculate the DP for different DXO triblock polymers with PCL-diol. These DP numbers shown in table 7.3 were the overall values and therefore should be divided by two in order to obtain the actual DP of the polymer chain attached to each hydroxyl group of the macro-initiator. For example the DP = 30 in No. 5 of table 7.3 showed that each hydroxyl group of the macro-initiator had PDXO chain of DP = 15. A representative \(^1\text{H-NMR}\) spectrum of poly (DXO-\text{b-CL-}b\text{-DXO}) is shown in figure 7.3.
Results and Discussion

Table 7.3: Lipase-CA catalyzed ROP of DXO in the presence of PCL-diol as macroinitiator

<table>
<thead>
<tr>
<th>No</th>
<th>Initiator</th>
<th>M/In</th>
<th>Con (%)</th>
<th>DP</th>
<th>DPd</th>
<th>M_n</th>
<th>M_w</th>
<th>M_z</th>
<th>PDI</th>
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<td>25</td>
<td>89.5</td>
<td>22</td>
<td>21</td>
<td>3700</td>
<td>8700</td>
<td>13900</td>
<td>1.6</td>
<td>-49.2</td>
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</tr>
<tr>
<td>2</td>
<td>PCL1250</td>
<td>40</td>
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<td>31</td>
<td>27</td>
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<td>42800</td>
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a monomer-to-initiator molar feed ratio. b determined by $^1$H-NMR of crude reaction mixture. c DP calculated by feed composition and monomer conversion using the formula $DP = (M/In) \times (Conversion/100)$. d determined by $^1$H-NMR of precipitated polymer. e obtained by SEC analysis with polystyrene standards. f obtained by DSC analysis

The $M_n$ values calculated from the DP numbers from $^1$H-NMR for all the samples are summarized in table 7.3 together with the other results. The DP was also calculated from the monomer-to-initiator molar feed ratio and the percentage monomer conversion using equation (7.1). The DP values obtained by the two methods were quite close to each other, as shown in table 7.3 columns 5 and 6, indicating the similar polymerization behavior as observed before.

SEC chromatograms of all the polymers showed an increase in molecular weight with increasing monomer-to-initiator molar feed ratio and a slight increase in the PDI value of poly(DXO-b-CL-b-DXO) was also observed. This could again be explained by the occurrence of trans-esterification reactions at the higher monomer-to-initiator ratio, where the higher viscosity and longer chains promoted such reactions. Figure 7.4 shows the SEC chromatograms of different poly(DXO-b-CL-b-DXO) samples (Nos. 1, 2 and 3 in table 7.3) in comparison to PCL1250. No peak at higher retention times was observed, indicating that all the PCL-diol was involved in initiating the polymerization.

An increase in $T_g$ with an increase in molecular weight of PDXO block was observed in the DSC thermograms of poly(DXO-b-CL-b-DXO), as shown in the table 7.3. The relative crystallinity of PCL middle block in the triblock polymer poly(DXO-b-CL-b-DXO) was found to decrease with increasing block length of the PDXO segments. This was due to the hindrance created by the PDXO segments to the crystallization of the PCL blocks.
Results and Discussion

Figure 7.3: $^1$H-NMR spectrum of poly (DXO-b-CL-b-DXO) synthesized by Lipase-CA catalyzed ROP of DXO in the presence of PCL-diol as initiator (No. 1, table 7.3)

Figure 7.4: SEC chromatograms depicting the increase in molecular weight of poly (DXO-b-CL-b-DXO) copolymers (Nos. 1, 2 and 3 in table 7.3) with increasing monomer-to-initiator ratio in comparison with the initiator PCL 1250

PCL is a semi-crystalline polymer having $T_m \sim 60^\circ C$ whereas PDXO is an amorphous polymer. In poly(DXO-b-CL-b-DXO) polymers, the PCL middle block showed a
Results and Discussion

tendency to crystallize which was affected by the length of the PDXO blocks. To check this effect of PDXO blocks on the crystallinity of PCL segment, samples (e.g. nos. 4, 5 and 6 in table 7.3) were analyzed by optical microscopy. As shown in figure 7.5, the presence of crystalline domains due to the PCL segment was significantly reduced as the PDXO block length was increased. As explained before, longer the PDXO chains, more hindrance it provided for the PCL to crystallize.

Figure 7.5: Optical microscope images of poly(DXO-b-CL-b-DXO) having different DP (Nos. 4, 5 and 6 in table 7.3) showing the relative change in the degree of crystallinity of the PCL segment of the triblock polymer (a) DP of PDXO block = 23, (b) DP of PDXO block = 30, (c) DP of PDXO block = 34

PCL-diol acted as a chain extender when it was used as an initiator to polymerize CL in the presence of Lipase-CA and PCL homopolymer was therefore finally generated. Six polymers having different DPs were synthesized as shown in table 7.4. The monomer conversion decreased with increasing monomer-to-initiator ratio following the similar trend as observed for DXO. The ratio of oxymethylene protons at 4.0 ppm and terminal methylene protons at 3.6 ppm was used to determine the DP of the PCL samples. The DP values obtained were subtracted by 10 (the repeat units counted for the PCL1250 macroinitiator) or by 16 (the repeat unit counted for the PCL2000 macroinitiator) to obtain the final values. The $M_n$ values calculated from these DP numbers are presented
Results and Discussion

in table 7.4. The DP values calculated using equation (7.1) were similar to those obtained by $^1$H-NMR (proton peak ratio method).

<table>
<thead>
<tr>
<th>No</th>
<th>Initiator</th>
<th>M/In$^a$</th>
<th>Con$^b$ (%)</th>
<th>DP$^c$</th>
<th>DP$^d$</th>
<th>$M_n^{d}$</th>
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<th>$W^{f}$ (%)</th>
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$^a$ monomer-to-initiator molar feed ratio. $^b$ conversion determined by $^1$H-NMR of crude reaction mixture. $^c$ DP calculated by feed composition and monomer conversion using the formula DP = (M/In) * (Conversion/100). $^d$ determined by $^1$H-NMR of precipitated polymer. $^e$ obtained by SEC analysis with polystyrene standards. $^f$ obtained by DSC analysis.

An increase in $M_n$ with increasing monomer-to-PCL-diol ratio was observed from SEC chromatograms and this confirmed the role of PCL-diol as an initiator. The PDI values for all the PCL samples were close to 1.3, indicating that the initiator had no effect on the molecular weight distribution and the absence of trans-esterification reactions. A decrease in relative crystallinity of PCL homopolymers was observed with an increase in the polymer chain length, for reasons similar to those discussed before.

7.3 Use of PEG as macro-initiator

Dihydroxyl-terminated PEG of different molecular weights (1000 and 2000 g/mol) were used to initiate the polymerization of DXO or CL with lipase-CA. In this way, the triblock polymers of DXO or CL with PEG, viz. poly (DXO-b-EG-b-DXO) or poly (CL-b-EG-b-CL), were synthesized. Six different triblock polymers of DXO were obtained by changing the monomer-to-initiator molar feed ratio as shown in table 7.5. The percentage monomer conversion and DP were calculated from $^1$H-NMR of the crude reaction mixture and the precipitated polymer sample, respectively. As can be seen in table 7.5, the monomer conversion decreased with an increase in monomer-to-initiator ratio. This observation follow the same trend as had been noticed before using different PCL-diols as initiators and therefore this could be explained by similar reasons. The DP for different PDXO samples was calculated by taking the ratio of the
peak intensity of oxymethylene protons at 4.2 ppm to that of the terminal methylene protons at 3.6 ppm. A representative $^1$H-NMR spectrum of poly (DXO-b-EG-b-DXO) is shown in figure 7.6. $M_n$ was calculated from these DP values and the results are presented in table 7.5. The DP was also calculated using equation (7.1) and compared with the values obtained by taking the ratio of proton peaks from $^1$H-NMR as described above. The values are presented in table 7.5 columns 5 and 6.

Table 7.5: Lipase-CA catalyzed ROP of DXO in the presence of PEG as macroinitiator

<table>
<thead>
<tr>
<th>No</th>
<th>Initiator</th>
<th>$M$/In</th>
<th>$\text{Con}^b$ (%)</th>
<th>$\text{DP}^c$</th>
<th>$\text{DP}^d$</th>
<th>$M_n^e$</th>
<th>$M_w^e$</th>
<th>$M_w^e$/SD</th>
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<td>8300</td>
<td>20500</td>
<td>43300</td>
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$^a$ monomer-to-initiator molar feed ratio. $^b$ conversion determined by $^1$H-NMR of crude reaction mixture. $^c$ DP calculated by feed composition and monomer conversion using the formula DP = ($M$/In) * ($\text{Conversion}$/100). $^d$ determined by $^1$H-NMR of precipitated polymer. $^e$ obtained by SEC analysis with polystyrene standards. $^f$ obtained by DSC analysis.

The expected increase in molecular weight of polymers with increasing monomer-to-initiator ratio was observed in the SEC results. An increase in dispersity was observed with an increase in DP of different poly (DXO-b-EG-b-DXO) samples due to occurrence of possible trans-esterification reactions. Figure 7.7 shows the SEC chromatograms of different poly (DXO-b-EG-b-DXO) samples (Nos. 4, 5 and 6 in table 7.5) together with PEG2000. The complete consumption of PEG2000 as initiator was revealed by the absence of any peak at higher retention times. The $T_g$ of PDXO blocks in poly (DXO-b-EG-b-DXO) increased with increasing PDXO block length, as can be seen in table 7.5.
Results and Discussion

Figure 7.6: $^1$H-NMR spectrum of poly(DXO-$b$-EG-$b$-DXO) synthesized by Lipase-CA catalyzed ring-opening polymerization of DXO in the presence of PEG as initiator (No. 1, table 7.5)

Figure 7.7: SEC chromatograms depicting the increase in molecular weight of poly(DXO-$b$-EG-$b$-DXO) (Nos. 4, 5 and 6 in table 7.5) copolymers with increasing monomer-to-initiator ratio in comparison with the initiator PEG 2000
Results and Discussion

Triblock polymers poly(CL-b-EG-b-CL) were synthesized when PEG was used as initiator for CL polymerization in the presence of Lipase-CA. Six different polymers of different DP were generated, as shown in table 7.6. The percentage monomer conversion decreased with increase in monomer-to-initiator ratio, as observed before. The ratio of methylene protons due to PCL at 4.0 ppm to methylene protons due to PEG at ~3.7 ppm was used to determine the DP of the different PCL triblock polymers. These DP values were used to calculate the $M_n$ of the polymers, which are presented in table 7.6. The DP values calculated using equation (7.1) were close to the values obtained by taking the ratio of proton peaks due to monomer and initiator.

Table 7.6: Lipase-CA catalyzed ring-opening polymerization of CL in the presence of PEG as macroinitiator

<table>
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<th>No</th>
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<th>$\text{DP}^d$</th>
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$^a$ monomer-to-initiator molar feed ratio. $^b$ determined by $^1$H-NMR of crude reaction mixture. $^c$ DP calculated by feed composition and monomer conversion using the formula $\text{DP} = (\text{M/In}) \times \left( \frac{\text{Conversion}}{100} \right)$. $^d$ determined by $^1$H-NMR of precipitated polymer. $^e$ obtained by SEC analysis with polystyrene standards. $^f$ obtained by DSC analysis

The $M_n$ of the triblock polymers, as obtained from SEC chromatograms, increased with increase in monomer-to-initiator molar ratio confirming PEG’s role as initiator. The polydispersity of the poly(CL-b-EG-b-CL) polymers did not change with changing the initiator or DP, showing the absence of the trans-esterification reactions. The relative crystallinity of PCL blocks in different poly (CL-b-EG-b-CL) polymers decreased with increasing PCL block length for reasons similar to those discussed before.
8. CONCLUSIONS

Easy to cast, smooth and elastic cross-linked films based on DXO and CL using 2,2’-bis-(ε-caprolactone-4-yl) propane (BCP) as the cross-linking agent were successfully obtained under relatively mild cross-linking conditions.

- Thermal, mechanical and surface properties of the films were easily controlled and tailored by varying the monomer composition and cross-link density. The films can be used as scaffolds for tissue engineering.

The Lipase-CA catalyzed polymerization of DXO is shown to be a useful tool to synthesize metal-free PDXO having both high molecular weight and high monomer conversion in a relatively short time under mild reaction conditions.

- The polymerization was found to have the characteristics of living polymerization.
- An initial activation by heating the enzyme to a high temperature was sufficient to start the polymerization, and that further monomer conversion could then occur even at room temperature.
- Water acted as initiator for the Lipase-CA catalyzed ROP of DXO.
- A higher enzyme loading led to an increased monomer conversion as well as polymer molecular weight. PDXO having \( M_n = 56000 \text{ g/mol} \) and \( M_w = 112000 \text{ g/mol} \) was obtained with 10.0 wt\% of Lipase-CA (with respect to monomer) at 60°C for 4 hours.
- The optimum polymerization temperature for Lipase-CA catalyzed ROP of DXO was 80°C.
- Lactones having the same ring size can have different specificity towards an enzyme.

Lipase-CA catalyzed ring-opening polymerization of 7-member lactones is a useful synthetic pathway to form terminal-functionalized polyesters or triblock polyesters using an alcohol as initiator.
Conclusions

- All the polymerizations were initiated by quantitative introduction of the initiator group at the polymer terminal (monohydroxyl initiator) or inside polymer chain (dihydroxyl initiator), yielding polyester in a versatile single-step reaction. The degree of polymerization of the polyester chain segments was controlled by varying the molar feed ratio of monomer to initiator.

- Macromers of PDXO or PCL containing terminal unsaturation, generated using 4-pentene-2-ol as initiator, will be useful to synthesize comb like polymers or for introducing hydrophilic or hydrophobic grafts into polyvinyls.

- Triblock copolymers of DXO or CL were synthesized using different dihydroxyl macro-initiators such as PCL-diol or PEG, where the hydrophilic or hydrophobic character of the copolymer was tailored by the choice of monomers and macro-initiators. This opened a novel pathway to synthesize aliphatic polyesters having amphiphilic character which can be used as slowly degrading sutures, temporary implants or drug delivery systems.
9. **FUTURE WORK**

- Enzyme-catalyzed ROP of lactones, DXO and CL, has been shown to be a versatile synthetic route to generate aliphatic polyesters. The method can be extended to polymerize several other possible combinations of lactone, lactide or cyclic carbonate monomers, where the polymers formed will be free from metallic impurities from organometallic catalysts.

- Besides bulk, enzyme-catalysis can be extended to solution, dispersion or emulsion modes of polymerization. Each polymerization mode will bring own advantages with them. The polymerization conditions can be further optimized to synthesize aliphatic polyesters of high molecular weight.

- Functional hydroxyl compounds used as initiators in enzyme-catalyzed polymerization of lactones can be used to generate aliphatic polyesters of vivid architectures such di-, tri- or multi-blocks, stars, comb, brushes and hyperbranched.

- Efforts have to be made to generate high molecular weight aliphatic polyesters. The polymers will then have sufficient strength to withstand the specific requirement of certain biomedical applications such as tissue implants, artificial cartilages and skin which require strong, elastic and tough polymers.
10. ACKNOWLEDGEMENT

My supervisor Professor Ann-Christine Albertsson is thanked for accepting me as her graduate student and for her excellent scientific guidance, encouragement and freedom for doing innovative research throughout my time at the department. It is her patience and continuous vigilance that made me a more confident person. Professor I. K. Varma is thanked for introducing me to the research group of Professor Albertsson.

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My friends outside the department are thanked for just being “my friends”.

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11. REFERENCES


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