Functional Polymers by Enzymatic Catalysis

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Technische Universiteit Eindhoven, op gezag van de Rector Magnificus, prof.dr.ir. C.J. van Duijn, voor een commissie aangewezen door het College voor Promoties in het openbaar te verdedigen op dinsdag 12 mei 2009 om 16.00 uur

door

Yan Xiao

geboren te Zhangjiagang, China
Dit proefschrift is goedgekeurd door de promotor:

prof.dr. C.E. Koning

Copromotoren:
Dr. A. Heise
en
dr.ir. A.R.A. Palmans

Xiao, Y.

A catalogue record is available from the Eindhoven University of Technology Library

ISBN: 978-90-386-1751-0

Copyright © 2009 by Yan Xiao

The work described in this thesis was financially supported by the Marie Curie Action RTN program “Biocatalytic Approach to Material Design” (BIOMADE; contract no. MRTN-CT-2004-505147).

Cover design: Yan Xiao and Paul Verspaget
Printed at the Universiteitsdrukkerij, Eindhoven University of Technology
# Table of Contents

## CHAPTER 1

Biodegradable Polymers Prepared by Enzymatic Catalysis .............1

1.1 Enzymatic catalysis .............................................................................................................2
1.1.1 Enzymatic catalysis and polymers ..................................................................................3
1.1.2 Ring opening polymerization of lactones ........................................................................6
1.2 Biodegradable elastomers ....................................................................................................10
1.2.1 Elastic microspheres in controlled drug delivery ...........................................................11
1.2.2 Biodegradable hydrogels ................................................................................................14
1.3 Outline and aim of the thesis .............................................................................................16

References ....................................................................................................................................19

## CHAPTER 2

Enzymatic Methacrylation: Lipase-catalyzed HEMA-initiated
Ring Opening Polymerization .........................................................23

Abstract ....................................................................................................................................23
2.1 Introduction ..........................................................................................................................24
2.2 Experimental part ................................................................................................................26
2.2.1 Materials ..........................................................................................................................26
2.2.2 Instrumentation ................................................................................................................26
2.2.3 Synthetic procedure .......................................................................................................28
2.3 Results and discussion .........................................................................................................29
2.3.1 Polyester structures from lipase-catalyzed HEMA-initiated ring-opening polymerization
..................................................................................................................................................29
2.3.2 Kinetic investigation of lipase-catalyzed ROP of PDL and CL initiated with HEMA...34
2.3.3 One-pot two-step synthesis of dimethacrylated polymers..............................................38

2.3 Conclusion ..........................................................................................................................40

References ....................................................................................................................................42

## CHAPTER 3

Enzymatic Acrylation: Lipase-Catalyzed HEA-Initiated Ring
Opening Polymerization .................................................................45
CHAPTER 4

Biodegradable Chiral Polyesters and Microspheres by Asymmetric Enzymatic Polymerization

Abstract

4.1 Introduction

4.2 Experimental part

4.2.1 Materials

4.2.2 Instrumentation

4.2.3 Synthetic procedure

4.3 Results and discussion

4.3.1 Asymmetric synthesis and degradation of chiral polyesters

4.3.2 Synthesis of chiral microspheres

4.3.3 Degradation of chiral microspheres

4.4 Conclusion

References
5.2 Experimental part ........................................................................................................94
  5.2.1 Materials ..................................................................................................................94
  5.2.2 Instrumentation .......................................................................................................94
  5.2.3 Synthetic procedure ...............................................................................................95

5.3 Results and discussion ................................................................................................98
  5.3.1 PMCL-b-PEG-b-PMCL with different chirality .........................................................98
  5.3.2 Network formation ..................................................................................................105
  5.3.3 Degradation study ..................................................................................................110

5.4 Conclusion ..................................................................................................................114

Reference ............................................................................................................................115

CHAPTER 6
Cumulated Advantages of Enzymatic and Carbene Chemistry for the Non-organometallic Synthesis of (co)Polyesters ...............117

Abstract ...............................................................................................................................117

6.1 Introduction ..................................................................................................................118

6.2 Experimental part .......................................................................................................119
  6.2.1 Materials ..................................................................................................................119
  6.2.2 Instrumentation .......................................................................................................120
  6.2.3 Synthetic procedure ...............................................................................................120

6.3 Results and discussion ..............................................................................................122
  6.3.1 General investigations ............................................................................................122
  6.3.2 “One-pot” reactions ...............................................................................................124
  6.3.3 PCL-b-PLA with different compositions by one pot reaction ..................................129

6.4 Conclusion ..................................................................................................................130

References ...........................................................................................................................131

Summary ..............................................................................................................................133

Acknowledgements ..........................................................................................................135

Curriculum Vitae ...............................................................................................................139
List of Publications .................................................................141
CHAPTER 1

Biodegradable Polymers Prepared by Enzymatic Catalysis

• A general introduction about enzymatic catalysis and biodegradable elastomers

• The outline and aim of the thesis
1.1 Enzymatic catalysis

In nature, enzymes are catalysts in metabolism and catabolism processes. The discovery of the first enzymes was dated back to the 1830s: diastase by Payen and Persoz and pepsin by Schwann. While the idea that enzymes could be used for a variety of commercial applications was always in the realm of possibility, it was only in the 1960s and early 1970s that commercial processes using enzymes were widely introduced. For example, carbohydrate-processing enzymes have been widely used in the food industry for the processing of corn, potatoes and other starches. In the past few decades, an increasingly important application of enzymes has been as a catalytic tool in the synthesis of specialty organic chemicals. Employing enzymes in organic synthesis has several advantages: (1) catalysis takes place and is efficient under mild reaction condition with regard to temperature, pressure, and pH, which often results in a remarkable energy efficiency; (2) high enantio-, regio- and chemoselectivity as well as regulation of stereochemistry are possible, providing development of new reactions to functional compounds for pharmaceuticals and agrichemicals; (3) enzymes are nontoxic natural catalyst with “green” appeal in commercial benefit and ecological requirement. One example among many is DSM’s biotechnological route to the antibiotic Cephalexin, which is performed on an industrial scale with high environmental and cost benefits as compared to the chemical synthesis (material savings 65 %; energy savings 65 %; cost reduction 50 %). As shown in Figure 1.1, the present route is greatly simplified compared to the past route by using acylase.
1.1.1 Enzymatic catalysis and polymers

In recent years researchers also investigated whether the advantages of enzyme catalysis be applied in polymer synthesis. *In vitro* enzymatic polymerization could provide new strategies for the manufacturing of useful polymers that are very difficult to produce by conventional chemical catalysis. According to their different functions, all enzymes are generally divided into six groups. Their catalytic character and some typical polymers produced by the respective enzymes are summarized in Table 1.1.³ ⁴ Notably, only three of them have been reported in enzymatic polymerization *in vitro*, *i.e.* oxidoreductases, transferases and hydrolases. Most of the oxidoreductases contain low-valent metals as the catalytic center.⁶ Some oxidoreductases, such as peroxidase, laccase and bilirubin oxidase, have been used as catalysts for the oxidative polymerizations of phenol and aniline derivatives to produce novel polyaromatics.⁷ ⁸ Transferases are enzymes transferring a group from one compound (donor) to another compound (acceptor). Several transferases such as phosphorylases and synthases have been found to be effective for catalyzing *in vitro* synthesis of polysaccharides and...
Hydrolases including glycosidases, lipases and proteases are enzymes catalyzing a bond-cleavage reaction by hydrolysis. They have been employed as catalysts for the reverse reaction of hydrolysis, leading to polymer production by a bond-forming reaction. Hydrolases are the most successful class of enzymes in polymer forming reactions.

Table 1.1 Classification of enzymes and typical polymers produced by respective enzymes.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Catalytic character</th>
<th>Typical polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidoreductases</td>
<td>Catalyze redox-reactions by electron transfer</td>
<td>Polyphenols, polyanilines, vinyl polymers</td>
</tr>
<tr>
<td>Transferases</td>
<td>Catalyze the transfer of a functional group, for example a methyl group or a glycosyl group, from donor to acceptor</td>
<td>Polysaccharides, cyclic oligosaccharides, polyesters</td>
</tr>
<tr>
<td>Hydrolases</td>
<td>Catalyze the hydrolysis of various bonds in order to transfer functional groups to water</td>
<td>Polysaccharides, polyesters, polycarbonates, poly(amaio acid)s</td>
</tr>
<tr>
<td>Lyases</td>
<td>Catalyze the cleavage of C-C, C-O, C-N and other bonds otherwise than by hydrolysis or oxidation</td>
<td>-</td>
</tr>
<tr>
<td>Isomerases</td>
<td>Catalyze either racemization or epimerization of chiral centers; isomerases are subdivided according to their substrates</td>
<td>-</td>
</tr>
<tr>
<td>Ligases</td>
<td>Catalyze the coupling of two molecules with concomitant hydrolysis of the diphosphate-bond in ATP or a similar triphosphate.</td>
<td>-</td>
</tr>
</tbody>
</table>

A lipase is an enzyme which catalyzes the hydrolysis of fatty acid esters, normally in an aqueous environment in living systems. It is also the most investigated enzyme for in vitro polymer synthesis including condensation and ring opening polymerization. Via self-condensation (A-B type or AA-BB type) and enzymatic polytransesterification, several polyesters have been successfully synthesized. However, much attention has been focused on the ring opening polymerization because of the diversity of commercially available cyclic monomers and the corresponding achievable polymers.
In addition to cyclic esters (Figure 1.2(a)), lipases have also been used to catalyze the ring opening polymerization (ROP) of cyclic carbonate, phosphate, and depsipeptide monomers,\(^{15-18}\) as shown in Figure 1.2 (b), (c), and (d), respectively. For example, several lipases including *Candida antarctica* lipase B, porcine pancreatic lipase and lipase AK were found to be effective for the ROP of trimethylene carbonate (TMC) and its derivates.\(^{19-21}\) Nevertheless, the lipase-catalyzed ROP of cyclic esters like lactones (Figure 1.2 (a)) and their derivatives (including alkyl substituted lactones and dioxanes) have been most extensively investigated due to their structural variety and the potential biodegradability of the corresponding polymers.\(^{22,23}\)

![Figure 1.2 Cyclic ester (a), carbonate (b), phosphate (c) and depsipeptide (d) monomers suitable for enzymatic ring opening polymerization (eROP).](image)

An enzyme that deserves special attention when discussing enzymatic ROP is the already mentioned *Candida antarctica* lipase B (CALB). Physically adsorbed on macroporous crosslinked beads of poly(methyl methacrylate) (Lewatit VP OC 1600, Bayer), this enzyme is commercially available as Novozym 435 from Novozymes.\(^{24}\) It is a highly versatile catalyst with activity towards a great variety of different substrates. The immobilized enzyme is thermostable and retains activity in various organic solvents. The recent breakthroughs in enzymatic ROP have been made possible to a large extent due to Novozym 435. Its success in enzymatic ROP (eROP) is partly based on its commercial availability and easy handling, making it a convenient catalyst, even for chemists with little knowledge on enzymology. Moreover, in many comparative polymerization studies Novozym 435 has the highest activity.

In a recent study, Gross *et al.* reported how the particle size of the immobilization resin influences CALB loading, fraction of active sites, and catalytic properties for polyester...
The results revealed that CALB adsorbed more rapidly on smaller beads. A nonuniform distribution with most enzymes present in the outer region of particles was found by IR microspectroscopy with 560-710 and 120 µm diameter resins. In contrast, as the resin particle size was decreased, the protein distribution became increasingly uniform throughout the resin particles. These results showed the benefits of systematic investigations of immobilization parameters to achieve enhanced enzyme-catalyst activities.

### 1.1.2 Ring opening polymerization of lactones

So far, nonsubstituted lactones with a ring size from 4 to 17 were subjected to ROP and gave corresponding polyesters (Scheme 1.1). Systematic studies have been carried out on the polymerizability of lactones of increasing ring sizes with Zn(Oct)$_2$ and lipases.$^{26-28}$ The ring strain, which decreases with increasing lactone size, eventually leads to faster propagation for more strained monomers in chemical polymerizations. In contrast, the polymerizability of lactones increases with increasing ring size when using the lipase of *Pseudomonas fluorescens* (Lipase PF).$^{26}$ Furthermore, Novozym 435-catalyzed ring opening polymerizations of lactones of varying ring sizes (6- to 13- and the 16-membered ring) demonstrate fascinating differences in their polymerization rates.$^{27}$ However, no obvious trend could be discerned in the reactivity difference. Several factors may play a role such as effects of basicity and dipole moment of the lactone and steric interactions with surrounding amino acid residues in the active site, but at the moment it remains concealed how important the relative contributions of each of these factors are. Nevertheless, Novozym 435 is a unique catalyst that enables the polymerization of lactones with a variable number of methylenes in their cyclic structure.
On the basis of reported results, it is believed that lipase-catalyzed ROP of lactones proceed by an enzyme-activated monomer mechanism. Taking ε-caprolactone as an example, Scheme 1.2 illustrates the catalytic process of the lipase. The active site of a lipase is generally formed by a catalytic triad consisting of serine, histidine and aspartate, which is electronically stabilized. An ester functions as substrate molecule and undergoes a nucleophilic attack by the primary alcohol group of serine in the active site (I in Scheme 1.2). Via the enzyme intermediate species (II in Scheme 1.2) the original alkoxy-group will be released, forming the so-called enzyme-activated monomer, EAM (III in Scheme 1.2). This is the key step determining the rate of the reaction. Subsequently, a nucleophile, e.g. a primary alcohol, water or a primary hydroxyl terminated polymer chain can attack this EAM-species, and via the new intermediate species (IV in Scheme 1.2) the final product is released, thereby regenerating the enzyme. In contrast to polymers with predictable molecular weights and low polydispersities obtained by organometallic “coordination-insertion” catalysts, the polydispersity index in most enzymatic polymerizations is close to 2 because of the unavoidable transesterification, in which all ester groups present in the system will participate.
Besides the generation of entirely metal-free products, which is crucial for biomedical applications, the most obvious advantage of enzymatic catalysis over chemical catalysis is enantioselectivity, which can be accomplished by eROP of substituted lactones. Those racemic lactones were polymerized to produce optically active polymers by selective reaction of the faster reacting enantiomer (enantioselectivity). The optically active slow reacting enantiomer remained as unreacted monomers. According to the monomer-activated mechanism, the lipase reacted with racemic lactones to produce the acyl-enzyme intermediates. However, the rate constants differed for the two enantiomers. Therefore, effects of ring size, position and length of the substituent of the racemic lactones in their lipase-catalyzed ROP have been investigated.33-37

(1) Ring size effect: Kobayashi et al. have found that in the polymerization of 6- and 7-membered lactones, the reaction behaviors of α-methyl-substituted lactones were
Biodegradable polymers prepared by enzymatic catalysis

relatively similar to those of the unsubstituted ones, while in the polymerization of α-substituted macrolides (13- and 16-membered), the polymerizability decreased by the introduction of the methyl substituent.\textsuperscript{33} More results of enantioselectivity and reaction rate on a range of ω-methylated lactones have also been reported.\textsuperscript{37}

(2) Substituent position effect: Of all the methyl-substituted 7-membered lactones (MeCL), 6-MeCL exhibits much slower polymerization kinetics than any other MeCL. Moreover, Novozym 435 shows S-selectivity for all methyl-substituted caprolactones except for 5-MeCL, where R-selectivity is observed. The three-dimensional structure of the faster reacting enantiomers reveals that there is an alternating orientation of the methyl group from 3- to 6-MeCL (Figure 1.3), suggesting an odd-even effect.\textsuperscript{34}

(3) Substituent length effect: 4-substituted-caprolactones, employing Novozym 435 as the biocatalyst, demonstrate dramatic differences in polymerization rates and selectivity depending on the size of the substituent. 4-EtCL polymerizes 5 times slower than 4-MeCl and 4-PrCL is even 70 times slower. The decrease in polymerization rate is accompanied by a strong decrease in enantioselectivity. Interestingly, Novozym 435 displays S-selectivity for 4-MeCL and 4-EtCL in the polymerization reaction, but the enantioselectivity is changed to the (R)-enantiomer in the case of 4-PrCL.\textsuperscript{35}

\textbf{Figure 1.3} Structures of the faster reacting enantiomers in Novozym 435 catalyzed ROP of the respective monomer mixtures.

It has been reported that highly enantioenriched polymers can be synthesized from substituted lactones by enzymatic ROP.\textsuperscript{37, 38} Enantiomeric purity >95\% has been realized for poly (4-MeCL) and poly (4-EtCL).\textsuperscript{35, 36, 39} Moreover, three ω-methylated lactones (7-MeHL, 8-MeOL and 12-MeDDL) have yielded the enantiopure R-polyester with excellent enantiomeric excess (>99\%).\textsuperscript{37}
1.2 Biodegradable elastomers

Different from photodegradable, oxidatively degradable or hydrolytically degradable materials, biodegradable plastics undergo degradation from the action of naturally occurring microorganisms such as bacteria, fungi, and algae. Therefore, their applications have been extended to the sectors including medicine, packaging, agriculture, and the automotive industry. In general, synthetic biodegradable polymers offer greater advantages than natural ones in that they can be tailored to give a wider range of properties. Synthetic polymers also represent a more reliable source of raw materials. So investigations on the synthetic biodegradable polymers have taken a leading position over the past two decades. For example, the greatest advantage of these degradable polymers in biomedical application is that they are broken down to biologically acceptable molecules that are metabolized and removed from the body via normal metabolic pathways. Furthermore, the polymers must be biodegradable into Food and Drug Administration (FDA) - approved compounds. Degradation of the polymer does not produce inflammation (causing acid), but instead generates membrane-permeable products that allow all of the polymer’s byproducts to diffuse outside the cell. That means byproducts should not accumulate in a patient’s tissue and cause inflammation. However, for some applications, the inferior mechanical properties and unsatisfactory compatibility with cells and tissues limit the applicability of some biodegradation polymers.

An increasing number of investigations have been focused on biodegradable elastomers, which can be defined as elastomers prepared from biodegradable components as a potential biomaterial for tissue engineering and drug delivery applications. The mechanical properties of biodegradable elastomers can be designed for those of the elastic soft tissues such as blood vessels, cartilage, and smooth muscle, thereby providing a three-dimensional polymer scaffold to support cell growth and orient growth towards the generation of replacement tissue. In controlled drug delivery, the absence of crystalline parts in the cured biodegradable elastomers would be favorable for biodegradation and constant release.
1.2.1 Elastic microspheres in controlled drug delivery

The purpose behind controlling the drug delivery is to achieve more effective therapies while maintaining drug levels within a desired range and eliminating the potential for both under- and overdosing. In recent years, controlled drug delivery formulations and the polymers used in these systems have become much more sophisticated. Polymers have been designed to respond to the changes in the biological environment, i.e., deliver or stop to deliver drugs based on these changes. Materials have been developed to target the specific cell, tissue or site where the drug they contain is to be delivered. To be successfully used in controlled drug delivery formulations, a material must be free of toxicity and compatible with the body. It must also have an appropriate physical property, with minimal undesired deformation, and be readily processable. To some extent, an elastic material can be a good candidate to satisfy most of the requirements, as long as stiffness is not required.

There are two primary mechanisms by which drugs can be released from a delivery system: diffusion (or swelling followed by diffusion) through or degradation of the polymeric material. Any or all of these mechanisms may occur in a given release system. Diffusion occurs when a drug passes through the polymer that forms the controlled-release device. In case of hydrophilic drugs an aqueous solvent content can enable the drug to diffuse through the swollen network into the external environment. Swelling can be triggered by a change involving pH, temperature, or ionic strength in the environment surrounding the delivery system. Examples of diffusion-release systems are shown in Figure 1.4.
All of the previously mentioned diffusion systems are based on polymers that do not change their chemical structure. However, a great deal of attention and research effort is being concentrated on biodegradable polymers. These materials degrade within the body as a result of natural biological processes, eliminating the need to remove a polymeric drug delivery system after release of the active agent has been completed. Most biodegradable polymers are designed to degrade as a result of hydrolysis of the polymer chains into biologically acceptable and progressively smaller compounds. Degradation may take place through bulk hydrolysis, in which the polymer degrades in a fairly uniform manner throughout the matrix, as shown schematically in Figure 1.5 (a). For some degradable polymers, most notably the polyanhydrides and polyorthoesters, the degradation occurs only at the surface of the polymer, resulting in a release rate that is proportional to the surface area of the drug delivery system (Figure 1.5 (b)). No matter which type of degradation proceeds, the most common formulation for these biodegradable materials is that of microparticles, which have been used in oral delivery systems and, even more often, in subcutaneously injected delivery systems.
Biodegradable polymers prepared by enzymatic catalysis

Figure 1.5 Drug delivery from (a) bulk-eroding and (b) surface-eroding biodegradable systems.\textsuperscript{44}

Spherical elastomers can be prepared as either thermoplastic or cured materials. As thermoplastic elastomers generally possess crystalline or high glass transition temperature regions which usually resist degradation, these materials degrade in a heterogeneous way,\textsuperscript{45, 46} which may lead to a non-controlled release system. For example, PCL microspheres have been reported to preferentially degrade in their amorphous domains, as shown in Figure 1.6.\textsuperscript{47} On the other hand, amorphous, cured elastomers can provide more linear and more homogenous degradation with time, maintenance of form stability, and tightly controlled network architecture, which are all advantages of biodegradable elastomers desired for controlled drug delivery. For these reasons, the focus of this thesis is on cured biodegradable elastomers.
1.2.2 Biodegradable hydrogels

Generally speaking, hydrophobic polymers like aliphatic polyesters degrade very slowly by simple hydrolysis under human body conditions. If they are copolymerized with a hydrophilic polymer like poly(ethylene glycol) (PEG), the hydrophilicity and biodegradation can be improved. Therefore, amphiphilic block copolymers or hydrogels have been extensively developed in drug delivery and tissue engineering. Polymer micelles formed from amphiphilic block copolymers by self-assembling have been reported to entrap drugs in the core for the targeted delivery.\(^\text{48}\) For intravenous application, it is critical that the micelles are stable, \(i.e.,\) they should have low critical micelle concentrations (CMC). Otherwise, the micelles will dissociate into unimers upon dilution in the bloodstream, causing nontargeted and excessively instantaneous drug release and toxicity.\(^\text{49}\) One of the strategies used to increase the stability of micelles is to crosslink the substrate, \(i.e.,\) synthesize crosslinked hydrogels. Hydrogels are hydrophilic, three-dimensional networks, which are able to absorb a large amount of water or biological fluids, and thus resemble, to a large extent, a biological tissue. Crosslinks have to be present to avoid dissolution of the hydrophilic polymer chains/segments into the aqueous phase. A great variety of chemical and
physical methods to establish crosslinking has indeed been used to prepare hydrogels.\textsuperscript{50} In chemically crosslinked gels, covalent bonds are present between different polymer chains. In physically crosslinked gels, dissolution is prevented by physical interactions, which exist between different polymer chains. The chemical methods consist of radical curing, reaction of complementary groups present on different polymer chains, high-energy irradiation and applying enzymes. Physically crosslinked hydrogels can be obtained from ionic interactions, crystallization, amphiphilic block and graft copolymers, hydrogen bonds and protein interactions. For hydrogels of synthetic polymers, one of the most promising crosslinking methods is photoinitiated (co)polymerization of the diacrylates with hydrophobic and hydrophilic segments. Hydrogels can be classified as homopolymer or copolymer networks, based on the method of preparation. They can also be classified, based on the physical structure of the networks, as amorphous, semi-crystalline, hydrogen-bonded structures, supermolecular structures and hydrocolloidal aggregates.\textsuperscript{51} Since it is advantageous for many applications that the hydrogels are biodegradable, labile bonds are frequently introduced into the gels. The labile bonds can be broken under physiological conditions, either enzymatically or chemically, in most of the cases by hydrolysis. Biodegradable polymers developed include poly (\(\alpha\)-hydroxyesters), polyanhydrides, polyorthoesters and poly (\(\alpha\)-amino acids). Among those biodegradable polymers, the most thoroughly investigated and used bioerodible polymer is of the poly (\(\alpha\)-hydroxyester) type, such as poly (lactic acid) (PLA), poly (glycolic acid) (PGA) and poly (LA-co-GA) which have already been approved as implantable polymers by FDA, just as the non-degradable PEG.\textsuperscript{52} Therefore, hydrogels based on interpenetrating polymer networks (IPN) of PEG and these biodegradable polymers can exhibit the desired biocompatibility and are therefore widely used in biomedical applications. The nature of the degradation products formed can be tailored by a rational and proper selection of the hydrogel building blocks.
1.3 Outline and aim of the thesis

Biodegradable elastomers have multiple potential uses in biomedical areas, particularly in the fields of tissue engineering and controlled drug delivery. An effective biodegradable, crosslinked elastomer for these purposes would be amorphous, should have aimed at readily alterable degradation rates and mechanical properties. The material should have a glass transition temperature below body temperature and processing into a variety of geometries should be easy. Its gel content should be high, reflecting efficient crosslinking. The material should also be biocompatible. Enzymatic polymerization has opened efficient routes in organic synthesis of functional molecules utilizing enzymatic selectivity, low energy consumption and cleanness. In the past few decades much attention has also been focused on enzymatic synthesis of polymers. However, in the majority of cases the reported materials can also be obtained by using traditional chemical catalysts. Therefore, the aim of the thesis is to synthesize curable biodegradable elastomers using the unique advantages of enzymes, for example its regio- and enantioselectivity. This can potentially open novel routes to materials, which are very difficult or even impossible to achieve by conventional chemical procedures. Moreover, taking advantage of enzymatic catalysis also offers metal-free routes to materials which can be potentially used in biomedical application. The micro- and macro-properties of all polymers are extensively investigated to offer adequate information for further in vivo study.

Chapter 1 starts with an overview of enzymatic ring opening polymerization. The mechanism of lipase-catalyzed transesterification is reviewed and the synthesis of optically pure polymers by asymmetric enzymatic polymerizations is discussed. Subsequently, biodegradable microspheres and hydrogels are discussed by highlighting different methods for the formation and degradation of materials for drug carrier and scaffold applications.

Chapters 2 and 3 describe the in situ enzymatic synthesis of (meth)acrylated polyesters by ring opening polymerization. These (meth)acrylate-terminated polyesters are important building blocks for crosslinked coatings or microspheres. The aim is to
answer the question whether enzymatic (meth)acrylation provides a feasible process for the production of (meth)acrylated polymers. This work is subdivided into two chapters with different emphasis.

Chapter 2 deals with 2-hydroxyethyl methacrylate (HEMA)-initiated ring opening polymerization of ε-caprolactone (CL) and ω-pentadecalactone (PDL). Instead of the expected mono-functionalized products, a number of different telechelic polymers with various end-group combinations were observed. Our kinetic studies show that the lipase B from *Candida antarctica* (CALB) does not discriminate between carbonyl bonds of the monomers, the polymers or the initiators, and transesterification reactions can thus not be prevented. However, when HEMA-initiation is combined with vinyl methacrylate end-capping, well-defined dimethacrylated polymers as curable precursors for network formation can be prepared.

In chapter 3, 2-hydroxyethyl acrylate (HEA) and HEMA are compared as initiators in CALB-catalyzed ring opening polymerization (ROP) of CL and PDL. The results presented in this study confirm that lipase-catalyzed ROP using HEA or HEMA as initiators leads to polymers with a mixed composition of end-groups. Large differences in lipase-catalyzed acyl transfer reaction rates between HEA and HEMA end-groups were observed (10-15 fold difference!) in which HEA was more prone to acyl transfer due to the less sterically hindered structure.

In chapter 4 a method is developed to synthesize chiral microspheres obtained from amorphous aliphatic polyesters, with the aim to use chirality to program polymer degradation. By enzymatic enantioselective kinetic resolution polymerization from racemic monomers, hydroxyl-terminated (R)-, (S)- and racemic poly(4-methyl-ε-caprolactone) (PMCL) were successfully synthesized. Preliminary degradation experiments with CALB show that the degradation rate can be tuned by the polymer chirality. Chiral microspheres with a diameter around 40 microns were obtained after acrylation of the polymers and subsequent *in situ* crosslinking in an oil-in-water (O–W) emulsion evaporation approach.

With the aim of tuning the polarity and degradation rate of polyester-based particles, chapter 5 describes the synthesis of semi-interpenetrating polymer network (SIPN)
hydrogels in the presence and with incorporation of poly(ethylene glycol) (PEG) as an extension of chapter 4. Two network formation methods were performed: (1) PEG diacrylate and PMCL (or PCL) diacrylate; (2) PMCL-\(b\)-PEG-\(b\)-PMCL (or PCL-\(b\)-PEG-\(b\)-PCL) diacrylate. Each method for PMCL-based system results in three hydrogels with different chirality. Properties and degradation behaviors were studied for all of the gels.

In chapter 6 we propose a non-organometallic synthesis of PCL-PLA copolymer by taking advantage of enzymatic and carbene catalysis. Enzymes do polymerize lactones but no lactides. Carbenes, on the other hand, are highly active catalysts for the polymerization of lactides. Blank reactions were performed to check the activity of both catalysts towards each monomer. Although mutual inhibition took place in most of the preformed blank reactions, PCL-PLA block copolymer was successfully synthesized by adding the reactants in the right sequence.
References

2002, 35, (11), 4266-4270.
CHAPTER 2

Enzymatic Methacrylation: Lipase-catalyzed HEMA-initiated Ring Opening Polymerization

Abstract

2-Hydroxyethyl methacrylate (HEMA) was used as initiator for the enzymatic ring opening polymerization (ROP) of ω-pentadecalactone (PDL) and ε-caprolactone (CL). The lipase B from Candida antarctica was found to catalyze the cleavage of the ester bond in the HEMA-end group of the formed polyesters, resulting in two major transesterification processes, methacrylate transfer and polyester transfer. This resulted in a number of different polyester methacrylate structures, such as polymers without, with one and with two methacrylate end-groups. However, when combined with end-capping, well defined dimethacrylated polymers (PPDL, PCL) were prepared.
2.1 Introduction

The biocompatibility and biodegradability of aliphatic polyesters makes this class of polymers important materials for biomedical applications.\textsuperscript{1-4} Among the most studied aliphatic polymers are polylactones, for example, poly(\(\varepsilon\)-caprolactone) (PCL), which has been investigated in applications ranging from implant materials to drug delivery materials. Besides their favorable biocompatibility, a reason for the attractiveness of PCL is its straightforward and versatile synthetic accessibility. Commonly, metal mediated ring opening polymerization is applied for the synthesis of PCL, which allows for the control of the molecular weight, polymer architecture and polydispersity. Details can be found in recent reviews by Albertsson et al.\textsuperscript{1} and Dubois et al.\textsuperscript{5}

Enzymatic ring opening polymerization provides an alternative route to aliphatic polyesters.\textsuperscript{6-8} In particular lipases have shown exceptional activity in the ring-opening polymerization of cyclic esters. Unlike with metal catalysts, this activity is not limited to small and medium ring size lactones like \(\varepsilon\)-caprolactone, where the release of ring strain is the driving force for the polymerization, but extends to macrocyclic esters. A comparative study by Duda \textit{et al.} has shown that the larger the lactone ring size the lower its activity in chemical ROP, while the reverse trend was observed in enzymatic ring opening polymerization (eROP).\textsuperscript{9} A detailed investigation of the underlying principles of the reactivities of lactones of increasing ring size in eROP has recently been published by van der Mee \textit{et al.}\textsuperscript{10}

One interesting macrocyclic monomer is \(\omega\)-pentadecalactone (PDL). It has been reported, that PDL can be polymerized to high conversions within short reaction times.\textsuperscript{11-13} The physical properties of poly(\(\omega\)-pentadecalactone) (PPDL) are similar to those of low density polyethylene.\textsuperscript{14, 15} However, the presence of ester bonds along the polymer chain makes it potentially biodegradable and thus an interesting candidate for biomedical applications.

The specific interest of this research is in the functionalization of these polymers to allow further reactions, such as the synthesis of macroinitiators, macromonomers or telechelics for the incorporation into polymer networks. On a more fundamental level we aim to develop a better understanding of enzymatic polymerization from both the
enzymology point of view as well as the mechanistic perspective. This interdisciplinary understanding will ultimately be necessary to apply enzymatic polymerization for engineering new functional polymers.

A very important aspect in the synthesis of functional polymers is end-group functionalization. For metal-mediated ROP a functional end-group can easily be introduced by the initiator method, i.e., the addition of a nucleophile (alcohol or amine). The latter acts as a true initiator and builds into the polymer chain as an end-group.\(^1\)\(^5\)

Due to the controlled character and the polymerization mechanism, i.e., end-group activation, this process is mostly free of side-reactions up to high monomer conversion and yields high degrees of initiator incorporation. Moreover, polymer molecular weights are determined by the monomer to initiator ratio. An example is the synthesis of PCL acrylates by metal-mediated ROP with hydroxyethyl acrylate (HEA) and hydroxyethyl methacrylate (HEMA) as initiators.\(^1\)\(^6\)-\(^1\)\(^8\) The obtained materials were further used to make graft copolymers in a controlled radical polymerization. Similar approaches to end-functionalization were reported for eROP. In some cases this was done to study reaction kinetics of eROP. It was concluded that an initiation profile similar to chemical ROP can be obtained under ideal conditions, i.e., the molecular weights of the polymers were determined by the monomer to initiator ratio.\(^1\)\(^9\),\(^2\)\(^0\)

Moreover, reactive end-groups were introduced by this method. For example, previous researchers successfully introduced initiators for controlled radical polymerization in the eROP of \(\varepsilon\)-caprolactone\(^2\)\(^1\)-\(^2\)\(^3\) and thiol end-groups for UV-induced radical cross-linking.\(^2\)\(^4\),\(^2\)\(^5\) Using an appropriate end-capper also \(\omega\)-functionality can be introduced enzymatically.\(^2\)\(^6\)-\(^2\)\(^8\)

Up to now little is known about mechanistic and kinetic aspects of the (meth)acrylation by the initiator method in eROP. Three reports describe the eROP of CL and PDL, respectively, by HEMA, resulting in the formation of HEMA end-capped macromonomers.\(^2\)\(^9\)-\(^3\)\(^1\) As opposed to metal-mediated ROP, eROP proceeds via an activation of carbonyl bonds by forming an enzyme-activated substrate complex (acyl enzyme). In this reaction the lipase does not discriminate between carbonyl bonds of the monomer (lactone), the polymer or the initiator and transesterification reactions can
thus not be prevented. Since HEMA comprises an ester bond one should expect, depending on the reaction conditions, a mixture of products with various end-groups as a consequence of the transesterification reactions.\textsuperscript{12, 23} This can be an advantage in the \textit{in situ} formation of reactive polymer mixtures, provided the process is fundamentally understood. We therefore started to investigate the one pot enzymatic synthesis of polyester acrylates and methacrylates. In this process a lipase-catalyzed ROP is carried out in the presence of a (meth)acrylating nucleophile. The results described in this chapter concern the polymerization of CL and PDL in the presence of HEMA.

\section*{2.2 Experimental part}

\subsection*{2.2.1 Materials}

Novozym 435 (\textit{Candida antarctica} lipase B immobilized on acrylic resin) was purchased from Novozymes A/S, Denmark and dried in a vacuum oven at 50 °C overnight before use. All chemicals were purchased from Aldrich. CL was distilled over CaH\textsubscript{2} and stored over molecular sieves and PDL was dried under vacuum before use.

\subsection*{2.2.2 Instrumentation}

\textsuperscript{1}H NMR spectra were recorded on a Bruker AM 400 and a Bruker AM 500. CDCl\textsubscript{3} containing 1 vol. \% TMS was used as solvent.

The following equipment was used for SEC analysis: A Waters 717 plus Autosampler, Waters model 510 apparatus equipped with 3 PL gel 10 \( \mu \)m mixed-B columns, 300 x 7.5 mm. Spectra were recorded with a PL-ELS 1000 evaporative light scattering detector, connected to an IBM compatible PC. Millenium software was used to process the data (version 3.05.01). The eluent consisted of HPLC grade chloroform (95 \% v/v) and methanol (5 \% v/v). SEC samples were prepared as 0.5 mg/ml solutions using the
Enzymatic methacrylation: HEMA-initiated eROP

eluent. The molecular weights were calibrated against polystyrene standards. MALDI-ToF-MS analysis was conducted on a Bruker UltraFlex MALDI-ToF-MS with SCOUT-MTP Ion Source (Bruker Daltonics, Bremen) equipped with a N_2 laser (337 nm), a gridless ion source and reflector design. All spectra were acquired using a reflector-positive method with an acceleration voltage of 25 and a reflector voltage of 26.3 kV. Calibration was performed in order to secure good mass accuracy. As for the samples, solutions of 2–5 x 10^{-3} M in CHCl_3 were prepared. The matrix utilized was 9-nitroanthrazene. Matrix solutions were prepared as 0.1 M solutions in THF. The samples were prepared as sample-matrix-Na solutions, employing a 0.1 M Na solution in THF. The preparation protocol included mixing of 5 µL of sample with 20 µL of matrix. Then 1 µL of the mixture was spotted on the MALDI target and was left to crystallize at room temperature (the THF was evaporated). Normally, 50 pulses were acquired for each sample. In order to achieve good mass accuracy and resolution, the analyses were performed at the laser threshold of each individual matrix/sample combination.

The calculation is based on $^1$H NMR signals: (1) HEMA initiated eROP of PDL. For the calculation of monomer conversion, $^1$H NMR signals at 4.14 ppm and 4.05 ppm were used. For the calculation of the methacrylate group distribution over time, the unique signals at 6.10 ppm (methacrylated end-hydroxyl), 6.13 ppm (HEMA end-group, due to the initiation) and 6.16 ppm (non-reacted HEMA) were used. The calculation of the 1,2-ethanediol group distribution over time was done using the signals at 4.34 ppm (HEMA end group), 4.28 ppm (dil residue within the polyester chain) and 4.22 ppm (dil with one free hydroxyl end-group). The distributions of all polymer end-groups over time were calculated using the signals at 4.34 ppm (HEMA end-group), 4.22 ppm (dil with one free hydroxyl end-group), 3.64 ppm (opened monomer hydroxyl end-group) and 6.10 ppm (methacrylated end-hydroxyl); (2) HEMA initiated eROP of CL. For the calculation of monomer conversion, $^1$H NMR signals at 4.25 ppm and 4.05 ppm were used. For the calculation of the methacrylate group distribution over time, the unique signals at 6.06 ppm (methacrylated end-hydroxyl), 6.12 ppm (HEMA end-group, due to the initiation) and 6.16 ppm
(non-reacted HEMA) were used. The calculation of the 1,2-ethanediol group distribution over time was done using the signals at 4.31 ppm (HEMA end group), 4.25 ppm (diol residue within the polyester chain) and 4.22 ppm (diol with one free hydroxyl end-group). The distributions of all polymer end-groups over time were calculated using the signals at 4.31 ppm (HEMA end-group), 4.22 ppm (diol with one free hydroxyl end-group), 3.64 ppm (opened monomer hydroxyl end-group) and 6.06 ppm (methacrylated end-hydroxyl).

### 2.2.3 Synthetic procedure

**A. HEMA initiated ROP of PDL for 24 h (Scheme 2.1A):** HEMA (200 µl, 1.6 mmol) was inject into PDL (2 g, 8.33 mmol) in a 15 mL round reaction flask. Addition of 40 mg of dry Novozym 435 started the reaction that was allowed to run for 24 h.

**B. Kinetic studies of HEMA initiated ROP:** PDL (5 g; 20.8 mmol) or CL (5 g; 43.8 mmol) was mixed with 504 µl (4.1 mmol) and 1.06 ml (8.7 mmol) of HEMA, respectively, in a 25-mL round-bottom reaction flask. The molar ratio between initiator and monomer was 1:5. The reaction was started by the addition of 100 mg of Novozym 435. Samples were taken every hour up to 7 h and after 24, 48 and 72 h. The samples of the reaction were filtered to remove any trace of enzyme and were subsequently mixed with CDCl₃ and analyzed by ¹H NMR.

**C. Dimethacrylated polymers:** The reaction was initially run at the same conditions as reaction B (the molar ratio between initiator and monomer was 1:10 for CL). After 24 h, vinyl methacrylate (407 µl, 3.2 mmol) was added to the reaction mixture and the reaction was allowed to run for another 48 h.

All reactions (A-C) were run at 80 °C under magnetic stirring. Reactions A and C were stopped by filtering off the enzyme. The products were precipitated in dry-ice cooled methanol and the polymers were filtered off by glass microfiber filters, and washed with dry-ice cooled methanol. The polymers were dried before being analyzed by ¹H nuclear magnetic resonance (NMR), matrix-assisted laser desorption/ionization
time-of-flight mass spectrometry (MALDI-ToF-MS) and size exclusion chromatography (SEC).

$^1$H NMR (400 MHz, CDCl$_3$, δ in ppm) for HEMA initiated PCL:
5.58 and 6.10 ppm (1H, s, $CH_2=C(CH_3)C(O)OCH_2CH_2OCH_2-$) and 5.52 and 6.06 ppm (1H, s, $-CH_2CH_2OC(O)C(CH_3)=CH_2$), 4.31 ppm (4H, m, $CH_2=C(CH_3)C(O)OCH_2CH_2OCH_2-$), 4.25 ppm (4H, s, $-CH_2C(O)OCH_2CH_2OC(O)C(CH_3)=CH_2$), 4.22 ppm (2H, m, HOCH$_2$CH$_2OC(O)CH_2-$), 4.14 ppm (2H, t, $-CH_2CH_2OC(O)C(CH_3)=CH_2$), 4.05 ppm (2H, t, $-CH_2CH_2OCO-$), 3.80 ppm (2H, m, HOCH$_2$CH$_2OC(O)C(CH_3)=CH_2$), 3.62 ppm (2H, t, $-CH_2CH_2OH$), 2.28 ppm (2H, t, $-OC(O)CH_2CH_2-$), 1.58-1.66 ppm (4H, m, $-CH_2CH_2CH_2CH_2CH_2-$), 1.32-1.41 ppm (20H, m, $-CH_2CH_2CH_2CH_2CH_2-$).

$^1$H NMR (500 MHz, CDCl$_3$, δ in ppm) for HEMA initiated PPDL:
5.60 and 6.13 ppm (1H, s, $CH_2=C(CH_3)C(O)OCH_2CH_2OCH_2-$) and 5.55 and 6.10 ppm (1H, s, $-CH_2CH_2OC(O)C(CH_3)=CH_2$), 4.34 ppm (4H, m, $CH_2=C(CH_3)C(O)OCH_2CH_2OCH_2-$), 4.28 ppm (4H, s, $-CH_2C(O)OCH_2CH_2OC(O)CH_2-$), 4.22 ppm (2H, m, HOCH$_2$CH$_2OC(O)CH_2-$), 4.14 ppm (2H, t, $-CH_2CH_2OC(O)C(CH_3)=CH_2$), 4.05 ppm (2H, t, $-CH_2CH_2OCO-$), 3.83 ppm (2H, m, HOCH$_2$CH$_2OC(O)CH_2-$), 3.64 ppm (2H, t, $-CH_2CH_2OH$), 2.28 ppm (2H, t, $-OC(O)CH_2CH_2-$), 1.61-1.69 ppm (4H, m, $-CH_2CH_2(CH_2)_{10}CH_2CH_2-$), 1.18-1.39 ppm (20H, m, $-CH_2CH_2(CH_2)_{10}CH_2CH_2-$).

### 2.3 Results and discussion

#### 2.3.1 Polyester structures from lipase-catalyzed HEMA-initiated ring-opening polymerization

In order to obtain methacrylated aliphatic polyester macromonomers, 2-hydroxyethyl methacrylate (HEMA) was used as initiator for the lipase-catalyzed ring-opening polymerization of ω-pentadecalactone (PDL) and ε-caprolactone (CL) in bulk at 80 °C
for 24 h (Scheme 2.1A). In accordance with literature reports we mainly obtained polymers with one HEMA end-group and one hydroxyl end-group (1).\textsuperscript{26-28} However, closer inspection of the reaction products revealed the formation of several other polymer products in this reaction. It was detected, by \textsuperscript{1}\textit{H} NMR and MALDI-ToF-MS, that the lipase not only catalyzed the HEMA-initiated ROP but also the cleavage of the ester bond within the HEMA-moiety of the polymer. This cleavage resulted in two major types of transesterification (acyl transfer) reactions: methacrylate transfer and polyester transfer (Scheme 2.1B, C). The methacrylate transfer (Scheme 2.1B) led to polymers with four different end-group structures; HEMA end-group (1, 3); hydroxyl end-group (1, 2); 1,2-ethanediol end-group (2, 3); methacrylated hydroxyl end-group (3). Furthermore, as a result of the polyester transfer reaction (Scheme 2.1C), the 1,2-ethanedioxy moiety was found to be present within the polyester chain (4).
Scheme 2.1 (A) Ring-opening polymerization of PDL (m = 11) and CL (m = 2) initiated by HEMA. (B) Methacrylate transfer from the HEMA end-group of the polymer to the hydroxyl end-group of the polymer. (C) Polyester transfer to the hydroxyl group of the residual 1,2-ethanediol end-group.
The presence of these structures was confirmed for both polymers, i.e., PCL and PPDL. The characteristic peaks of the repeating units and end-groups of PPDL and PCL were observed by $^1$H NMR. Figure 2.1 shows the example of PPDL: the chemical shifts of the two methacrylate end-groups were assigned as $h_1, j_1$ and $a$ (HEMA end-group) and $h_2, j_2$ and $d$ (methacrylated end-hydroxyl of the polymer). Characteristic peaks of the
three 1,2-ethanediol polyester structures were assigned: HEMA-moiety (peak a in Figure 2.1); 1,2-ethanediol end-group (peaks c and f in Figure 2.1); Diol residue within the polyester chain (peak b in Figure 2.1). The methylene adjacent to the hydroxyl end-group was assigned as g. From MALDI-ToF-MS analysis three main product distributions were observed, each with a repeating interval of 240 Da, being the mass of one PDL monomer residue (Figure 2.2A). The differences between the series of main products were 68 Da, which matches with the mass of a methacrylate group. In Figure 2.2, the peaks corresponding to polymers containing one methacrylate end-group are labelled with II. Peaks corresponding to polymers with two methacrylate ends are labelled with III, while those corresponding to polymers without methacrylate ends have the label I.

These results confirm that transesterification reactions are prominent side reactions in enzymatic ROP and consequently the use of ester containing nucleophiles (“initiators”) has its limitations for the synthesis of well-defined macromonomers. On the other hand, this opens opportunities for the in situ synthesis of functional polymers, provided that the factors influencing the frequency and extend of the transesterification reactions are understood.
Figure 2.2 MALDI-ToF-MS spectra of PPDL obtained by CALB-catalyzed HEMA-initiated ROP of PDL at 80 °C. (A) Product after 24 h (precipitated in dry-ice cooled MeOH, washed and dried). Samples for kinetics investigation (B) 4 h and (C) 72 h (no purification). The mass distribution I represents polymers without methacrylate end-group, and the mass distribution II represents polymers with one methacrylate end, while the mass distribution III represents polymers with two methacrylate ends.

2.3.2 Kinetic investigation of lipase-catalyzed ROP of PDL and CL initiated with HEMA

In order to get a better understanding of the eROP reaction described above, we performed a kinetic investigation of the HEMA-initiated ROP of PDL and CL using $^1$H NMR and MALDI-ToF-MS. The kinetics of the ROP, the methacrylate transfer and the polyester transfer were investigated. The content of the different end-groups in the polyester products were analyzed as a function of time.

In Figure 2.3A, the ROP of PDL using HEMA as an initiator was followed as a function of time. The conversion of PDL and the distribution of the methacrylate moiety (the consumption of HEMA (initiator), the formation of HEMA initiated
polymers (ROP) and the formation of polymers with a methacrylated end-hydroxyl group (methacrylate transfer)) were quantified with $^1$H NMR and plotted as a function of time. At the beginning of the polymerization, the ROP was the major process (Scheme 2.1A): 80% of the initiator was consumed after 1 hour, resulting in HEMA-initiated polymers (1). When the monomer (PDL) and most of the initiator were consumed, the transfer of the methacrylate moiety in the HEMA end-group of the initially formed polymer (1) to the hydroxyl end-group of the polymer, resulting in polymers (2) and (3), became significant (Scheme 2.1B). This can be seen as a decrease in the concentration of polymers with a HEMA end-group and an increase in the concentration of polymers with a methacrylated end-hydroxyl group (Figure 2.3A). Similar results were observed using CL (Figure 2.3B). The conversion of HEMA and consumption of monomer proceeded faster with PDL than with CL, which is in agreement with the literature where it has been shown that CALB displays higher activity in ROP of PDL than of CL. 

![Figure 2.3](image)

Figure 2.3 Kinetic studies of CALB-catalyzed ROP of PDL (A) and CL (B) initiated with HEMA. Consumption of the lactone (—△—) with time. Distribution of the methacrylate group with time: Consumption of HEMA (—■—); The formation of HEMA-initiated polymers by ROP (—●—); Formation of polymers with methacrylated end-hydroxyl group by methacrylate transfer (—▲—).

The methacrylate transfer was faster for PPDL as compared with PCL and after 72 h, 79% of the initial amount of the methacrylate group was found on the PPDL hydroxyl
end-group as compared with 43% for PCL. This difference in methacrylate transfer activity is probably due to the difference in monomer consumption between CL and PDL. PDL was fully consumed after 4 h while 30% of CL still remained after 7 h, which could compete with the methacrylate transfer process, i.e., slowing it down.

The distribution of the 1,2-ethanediol group in the polymer products during the reaction is presented in Figure 2.4. As a result of the methacrylate transfer, polymers with 1,2-ethanediol end-groups (2) were concomitantly produced (Scheme 2.1B). This opens the possibility of a polyester transfer to the hydroxyl group of the diol, resulting in polymers with the 1,2-ethanediol moiety fully incorporated within the polyester main chain (4) (Scheme 2.1C). At the beginning of the reaction the diol residue was exclusively present as an integral part of the HEMA end group (Figure 2.4).

![Figure 2.4](image_url)

**Figure 2.4** The distribution of the 1,2-ethanediol moiety (originating from HEMA) with time during CALB-catalyzed ROP of PDL (A) and CL (B) initiated with HEMA: 1,2-ethanediol within the HEMA end-group ( ), 1,2-ethanediol end-group ( ), 1,2-ethanediol incorporated within the polyester chain ( ).

After 2 h significant transmethacrylation started to take place as evident from the steep decrease of the concentration of the HEMA end-group. Correspondingly, the concentration of the 1,2-ethanediol end-group and of the 1,2-ethanediol within the
polyester chain increased to 30% and 48%, respectively, after 72 h. Similar trends were observed for both PDL (Figure 2.4A) and CL (Figure 2.4B).

In Figure 2.5A, the relative contents of all end-groups in PPDL are plotted as a function of time. At the beginning of the polymerization, the ROP was the major process (Scheme 1A) resulting in polymers with both HEMA and hydroxyl end-groups in a 1:1 ratio. With time the transesterification processes became dominant, resulting in polymers containing a mixture of end-groups (Scheme 1). After 72 h the relative contents of end-groups in PPDL were as follows: 11% HEMA end-groups (1, 3); 21% hydroxyl end-groups (1, 2, 4); 15% 1,2-ethanediol end-groups (2, 3); 53% methacrylated end-hydroxyl groups (3, 4). A similar trend was observed with CL (Figure 2.5B).

![Figure 2.5](image)

**Figure 2.5** The relative content of end-groups in PPDL(A) and PCL(B) with time, obtained in CALB-catalyzed ROP of PDL initiated with HEMA: HEMA end-group (●); 1,2-ethanediol end-group (□); Hydroxyl end-group (○); Methacrylated hydroxyl end-group (▲).

From $^1$H-NMR analysis the methacrylate end-groups (HEMA end-group and methacrylated end-hydroxyl) after 72 h, constituted 64% of the total end-groups in the polymer while the hydroxyl containing end-groups (hydroxyl end-group and 1,2-ethanediol end-group) add up to 36%. This difference was also confirmed by MALDI-ToF-MS. For the sample taken at 4 h (Figure 2.2B), it was observed that the
largest group of peaks corresponds to HEMA-initiated polymers. On the other hand, for
the sample after 72 h (Figure 2.2C), the largest group of peaks was corresponding to
polymers with two methacrylate end-groups. This can possibly be explained by the
liberation of 1,2-ethanediol from polymer (2) by a transesterification process. Evidence
for this was obtained from $^1$H NMR analysis of the product samples, which shows the
free 1,2-ethanediol group (peak $n$, Figure 2.1B). The diol is poorly soluble in PPDL and
might thus not be easily incorporated into the polyester, resulting in a net loss of
1,2-ethanediol in the polyesters. This leads to a decrease in the number of hydroxyl
end-groups and an increase of the methacrylate end-groups in the polymers.
Furthermore, ethylene glycol dimethacrylate was also produced, possibly by
methacrylation of HEMA (peak $k$, Figure 2.1B).

The results clearly show the kinetics of the ROP and the transesterification processes
when using an initiator like HEMA with a cleavable ester group in lipase-catalyzed
ROP of PDL and CL. While the transesterification processes occur at moderate
frequency at low monomer conversion, it becomes dominant at longer reaction times.
This clearly shows the difficulties in getting well-defined macromonomers using this
procedure as dimethacrylated polyesters are produced even at low conversions.

### 2.3.3 One-pot two-step synthesis of dimethacrylated polymers

By using HEMA as initiator for the enzymatic ring-opening polymerization of CL and
PDL, polyesters with a mixture of end-group combinations were obtained as described
above (Scheme 2.1). In order to prepare fully methacrylated material, for use as
building blocks for polymer networks, we attempted a one-pot procedure for the
HEMA-initiated ROP reaction combined with vinyl methacrylate end-capping
(methacrylation) via a second step (Table 2.1). By $^1$H NMR, full conversion of the
hydroxyl ends was observed since the peaks at 3.64 ppm (methylene group adjacent to
the hydroxyl end) and at 3.83 ppm (1,2-ethanediol end-group) had disappeared (Figures
2.6A, 2.6B). The MALDI-ToF-MS spectrum of the resulting polymer showed only the
distribution that corresponds to polymers with two methacrylate ends. The mass differences between the peaks were 240 Da (Figure 2.7A) and 114 Da (Figure 2.7B), which correspond to the molecular weight of one PDL and CL unit, respectively. The ease, by which functionality can be added to PPDL and PCL, implies that such macromonomers can form the basis for polymer network formation. Work is currently in progress regarding the properties of networks made from these macromonomers, i.e. the novel PPDL dimethacrylate.

Figure 2.6 $^1$H NMR spectra of CALB catalyzed ROP reaction initiated with HEMA and end-capped with vinyl methacrylate via a second step, but in one pot: (A) PDL ($m = 11$); (B) CL ($m = 2$).
Figure 2.7 MALDI-ToF-MS spectra of CALB-catalyzed ROP reaction initiated with HEMA and end-capped with vinyl methacrylate in a second step, but in one pot: (A) PDL (m = 11); (B) CL (m = 2).

Table 2.1 Dimethacrylated PCL and PPDL.

<table>
<thead>
<tr>
<th>Product</th>
<th>Ratio I:M:T&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reaction time (h)</th>
<th>Fraction of dimethacrylation (%)</th>
<th>$M_n$ (Da)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>$M_w/M_n$&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL</td>
<td>1:5:2</td>
<td>24</td>
<td>&gt;95%</td>
<td>4000</td>
<td>1.55</td>
</tr>
<tr>
<td>PPDL</td>
<td>1:5:2</td>
<td>24</td>
<td>&gt;95%</td>
<td>11000</td>
<td>1.39</td>
</tr>
</tbody>
</table>

<sup>a</sup>I = initiator, T = terminator, M = monomer. The ratio is in mol/mol. <sup>b</sup>Values obtained from size exclusion chromatography.

2.3 Conclusion

In this chapter we investigated the methacrylation of PCL and PPDL by HEMA in a lipase-catalyzed ROP. While the ROP of the monomer initiated by HEMA was the predominant reaction at low monomer conversion, increasing transesterification occurred at longer reaction times. This led to a number of different telechelic polymers
with various end-group combinations. This transmethacrylation process was found to be faster with PDL than with CL, which might be due to the overall faster reaction kinetics of the macrocyclic monomer. The results show that initiators with cleavable ester groups are of limited use to obtain well-defined mono-functionalized macromonomers. Due to the enzyme-catalyzed transesterification process, reaction times should be short since dimethacrylated polymers are detected even at low conversion. On the other hand, fully dimethacrylated polymers (PPDL, PCL) were prepared by combining HEMA-initiation eROP with enzyme-catalyzed end-capping using vinyl methacrylate in a second step, but in one pot.
References

24. Hedfors, C.; Ostmark, E.; Malmstrom, E.; Hult, K.; Martinelle, M.
CHAPTER 3

Enzymatic Acrylation: Lipase-Catalyzed HEA-Initiated Ring Opening Polymerization

Abstract

2-Hydroxyethyl acrylate (HEA) and 2-hydroxyethyl methacrylate (HEMA) were compared as initiators in lipase (Candida antarctica lipase B) catalyzed ring-opening polymerization (ROP) of ε-caprolactone (CL) and ω-pentadecalactone (PDL). The results presented in this study confirm that lipase catalyzed ROP using HEA or HEMA as initiators leads to polymers with a mixed composition of end-groups: HEA/HEMA end-group; 1,2-ethanediol end-group; hydroxyl end-group; acrylated/methacrylated hydroxyl end-group. This is due to the involvement of the ester bonds in the HEA and HEMA moieties in transacylation processes. Large differences in lipase catalysed acyl transfer reaction rate between HEA and HEMA end-groups were observed (10-15 fold) in which HEA was more prone to acyl transfer due to the less hindered structure.

This chapter is based on:
the collaboration work with the group of Prof. K. Hult in Royal Institute of Technology, Sweden;
3.1 Introduction

Polyester acrylates for medical applications are almost exclusively synthesized by metal-mediated ring opening polymerization (ROP) and subsequent acrylation of the polyester hydroxyl end-group by acryloyl chloride or anhydride. While this is an accepted procedure, it leaves the problem of purification of the polymer from the metal catalyst as well as the acrylation by-product (excess acryloyl chloride and chloride salt). Alternatively, acrylate-functional initiators can be employed in the ROP, for example, 2-hydroxyethyl acrylate (HEA) and 2-hydroxyethyl methacrylate (HEMA) giving access to mono-acrylated polymers.

Enzymatic ROP offers an alternative route to aliphatic polyesters, thereby eliminating the use of metal catalysts. As opposed to metal-mediated ROP, enzymatic ROP proceeds via an activation of carbonyl bonds by forming an enzyme-activated substrate complex (acyl enzyme). In this reaction the lipase does not discriminate between carbonyl bonds of the monomer (lactone), the polymer or the initiator and transesterification reactions can thus not be prevented. The initiation process in an enzymatic ROP and its influence on the polymer structures was recently investigated by de Geus et al. However, using an appropriate end-capper, also ω-functionality can be introduced enzymatically. Fully dimethacrylated polymers (PPDL, PCL) were prepared by combining HEMA-initiation ROP with end-capping using vinyl methacrylate. Therefore, it is reasonable to assume that fully diacrylate polyesters could also be enzymatically synthesized by combining HEA-initiation ROP with end capping using vinyl acrylate.

In chapter 2 we could show that in the HEMA-initiated ROP of PDL and CL the ester bond within HEMA participates in the transesterification reactions, resulting in a mixture of polymer products with various end-groups such as polymers without, with one and with two methacrylate end-groups. Furthermore, the 1,2-ethanediol moiety (from HEMA) was found within the polyester main chains. The extent and thus the distribution of end-groups were found to depend on the reaction time and monomer used. After 72 h, 79 % of the initial amount of the methacrylate moiety (from HEMA)
was situated on the hydroxyl end-group of the poly-(ω-pentadecalactone) (PPDL). In the case of PCL only 48% of the intact HEMA end-group was found at the polymer chain end. We now extend our investigation of the acrylation of aliphatic polyesters, obtained from enzymatic ROP, by a systematic comparison of initiation from HEA and HEMA. The results show distinct differences between the two initiators, which can be used as guidelines in the enzymatic acrylation of polyesters.

3.2 Experimental part

3.2.1 Materials

Novozym 435 (*Candida antarctica* lipase B immobilized on acrylic resin) was purchased from Novozymes A/S, Denmark and dried in a vacuum oven at 50°C overnight before use. All chemicals were purchased from Aldrich and used as received unless otherwise mentioned. CL was distilled from CaH$_2$ and stored over molecular sieves. PDL was dried under vacuum before use.

3.2.2 Instrumentation

$^1$H NMR spectra were recorded on a Bruker AM 400 and a Bruker AM 500. CDCl$_3$ containing 1 vol. % TMS was used as solvent.

MALDI-ToF-MS analysis was conducted on a Bruker UltraFlex MALDI-ToF-MS with SCOUT-MTP Ion Source (Bruker Daltonics, Bremen) equipped with a N$_2$ laser (337 nm), a gridless ion source and reflector design. All spectra were acquired using a reflector-positive method with an acceleration voltage of 25 and a reflector voltage of 26.3 kV. Calibration was performed in order to secure good mass accuracy. Sample solutions of 2–5 mM were prepared in CHCl$_3$, while the matrix (9-nitroanthrazene) solutions were prepared as 0.1 M solutions in THF containing 0.1 M of sodium trifluoroacetate. The preparation protocol included mixing of 5 µL of sample with 20
µL of matrix. Then 1 µL of the mixture was spotted on the MALDI target and was left to crystallize at room temperature (the THF was evaporated). Normally, 50 pulses were acquired for each sample. In order to achieve good mass accuracy and resolution, the analyses were performed at the laser threshold of each individual matrix/sample combination.

The calculation is based on \textsuperscript{1}H NMR signals: (1) HEMA initiated eROP of PDL. For the calculation of monomer conversion, \textsuperscript{1}H NMR signals at 4.14 ppm and 4.05 ppm were used. For the calculation of the acrylate group distribution over time, the unique signals at 4.14 ppm (acylated end-hydroxyl), 4.34 ppm (HEA end-group, due to the initiation) and 3.85 ppm (non-reacted HEA) were used. The calculation of the 1,2-ethanediol group distribution over time was done using the signals at 4.34 ppm (HEA end group), 4.28 ppm (diol residue within the polyester chain) and 4.22 ppm (diol with one free hydroxyl end-group). The distributions of all polymer end-groups over time were calculated using the signals at 4.34 ppm (HEA end-group), 4.22 ppm (diol with one free hydroxyl end-group), 3.64 ppm (opened monomer hydroxyl end-group) and 4.14 ppm (acylated end-hydroxyl); (2) HEMA initiated eROP of CL. For the calculation of monomer conversion, \textsuperscript{1}H NMR signals at 4.25 ppm and 4.05 ppm were used. For the calculation of the acrylate group distribution over time, the unique signals at 4.11 ppm (acylated end-hydroxyl), 4.32 ppm (HEA end-group, due to the initiation) and 3.85 ppm (non-reacted HEA) were used. The calculation of the 1,2-ethanediol group distribution over time was done using the signals at 4.32 ppm (HEA end group), 4.23 ppm (diol residue within the polyester chain) and 3.77 ppm (diol with one free hydroxyl end-group). The distributions of all polymer end-groups over time were calculated using the signals at 4.32 ppm (HEA end-group), 3.77 ppm (diol with one free hydroxyl end-group), 3.59 ppm (opened monomer hydroxyl end-group) and 4.11 ppm (acylated end-hydroxyl).
### 3.2.3 Synthetic procedure

Kinetic studies of HEA-initiated ROP: PDL (5 g; 20.8 mmol) or CL (5 g; 43.8 mmol) was mixed with 0.480 ml (4.1 mmol) and 1.01 ml (8.7 mmol) of HEA, respectively, in a round-bottom reaction flask at 80 °C. The molar ratio between initiator and monomer was thus 1:5. Then the reaction was started by the addition of 100 mg of Novozym 435 and kept at 80 °C under magnetic stirring. Samples were taken every hour for the first 7 h of the reaction and after 24, 48 and 72 h, respectively. The samples taken from the reaction were filtered to remove any trace of enzyme and were subsequently dissolved in CDCl₃ and analyzed by ¹H NMR.

¹H NMR (400 MHz, CDCl₃, δ in ppm) for HEA-initiated poly-CL:

5.82 and 6.38 ppm (1H, s, CH₂=CHC(O)OCH₂CH₂OCH₂⁻) and 5.78 and 6.33 ppm (1H, s, -CH₂CH₂OOC(O)CH=CH₂), 4.32 ppm (2H, m, CH₂=CHC(O)OCH₂CH₂OCH₂⁻), 4.28 ppm (2H, m, CH₂=CHC(O)OCH₂CH₂OCH₂⁻), 4.23 ppm (4H, s, -CH₂C(O)OCH₂CH₂OC(O)CH₂⁻), 4.19 ppm (2H, m, HOCH₂CH₂OC(O)CH₂⁻), 4.11 ppm (2H, t, -CH₂CH₂OC(O)CH=CH₂), 4.05 ppm (2H, t, -CH₂CH₂OOC⁻), 3.77 ppm (2H, m, HOCH₂CH₂OC(O)CH₂⁻), 3.59 ppm (2H, t, -CH₂CH₂OH), 2.28 ppm (2H, t, -OC(O)CH₂CH₂⁻), 1.58-1.66 ppm (4H, m, -CH₂CH₂CH₂CH₂CH₂⁻), 1.32-1.41 ppm (2H, m, -CH₂CH₂CH₂CH₂CH₂⁻).

¹H NMR (500 MHz, CDCl₃, δ in ppm) for HEA initiated poly-PDL:

5.84 and 6.43 ppm (1H, s, CH₂=CHC(O)OCH₂CH₂OCH₂⁻) and 5.79 and 6.37 ppm (1H, s, -CH₂CH₂OOC(O)CH=CH₂), 4.34 ppm (2H, m, CH₂=CHC(O)OCH₂CH₂OCH₂⁻), 4.30 ppm (2H, m, CH₂=CHC(O)OCH₂CH₂OCH₂⁻), 4.28 ppm (4H, s, -CH₂C(O)OCH₂CH₂OC(O)CH₂⁻), 4.22 ppm (2H, m, HOCH₂CH₂OC(O)CH₂⁻), 4.14 ppm (2H, t, -CH₂CH₂OC(O)CH=CH₂), 4.05 ppm (2H, t, -CH₂CH₂OOC⁻), 3.83 ppm (2H, m, HOCH₂CH₂OC(O)CH₂⁻), 3.64 ppm (2H, t, -CH₂CH₂OH), 2.28 ppm (2H, t, -OC(O)CH₂CH₂⁻), 1.61-1.69 ppm (4H, m, -CH₂CH₂CH₂CH₂CH₂⁻), 1.18-1.39 ppm (20H, m, -CH₂CH₂(CH₂)₁₀CH₂CH₂⁻).
3.3 Results and discussion

3.3.1 Polyester structures

In order to rationalize the action of 2-hydroxyethyl acrylate (HEA) as compared with 2-hydroxyethyl methacrylate (HEMA) as initiator in lipase-catalyzed ROP, a comparative investigation was performed using PDL and CL as monomers. The HEA-initiated ROP, using Candida antarctica lipase B (CALB), was found to follow the reaction pathways shown in Scheme 3.1: (A) HEA-initiated ROP generating polymer (1); (B) Acyl transfer of the acrylate group of polymer (1) to the hydroxyl end-group of the polymer generating polymers (2) and (3); (C) Acyl transfer of a polyester chain to polymer (2) generating polymer (4). This is in full analogy with the processes found when using HEMA as initiator.\(^{38}\)

In lipase catalysis a covalent intermediate, acyl-enzyme, is formed by a nucleophilic attack on the carbonyl carbon (in the ester to be cleaved) by the active nucleophile (serine) situated in the active-site of the enzyme. In Scheme 3.1, three different acyl enzymes are illustrated: (A) Lipase acylated by the opened monomer; (B) Lipase acylated by the (meth)acrylate moiety; (C) Lipase acylated by a polyester moiety. The formed acyl-enzyme is subsequently deacylated: (A) by the initiator (initiation) or the growing polyester (propagation); (B) by the hydroxyl end-group of the polymer; (C) by the ethanediol moiety of the polymer.

The obtained polyester structures were characterized using \(^1\)H NMR and MALDI-ToF-MS as a function of reaction time, which allowed for the identification and quantification of the reaction products with various end-groups. As a representative example, Figure 3.1 shows the \(^1\)H NMR spectrum of a PPDL sample taken after 4 h of reaction time in the presence of HEA. The chemical shifts of the two acrylate end-groups were assigned as \(m_1, n_1\) and \(o_1\) (HEA end-group) and \(m_2, n_2\) and \(o_2\) (acylated end-hydroxyl of the polymer). Characteristic peaks of the three 1,2-ethanediol polyester structures were also assigned: HEA-moiety (peak \(b\) and \(c\)); 1,2-ethanediol end-group (peaks \(j\) and \(f\)); diol residue within the polyester chain (peak \(e\)). The methylene adjacent to the hydroxyl end-group was assigned as \(l\). From
MALDI-ToF-MS analysis three main product distributions were observed, each with a repeating interval of 240 Da corresponding to the mass of one PDL monomer residue. The differences between the series of main products were 56 Da, which matches with the mass of an acrylate group (Figure 3.2). The careful analysis of the reaction products allowed us (i) to investigate whether the lipase, CALB, displayed different activities towards HEA as compared with HEMA as acyl acceptor (initiator in reaction A, Scheme 3.1) and acyl donor (acyl transfer in reaction B, Scheme 3.1), and (ii) to relate this to the different structures of both initiators (hydrogen versus methyl group in \( \alpha \)-position to the carbonyl moiety) and the individual reaction kinetics of the two monomers.

**Scheme 3.1** (A) CALB-catalyzed ROP of PDL (\( m =11 \)) and CL (\( m = 2 \)) initiated by HEA or HEMA. (B) Acrylate transfer from the HEA (HEMA) end-group of the polymer to the hydroxyl end-group of the polymer. (C) Polyester transfer to the hydroxyl group of the residual 1,2-ethanediol end-group.
Figure 3.1 $^1$H NMR spectrum of PPDL obtained by CALB-catalyzed HEA-initiated ROP of PDL at 80 °C. Sample taken after 4 h reaction time (no purification).
3.3.2 HEA and HEMA initiation kinetics

In Figure 3.3, the conversion of both lactones (PDL and CL) and the consumption of initiator (HEA or HEMA) is plotted against time. In agreement with the literature, the enzymatic polymerization of PDL was faster than that of CL. In bulk conditions, PDL was fully converted into polymer after 3 h, in the presence of HEA or HEMA, while a reaction time of more than 40 h was required to reach a conversion of over 90% in the CL polymerization. Thus, CALB-catalyzed ROP was about 7 fold more efficient using PDL as monomer as compared with CL (taking into account the double amount of moles in bulk CL as compared to bulk PDL).
Figure 3.3 Kinetic studies of CALB-catalyzed ROP of PDL (A) and CL (B) initiated with HEA or HEMA. Conversion of the lactone with time; HEA-initiated (■); HEMA-initiated (□). The amount of free initiator; HEA (●) and HEMA (○).

Figure 3.4 The degree of polymerisation (Dp) of PPDL and PCL with time in CALB-catalyzed ROP of PDL (A) and CL (B) initiated with HEA (●) or HEMA (○).

The consumption of HEA and HEMA follow a similar pattern for PDL (Figure 3.3A) and with CL (Figure 3.3B). Further, the conversions of PDL display a similar rate profile, irrespective of using HEA or HEMA as initiator. In the case of CL the conversion of monomer was somewhat faster with HEMA than with HEA. Probably the transesterification reaction, transferring the acrylate moiety from the HEA moiety to the end-hydroxyl group of the polymer, Scheme 3.1B, competes with the ROP, thus slowing it down. In the case of HEMA, the methacrylate transfer reaction was much slower, thus, much less competition was encountered. In Figure 3.4, the degree of
polymerisation (Dp) is plotted versus time. The increase in Dp follows the same trend as the reaction kinetics, namely a fast increase in Dp for PPDL and a much slower increase for PCL. The lower Dp for HEA-initiated PCL (Figure 3.4B) correlates with the lower conversion of CL in Figure 3.3B.

A similar reaction rate profile was observed for HEA and HEMA, meaning that CALB used these initiators with almost similar efficiency in the enzymatic ROP. The structural difference between HEA and HEMA is situated quite far from the nucleophilic hydroxyl group of the initiators and the additional methyl group in HEMA did not result in any major steric interaction in the active site of CALB, which might resulted in lowering the initiation efficiency of HEMA. However, these experiments do not allow any conclusion on the obtained molecular structures and thus on the mode of action in these reactions with respect to transacylation reactions.

3.3.3 Kinetics of acyl transfer of acrylate and methacrylate moieties

![Figure 3.5](image)

**Figure 3.5** Kinetic studies of CALB-catalyzed ROP of PDL (A) and CL (B) initiated with HEA or HEMA. Distribution of the acrylate or methacrylate group with time (Free initiator, HEA/HEMA, not shown, see Figure 3.3): The formation of HEA- (●) or HEMA- (○) initiated polymers (1) by ROP. Formation of polymers (3) with acrylated or methacrylated end-hydroxyl group by acrylate (■) or methacrylate transfer (□).
In Figure 3.5, the formation of HEA/HEMA-initiated polymers (1) (process A in Scheme 3.1) and the formation of polymers (3) with acrylated/methacrylated end-hydroxyl groups (process B in Scheme 3.1) is shown. In Figure 3.5A, two regimes can clearly be identified. During the first 1-2 hours the ROP of PDL, initiated with HEA or HEMA, was the predominating reaction (process A in Scheme 3.1) resulting in HEA- (HEMA-) initiated polymers (1).

As illustrated in Figure 3.5A, the amount of these polymers reached a maximum level of ca. 50% of the total amount of HEA present and ca. 80% of the total amount of HEMA present, respectively. This is followed by a decrease of the HEA (HEMA) end-groups in polymers (1) by an acrylate (methacrylate) transfer process to the end-hydroxyl group of polymers (2), generating polymers (3) (process B, Scheme 3.1). This suggests that process B becomes dominant as compared to process A at this point.

The drop in the amount of HEA/HEMA end-groups roughly coincides with the point of full monomer conversion. While the initiation process and the lactone conversion were concluded to be very similar for both HEA and HEMA, the rate of the lipase-catalyzed acrylate transfer reaction was found to be 10-15 fold faster as compared to the rate of the methacrylate transfer reaction. Thus, when using HEMA as initiator, the ROP was the major event at low conversion with the formation of HEMA-initiated polymers, with only a low amount of polymer (3) in the beginning of the ROP due to the low activity in the methacrylate transfer reaction (Figure 3.5, Scheme 3.1B). However, in the case of using HEA as initiator the high activity of the acrylate transfer resulted in high levels of polymer (3), with acrylated end-hydroxyl group, even at the beginning of the ROP. After 3h more than 50% of the initial amount of the acrylate group, originating from HEA, was found on the PPDL hydroxyl end-group. On the other hand, less than 5% of the methacrylate group was found on the end-hydroxyl group when using HEMA. It appears that the initial phase of the polymerization is kinetically controlled, while due to transesterification reactions in the later stage the thermodynamic equilibrium is reached. The system is in a total equilibrium between monomer, polymer and all end-groups. A similar trend was found when using CL as monomer, with the difference that the ROP of CL is slower and thus monomer is
available for a longer period of time. Consequently, in this case transacylation reactions on HEA/HEMA also occur with a lower rate. Nevertheless, the HEA reaction appears to have reached the thermodynamic equilibrium within the reaction time of 70 h, while the slower HEMA transacylation did not (Figure 3.5B). This difference in acyl transfer rate between an acrylate and a methacrylate moiety on a polymer is probably due to a combined effect of 1) steric interaction of the methyl group in the HEMA moiety in the active site of CALB, making it less efficient as acyl donor, and 2) lower chemical reactivity of the HEMA group in acyl transfer reactions. It is well known that a $\alpha$-methyl moiety in acid/esters will lower the chemical reactivity in acyl transfer reactions.\(^{39}\)

### 3.3.4 Polyester transfer

As a result of the acrylate/methacrylate transfer reaction, polymers with 1,2-ethanediol (ED) end-groups (2) were produced (Scheme 3.1B). The distribution of the 1,2-ethanediol groups in the polymer products during the reaction with HEA and HEMA is presented in Figure 3.6 for PDL. For example, it can be seen that initially the majority of ED is present in the HEA end-group itself (ca. 60\%) (Figure 3.6A). After a reaction time of about three hours, transacylation of the ED occurs and gradually reduces the percentage of ED in these end-groups. Concurrently, the incorporation of ED as polymer end-groups (Figure 3.6B) leads to hydroxyl functional polymers (2) (Scheme 3.1). Finally, these are increasingly incorporated into the PPDL chains up to 55\% (Figure 3.6C) in the thermodynamic equilibrium situation, resulting in dihydroxy functional polymers (4) (Scheme 3.1).

As a result of the different reaction rates of acrylate and methacrylate transfer, the generation of polymers with ED end-groups was faster when using HEA than when using HEMA (Figure 3.6). Consequently, the faster production of polymer (2) using HEA resulted in a faster polyester transfer to the hydroxyl group of the diol, resulting in polymers with the ED moiety fully incorporated within the polyester chain (4). Again, a
similar yet slower process was observed in the polymerization of CL (Figure 3.7).

![Graph A](image)
![Graph B](image)
![Graph C](image)

**Figure 3.6** The distribution of the 1,2-ethanediol (ED) moiety originating from HEA (■) or HEMA (□) with time during CALB-catalyzed ROP of PDL. A: ED within the HEA/HEMA end-group of polymer (1); B: ED end-group of polymer (2); C: ED incorporated within the polyester chain of polymer (4).
Enzymatic acrylation: HEA-initiated eROP

Figure 3.7 The distribution of the 1,2-ethanediol (ED) moiety originating from HEA (■) or HEMA (□) with time during CALB-catalyzed ROP of CL. A: ED within the HEA/HEMA end-group; B: ED end-group; C: ED incorporated within the polyester chain.

3.3.5 End-group structures

From the presented data a picture of the relative contents of all end-groups in PPDL and PCL can be drawn as a function of time. Figure 3.8A shows that in the polymerization of PDL in the presence of HEA, the highest concentration of HEA end-groups was around 43% after 1 h of reaction time. This coincides with the time required for total conversion of the monomer and can be considered kinetically controlled (Figure 3.3). Subsequently, transacylation under thermodynamic control results in the reshuffling of the end-groups until an equilibrium situation is reached. In the case of HEA/PDL the
polymer end-groups were 62% acrylate, 16% hydroxyl, 12% ED (hydroxyl) and 10% HEA. Interestingly, about the same equilibrium composition seems to be reached in the HEMA/PDL polymerization, yet at a slightly slower rate due to the more hindered structure of HEMA (Figure 3.8B). It has to be noted that NMR analysis gives an overall picture and does not allow any conclusion on how these end-groups are combined in one polymer molecule. It is most likely that mixtures of mono and diacrylates (one HEA, one acrylate end-group), and mono and diol polymers are obtained.

![Figure 3.8](image_url)

**Figure 3.8** The relative content of end-groups in PPDL with time, obtained in CALB-catalyzed ROP of PDL initiated with HEA (A) and HEMA (B): HEA/HEMA end-group (•); 1,2-ethanediol end-group ( ); Hydroxyl end-group (■); Acrylated/methacrylated hydroxyl end-group (▲).

For the slower reacting CL a similar result was obtained for HEA, with 43% acrylate, 30% hydroxyl, 15% ED (hydroxyl) and 12% HEA end-groups (Figure 3.9). While the equilibrium composition for this reaction appears to be reached within the experiment time, this seems not to be the case for the HEMA/CL reaction. However, even though this reaction has a slower equilibration it is reasonable to assume that ultimately a similar end-group composition will be reached.
Figure 3.9 The relative content of end-groups in PCL with time, obtained in CALB-catalyzed ROP of CL initiated with HEA (A) and HEMA (B): HEA/HEMA end-group (◊); 1,2-ethanediol end-group (●); Hydroxyl end-group (■); Acrylated/methacrylated hydroxyl end-group (▲).

3.4 Conclusion

When using initiators with cleavable ester bonds in enzymatic ROP, such as HEA or HEMA, polymers with a mixed composition of end-groups will be obtained. This is due to the involvement of the ester bonds of the HEA and HEMA moieties in transacylation processes. While in both cases the amount of HEA and HEMA end-groups could be maximized by short reaction times, a defined (meth)acrylation by this approach was not possible. The dynamics of polyester structures due to lipase-based acyl transfer reactions resulted in a thermodynamic equilibrium distribution of end-groups in the case of PDL, which most probably will also be the case for CL when longer reaction times are applied. Large differences in acyl transfer reaction rates between HEA and HEMA were observed (15 fold) in which the HEA was more prone to acyl transfer due to the less hindered structure. Our results show that transesterification reactions are present at high activity throughout the enzymatic ROP process and have to be seriously taken into account when performing an enzyme-catalyzed ROP.
References

CHAPTER 4

Biodegradable Chiral Polyesters and Microspheres by Asymmetric Enzymatic Polymerization

Abstract

Materials with selective bio-responsiveness could have potential in medical applications. In this chapter chiral microspheres were obtained from non-crystalline aliphatic polyesters with the aim to use chirality to program polymer microsphere degradation. By enzymatic enantioselective kinetic resolution polymerization from racemic monomers, hydroxyl-terminated \((R)\)-, \((S)\)- and racemic poly(4-methyl-\(\varepsilon\)-caprolactone) (PMCL) were successfully synthesized. Preliminary degradation experiments with *Candida antarctica* Lipase B show that the degradation rate can be tuned by the polymer chirality. Chiral microspheres around 40 microns were obtained after acrylation of the polymers and subsequent in-situ cross-linking in an oil-in-water (O/W) emulsion evaporation approach.

This chapter is based on:
4.1 Introduction

Polymers have established themselves as versatile materials in biomedical applications, for example as biodegradable polymer materials for tissue engineering and drug delivery. Biodegradable polymers provide a significant advantage over non-degradable polymers, since their use circumvents the requirement of removal. The preferred application of biodegradable polymers for drug delivery is in the form of polymer microspheres. Microspheres can overcome many of the shortcomings of conventional drug delivery systems as they can be ingested or injected; they can be tailored for desired release profiles and in some cases can even provide organ-targeted release.

Besides drug diffusion through the particle, the degradation of the drug-loaded particle is one of the main release mechanisms. The latter depends partly on the physical properties of the microsphere as surface morphology, porosity and size (distribution) significantly impact the degradation behavior. For example, Albertsson showed that polymer degradation occurred first in the amorphous regions of microspheres followed by a slower degradation in the crystalline regions. Park reported that microspheres consisting of high molecular weight polymers need to be longer degraded than corresponding microspheres prepared from low molecular weight material. The controlled degradation and thus release of medication from polymer microspheres is consequently achievable by manipulating the properties of the microsphere itself, as well as the physical and chemical properties of the polymer. Significant research has been devoted to the latter and a number of natural polymers, such as cellulose, chitin, and chitosan were employed. By far the most dominating class of polymers investigated for degradable drug delivery systems are aliphatic polyesters, such as polyglycolide, polylactide and poly(ε-caprolactone) (PCL) and their copolymers. In particular, PCL is a promising candidate for controlled release applications due to its physical properties and high permeability to low molecular weight species at body temperature. A disadvantage of PCL is its high crystallinity, which results in a very slow degradation. Generally, optimization of the polymer degradation profile can be
achieved by copolymerization. Examples are copolymers of ε-caprolactone with 1,5-dioxepan-2-one (DXO) and lactides/glycolides, for which it has been demonstrated that the composition of the copolymer determines the polymer degradation.\textsuperscript{46} The drawback of this method is that changing the copolymer composition automatically changes its physical and thermal properties, such as the crystallinity, $T_g$ and $T_m$. The ideal biomaterial should thus be amorphous, possess readily adjustable degradation rates with a $T_g$ below body temperature and be easily processable into various shapes. Recently, curable biodegradable elastomers have been reviewed as they can fulfill these requirements.\textsuperscript{47}

We are aiming to develop amorphous bio-responsive polymers in which the response can be tuned without compromising the physical properties by avoiding copolymerization. Our approach is to take advantage of chirality as a tool to program polymer reactivity in the presence of a stereoselective enzyme. A recent study in our project reported the first example of an enzyme-responsive material by enzymatic stereoselective modification of chiral polymer side chains.\textsuperscript{48} The induced property change of the polymer was dependent on its enantiomeric composition. Now the concept is extended in this chapter to biodegradable chiral polymer microspheres (Scheme 4.1). That enzymatic degradation rates depend on the stereochemical composition of the polymer has been shown for polyesteramides containing L- and L,D-alanine, polyhydroxybutyrate stereoisomers and polylactides.\textsuperscript{44, 49, 50} However, all of these materials are semi-crystalline and it has been stated that the effect of crystallinity usually dominates the degradation.

![Scheme 4.1](image)

**Scheme 4.1** Polymers and microspheres with different enantiomeric composition which is hypothesized to tune degradation. $S$ and $R$ stand for $S$ and $R$ enantiomer, respectively.
For our approach, we have selected poly (4-methyl-ε-caprolactone) (PMCL). This polymer is structurally related to PCL, as shown in Scheme 4.2, but fully amorphous ($T_g = -68^\circ C$). This makes it an interesting material for application in biodegradable microspheres, as the degradation should be independent of the ratio between crystalline and amorphous regions. Equally importantly for our aim: it contains a chiral center. The degradation of PCL proceeds via hydrolysis of the backbone ester bonds as well as by enzymatic attack. With *in vitro* experiments, Wang showed that the degradation of PCL microspheres is accelerated in the presence of lipase. It is reasonable to assume a similar degradation behavior for PMCL, with the advantage of PMCL being fully amorphous with chiral centers along the polymer backbone. While the hydrolytic, acid- or base-catalyzed degradation will not be affected by the chirality of the polyester, enzymes are stereoselective. Thus we hypothesize that the chirality of the polyester might be a way to tune the rate of the *in vivo* degradation of polymers. This is of course a very simplified view on a highly complex process. For example, chemical and enzymatic processes might happen simultaneously, and in the body enzymes with opposite stereoselectivity might be involved in the polymer degradation. The proof of feasibility of the proposed approach, and thus the validation of our hypothesis, requires investigations on several levels involving the development of a synthetic route to chiral microspheres and the proof of selectivity in their degradation (*in vitro* and *in vivo*).

**Scheme 4.2** Chemical structure of ε-caprolactone (CL) and two enantiomers (R and S) of 4-methyl-ε-caprolactone (MCL).

This chapter focuses on the first step to obtain bio-responsive microspheres, *i.e.*, the synthetic approach to chiral PMCL microspheres by stereoselective enzymatic ring
opening polymerization (ROP). Furthermore, preliminary experimental results show that the enzymatic selectivity is retained under hydrolysis conditions.

4.2 Experimental part

4.2.1 Materials

All chemicals were purchased from Aldrich and used without further purification unless otherwise noted. Toluene and dichloromethane were dried over aluminum oxide and stored on molecular sieves, which were pre-dried in an oven at 150 °C before use. Novozym 435 and Novozym CALB L (a lipase B from *Candida antarctica* produced by submerged fermentation of a genetically modified Aspergillus oryzae microorganism) were obtained from Novozymes A/S, Denmark.

4.2.2 Instrumentation

Size exclusion chromatography (SEC) was performed on a Waters GPC equipped with a Waters Model 510 pump, using a set of two linear columns (Mixed C. Polymer Laboratories, 30 cm, and 40 °C). Detection was performed using a Waters Model 2414 refractometer. THF was used as eluent with a flow rate of 1.0 ml/min. All samples were diluted to 1.0 mg/ml in THF and filtrated using 0.2 µm syringe filters. The molecular weights of all polymers were calculated based on polystyrene standards. $^1$H NMR spectroscopy was performed using a VARIAN 400 NMR at 20 °C. Samples were diluted in CDCl$_3$ to 30-50 mg/ml. Data were processed using VNMR-software. MALDI-ToF-MS analysis was carried out on a Voyager DE-STR from Applied Biosystems using Trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]-malononitril (DCTB) as matrix material and potassium trifluoroacetate as ionization agent. All spectra were recorded in the reflector mode. Samples were prepared using 1 mg/ml of polymer in THF. The ratio of polymer sample to matrix was 1:5 (w/w %).
Scanning Electron Microscopy was performed using a Philips (now Fei Co.) environmental scanning electron microscope XL-30 ESEM-FEG in the high vacuum mode at an acceleration voltage of 3 kV. FTIR spectra were acquired with a Biorad FTS 6000 spectrometer with a Golden Gate Single reflection High Temperature Diamond ATR (specac). MCL polymerizations were followed by chiral gas chromatography (GC) with a Shimadzu 6C-17A GC equipped with an FID employing a Chrompack Chirasil-DEX CB (DF) 0.25) column. Injection and detection temperatures were set at 300 and 325 °C, respectively. Separations were done under isothermal conditions with the column temperature set at 125 °C for MCL, baseline separation of the enantiomers was achieved. All samples were measured in duplo using a Shimadzu AOC-20i autosampler. The enantiomeric excess of the monomer (ee\(_m\)) was calculated as follows: 
\[ ee_m = \frac{(R - S)}{(R + S)} \]
where \( R \) and \( S \) represent the areas of the GC peaks of the unreacted (\( R \))- and (\( S \))-enantiomer, respectively. The enantiomeric excess of the polymer (ee\(_p\)) was calculated from ee\(_m\) and conversion (\( c \)) according to the following equations:
\[ ee_p = \frac{(S_p - R_p)}{(S_p + R_p)} \]
\[ S_p = 0.5 - [(1-c)^*S/(S+R)] \] and \[ R_p = 0.5 - [(1-c)^*R/(S+R)] \]. 
The enantiomeric ratio (E-ratio),\(^{53}\) which in terms of conversion (\( c \)) and ee\(_m\) is expressed as 
\[ E = \ln[(1 - c)(1 - ee_m)]/\ln[(1 - c)(1 + ee_m)] \], was fitted using Origin 6.0 employing the nonlinear curve fit option and rewriting the formula to 
\[ c = 1 - [(1 - ee_m)/(1 + ee_m)^E/(1/E - 1)] \]. 
Optical rotations were measured in dichloromethane on a Jasco DIP-370 polarimeter at a wavelength of 590 nm (NaD-line) with 5 cm cuvettes at room temperature (concentration: 0.1 g ml\(^{-1}\)).

### 4.2.3 Synthetic procedure

**A. 4-Methyl-\( \varepsilon \)-caprolactone (MCL)**

MCL was synthesized by Baeyer-Villiger oxidation of 4-methylcyclohexanone according to a literature procedure.\(^{54}\) 4-Methylcyclohexanone (25 gr, 223 mmol) was dissolved in chloroform (200 ml) and dropped slowly (over approx. 1 hr) via a dropping funnel to a stirred suspension of \( m \)-chloroperbenzoic acid (\( m \)-CPBA) (63 gr, 75% pure)
in chloroform (300 ml). The reaction was done at room temperature. The resulting suspension was stirred overnight. A sample was taken and measured with $^1$H-NMR to make sure all ketone was converted. If the reaction proceeds very slowly, the reaction can be performed under reflux conditions. The solids were filtered over celite and the filtrate was washed with saturated Na$_2$CO$_3$ solution (3*200 ml) and saturated NaCl solution (1*200 ml). Then the organic layer was dried with MgSO$_4$, filtered and evaporated in vacuo. The resulting clear liquid was distilled over CaH$_2$ (0.56 Torr., 67°C). The yield was about 80% of clear liquid.

B. Poly-$\varepsilon$-caprolactone (PCL) diol

Novozym 435 (10 wt. % with respect to monomer) and 1,6-hexanediol (1.04 g; 8.76 mmol) were weighed into a flask. Then, the flask was equipped with a stirring bar and put overnight in a vacuum oven at 50 °C. The vacuum oven was flushed with N$_2$ and molecular sieves were added before the flask was sealed with a rubber septum. The reaction was started by adding 37.9 ml of a 4.5 M stock solution of CL and toluene through the septum and stirring at 60 °C. Samples were taken at regular time intervals and analyzed with $^1$H NMR, GC and SEC. The reaction was stopped after 3 hours, diluted with dichloromethane and filtered to remove the Novozym 435. The polymer was precipitated in cold methanol and dried under vacuum in an oven at 40 °C. A yield of 17.5 g (85 %) was realized. $^1$H NMR (CDCl$_3$): $\delta$ 4.08 (t, CH$_2$OCO), 3.63 (t, CH$_2$OH end-group), 2.32 (t, CH$_2$COO), 1.68 (m, CH$_2$), 1.41 (m, CH$_2$).

C. Poly-4-methyl-$\varepsilon$-caprolactone (PMCL) diols

(1) Preparation of poly-(S)-4-methyl-$\varepsilon$-caprolactone. Novozym 435 (10 wt. % relative to monomer) and hexanediol (230 mg; 1.96 mmol) were weighed into a flask. Then, the flask was equipped with a stirring bar and put overnight in a vacuum oven at 50 °C. The vacuum oven was backfilling with N$_2$ and molecular sieves were added before the flask was sealed with a rubber septum. The reaction was started by adding 20 ml of a 4.0 M stock solution of MCL and toluene through the septum and stirring at 45 °C. Samples were taken at regular time intervals and analyzed with $^1$H NMR, GC and SEC. The
reaction was stopped after 3 hours by filtrating Novozym 435 after the dilution of dichloromethane. The polymer was precipitated in cold heptane and dried under vacuum in an oven at 40 °C. Yield: 3.1 g (30 % with respect to total monomer).

(2) Preparation of poly-(R)-4-methyl-ε-caprolactone. The filtrate in cold heptane from (1), containing 50% of unreacted MCL, was concentrated and distilled from CaH₂ under reduced pressure. The monomer obtained gave an R-enriched enantiomeric purity of 95%. A stock solution of 5.16g (40.2 mmol) (R)-MCL in 5 ml toluene was injected into a flask with pre-dried Novozym 435 (10 wt. % relative to monomer) and hexanediol (235 mg, 2.0mmol). The polymerization ran for 48 h to ensure high conversion of (R)-MCL. The polymer was obtained after precipitation in cold heptane and drying in a vacuum oven at 40 °C. Yield: 3.6 g (70 %).

(3) Preparation of poly-(R, S)-4-methyl-ε-caprolactone. The reaction was performed following the same procedure as (2) except that the monomer in the stock solution was a racemic mixture of (S)- and (R)-MCL instead of (R)-MCL. Yield: 3.6 g (72 %). ¹H NMR PMCL (CDCl₃): δ 4.15 (t, CH₂OCO), 3.68 (t, CH₂OH end-group), 2.2-2.45 (m, CH₂COO), 1.4-1.8 (m, CH₂, CH), 0.8-1.1 (d, CH₃).

D. Acrylation of PCL (or PMCLs) diol

PCL (1.8 g; ca. 0.98 mmol) or PMCL (1.5 g; ca. 0.98 mmol) was dissolved in 20 ml dichloromethane in a 100 ml 3-necked flask. Triethylamine (0.40 ml; 5.8 mmol) was added afterwards and the mixture was stirred in an ice bath. Acryloyl chloride (0.32 ml, 3.1 mmol) dissolved in 20 ml dichloromethane was dropped very slowly into the mixture. After that, the reaction was kept stirring at room temperature overnight. The solid was removed by filtration and the filtrate was washed with saturated Na₂CO₃ solution (3 times) and NaCl solution (1 time), dried over MgSO₄. The diacrylate was collected by evaporating the solvent in vacuo. Yield: 1.2 g (60 %).

¹H NMR (CDCl₃): PCL diacrylate: δ 5.5-6.1 (m, CH₂=CHCOO), 4.15 (t, CH₂OCOCH=CH₂), 4.08 (t, CH₂OCO), 2.32 (t, CH₂COO), 1.68 (m, CH₂), 1.41 (m, CH₂). PMCL diacrylate: δ 5.5-6.1 (m, CH₂=CHCOO), 4.22 (t, CH₂OCOCH=CH₂), 4.15 (t, CH₂OCO), 2.2-2.45 (m, CH₂COO), 1.4-1.8 (m, CH₂, CH), 0.8-1.1 (d, CH₃).
E. Polymer degradation
The synthesized enantioenriched PMCLs (0.5 g) were dissolved in 5 ml toluene. Novozym 435 (0.5 g) and 0.5 g of benzyl alcohol were added and the mixtures were stirred at 60 °C for 24 hours. Samples were taken at regular intervals for NMR and SEC analysis.

F. Formation of microspheres
1 g PCL (or PMCL) diacrylate and 2, 2-dimethoxy-2-phenylacetophenone (photo initiator) (ca. 20:1 molar ratio with respect to acrylate groups) were dissolved in 10 ml dichloromethane. The mixture was emulsified under stirring with a 0.25 % w/w polyvinyl alcohol (PVA, M_w=13000-23000 g mol^{-1}) solution to form an oil-in-water (O/W) emulsion. At the same time, the open reactor was exposed to UV-light (HPR125W, wavelength=350-400nm). Radiation was continued for 1.5 h at a stirring speed of about 500 rpm, to evaporate the organic solvent. The crosslinked particles were collected by filtration. These microspheres were extracted with water and dichloromethane, respectively, for 3 times and dried in a vacuum oven at 40 °C. This yielded around 0.46 g (46 %) cross-linked particles.

G. Microspheres degradation
Chemical degradation: 20 mg crosslinked microspheres and 20 ml phosphate buffered saline (PBS) solution (pH=7.4) were added in a degradation tube, which was then incubated in an oven at 37 °C. Enzymatic degradation: 20 mg crosslinked microspheres, 1 ml Novozym CALB L and 20 ml PBS solution (pH=7.4) were added in a degradation tube, which was then incubated in an oven at 37 °C. Samples were taken out from time to time for drying and SEM measurement.
4.3 Results and discussion

A convenient approach to obtain polymer microspheres is the O/W solvent evaporation technique. Usually, the polymer is dissolved in a volatile organic solvent, e.g., methylene chloride. This oil phase is then added drop-wise to a water phase containing a stabilizer like polyvinyl alcohol under vigorous stirring. After forming a stable emulsion, the polymer precipitates in the dispersed phase as the solvent is removed by evaporation. The hardened microspheres can then be collected, washed and dried. While this technique works well for crystalline or semi-crystalline polymers, which form solid microspheres at room temperature, it cannot be applied to the formation of PMCL microspheres due to its fully amorphous character and its rubbery nature at room temperature ($T_g$: -68 °C). In order to obtain stable PMCL microspheres, we had to modify the process such that the polymer microspheres would be crosslinked in the oil phase prior to evaporation of the organic solvent. This was achieved by UV-crosslinking of PMCL diacrylates in the presence of a photoinitiator during the O/W solvent evaporation. While photopolymerization is a method that has been applied to make biodegradable networks, to our knowledge such approach has not yet been described for the synthesis of amorphous microspheres. For comparison we synthesized both PCL and chiral PMCL microspheres by this approach.

4.3.1 Asymmetric synthesis and degradation of chiral polyesters

Scheme 4.3 depicts the reaction scheme for the synthesis of chiral PMCL diacrylates. The key step is the enantioselective enzymatic ring opening polymerization (ROP) of MCL to afford enantioenriched polyester diols. Successful synthesis requires the control of two aspects of the polymerization. (1) End-group control to warrant high yield of telechelic dihydroxy polyesters and (2) control over the enantioselectivity of the reaction to yield polymers of high enantiopurity.
Scheme 4.3 Synthesis of chiral polymer precursors for the formation of chiral crosslinked microspheres. Reaction conditions: 1: (R,S)-MCL, hexandiol, Novozym 435, reaction stopped at 50 % conversion; 2: (R)-MCL, hexandiol, Novozym 435, 90 % conversion; 3: (R,S)-MCL, hexanediol, Novozym 435, 91 % conversion.

In analogy to end-group control in metal-mediated ROP, the addition of a nucleophile or initiator is a feasible strategy to introduce the desired end-groups in enzymatic ROP (initiator method). However, due to the mechanistic nature of enzymatic ROP, equilibrium between ROP and transesterification is usually established during the course of the polymerization. The obtained polymers can thus contain various amounts of cyclic ester and polymers lacking the initiator end-group (e.g. by water initiation). Nevertheless, it has been shown that by this method good end-group control is achievable with hydroxy-initiators under optimized conditions.\textsuperscript{23, 25, 27, 29, 30, 63, 64} Since the end-group control of telechelic dihydroxyl polyesters by enzymatic ROP has not been extensively studied yet,\textsuperscript{65, 66} we first attempted the enzymatic ROP of CL in the presence of hexanediol by MALDI-ToF-MS. A complete monomer conversion was obtained after 3 hours as confirmed by \textsuperscript{1}H NMR spectroscopy (Figure 4.1). MALDI-ToF-MS spectra only show one main distribution of peaks with a mass
difference of 114 Da between the individual peaks, which corresponds to the monomer unit (Figure 4.2). Both NMR and MALDI results confirm that the initiation had exclusively occurred from hexanediol.

![Figure 4.1](image1.png)

**Figure 4.1** $^1$H-NMR spectrum of telechelic poly-(ε-caprolactone) diol.

![Figure 4.2](image2.png)

**Figure 4.2** MALDI-ToF-MS spectrum of telechelic poly-(ε-caprolactone) diol.

Encouraged by the good results, we then applied the same reaction conditions to the enzymatic ROP of MCL. The goal was to synthesize three polymers of similar molecular weight but with different chirality in the polymer backbone. Since the
separation of the two enantiomers of MCL and subsequent individual polymerization is not possible, the polymers were synthesized by asymmetric chiral resolution polymerization from the racemic monomer mixture. Lipases like *Candida antarctica* lipase B (CALB) are perfectly suited to catalyze this polymerization.\textsuperscript{22,67,68} It has been reported that immobilized CALB (Novozym 435) has a stereoselective preference for the (S)-enantiomer of MCL under optimized conditions. An enantiomeric excess (ee\textsubscript{p}) of the monomer in (S)-configuration of 90 % was found in the polymer. From that, an E-ratio of 16.9 was calculated for this polymerization.\textsuperscript{28} The latter is a measure for the enantioselectivity of a reaction under specific reaction conditions in which a higher value represents a higher selectivity. However, all previous reports were limited to kinetic studies and do not describe the synthesis nor the evaluation of larger quantities of materials. The challenge in obtaining the two chiral polymers in sufficient quantities is to conduct the polymerization on a larger scale, while retaining a sufficiently high E-ratio. This requires to precisely monitor the monomer conversion, such that the reaction can be stopped once the (S)-enantiomer has been fully consumed. Figure 4.3 shows the corresponding conversion plot of the polymerizations obtained from chiral GC. From the figure it can be derived that the conversion of the (S)-MCL (49 % total conversion) was completed after 2 h. The enantiomeric excess of the monomer (ee\textsubscript{m}) and the polymer (ee\textsubscript{p}) both reached a maximum of 0.85 and 0.80. From this, an E-ratio of 21.4 ± 3.4 was calculated by fitting the conversion data. This is slightly higher than the value reported earlier and confirms that the high selectivity of the polymerization was preserved under the conditions applied on preparative scale. Thus, the poly-(S)-MCL was readily accessible by stopping the reaction at 50 % monomer conversion (route 1, Scheme 4.3). After precipitation of the reaction mixture in heptane, a liquid polymer was recovered with a molecular weight of 3,050 g mol\textsuperscript{-1} (SEC). An optical rotation of this polymer of -4.36° was measured in agreement with its (S)-enriched structure. Moreover, the \textsuperscript{1}H NMR analysis is in agreement with the desired diol structure (Figure 4.4). Calculation of the molecular weight based on NMR end-group analysis results in a M\textsubscript{n} of 2,920 g mol\textsuperscript{-1}, in agreement with the M\textsubscript{n} in SEC calibrated with polystyrene. Further confirmation for the polymer structure was
obtained from MALDI-ToF-MS, which shows only peaks representative of PMCL diol (Figure 4.5). It is remarkable, that no water-initiated polymers and only traces of cyclic polymers are observed in the spectra. While the precipitation of the samples certainly has removed low molecular weight fractions to a certain extend, cyclic structures are usually observed up to high molecular weights under the applied reaction conditions.\textsuperscript{23}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure4_3}
\caption{Polymerization of 4-methyl-\(\varepsilon\)-caprolactone using 1,6-hexanediol as initiator (Novozym 435). (A) Monomer conversion as a function of reaction time. The dashed line indicates 50 \% total monomer conversion. (B) Monomer conversion for enzymatic ROP of 4-MCL at 45 °C as a function of enantiomeric excess of the recovered monomer (ee\(_m\)), dotted line represents best fit to determine E-ratio (R\(^2\)=0.997; E = 21.4 \pm 3.5).}
\end{figure}
Biodegradable chiral polyesters and microspheres

Figure 4.4 $^1$H NMR spectra of telechelic poly-((S)-4-methyl-$\varepsilon$-caprolactone) diol (1) and poly-((S)-4-methyl-$\varepsilon$-caprolactone) diacrylate (2).

Figure 4.5 MALDI-ToF-MS spectra of telechelic poly ((S)-4-methyl-$\varepsilon$-caprolactone) diol (A) and poly-((S)-4-methyl-$\varepsilon$-caprolactone) diacrylate (B) showing an increase of molar masses of 110 Da due to addition two acrylate end-groups. Small distributions in both spectra at low molecular weight represent cyclic polymers.
For the synthesis of the poly-(R)-MCL the non-polymerized monomer from the previous reaction was recovered from the precipitation filtrate and purified by distillation. According to chiral GC, the enantiomeric purity of the (R)-enriched MCL was 95%. This monomer was polymerized with Novozym 435 in the presence of 1,6-hexanediol, similar to the (S)-enantiomer. Due to the lower reactivity of this enantiomer, the reaction was conducted for 48 h to ensure a high monomer conversion. A polymer with a SEC molecular weight of $M_n=2,900$ g mol$^{-1}$ (NMR: $M_n=2,600$ g mol$^{-1}$) was recovered, which is close to the value of $3,050$ g mol$^{-1}$ obtained for poly-(S)-MCL. While the diol structure was confirmed by $^1$H NMR and MALDI analysis, the optical rotation of this polymer of $+5.95^\circ$ confirms the high enrichment in (R)-monomer.

Poly-(R,S)-MCL was synthesized by carrying out the enzymatic ROP of a racemic mixture of (S)- and (R)-MCL to 91.2 % monomer conversion (route 2, Scheme 4.3). A polymer with an SEC molecular weight of $M_n=3,200$ g mol$^{-1}$ (NMR: $M_n=3,100$ g mol$^{-1}$) was obtained, again close to the molar masses of both chiral polyesters, and results of $^1$H-NMR as well as MALDI-ToF-MS analysis coincide with the results of poly-(S)-MCL. The optical rotation of this polymer was $-0.5^\circ$, which confirms the near-racemic nature of the polymer. While it is not possible to verify enantiomeric randomness of the polymer, it is reasonable to assume that due to the high degree of transesterification in enzymatic ROP randomization occurs. In order to facilitate this process the reaction was continued for 48 h at 45 °C until full conversion was reached (Table 4.1).

All synthesized PMCLs were fully amorphous and have similar $T_g$’s between -66 and -69 °C. As they only differ in their stereochemistry, they are chemically indistinguishable and only the interaction with a stereoselective catalyst, like an enzyme, will result in a distinguishable property change, e.g. degradation kinetics. In order to investigate the effect of the polymer chirality on their biodegradation behavior, all enantio-enriched polymers were subjected to preliminary degradation experiments. In a first set of experiments we choose the same lipase as for the synthesis, namely CALB. All polymers were dissolved in toluene in the presence of an excess of benzyl
alcohol (BA) as nucleophile and Novozym 435 (Scheme 4.4). Benzyl alcohol (BA) was used since it allows the convenient monitoring of the polymer degradation by following the benzyl ester proton signal in $^1$H NMR. Figure 4.6 reveals the occurrence of the degradation by showing benzyl ester proton signal shift (from 4.8 ppm to 5.2 ppm) during degradation. Therefore, the conversion of benzyl alcohol can be simply calculated by $I_{5.2\text{ppm}}/(I_{4.8\text{ppm}}+I_{5.2\text{ppm}})*100\%$ (I stands for intensity which was integrated from peaks in NMR), which to some extent represents the degradation rate of PMCL.

As becomes evident from Figure 4.7, the degradation is the fastest for poly-(S)-MCL and the slowest for poly-(R)-MCL, while the degradation of poly-(R,S)-MCL showed intermediate kinetics. The results indicate that the lipase stereoselectivity is retained under degradation conditions. More detailed studies on degradation in aqueous environment will be shown in the later sections in order to mimic in vivo degradation.

Nevertheless, the preliminary results provide first support for the hypothesis that chirality can be used to program the degradation of synthetic polymers.

Table 4.1 Telechelic PCL and chiral PMCL diols from initiation of CL and MCL with 1,6-hexanediol.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$M_n^{a}$ SEC (g mol$^{-1}$)</th>
<th>PDI$^{a}$</th>
<th>$M_n^{b}$ NMR (gmol$^{-1}$)</th>
<th>$T_g$ (°C)</th>
<th>Optical rotation (°)</th>
<th>Degree of acrylation$^{c}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL</td>
<td>3,270</td>
<td>1.50</td>
<td>3,580</td>
<td>- 57.2</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>PMCL(S)</td>
<td>3,050</td>
<td>1.34</td>
<td>2,920</td>
<td>- 66.3</td>
<td>- 4.36</td>
<td>99</td>
</tr>
<tr>
<td>PMCL(R,S)</td>
<td>3,200</td>
<td>1.53</td>
<td>3,100</td>
<td>- 68.6</td>
<td>- 0.51</td>
<td>99</td>
</tr>
<tr>
<td>PMCL(R)</td>
<td>2,900</td>
<td>1.47</td>
<td>2,600</td>
<td>- 66.3</td>
<td>+ 5.95</td>
<td>99</td>
</tr>
</tbody>
</table>

$^{a}$ Determined by SEC calibrated with polystyrene standards. $^{b}$ Calculated from $^1$H NMR: $M_n = 118 + I_{4.05\text{ppm}}/I_{3.65\text{ppm}}*2*128$ (or 114). $^{c}$ Calculated from $^1$H NMR: degree of acrylation (%) = $I_{4.2\text{ppm}}/(I_{4.2\text{ppm}} + I_{3.65\text{ppm}})*100\%$. 


Scheme 4.4 Principle of PMCL degradation experiments, which were carried out with CALB using a large excess of benzyl alcohol in toluene.

Figure 4.6 $^1$H NMR spectra of (1) mixture of PMCL and excess benzyl alcohol before degradation; (2) mixture of oligomers during degradation.
Figure 4.7 Kinetic of Novozym 435 catalyzed degradation of chiral and racemic PMCL in the presence of benzyl alcohol as a nucleophile. Degradation is expressed as benzyl alcohol conversion.

4.3.2 Synthesis of chiral microspheres

Encouraged by the degradation results we developed a synthetic route towards chiral microspheres. As explained above, the low $T_g$ of the PMCL does not allow the formation of stable microspheres by the standard O/W technique. We therefore modified the protocol with the aim to crosslink the polymers in the emulsion state by photopolymerization. Therefore, all polymer diols were first acrylated with acryloylchloride. The progress of the reaction was followed by $^1$H NMR spectroscopy, which shows the complete shift of the signal of the methylene group adjacent to the hydroxyl end-group from 3.65 ppm to 4.1 ppm upon acrylation. Moreover, a cluster of signals between 5.5 and 6.5 ppm, characteristic of the acrylate double bond protons, appears in the spectrum (Figure 4.4(2)). Further evidence for the quantitativeness of the reaction was obtained from MALDI-ToF-MS. All observed peaks are in agreement with polymers having two acrylate units as end-groups. No residual peaks of polymer diols or mono-acrylated polymers were detected (Figure 4.5B).

With well-defined diacrylates, (mini-)emulsion polymerization of PCL macromonomer was first attempted to achieve crosslinked microspheres. A stable emulsion
could be maintained at room temperature after ultrasonication of a PCL diacrylate / toluene / sodium dodecyl sulfate (SDS, a surfactant) / water system. However, precipitates were found when the reaction was started by increasing the temperature and after adding initiator (azo-bis-isobutyronitrile, AIBN or potassium persulfate, KPS). These precipitates were soluble in THF or dichloromethane and contained mainly unreacted PCL diacrylate, as determined from NMR. The result led us to believe that crosslinking did not occur and increased temperature might destabilize the emulsion. Therefore, the use of thermo-sensitive initiator should be avoided and a non-temperature-induced initiation like UV irradiation could be considered.

According to literature, PCL microspheres without crosslinking have been prepared by the so-called O/W emulsion solvent evaporation method. The feasibility of the photo crosslinking process in such a system was then first tested, as illustrated in Figure 4.8. In view of potential biomedical applications 2,2-dimethoxy-2-phenylacetophenone was selected as a photo initiator, since it has approval for medical applications. The O/W emulsion was obtained by dissolving PCL diacrylate and photo initiator (molar ratio 10:1) in dichloromethane. The mixture was then added to water and emulsified under stirring with polyvinyl alcohol (PVA) in water (0.25% w/w). By exposing the open reactor to UV-light (HPR125W) the crosslinked particles were formed. A conversion of ca. 90% of the double bonds was estimated, which was concluded from solid state $^1$H NMR analysis, which showed the almost complete disappearance of the characteristic acrylate signals (5.5 – 6.5 ppm), and from IR spectroscopy based on the diminishing of the characteristic double bond absorptions at 1,625 cm$^{-1}$ and 830 cm$^{-1}$ (Figure 4.9).
Biodegradable chiral polyesters and microspheres

Figure 4.8 An illustration of the microsphere formation procedure: O/W emulsion solvent evaporation combined with photo crosslinking.

Figure 4.9 ATR-FTIR spectra of diacrylated Poly-(S)-MCL (bottom) and corresponding microspheres after UV curing (top). Arrows indicate the (almost) absence of the characteristic double bond absorptions at 1,625 cm\(^{-1}\) and 830 cm\(^{-1}\).

In the case of PCL, the microspheres were recovered as a white free-flowing powder. The SEM images (Figure 4.10) show particles of uniform spherical shape with a smooth surface. Although the overall diameter of these particles ranges from a few
microns to 80 microns, most of them are around 40 microns (visual estimation). Moreover, DSC analysis showed that $T_g$ had increased significantly from -57.2 °C to -26.4 °C due to the crosslinking reaction. The same procedure was then applied to synthesize the chiral particles from the PMCL diacrylates. The crosslinked microspheres were purified by washing with water and CH$_2$Cl$_2$ several times to remove stabilizer and unreacted acrylates. After drying, fluffy white powders were obtained. As in the case of the PCL, these particles did not dissolve in organic solvents, which in addition to the spectroscopic analysis provides evidence for the crosslinking. Inspection of the SEM pictures (Figure 4.10) shows that the surface of the obtained chiral microspheres is less smooth than that of the PCL microspheres. We tend to explain this with the low $T_g$ of the polymers and the absence of crystallinity, but it might also be a consequence of the non-optimized emulsification procedure.

![Figure 4.10 SEM images of cross-linked PCL microspheres (top images) and cross-linked chiral Poly-(S)-MCL microspheres (bottom images).](image)

### 4.3.3 Degradation of chiral microspheres

Although the *in vitro* degradation behavior of PCL microspheres has been extensively
studied,\textsuperscript{3,52} the chiral amorphous PMCL microspheres degradation is for the first time shown in this work. The experiments were carried out either in a PBS solution for chemical degradation or in a CALB/PBS solution for enzymatic degradation. At the same time, the behavior of crosslinked poly-(R,S)-MCL and poly-(S)-MCL microspheres were compared under each condition. Herein SEM was employed as the main method to monitor the surface morphology during the degradation, as shown in Figure 4.11. In the first two rows the images describe that poly-(R,S)-MCL microspheres degraded within two years both chemically and enzymatically. It is shown that the surface morphology has hardly changed after one year, both for chemical and enzymatic degradation. However, the images in the third column taken after two years show a difference between chemical and enzymatic degradation. The surface morphology is maintained for the microspheres in the PBS solution, while the roughness started disappearing and a few cracks appeared on the surface of the ones in the CALB/PBS solution. This phenomenon agrees with the results reported in literature that chemical degradation follows a bulk erosion procedure while enzymatic degradation is chose to a surface erosion one.\textsuperscript{69,70} A similar tendency was found for the degradation of poly-(S)-MCL microspheres (Figure 4.11, row 3 and 4) where no surface transformation was observed after one year, while the surface of chemically and enzymatically degraded spheres differed after two years. It is again confirmed that enzymatic degradation undergoes surface erosion by destroying the original morphology and that chemical degradation occurs through bulk erosion and causes retention of the initial shape.

Chemical degradation behavior of poly-(R,S) and (S)-MCL microspheres was compared in rows 1 and 3 of Figure 4.11. It is found that the morphology of poly-(R,S) and (S)-MCL microspheres was identical either after one or two years. So the hydrolytic process catalyzed by base or acid was not affected by chirality. However, discrimination was discovered between poly-(R,S) and (S)-MCL microspheres after two years in CALB/PBS solution. Comparing row 2, in column 3 with row 4, in column 3 (Figure 4.11), more cracks and erosions are detected in poly-(S)-MCL microspheres than in poly (R,S) MCL ones. Therefore, it appeared that poly-(S)-MCL microspheres
exhibit a faster degradation than poly-(R,S)-MCL ones in an enzyme solution. This agrees with the results of the PMCL degradation in toluene described previously. Enantioselectivity of CALB seems still retained during microspheres degradation based on surface monitoring data. However, it is too early to conclude that enzymatic degradation is affected by chirality. Much more evidence is required in addition to the observed surface change. Furthermore, despite the complete amorphous character, PMCL microspheres exhibit a slow degradation behavior probably due to low water accessibility and crosslinking.

![Figure 4.11](image_url) SEM images of PMCL microspheres during degradation.

86
4.4 Conclusion

By enzymatic kinetic resolution polymerization of racemic 4-methyl-ε-caprolactone (MCL) initiated from 1,6-hexandiol, two enantioenriched aliphatic polyesters, i.e., poly-(S)- and poly-(R)-MCL and the racemic poly-(R,S)-MCL were obtained. Analysis confirmed that molecular weights of the polymers were almost identical. Moreover, the polymers were fully amorphous with similar T_g values. Preliminary degradation experiments with *Candida antarctica* lipase B in toluene confirmed that the degradation rate is dependent on the chirality of the polymer. After acrylation, crosslinked chiral polymer microspheres were synthesized by a modified O/W emulsion photopolymerization. PCL and chiral PMCL particles showed a uniform spherical shape with a diameter ranging from a few microns to 80 microns. Amorphous chiral microspheres open the possibility to study the *in vitro* and *in vivo* enzymatic degradation as a function of chirality, without the influence of crystallinity. While the degradation of the microspheres is slow, initial results suggest a different degradation rate as a function of the microsphere chirality. However, more experiments are required to support a final conclusion.
References

CHAPTER 5

Bio-erodible Semi-Interpenetrating Networks (SIPNs) from PEG and PCL/PMCL

Abstract

Semi-interpenetrating polymer networks composed of poly(ethylene glycol) (PEG) and poly-ε-caprolactone (PCL) / poly-4-methyl-ε-caprolactone (PMCL) were synthesized and characterized to obtain bio-erodible hydrogels. Two types of network formation, including (A) crosslinking of two homopolymer diacrylates and (B) crosslinking of one block copolymer diacrylate, were investigated. Furthermore, a PMCL-based hydrogel is advantageous over a PCL-based one because of its low glass transition temperature and oily morphology, which contribute to the homogeneity and easy-processability of the gel.
5.1 Introduction

The name hydrogel in this chapter refers to a polymer network obtained by copolymerization or crosslinking of a hydrophilic polymer in the presence of an additional, degradable polymer. Different from traditional polymers, the high water content and crosslinked structure of hydrogels mimic the natural environment of articular cartilage with an extracellular matrix consisting of crosslinked collagen and proteoglycans in the aqueous phase. Thus various hydrogels derived from biodegradable polymers have been used to make medical devices for drug delivery systems, tissue engineering, medical sensors and so on, for which biocompatibility, biodegradability and non-toxicity are extremely important, in addition to physicochemical properties. For example, Park et al. have reported that biodegradable elastic hydrogel scaffolds based on poly(ethylene glycol)/poly(ε-caprolactone) (PEG/PCL) were fabricated as a delivery vehicle of rabbit chondrocytes for the formation of neocartilage. Yang et al. prepared semi-interpenetrating polymer networks, also consisting of PCL and PEG macromer, to improve tensile properties in developing biodegradable sutures. Understanding, predicting, and manipulating the degradation profile of these hydrogels becomes very important when attempting to design an appropriate material for these applications. In the ideal situation, the hydrogel degradation would be tuned to reflect the rate at which a certain application is required.

Crystallinity is considered as being one of the most important factors to cumber degradation, as reported by many researchers. Wang et al. observed that the amorphous parts of PCL microspheres were completely degraded after nine weeks while the crystalline domains were retained. This promoted us to investigate the idea of using completely amorphous aliphatic polyesters to accelerate degradation. However, as discussed in Chapter 4, the amorphous PMCL-based microspheres still showed a slow degradation, either in phosphate buffered saline (PBS) or in enzyme solution. Presumably, the dominant reason is the poor water accessibility, which makes the ester bonds difficult to cleave. Thus, a more water-accessible material without crystalline
domains should be ideal to achieve the desired degradation. PEG, a known water-soluble polymer, has been widely used in the formation of hydrogels for biomedical applications despite its non-degradable character.\textsuperscript{76} Amphiphilic hydrogels consisting of both aliphatic polyesters and PEG are therefore expected to improve water penetration into the material, whilst maintaining degradability of the polyester component. Furthermore, the application of amorphous polyester as a precursor for crosslinking results in a low processing temperature.

In addition to crystallinity, chirality could be another parameter to tune the degradation.\textsuperscript{77} PMCL diols and diacrylates with different chirality have been successfully synthesized by enzymatic polymerization, as demonstrated in Chapter 4. Here we follow two strategies to form the chiral hydrogels, both resulting in different network structures: (1) crosslinking of PEG diacrylate and PMCL diacrylate (Scheme 5.1 (A)); (2) crosslinking of PMCL-\textit{b}-PEG-\textit{b}-PMCL diacrylate (Scheme 5.1 (B)). Considering that two chiral and one racemic PMCL can be synthesized, each method results in three hydrogels with different chirality. In this chapter their properties and degradation behavior are compared with those of PCL-based hydrogel. A most promising hydrogel formation method is proposed and a degradation-tunable material is obtained.

Scheme 5.1 Two types of interpenetrating polymer network structures obtained from PEG and PMCL: (A) PEG diacrylate and PMCL (or PCL) diacrylate network; (B) PMCL-\textit{b}-PEG-\textit{b}-PMCL (or -PCL) diacrylate network.
5.2 Experimental part

5.2.1 Materials

All chemicals were purchased from Aldrich and used without further purification unless otherwise noted. PMCL diacrylates with different chirality, i.e. poly-(S)-MCL diacrylate, poly-(R)-MCL diacrylate and poly-(R,S)-MCL diacrylate were synthesized via 1,6-hexanediol-initiated enzymatic ring opening polymerization and acrylation as described in Chapter 4. Diethyl ether and toluene were dried over alumina and stored over molecular sieves. Acryloyl chloride was obtained from Fluka. Novozym 435 (*Candida antarctica* lipase B immobilized on acrylic resin) was purchased from Aldrich, and it was dried in a vacuum oven before use. PEG diol (M<sub>n</sub>=600 g mol<sup>-1</sup>) and PEG diacrylate (M<sub>n</sub>=700 g mol<sup>-1</sup>) from Aldrich were used. Phosphate buffered saline (PBS) solution (0.01M, pH=7.4) was prepared by dissolving a pouch of PBS powder (from Sigma) in 1 L distilled water. Novozym CALB L, a lipase B from *Candida antarctica*, produced by submerged fermentation of a genetically modified Aspergillus oryzae microorganism, was obtained from Novozymes A/S. Lipase PS from *Pseudomonas cepacia* was purchased from Aldrich.

5.2.2 Instrumentation

Size exclusion chromatography (SEC) was performed on a Waters GPC equipped with a Waters Model 510 pump, using a set of two linear columns (Mixed C. Polymer Laboratories, 30 cm, and 40 °C). Detection was performed using a Waters Model 2414 refractometer. THF was used as eluent with a flow rate of 1.0 ml min<sup>-1</sup>. All samples were diluted to 1.0 mg ml<sup>-1</sup> in THF and filtrated using 0.2 µm syringe filters. The molecular weights of all polymers were calculated based on polystyrene standards. ¹H-NMR spectroscopy was performed using a VARIAN 400 NMR at 20 °C. Samples were diluted in CDCl<sub>3</sub> to 30–50 mg ml<sup>-1</sup>. Data were processed using VNMR software. FTIR spectra were acquired with a Biorad FTS 6000 spectrometer with a Golden Gate
Single reflection High Temperature Diamond ATR (specac). MCL polymerizations were followed by chiral gas chromatography (GC) with a Shimadzu 6C-17A GC equipped with an FID employing a Chrompack Chirasil-DEX CB (DF 0.25) column. Injection and detection temperatures were set at 300 and 325 °C, respectively. Separations were done under isothermal conditions with the column temperature set at 125 °C for MCL. Baseline separation of the enantiomers was achieved. All samples were measured in duplo using a Shimadzu AOC-20i autosampler. The enantiomeric excess of the monomer (ee$_m$) was calculated as shown in chapter 4.

### 5.2.3 Synthetic procedure

A. *Synthesis of PCL-b-PEG-b-PCL diol and PMCL-b-PEG-b-PMCL diols with different chirality*

1. Preparation of PCL-b-PEG-b-PCL diol. Novozym 435 (10 wt. % relative to monomer) and PEG diol (1.2 g; 2 mmol) were weighed into a flask. Then, the flask was equipped with a stirring bar and put overnight in a vacuum oven at 50 °C. The vacuum oven was backfilling with N$_2$ and the flask was taken out with a sealed rubber septum. The reaction was started by adding 10 ml dry toluene and CL (4.56 g, 40.0 mmol) through the septum and stirring at 60 °C. Samples were taken at regular time intervals and analyzed with $^1$H NMR, GC and SEC. The reaction was stopped by filtering Novozym 435 with the dilution of dichloromethane. The residue was precipitated in cold methanol and dried under vacuum in an oven at 40 °C. Yield: approximately 78 %.

2. Preparation of Poly(S)MCL-b-PEG-b-Poly(S)MCL diol. Novozym 435 (10 wt. % relative to monomer) and PEG diol (1.17 g; 1.96 mmol) were weighed into a flask. Then, the flask was equipped with a stirring bar and put overnight in a vacuum oven at 50 °C. The vacuum oven was backfilling with N$_2$ and the flask was taken out with a sealed rubber septum. The reaction was started by adding 10 ml dry toluene and MCL (10.3 g, 80.4 mmol) through the septum and stirring at 45 °C. Samples were taken at regular time intervals and analyzed with $^1$H-NMR, GC and SEC. The reaction was
stopped after 3 hours at 45% monomer conversion by filtering Novozym 435 after dilution with dichloromethane. The filtrate was concentrated by a rotary evaporator and purified by vacuum distillation (67 °C, 0.1 Torr.) The residue was precipitated in cold heptane and dried under vacuum in an oven at 40 °C. Yield: approximately 28 % with respect to total monomer.

(3) Preparation of Poly(R)MCL-b-PEG-b-Poly(R)MCL diol. The distilled fraction from (2), containing 50% of unreacted MCL, gave an R-enriched enantiomeric purity of 90%. A stock solution of 5.0 g (39.1 mmol) (R)-MCL in 5ml toluene was injected into a flask with pre-dried Novozym 435 (10 wt. % relative to monomer) and PEG diol (1.17 g, 1.95 mmol). The polymerization ran for 48 hours at 60 °C to ensure high conversion of (R)-MCL. The polymer was obtained after precipitation in cold heptane and drying in a vacuum oven at 40 °C. Yield: 68%.

(4) Preparation of Poly(R,S)MCL-b-PEG-b-Poly(R,S)MCL diol. The reaction was performed following the same procedure as (3) except that the monomer in the stock solution was a racemic mixture of (S) and (R)-MCL instead of (R)-MCL. Yield: 71 %.

B. Acrylation of PMCL-b-PEG-b-PMCL diols
1.0 mmol (2.0g or 4.0g) of PMCL-b-PEG-b-PMCL (or PCL-b-PEG-b-PCL diol) was dissolved in 10 ml dry diethyl ether in a 100 ml 3-necked flask. Then 0.20 ml (4.4 mmol) of triethylamine was added and the mixture was stirred in an ice bath. Subsequently 0.32 ml acryloyl chloride (4.0 mmol) dissolved in 15 ml dichloromethane was dropped very slowly into the mixture. After that, the reaction was kept stirring at room temperature overnight. The solid was removed by filtration and the filtrate was concentrated by evaporating the solvent in vacuo. Yield: 40%.

C. Enzymatic degradation of PMCL-b-PEG-b-PMCL diols
50 mg PMCL-b-PEG-b-PMCL diol was mixed with 5 ml distilled water (10mg ml⁻¹) in a vial. An emulsion was formed after an intensive shaking. 5 mg non-immobilized CALB was then added to the emulsion and the vial was put into an oven at 37 °C. Samples were taken out from time to time for DLS and SEC measurements. SEC
samples were prepared by freeze drying overnight before dissolving in THF.

**D. Crosslinking of PEG diacrylate and PCL (or PMCL) diacrylate**

0.56 g PCL (or PMCL) diacrylate (ca. 0.2 mmol), 0.14 g PEG diacrylate (0.2 mmol) and 20.5 mg 2,2-dimethoxy-2-phenylacetophenone (photo initiator) (10 : 1 molar ratio with respect to acrylate groups) were dissolved in 2 ml dichloromethane. The mixture was then added into defined wells (1.5 cm in diameter and 0.5 cm in depth) of a Teflon plate, which was then replaced into a quartz-top box under the nitrogen flow (Figure 5.1). After the complete removal of dichloromethane, a homogenous mixture was obtained. The box was exposed to UV-light (HPR125W, wavelength = 350–450 nm) at room temperature for three hours, after which a disk-shaped hydrogel was obtained. The gel content was measured by comparing the weight of the hydrogel extracted with water and dichloromethane ($W_i$) and the weight of the original hydrogel ($W_0$). The value was $W_i/W_0*100\%$. The water content was calculated by $(W_j-W_0)/W_0*100\%$. $W_j$ was the weight of the hydrogel which had absorbed water for 24 hours.

**E. Crosslinking of PMCL-b-PEG-b-PMCL diacrylate**

0.65 g PMCL-b-PEG-b-PMCL (or PCL-b-PEG-b-PCL) (ca. 0.2 mmol) and 10.2 mg 2,2-dimethoxy-2-phenylacetophenone (photo initiator) (10 : 1 molar ratio with respect to acrylate groups) were dissolved in 2 ml dichloromethane. Then the same crosslinking procedure as described under D was applied. The gel and water content were also measured in the same way as described under D.
Figure 5.1 UV curing box was equipped with a mold under N₂ protection. UV light was applied from a height of 10 cm above the box cover. Crosslinking was performed at room temperature.

F. Degradation of hydrogels in PBS and enzyme solutions

20 mg hydrogel was weighed in a vial followed by the addition of the degradation solutions: (1) 2 ml phosphate buffered saline (PBS) solution (pH=7.4); (2) 1 mg/ml of lipase PS/PBS solution (pH=7.4). The vial was sealed and put into the oven at 37 °C. Every two weeks the hydrogel was filtrated from the solution and freeze dried overnight before it was weighed. Then the solution was replaced by a freshly prepared solution with the same concentration. The weight loss was calculated by \((W_0-W_k)/W_0\times100\%\). \(W_0\) was the weight of the original hydrogel before degradation; \(W_k\) was the weight of the dried hydrogel during degradation.

5.3 Results and discussion

5.3.1 PMCL-b-PEG-b-PMCL with different chirality

While the amphiphilic block copolymers were initially intended for crosslinking in SIPN hydrogels, study of the literature suggested another application, i.e. in degradable micelles. Block copolymer micelle-based delivery systems have been used to improve the therapeutic efficiency of many drugs. Amphiphilic molecules such as
Bio-erodible SIPNs from PEG and PCL/PMCL

PEG-PCL di or tri-block copolymers were suggested as useful tools for targetable bone imaging and anticancer drug carrier.\textsuperscript{80, 81} The influence of PEG and PCL chain length on the physical properties of micelles has been widely explored.\textsuperscript{82, 83} However, the extreme hydrophobicity and semi-crystalline nature of PCL often necessitates the use of elevated temperature and/or organic solvent to aid the dissolution and self-assembly of the polymer. In contrast, dispersions of PMCL-\textit{b}-PEG-\textit{b}-PMCL in water generate micelles at moderate temperatures without the use of a cosolvent. Up to now, only a few studies on the synthesis and morphology of PEG-PMCL block polymer were reported.\textsuperscript{84-87} To the best of our knowledge, enzymatic synthesis of PMCL-\textit{b}-PEG-\textit{b}-PMCL and its chirality have never been studied. In this chapter, initial experiments for the synthesis of PMCL-\textit{b}-PEG-\textit{b}-PMCL micelles with different chirality were investigated and preliminary degradation experiments were carried out.

Instead of 1,6-hexanediol (Chapter 4), PEG diol (600 g mol\textsuperscript{-1}) was utilized as a macroinitiator in the asymmetric chiral resolution polymerization of a racemic MCL mixture. The goal was to synthesize PMCL-\textit{b}-PEG-\textit{b}-PMCL diols with different chirality in a similar strategy (Scheme 5.2) as described in Chapter 4. The same reaction conditions were applied and Novozym 435 kept a stereoselective preference for the (\textit{S})-enantiomer of MCL. However, the enantiomeric excess of the monomer (ee\textsubscript{m}) as well as the enantiomeric excess of the polymer (ee\textsubscript{p}) calculated from chiral GC only reached 0.70 and 0.62, respectively. The values are much lower than those (0.85 and 0.80) obtained in Chapter 4 when 1,6-hexanediol was used. Moreover, an E-ratio of 15.3 confirmed a relatively low enantioselectivity of the polymerization when PEG diol was used. The reaction was stopped at 45\% conversion and unreacted (\textit{R})-monomer was distilled from the mixture. A liquid polymer of Poly(\textit{S})MCL-\textit{b}-PEG-\textit{b}-Poly(\textit{S})MCL diol was recovered with a molecular weight of 1,800 g mol\textsuperscript{-1}, which was much lower than the theoretical M\textsubscript{n} according to the monomer/initiator feed ratio. Generally the polymerization with PEG diol was slower and not as efficient as with hexanediol. Therefore, the chance of randomization was higher, which might explain the lower ee\textsubscript{m} and ee\textsubscript{p}. Despite of the poor enantioselectivity in this macroinitiaton, an optical rotation of the polymer of -2.8° was measured in agreement with its (\textit{S})-enriched
structure. For the synthesis of Poly(R)MCL-b-PEG-b-Poly(R)MCL, distilled (R)-MCL with an enantiomeric purity of 90% was polymerized by Novozym 435 in the presence of PEG diol. The reaction was kept for 48 hours at 60 °C to ensure a high monomer conversion due to the lower reactivity of this enantiomer. SEC measurement revealed a molecular weight of 1,950 g mol⁻¹, which is similar to 1,800 g mol⁻¹ obtained for Poly(S)MCL-b-PEG-b-Poly(S)MCL but lower than the theoretical value (3,160 g mol⁻¹). The optical rotation of this polymer was +3.8°, confirming a high enrichment in (R) enantiomer. Poly(R,S)MCL-b-PEG-b-Poly(R,S)MCL was synthesized by carrying out the eROP of a racemic MCL mixture to a monomer conversion of 85%. The optical rotation of this polymer was 0°, which agrees with the racemic nature of the polymer. The SEC molecular weight of 2,400 g mol⁻¹ was slightly higher than the ones obtained for both chiral block polymers but still is in a comparable range.

**Scheme 5.2** Synthetic strategy for PMCL-b-PEG-b-PMCL diols with different chirality (1) Poly(S)MCL-b-PEG-b-Poly(S)MCL diol; (2) Poly(R,S)MCL-b-PEG-b-Poly(R,S)MCL diol; (3) Poly(R)MCL-b-PEG-b-Poly(R)MCL diol.
In order to clarify the formation of block polymers, Poly(R,S)MCL-b-PEG-b-Poly(R,S)MCL diol was taken as an example for SEC and ¹H-NMR analysis, which gave similar results for the other two PEG/PMCL copolymers with different chirality. In Figure 5.2, a significant molecular weight shift was found between the original PEG initiator and the final PMCL-b-PEG-b-PMCL, indicating that MCL monomer was successfully polymerized by macroinitiation. However, the block copolymer trace is quite broad and overlaps with the PEG trace, which means that the presence of block copolymers with low content of MCL (and even pure PEG) is likely. More evidence was obtained from ¹H-NMR spectra as shown in Figure 5.3 where characteristic peaks of both PEG and PMCL were precisely assigned. It should be emphasized that peak “c” at 4.25 ppm was corresponding to the PEG protons next to a PMCL block. Therefore, it is confirmed that PEG diol had performed as a macroinitiator for the eROP of MCL, eventually leading to the desired amphiphilic block polymer (PMCL-b-PEG-b-PMCL). However, the material is not very well defined in terms of molecular composition.

Figure 5.2 SEC traces of PEG initiator and the final PMCL-b-PEG-b-PMCL.
Table 5.1 summarizes all the characteristic data of the abovementioned three polymers. It shows that we successfully obtained three PMCL-\(b\)-PEG-\(b\)-PMCL diols with a similar molecular weight (~2,000 g/mol), but different in optical rotation (chirality). As a comparison, PCL-\(b\)-PEG-\(b\)-PCL diol was synthesized by eROP from the PEG diol with the same monomer to initiator ratio of 20:1. However, the SEC molecular weight of 4,000 g mol\(^{-1}\) was higher than the theoretical \(M_n\) of 2,880 g mol\(^{-1}\) and that of the chiral block copolymers. Even though the difference in hydrodynamic volume between PCL and PMCL has to be considered when comparing the molecular weights, the
results suggest that the higher reactivity of \( \varepsilon \)-caprolactone results in higher molecular weight. Different from using a small initiator as 1,6-hexanediol, macroinitiation by polar PEG diol provides less control over the molecular weight.

As all synthesized PMCL-\( b \)-PEG-\( b \)-PMCL diols were chemically indistinguishable, their degradation behavior can only be distinguished by bringing them in contact with a stereoselective catalyst like an enzyme. Thus preliminary degradation experiments were carried out with the same lipase (CALB) as used for the synthesis to investigate the effect of polymer chirality on the degradation. Different from PCL-\( b \)-PEG-\( b \)-PCL which was precipitated in water, all the PMCL-\( b \)-PEG-\( b \)-PMCL diols spontaneously formed an “emulsion” when added to the aqueous CALB solution (Figure 5.4A). This is of great interest that biodegradable micelles based on PEG/PMCL amphiphilic polymer could be formed in water without additional solvent and at a relatively low PEG concentration, which are limitations for the semi-crystalline polymer (e.g. PCL) system. Since no evidence of micellar formation was found for PCL-\( b \)-PEG-\( b \)-PCL, the degradations were performed only for the PMCL-based “emulsions” in a 37°C oven and monitored by SEC and DLS. The changes of molecular weight and particle size with degradation time are shown in Figures 5.5 and 5.6, respectively. It can be seen in Figure 5.5 that the molecular weight loss during degradation of the three polymers displayed the same tendency, irrespective of the chirality. All the samples experience a fast degradation in the first two days with a sharp molecular weight decrease. This is followed by a slight molecular weight increase. This behavior has also been described in the literature and is explained by the fact that CALB acts as a catalyst both for polymerization and degradation.\(^8\)\(^8\) The slight molecular weight increase could be a consequence of the reversible character of the reaction. The samples were then completely degraded in 13 days. The molecular weight of the final residue remained around 600 g/mol, which was exactly the molecular mass of the non-degradable PEG diol. Figure 5.6 shows the DLS results for the particle size evaluation, which indicates that the micellar morphology changes to some extent during the degradation process. It is shown that particle sizes generally decreased during degradation for all the emulsions of (\( S \)), (\( R \)) and (\( R,S \)). This decrease may be attributed to the collapse of the micelle core,
formed by PMCL, which degraded with time. Again, there is no obvious effect of chirality on particle size change. However, the micelle structures were greatly destroyed after 24 hours, as shown by the much more clear systems in Figure 5.4B. It has to be noted that any more quantitative discussion of the development of the micellar structures would require a more systematic study. This was not within the scope of this work and these data should thus just be interpreted qualitatively. It can be concluded, however, that PMCL-containing block copolymers can be an interesting alternative to PCL, as their amorphous character aids processability and enhances biodegradation speed. Moreover, the effect of chirality on degradation of these systems is negligible, which might be a consequence of the low enantiomeric excess of the chiral blocks.

**Figure 5.4** Non-crosslinked PMCL-b-PEG-b-PMCL diols (left: Poly(S)MCL-b-PEG-b-Poly(S)MCL diol; middle: Poly(R,S)MCL-b-PEG-b-Poly(R,S)MCL diol; right: Poly(R)MCL-b-PEG-b-Poly(R)MCL diol) in aqueous enzyme solutions (A) 0 hour; (B) 24 hours.
Figure 5.5 Molecular weight of non-crosslinked PMCL-b-PEG-b-PMCL diols plotted against degradation time (1 mg ml\(^{-1}\) CALB solution). Data obtained from SEC analysis.

Figure 5.6 Particle size obtained from DLS plotted against degradation time for PEG-b-PMCL diols.

5.3.2 Network formation

The above discussed micellar morphologies represent a dynamic system, which often
has limited applicability in drug delivery. Crosslinking hydrogels consisting of PEG and PMCL can easily overcome this shortcoming. Reported by several authors, semi-interpenetrating polymer networks (SIPNs) composed of PCL and PEG macromers with acrylate groups have been synthesized and characterized with the aim of obtaining bio-erodible hydrogels for release studies. Similar SIPNs were constructed by crosslinking of acrylate terminated PEG/PCL block polymers. However, the two main methods of making PCL-based hydrogels were restricted to the use of cosolvents or increased temperature, which could be limiting factors for in situ gel formation. As amorphous polyester, PMCL has a relatively low T\textsubscript{g} and an oily character with good mobility at low temperatures, which contributes to the ease of gel formation at a low temperature, even without applying organic cosolvents. Two methods of network formation were applied to PMCL (or PCL for comparison), as proposed in Scheme 5.1.

### Table 5.2 Parameters of networks (A) and (B) consisting of PEG and PCL/PMCL building blocks.

<table>
<thead>
<tr>
<th>Network Code</th>
<th>Composition</th>
<th>Feed ratio (wt./wt.)</th>
<th>Real ratio (wt./wt.)\textsuperscript{a}</th>
<th>Gel Content\textsuperscript{b} (wt. %)</th>
<th>Water Content\textsuperscript{c} (wt. %)</th>
<th>T\textsubscript{c}\textsuperscript{d}</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>PEG/PCL diacrylates</td>
<td>20:80</td>
<td>20:80</td>
<td>87.7</td>
<td>12.6</td>
<td>30 °C</td>
</tr>
<tr>
<td>A2</td>
<td>PEG/PMCL diacrylates</td>
<td>20:80</td>
<td>20:80</td>
<td>92.0</td>
<td>14.8</td>
<td>Amorphous</td>
</tr>
<tr>
<td>B1</td>
<td>PCL\textsubscript{-}b-PEG-b-PCL diacrylate</td>
<td>20:80</td>
<td>~15:85</td>
<td>61.2</td>
<td>5.9</td>
<td>28 °C</td>
</tr>
<tr>
<td>B2</td>
<td>PMCL\textsubscript{-}b-PEG-b-PMCL diacrylate</td>
<td>20:80</td>
<td>~15:85</td>
<td>93.5</td>
<td>6.7</td>
<td>Amorphous</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The real ratio of PEG/PCL (or PMCL) was determined by \textsuperscript{1}H NMR as shown in Figure 5.3; \textsuperscript{b,c} the calculation of the gel and water content is mentioned in the synthetic procedure D; \textsuperscript{d} crystallization temperature was determined by DSC during decreasing temperature at 10\textdegree C/min.
All polymer diols were first acrylated with acryloyl chloride before crosslinking as illustrated in Chapter 4. Each of them achieved a high acrylation degree of above 90%, which allows the further comparison of the hydrogels. Table 5.2 summarizes the networks formed from different diacrylates of PEG and PCL/PMCL. A1 and A2 are semi-interpenetrating polymer networks (SIPNs) from PEG/PCL diacrylates and PEG/PMCL diacrylates, respectively. After mixing of PEG diacrylate (M_n=700 g mol⁻¹) and PMCL diacrylate (M_n≈2000 g mol⁻¹) in a 1:1 mole ratio in the mold, UV irradiation was applied in the presence of a photo initiator for 3 hours before a disk-shaped hydrogel was obtained (A2 in Figure 5.7). For comparison, PCL diacrylate with a similar M_n was also crosslinked with PEG diacrylate in a 1:1 mole ratio to create a similarly shaped hydrogel (A1 in Figure 5.7). It can be seen that the transparency and homogeneity of A2 is better than that of A1. This agrees with our hypothesis that PMCL with low T_g and high mobility contributes to the homogeneity of the gel. Moreover, a crystalline peak was found by DSC analysis of A1, contrary to A2, indicating that crystalline domains remained after crosslinking in the PCL network, while PMCL network is completely amorphous. This difference is expected to influence the rates of the biodegradation process. FTIR spectra confirmed a complete conversion of acrylate groups, which was evidenced by the disappearance of the peaks at 1625 cm⁻¹ and 830 cm⁻¹ for both A1 and A2 (Figure 5.8). The gel content of A1 and A2, determined by solvent extraction with dichloromethane, gave relatively high values of 87.7% and 92.0%, which indicates the efficient crosslinking. The latter is slightly higher than the former, probably due to the higher efficiency of crosslinking of PMCL. The water content is dependent on the weight fraction of PEG due to the hydrophilicity of PEG. Therefore, both A1 and A2 have similar water absorption (12.6% and 14.8%) since they contain similar PEG fractions.
Chapter 5

Figure 5.7 Hydrogels formed after 3-hour UV irradiation: A1, crosslinked from PEG and PCL diacrylates; A2, crosslinked from PEG and (R,S)PMCL diacrylates; B1, crosslinked from PCL-\(b\)-PEG-\(b\)-PCL diacrylate; B2, crosslinked from (R,S)PMCL-\(b\)-PEG-\(b\)-(R,S)PMCL diacrylate.

Figure 5.8 FTIR spectra of four types of cross-linkable precursors before and after crosslinking: A1, PEG and PCL diacrylate; A2, PEG and (R,S)PMCL diacrylate; B1, PCL-\(b\)-PEG-\(b\)-PCL diacrylate; B2, (R,S)PMCL-\(b\)-PEG-\(b\)-(R,S)PMCL diacrylate.
Networks B1 and B2 were made in another way, namely by crosslinking of PCL-\textit{b}-PEG-\textit{b}-PCL or PMCL-\textit{b}-PEG-\textit{b}-PMCL diacrylates, which were acrylated by acryloylchloride reaction with PCL-\textit{b}-PEG-\textit{b}-PCL or PMCL-\textit{b}-PEG-\textit{b}-PMCL diols. B1 and B2 were obtained after 3 hours UV irradiation of the diacrylates as shown in Figure 5.7. Similar to the A-series, B2 shows a more transparent and homogenous morphology than B1, which again indicates that PMCL is advantageous over PCL in network formation. Consequently, B2 kept amorphous while A2 remained crystalline as determined by DSC. FTIR spectra of B2 were in agreement with the one of network (A), where acrylate groups were completely converted as evident from the disappearing bands at 1625 cm\(^{-1}\) and 830 cm\(^{-1}\). However, the acrylate group in B1 was not completely converted, since the band at 830 cm\(^{-1}\) was still visible after crosslinking. This may be caused by a less efficient UV curing for PCL-\textit{b}-PEG-\textit{b}- PCL diacrylate than for PMCL-\textit{b}-PEG-\textit{b}-PMCL diacrylate because of the lower mobility of the former. The gel content in B2 is much higher than the one in B1 as shown in the appearance of Figure 5.7 where B2 has more homogeneity and transparency than B1, indicating a more efficient crosslinking in B2. The water content of B1 and B2 also gave similar values of 5.9% and 6.7%, respectively, because of the comparable PEG weight fraction. By comparing A1 with B1, (or A2 with B2), the two different network formation methods were evaluated. As indicated above, the final network properties hardly showed any difference between A1 and B1 (or A2 and B2) with the exception of the water content. That is to say, crosslinking of the mixture of two diacrylates almost gave the same effect as crosslinking a block copolymer diacrylate. However, water content of network B is generally lower than that of network A due to the lower PEG fraction of B1 and B2. Although the feed ratio of PEG and MCL in block polymer synthesis was kept at 20/80, which was consistent with PEG/PMCL ratio in network A, the low conversion caused by inefficient macro initiation led to a PEG fraction below the feed ratio. Taking the synthetic procedure into account, network A (crosslinking of mixture of two diacrylates) has advantages over network B (crosslinking of a block polymer diacrylate) due to its more controllable and simplified manner. Moreover, the ratio PEG/PCL or PEG/PMCL can easily be varied.
In addition to the difference between PCL and PMCL, or between the nature of the network A or B, the networks based on PMCL resulted in three different networks differing in chirality but similar in chemical structure. For example, network A2 can be built up from PEG diacrylate and three PMCL diacrylates, i.e., (S)PMCL, (R,S)PMCL or (R)PMCL diacrylate, which have been obtained in Chapter 4. Analogously, network B2 also led to three types of hydrogels including crosslinked (S)PMCL-b-PEG-b-(S)PMCL, (R,S)PMCL-b-PEG-b-(R,S)PMCL and (R,S)PMCL-b-PEG-b-(R,S)PMCL diacrylate. Although optical rotation characterizes the chirality of these crosslinkable precursors of PMCL, there is no direct method to distinguish crosslinked networks with different chirality. Since Novozym 435 has been known as an enantioselective catalyst for MCL polymerization, it could also be applied for the degradation of the corresponding polyesters to discriminate the chirality of the network. Therefore, we hypothesized that it is possible to tune the enzymatic degradation rate by controlling chirality without changing the physical properties of the hydrogel.

### 5.3.3 Degradation study

There has been great emphasis on PEG/PCL hydrogels with encapsulated drugs for drug release. The latter largely depends on the degradation rate of these hydrogels. Therefore, investigation of the degradation of novel hydrogels to some extent guides their potential biomedical application. Figure 5.9 shows the degradation profiles for networks A and B against incubation time in either PBS or 1 mg/ml lipase PS (Pseudomonas cepacia)/PBS solutions, pH 7.4 at 37°C, which has been considered as a typical recipe for enzymatic degradation. These results show that the degradation occurred faster with enzyme than without enzyme, especially for PMCL networks (A2 and B2). It is consistent with literature reports that the enzyme accelerates the polyester degradation due to its catalytic cleavage of ester bonds. Moreover, A2 degraded faster than B2 in both solutions in agreement with the different water contents (see Table 5.2). This result is attributed to the hydrophilic nature of PEG, which increases the affinity of
water for the polymer matrix. It is reasonable to assume that the enhanced degradation is due to the higher PEG fraction in A2 compared to B2. In addition to the different degradation behaviors within PMCL-based hydrogels, distinctions were also found when they are compared to PCL-based hydrogels. It can be concluded from Figure 5.9 that whether chemically or enzymatically, PEG/PMCL networks (A2 and B2) degrade faster than PEG/PCL ones (A1 and B1). As indicated in literature, amorphous parts undergo a faster degradation than crystalline ones. It is therefore reasonable that faster degradation is obtained in PEG/PMCL networks where crystallinity is absent.

![Figure 5.9](image)

**Figure 5.9** Weight loss plotted against degradation time of network types A1, A2, B1 and B2 in PBS solution (left) and in 1 mg/ml lipase PS/PBS solution (right), pH=7.4. A2 and B2 refer to (R,S)PMCL-based networks. Each point represents the mean ±S.D. of at least three experiments.
Figure 5.10 Weight loss plotted against degradation time of networks of type A2 with different chirality in 1 mg/ml lipase PS/PBS solution, pH=7.4. Each point represents the average value of at least three experiments.

Figure 5.11 Weight loss was plotted against degradation time of network B2 with different chirality in 1 mg/ml lipase PS/PBS solution, pH=7.4. Each point represents the average value of at least three experiments.
Since each of the network types A2 and B2 resulted in three networks with different chirality, their degradation was performed in enzyme solution (lipase PS/PBS) to discriminate the rate. Figure 5.10 represents the PEG/PMCL diacrylates network (A2) degradation behavior within 130 days. Except for a much slower degradation of A2(R,S), A2(S) and A2(R) underwent a similar weight loss, irrespective of the chirality. Figure 5.11 shows preliminary degradation results for network types B2, which consists of B2(S), (R) and (R,S). Similar to the previous result of A2, B2(R,S) also degraded more slowly compared to the other two chiral networks. However, it is difficult to tell the difference between (S) and (R) because A2(S) degraded faster than A2(R) while B2(S) degraded more slowly than B2(R), as shown in Figures 5.10 and 5.11. Our initial experiments only indicate that racemic PMCL-based hydrogels, which was used to compare with PCL-based ones (see Figure 5.9), performed a slower degradation than the chiral ones in Lipase PS. Therefore, it can be concluded that PMCL-based hydrogels generally degrade faster than PCL-based ones even if the chirality is taken into account. This indicates that the amorphous character dominates over the chirality for the discrimination of the degradation rate for the applied degradation conditions. Moreover, the degradation behavior of the PMCL-based hydrogels in lipase PS is different from the behavior of PMCL polymers (or particles) in CALB (see Chapter 4) where a rate of (S)>(R,S)>(R) is retained. This difference could be caused by the different enzymes used for the degradation study. However, more work should be carried out to investigate if racemic hydrogels indeed degrade faster than chiral ones in lipase PS solution. Our preliminary results only show this tendency without extensive scientific evidence.
5.4 Conclusion

To improve water affinity and ultimately enhance biodegradability, amphiphilic triblock polymers and semi-interpenetrating polymer network (SIPN) hydrogels based on PEG and PCL (or PMCL) were investigated. PMCL-\textit{b}-PEG-\textit{b}-PMCL with different chirality was prepared by eROP using PEG diol initiator, which results in lower enantioselectivity and less control over the molecular weight as compared to small initiators. Biodegradable micelles could be formed from PEG/PMCL block polymer with low PEG fraction in the absence of cosolvent, which are limitations for PCL-based block polymer. SIPN hydrogels were built up in the way either (A) to crosslink a mixture of two diacrylates or (B) to crosslink one block polymer diacylate. However, network A has advantage over network B due to its more controllable and simplified synthetic procedure, as well as the large variety of PEG/PCL (or PMCL) ratio. Moreover, due to its amorphous morphology and low $T_g$, PMCL might be an alternative to PCL with respect to easier processability and higher biodegradability. However, an effect of chirality can not be seen although more experiments are necessary to come to a final conclusion. If there is no effect of chirality the PMCL should be made by chemical catalysis because it is easier to control.
Reference

CHAPTER 6

Cumulated Advantages of Enzymatic and Carbene Chemistry for the Non-organometallic Synthesis of (co)Polyesters

Abstract

Enzyme- and carbene-catalyzed ring opening polymerization can be combined in a one-pot reaction for the metal-free synthesis of degradable block copolymers. The compatibility test of both monomers and catalysts provides the experimental proof to design an elegant route for the synthesis. Desired PCL-b-PLA was obtained by controlling monomer feed ratio and sequence of addition.

This chapter is based on: the collaboration work with the group of Prof. P. Dubois in University of Mons-Hainaut, Belgium; Xiao, Y.; Coulembier, O.; K.; Koning, C. E.; Heise, A.; Dubois, Ph. Chem. Comm. In press.
6.1 Introduction

Aliphatic polyesters such as polylactide (PLA) and poly-\(\varepsilon\)-caprolactone (PCL) have an increasing presence among biodegradable polymers in various medical applications.\(^9\)

In many cases, random or block copolymers are synthesized so as to advantageously modify the polymer properties. Commonly PCL and PLA and their (block) copolymers are produced by organometallic, \(e.g.,\) tin(II) 2-ethyl hexanoate catalyzed ring-opening polymerization (ROP) of the corresponding cyclic esters.\(^9\) However, biomaterials are among the most sensitive materials with respect to product safety and purity. While tin(II) 2-ethyl hexanoate is approved for biomedical grade polymers, there is an increasing pressure on its use for medical-grade polymers due to some possible toxicity issues. The development of organometallic-free alternatives is thus of high interest in the biomedical and polymer community.

Two promising metal-free alternatives are the enzyme- and carbene-catalyzed ROP, respectively. The chemistry of \(N\)-heterocyclic carbenes (NHCs) has become a major area of research as potent nucleophilic organic catalysts for a wide variety of organic transformations. It has been recently demonstrated that 1,3,4-triphenyl-4,5-dihydro-\(1H\)-1,2,4-triazol-5-ylidene carbene catalyzes the ROP of lactide (LA) in the presence of alcohol initiators.\(^1\)\(^0\) These polymerizations proceed with first-order kinetics and exhibit a linear correlation between molecular weight and conversion and can yield polymers with low polydispersity indices (PDI) around 1.1. The exceptional control observed in this system is attributed to the reversible formation of a dormant alkoxyl triazoline, which keeps both the free carbene and the alcohol chain ends at a low concentration, thereby minimizing the rate of transesterification of the polymer chains. Unfortunately, the cited carbene-catalyzed polymerization of \(\varepsilon\)-caprolactone (CL) was not successful yet.

Lipases like immobilized \textit{Candida antarctica} lipase B (CALB), on the other hand, have proven high catalytic activity in the ROP of lactones of all ring sizes.\(^7\)\(^,\)\(^8\)\(^,\)\(^1\)\(^0\) In contrary to carbenes, the CALB catalyzed ROP of L-lactides was so far unsuccessful. While the reversible inhibition of the CALB by the lactide has been suggested\(^1\)\(^0\)\(^2\) also the fact
ROP of the monomer produces a chiral secondary alcohol end-group largely prevents further propagation.

While both metal-free ROPs offer certain advantages, they seem to be highly complementary in the classes of monomers they can polymerize. CALB does polymerize lactones but no lactides. Carbenes, on the other hand, are highly active catalysts for the polymerization of lactides but not for lactones. This prompted us to investigate whether the chemoenzymatic combination of both catalysts is possible in order to overcome the individual limitations and develop a completely metal-free ROP to biomedically relevant degradable aliphatic polyesters (Scheme 6.1). While chemoenzymatic polymerizations using CALB and controlled polymerizations have been reported, those either involve an organometallic metal catalyst or produce non-degradable block copolymers.21-23, 103, 104

Scheme 6.1 Synthetical strategy towards block copolyester by combining enzymatic (E) and carbene (C) catalysed ROP.

6.2 Experimental part

6.2.1 Materials

ε-Caprolactone (CL) was purchased from Aldrich, distilled over CaH₂ and stored over molecular sieves. Novozym 435 (E) was obtained from Novozymes A/S, Denmark.
L-Lactide (LA) was obtained from Purac, recrystallized from toluene three times prior to use and stored in a glovebox. Benzyl alcohol and toluene were distilled over CaH$_2$. 1,3,4-Triphenyl-4,5-dihydro-$1H$-1,2,4-triazol-5-ylidene was obtained after thermolysis of its methanol adduct (Acros) at 90 °C for 12 hours. Carbon disulfide (Acros) was dried on molecular sieves (4Å) for 12 hours.

### 6.2.2 Instrumentation

Gel permeation chromatography was performed in tetrahydrofuran on a Waters chromatograph equipped with four 5 µm Waters columns (300 mm × 7.7 mm) connected in series with increasing pore size (10, 100, 1000, 10$^5$, 10$^6$ Å). Polystyrene samples of known molecular weight were used as calibration standards. A Waters 410 differential refractometer and 996 photodiode array detector were employed. THF was used as eluent with a flow rate of 1.0 ml/min. All samples were diluted to 1.0 mg/ml in THF and filtrated using 0.2 µm syringe filters. $^1$H- and $^{13}$C-NMR spectroscopy was performed using a VARIAN 400 NMR at 20 °C. Samples were diluted in CDCl$_3$ to 30-50 mg/ml. Data were processed using VNMR-software.

### 6.2.3 Synthetic procedure

**A. General procedure for Novozym 435 polymerization of $\varepsilon$-caprolactone.**

Table 6.1, Entry 1. In a previously flamed and purged round bottom flask, a stock solution of benzyl alcohol (0.5 ml, 4.8 mmol) and toluene (25.5 ml) was prepared as initiator and solvent for each reaction ([I]$_0$ = 0.18 M). In a secondary flamed and purged flask, 0.02 g Novozym 435 (E) (10 wt.% of monomer) was dried with a stirring bar under vacuum at 40 °C overnight. The reaction was started by adding 0.2 g $\varepsilon$-caprolactone (1.8 mmol) and 0.19 ml stock solution (3.5 x 10$^{-5}$ mol) under nitrogen flow. The mixture was kept stirring at 90 °C for 6 hours, followed by the precipitation in cold methanol (yield: 70 %).
B. General procedure for carbene polymerization of L-lactide

Table 6.1, Entry 4. In a glove-box, 0.2 g of L-lactide (1.4 mmol) was charged in a flamed and purged round bottom flask containing 9 mg of 5-methoxy-1,3,4-triphenyl-4,5-dihydro-1H-1,2,4-triazol-5-ylidene previously dried under vacuum for 12 hours at 90°C to generate the 1,3,4-triphenyl-4,5-dihydro-1H-1,2,4-triazol-5-ylidene active carbene (C). Outside of the box, 0.15 ml of an initiator stock solution of benzyl alcohol (0.5 ml, 4.8 mmol) in toluene (25.5 ml) was added (2.79 x 10^{-5} mol) under nitrogen by using a previously dried and purged syringe. After 6 hours at 90°C, the polymerization was quenched by addition of dried CS₂ (0.4 ml, 6.9 mmol) and the medium was precipitated in excess of cold methanol (ca. 20 ml). The recovered polymer was dried until constant weight (yield = 82%) and analyzed by SEC and NMR analysis.

C. General procedure for ε-caprolactone/L-lactide copolymerization.

Table 6.2, Entry 3. In a previously flamed and purged round bottom flask, a stock solution of benzyl alcohol (0.5 ml, 4.8 mmol) and toluene (25.5 ml) was prepared as initiator and solvent for each reaction ([I]₀ = 0.18 M). In a second flamed and purged round bottom flask, 0.18 g of Novozym 435 (10 wt % of monomers) was dried under vacuum for 12 hours at 40°C. At room temperature, 1 ml of ε-caprolactone (7.0 mmol) was added under nitrogen and 1.5 ml of the initiator stock solution was added (2.79 x 10^{-4} mol). The mixture was then kept stirring at 60 °C for 6 hours. Afterwards, the mixture was added by a preliminary purged capillary to 92 mg of 5-methoxy-1,3,4-triphenyl-4,5-dihydro-1H-1,2,4-triazol-5-ylidene and 1 g of L-lactide (6.9 mmol) previously incorporated in a purged and dried round bottom flask. The copolymerization was then initiated by thermal treatment at 90 °C. After 6 hours, the solution was filtered and precipitated in excess of cold methanol. The recovered polymer was dried until constant weight and analyzed by SEC and NMR analysis (yield: 62%).
6.3 Results and discussion

6.3.1 General investigations

The combined enzymatic-carbene polymerization was first addressed in a number of control experiments, which served to design a one-pot polymerization. All reactions were performed under identical reaction conditions with respect to concentration, polymerization time, temperature, and monomer-to-initiator ratio (Table 6.1). In the first experiment, CL was polymerized by the immobilized CALB enzyme (E) from benzyl alcohol at 90 °C for 6 hours, yielding PCL with a molecular weight of 10,400 g mol\(^{-1}\) (Table 6.1, entry 1). The PDI of 1.8 is in the typical range for enzymatic ROP due to the inevitable transesterification reactions. As anticipated, when CL was replaced by LA no polymerization reaction was observed (Table 6.1, entry 2), which is in agreement with the state of the art. The same experiments were then performed replacing the lipase by the 1,3,4-triphenyl-4,5-dihydro-1\(H\)-1,2,4-triazol-5-ylidene carbene catalyst (C) keeping all other conditions unchanged. As expected, LA was polymerized to a high conversion (\textit{ca.} 80 %) and a molecular weight of 7,700 g mol\(^{-1}\) with perfect end-group fidelity and a low PDI (1.1) according to the controlled character of this polymerization (Table 6.1, entry 4). On the other hand, the CL polymerization did not yield any polymer from the carbene catalyst under the same conditions (Table 6.1, entry 3). As suspected, and in contrast to the LA system, the CL polymerization from C did not proceed due to the sterically encumbered structure of the triazol-based carbene catalyst confirming the inactivity of the imidazolin-2-ylidene towards CL polymerization. These reactions show the distinct activities of the two-catalysts regarding both monomers. The next experiments were designed to find out the apparent complementarities and apply those towards the synthesis of CL/LA block copolymers.
Table 6.1 Results of LA and CL polymerization initiated from benzyl alcohol using enzymatic (E) and/or a carbene (C) catalysis. Polymerizations performed for a monomer concentration of 4 M for 6 hours at 90 ºC at a total monomer-to-initiator ratio of 50:1.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Cat.</th>
<th>Monomer</th>
<th>Mn (g/mol)$^a$</th>
<th>PDI$^a$</th>
<th>DPCL$^b$</th>
<th>DPLA$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E</td>
<td>CL</td>
<td>10,400</td>
<td>1.8</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>E</td>
<td>LA</td>
<td>-$^c$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>CL</td>
<td>-$^c$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>LA</td>
<td>7,700</td>
<td>1.1</td>
<td>-</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>CL, LA</td>
<td>-$^c$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>C</td>
<td>CL, LA</td>
<td>-$^c$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>E, C</td>
<td>CL</td>
<td>-$^c$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>E, C</td>
<td>LA</td>
<td>7,200</td>
<td>1.3</td>
<td>-</td>
<td>27</td>
</tr>
</tbody>
</table>

$^a$ Determined by SEC in THF with polystyrene standards. $^b$ Degree of polymerization (DP) is determined by $^1$H NMR analysis: DPCL=$[I_{4.1}/I_{3.65}]$; DPLA=$[I_{5.1}/(2 \times I_{4.35})]$. $^c$ No polymerization took place.

In order to avoid intermediate work-up procedures, it was the aim to conduct both polymerizations consecutively in one pot. The combination of two polymerization techniques in one pot requires a detailed understanding of the mutual interactions of both techniques. Copolymerization experiments were thus performed to test the compatibility of both techniques with respect to monomers and catalysts. CALB was first used to initiate the CL/LA copolymerization from benzyl alcohol. After 6 hours of reaction no polymerization was observed (Table 6.1, entry 5). This result is in agreement with those reported by Gross et al. who suggested that LA acts as a reversible inhibitor of CALB during its copolymerization with CL. Another probable reason is the formation of a secondary lactoyl alcohol chain-end, which could prevent further chain propagation.

Surprisingly, the carbene-catalyzed polymerization of LA did not occur in the presence of CL (Table 6.1, entry 6). To our knowledge, this phenomenon has not been reported yet and the latent mechanism is still under investigation. The reactions corresponding to entries 7 and 8 in Table 6.1 were designed to investigate the catalyst
compatibility. Therefore, the two catalysts were mixed with one of the monomers and a polymerization initiated. In these experiments the mixture of CL, lipase and carbene did not yield any polymer (Table 6.1, entry 7). This result suggests that the enzymatic ROP of CL is inhibited in the presence of the carbene. The exact mechanism of inhibition, e.g., blocking of the active site of the enzyme, or its reversibility is unknown yet. In contrast, no inhibition was observed when the carbene-catalyzed polymerization of LA was performed in the presence of the enzyme (Table 6.1, entry 8), which confirms that CALB has no influence on the catalytic activity of the carbene. In summary, a poor compatibility of the system was observed for the enzymatic ROP in the presence of LA and carbene, while for the carbene-catalyzed ROP a high acceptance for Novozym 435 and CL was shown when polymerizations were performed in toluene at 90 °C.

6.3.2 “One-pot” reactions

The results of the compatibility test presented above have to be considered when designing a “one-pot” reaction. With the mutual inhibition described, difficulties were expected in making the copolymer by simply mixing two monomers and two catalysts in “one-pot” at elevated temperature. A successful “one-pot” block copolymer synthesis must therefore be divided into two steps, in which each polymerization step is triggered individually. At temperatures well below 90 °C the triazolinium carbene forms a stable adduct with primary alcohols such as the benzyl alcohol used in our synthesis.\textsuperscript{100} We rationalized that this could bind the carbene so as to prevent inhibition of the enzyme. While enzymatic polymerization is still possible at low temperatures (water initiated),\textsuperscript{23} the carbene-catalyzed ROP of LA should be prevented and only initiated when the temperature is raised above 90 °C. We therefore attempted to vary the reaction temperature so as to allow first the enzymatic polymerization of CL at lower temperature (60 °C and 30 °C, respectively) from a mixture containing all reaction components and subsequently initiate the carbene-catalyzed polymerization of LA by increasing the temperature (Table 6.2, entries 1 and 2). In both cases only the
Cumulated advantages of enzymatic and carbene chemistry

formation of PLA homopolymer was observed, which suggests that the inhibition of the enzyme by the carbene is most likely due to blocking the active site (serine). This seems to overrule the alcohol-carbene adduct formation. It has to be noted that the previously observed inactivity of the carbene-catalyzed polymerization in the presence of CL (Table 6.1, entry 6) was not observed in this mixture. This suggests a more complex interaction between the components, which is still under investigation.

Table 6.2. Results of the chemoenzymatic “one-pot” reaction of LA and CL using enzymatic (E) and/or carbene (C) catalyst.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Mn (g/mol)(^a)</th>
<th>PDI(^a)</th>
<th>DP(_{CL})(^b)</th>
<th>DP(_{LA})(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C, E, CL, LA (60 °C, 2h)</td>
<td>5,500</td>
<td>1.5</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>(90 °C, 6h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>C, E, CL, LA (30 °C, 24h)</td>
<td>5,000</td>
<td>1.4</td>
<td>-</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>(60 °C, 24h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>E, CL (60 °C, 6h)</td>
<td>10,500</td>
<td>1.5</td>
<td>29</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>C, LA (90 °C, 6h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>C, LA (90 °C, 6h)</td>
<td>8,500</td>
<td>1.8</td>
<td>19</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>E, CL (60 °C, 6h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Determined by SEC in THF with polystyrene standards. \(^b\) Determined by \(^1\)H-NMR analysis: DP\(_{CL}\)=[I\(_4\,4.1\)/(I\(_3\,3.65\)+2I\(_4\,4.35\)]; DP\(_{LA}\)=[I\(_5\,5.1\)/(I\(_3\,3.65\)+2I\(_4\,4.35\)].

Due to the incompatibility of the enzymatic reaction with both the LA and the carbene, the synthesis of block copolymers must be achieved by sequential addition of reactants (Table 6.2, entry 3). Since carbene polymerization of LA is more controlled than enzymatic ROP, carbene macrorinitiation will result in a higher block copolymer yield. Thus, the enzymatic reaction has to be performed first. This was realized by first polymerizing CL in the presence of CALB at 60 °C for 6 h. During this time a quantitative CL conversion was achieved. Subsequently, the carbene, together with the LA, was added to the reaction flask. Further polymerization at 90 °C for 6 h yielded the expected block copolymer (Table 6.2, entry 3). An advantage of this procedure relies on
the unnecessary deactivation of the enzyme prior to the second reaction step, because the carbene itself acts as an internal inhibitor for the enzyme. This prevents enzyme-catalyzed transesterification reactions, which could lead to an undesired randomization of the block copolymer.

After the enzymatic polymerization of CL a polymer with a number average molecular weight of 5,300 g mol\(^{-1}\) was obtained. GPC analysis revealed a clear molecular weight shift to 10,500 g mol\(^{-1}\) after addition of the carbene and LA comonomer (Figure 6.1). This provides first evidence that the macroinitiation took place. Further proof could be found in the comparison of the \(^1\)H NMR spectra of the block copolymer and of a sample taken before the addition of carbene/LA. In particular the disappearance of the caproic \(\omega\)-hydroxymethylene end-group initially present at 3.65 ppm (Figure 6.2, d’) in the final product confirms that the hydroxyl end-groups of PCL were completely converted by initiating the polymerization of LA. Furthermore, in the spectrum of the block copolymer a signal at 4.35 ppm could be detected, which was assigned to the \(\omega\)-hydroxy end-group of the PLA block (Figure 6.2, A’). Further evidence for the block-like structure was obtained from \(^{13}\)C NMR analysis. Figure 6.3 shows the significant region of the relevant carbonyl signals. Only two main peaks at 169.5 ppm and 173.5, respectively, are observed. This confirms that only two types of ester bonds are present in the polymer, namely LA-LA and CL-CL. Random copolymers would have shown a plethora of additional peaks in between the precited carbon signals due to the formation of different triads based on caproyl and lactoyl units (LA-LA-CL, CL-LA-CL, etc …).
Figure 6.1 GPC traces of “one-pot” carbene/enzyme-catalyzed block copolymer synthesis (Table 6.1, entry 3): (A) PCL obtained after step 1 and (B) P(CL-\(b\)-LA).

Figure 6.2 \(^1\)H NMR spectra of “one-pot” carbene/enzyme catalysed block copolymer synthesis (Table 6.1, entry 3): (top) PCL obtained after step 1 and (bottom) P(CL-\(b\)-LA).
For the completion of “one-pot” reactions, the polymerization with an opposite sequence of monomer addition was carried out, i.e., LA was first polymerized followed by CL polymerization (Table 2, entry 4). The first step was realized at 90 ºC for 6 hours. A sample of the intermediate product (PLA) taken for SEC and NMR analysis showed a Mn of 6,600 g mol\(^{-1}\) and a DP of 18, respectively. As indicated in Table 1, entry 7, the carbene catalyst would inhibit the polymerization of CL. Therefore, CS\(_2\) was added to decompose the carbene prior to the addition of CL and Novozym 435. Subsequently, the enzymatic polymerization was continued for another 6 hours at 60 ºC after which the final product was recovered. A clear molecular weight increase to 8500 g mol\(^{-1}\) was detected. However, the conclusion that PLA-PCL copolymers were successfully synthesized still remains debatable despite the molecular weight shift. There were two issues suggesting that we might obtain two homopolymers instead of a block copolymer: (1) DP of LA increasing from 18 to 27 contributed to the molecular weight enhancement; (2) in principle, the hydroxyl end of PLA contains a secondary alcohol with S configuration, which is unable to initiate the ROP of CL. Moreover, \(^1\)H NMR spectra of the final product shows hydroxyl ends of both PCL (3.65 ppm) and PLA (4.3 ppm). This also indicates the formation of two homopolymers. More evidence was obtained by \(^{13}\)C-NMR spectra, similar to Figure 6.3, where peaks at 169.5 ppm and 173.5 ppm are found corresponding to the carbonyl groups in pure PCL and PLA.
respectively. Close to both peaks there are no additional signals, which imply hardly any CL-LA sequence formed. So, with the NMR analysis, the final product of Table 2, entry 4 can only be a mixture of homopolymers of PCL and PLA, or PCL-PLA block polymer. Mainly based on the presence of two hydroxyl end-groups of both PCL and PLA, and on the impossibility of secondary hydroxyl with $S$ configuration, we conclude that PCL and PLA homopolymers were finally achieved.

6.3.3 PCL-$b$-PLA with different compositions by one pot reaction

Taking advantage of the degradability of PLA and the permeability to drugs of PCL, PCL-$b$-PLA has been extensively studied for biomedical applications. However, most of PCL-PLA block copolymers were produced by organometallic compounds-catalyzed ROP, which may cause toxicity issues in medical-grade use. Therefore, the “PCL first” route previously described was developed as a standard metal-free way to synthesize PCL-$b$-PLA in which the PLA block was polymerized by carbene catalysis. Now we extend our investigation to the effect of composition. Three combinations of CL and LA were studied as shown in Table 6.3. The targeted degree of polymerizations of CL and LA were 15/35, 25/25 and 35/15 for entries 1, 2 and 3, respectively. It was observed that the DP values 13/38, 26/25 and 37/13, calculated from $^1$H NMR, are consistent with the targeted ones. SEC analysis revealed clear molecular shifts and $^1$H NMR showed the disappearance of PCL hydroxyl end groups for all entries, indicating that block copolymers were obtained. Depending on the different compositions, the amount of the molecular weight increase agreed with the DP of LA. Namely, when a higher DP of LA was targeted, a more pronounced shift of molecular weight in SEC. Based on NMR and SEC results, it can be concluded that the composition of the block copolymer can be simply controlled by the feeding ratio of the monomers. Therefore, the “PCL first” route offers an attractive non-organometallic synthetic method to achieve PCL-PLA block copolymers with different compositions.
<table>
<thead>
<tr>
<th>Entry</th>
<th>DP_{CL} Targeted</th>
<th>DP_{CL} Exp.</th>
<th>DP_{LA} Targeted</th>
<th>DP_{LA} Exp.</th>
<th>Mn (g/mol)</th>
<th>PCL</th>
<th>PCL-b-PLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>13</td>
<td>35</td>
<td>38</td>
<td>4,150</td>
<td>9,020</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>26</td>
<td>25</td>
<td>25</td>
<td>7,070</td>
<td>10,540</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>37</td>
<td>15</td>
<td>13</td>
<td>8,180</td>
<td>9,870</td>
<td></td>
</tr>
</tbody>
</table>

\(a\) Determined by SEC in THF with polystyrene standards. \(b\) Determined by \(^1\)H NMR analysis: \(DP_{CL} = [I_{4.1}]/(I_{3.65}+2I_{4.35})\); \(DP_{LA} = [I_{5.1}]/(I_{3.65}+2I_{4.35})\). \(c\) Samples taken out before LA polymerization.

### 6.4 Conclusion

A complete compatibility study of CL, LA, carbene and Novozym 435 was performed to test the possibility of “one-pot” chemoenzymatic polymerization of CL and LA. However, a poor compatibility of the system was observed for the eROP in the presence of LA and carbene, while for the carbene-catalyzed ROP, a high acceptance for Novozym 435 and CL was shown. Due to the mutual inhibition described, “one-pot” two-step reactions were designed to achieve well-defined block copolymers. Based on SEC and NMR analysis, it can be concluded that (1) the “one pot” two-step reactions triggered by temperature elevation end up exclusively with PCL homopolymer; (2) “PLA-first” route give a mixture of two homopolymers (PLA and PCL); (3) PCL-b-PLA can only be realized by “PCL-first” strategy, which allows carbene-catalyzed ROP initiated by the hydroxyl end of PCL. With “PCL-first” strategy, PCL-PLA block copolymers with different compositions could be obtained by controlling the monomer feed ratio.
References

Summary

Functional Polymers by Enzymatic Catalysis

Enzymes are precision catalysts from nature that can be used to create novel polymers and materials which are very difficult or even impossible to achieve by conventional chemical procedures. This is of particular importance in the field of biodegradable polymers, where materials are designed not only to ensure a healthier, cleaner and more sustainable world, but also to direct the development of novel functional materials for high performance areas such as biomedical applications. However, previous researchers have focused more on the exploitation of enzymes for \textit{in vitro} synthesis than on the fundamental understanding of enzymes for functional polymer synthesis. Therefore, the aim of this PhD project is (1) to use the special character of enzymatic catalysis to offer an advanced synthetic method for existing biodegradable polymers, and (2) to take advantage of the high selectivity in enzymatic catalysis to develop novel materials which have never been achieved before and explore their potential application in the biomedical area.

Chapters 2 and 3 aim to answer the question whether enzymatic acrylation provides a feasible process for the production of acrylated polymers. 2-Hydroxyethyl methacrylate (HEMA) or 2-hydroxyethyl acrylate (HEA) initiated ring opening polymerization of $\varepsilon$-caprolactone (CL) and $\omega$-pentadecalactone (PDL) were carefully investigated. Instead of the expected mono-functionalized products, a number of different telechelic polymers with various end-group combinations were observed. Our kinetic studies show that the lipase B from \textit{Candida antarctica} (CALB) does not discriminate between carbonyl bonds of the monomers, the polymers or the initiators, and transesterification reactions can thus not be prevented. Large differences in lipase-catalyzed acyl transfer reaction rates between HEA and HEMA end-groups were observed (10-15 fold difference!) in which HEA was more prone to acyl transfer due to
the less sterically hindered structure. However, when HEMA (or HEA) initiation is combined with vinyl methacrylate (or acrylate) end-capping, well-defined dimethacrylated (diacrylated) polymers as curable precursors for network formation can be prepared. This method provides pioneering insight into green enzymatic acrylation of biodegradable polymers.

Chapter 4 and 5 concern the development of novel chiral microspheres and hydrogels obtained from poly-4-methyl-ε-caprolactone (PMCL), with the aim to use chirality to program polymer degradation. Preliminary degradation experiments with CALB show that the degradation rate can be tuned by the polymer chirality. However, the chirality-based rate discrimination is not pronounced enough in the crosslinked materials for \textit{in vivo} application. To improve water accessibility of the biodegradable networks, semi-interpenetrating polymer network (SIPN) hydrogels based on polyethylene glycol (PEG) and PCL (or PMCL) were investigated. Due to its amorphous morphology and low $T_g$, PMCL might be an attractive alternative to PCL with respect to easier processability and faster degradation.

Combining advantages of enzymatic and carbene catalysis, chapter 6 describes a non-organometallic catalyst-based synthesis for copolyesters consisting of PCL and polylactide (PLA) blocks. While inhibition of the enzyme by the carbene was observed, a “one-pot” two-step reaction allowed the synthesis of well-defined block copolymers from LA and CL. With this, metal-free PCL-PLA block copolymers with different compositions could be obtained.
Finally the four-year journey comes to an end. It is a pleasure to express my gratitude to a great number of people whose contributions enabled me to complete this thesis successfully.

In the first place I would like to record my gratitude to my supervisors Prof. Cor Koning and Dr. Andreas Heise for their guidance, support and suggestions from the initial to the final level of this research. Special thanks owe to Andreas, whose vast knowledge of enzymatic polymerization has helped me understand this subject which was foreign to me before. More importantly, his continuous encouragement and confidence in me and my ability always drive me going further. Many thanks to Cor for giving me the opportunity to pursue my PhD in his group. I appreciated his positive attitude on my project and well-targeted training to develop my personal skills. Andreas and Cor, your kindness, patience and support over these four years have brought me such rapid progresses on my scientific achievements, personal development and European life experience, dank u wel! I am also highly thankful to Dr. Anja Palmans, for her valuable suggestions throughout this study and thorough corrections of my manuscripts.

I would like to extend my sincere gratitude to the core-committee members, Prof. Karl Hult, Prof. Philippe Dubois and Prof. Jan Meuldijk, for reading the manuscript and valuable comments. Also thanks to Prof. Steven Howdle for his initial recommendation to this PhD position and being member of my defense committee.

The collaboration work with Prof. Hult’s group in Royal Institute of Technology (KTH) and Prof. Dubios’ group in University of Mons-Hainaut (UMH) was extremely appreciated. Mohamad, thank you for your visit to Eindhoven with highly efficient lab-working, as well as data-processing and draft-writing. The papers and chapters
about HEMA and HEA would not have been possible without your great input. I would like to acknowledge Dr. Mats Martinelle for the informative discussion, the revision and submission of the manuscripts. I would also like to thank Dr. Olivier Coulembier for taking care of my short-stay in Mons and all the e-mail communications. His expertise in carbene chemistry and sophisticated skills in lab has definitely upgraded my knowledge and the level of the thesis.

I am also indebted to BIOMADE (Marie-Curie RTN programme), which offered me not only the financial support but also a European personal network. David (DSM), Iris, Chris, Mohamad, Neil, Marc, Dragos, Cristian, Silvia, Jason, katarzyna, Markus, Eva, Karl, Helmut, David (CIDETEC), Steven, Germ… The stimulative discussions and happy time during project meetings and the contact built up afterwards will be cherished in my memory for ever. I would also like to thank Mike de Leeuw and his Branching Tree Company for the financial support in the final year of my PhD study.

During these four years, I met a lot of people who helped me with experimental and analytical techniques outside of our group. Thanks to Ir. Otto van Asselen for the professional suggestions and Dr. Dong Weifu for the help on FTIR. Thanks to Dr. Han Wei for the wonderful SEM images. Thanks to Pit (SKT) for the UV-curing equipment. Thanks to Ing. Brahim Mezari for the extraordinary busy solid-state NMR analysis. Thanks to Sun Chunxia for TGA and Li Weizhen for DSC measurements. Also thanks to Dr. Bart van As, Dr. Lou Xianwen and Ir. Martijn Veld for the help on chiral GC.

Working in SPC is a pleasant and unforgettable experience for me. Thank you very much to all SPCers! My special gratitude goes to: Dr. Marshall Ming, Prof. Alex van Herk and Dr. Dirk-Jan Voorn for their professional advice and comments on emulsion polymerization; Dr. Matthijs de Geus for bringing me into the enzyme world and tremendous help in the initial stage of my PhD study; Ali for fruitful discussions about amphiphilic polymers and their cryo-TEM characterization; Marion and Henk for HPLC and MALDI measurements, especially the discussion about the possibility to
characterize chiral block copolymers; Raf for valuable suggestions on all of the complicated synthetic procedures; Joris and Mark for table-top SEM instruction; Hector for his patient explanation about DLS mechanism; My student Jessica for macrodiol synthesis; Donglin for reading my thesis drafts; Pleunie and Caroline for the assistance of my life in Eindhoven. I am very thankful to all the Heise group members: Matthijs, Rutger, David, Gaëtan, Gijs, Inge, Hemant and Bahar, for the helpful discussions and joyful lab-sharing.

I also appreciate the funny chat and friendly atmosphere created by my officemates (STO 1.43): Wouter, David, Jens, Rutger, Saskia, Ali, Timo, Gözde, Bahar, Hemant and Fachri. Special thanks to Rutger for his “fresh and fruity” greetings in the morning, his geographical introduction of the Netherlands and his effort for integrating me into the western European culture. Saskia, we were the only-two ladies in our office for a long time. Thank you for sharing with me the gossips, job-hunting experience, living-in-Eindhoven tips… Many thanks to Ali for the talks on various topics and answering me some stupid questions. Also thanks to Timo for the Dutch documents translation and technical consultant. David, thank you for your help with my English and lab work at the beginning of my PhD study.

In fact, I would not have carried out my PhD project abroad if I had not been supported and encouraged by my previous supervisors in Shanghai Jiao Tong University (Shanghai, China), namely Prof. Yan Deyue and Prof. Zhu Xinyuan. I am especially grateful to their wise advice on my scientific thinking and private life.

Collective and individual acknowledgments are also owed to my friends, whose presence provides me numerous encouragement, support, help and joy in the past four years. Many thanks go particular to Wang Ting, Huang Rubin, Tang Donglin, Jiang Zhouting, Yuan Ming, Li Zhili and Han Wei, with whom I shared most of memorable moments in Eindhoven. Also thanks to Lou Xianwen, Marshall, Tian Mingwen, Xue Lijing, Langee, Dong Weifu, Wang Lili, Sun Chunxia, Li Weizhen, Shuffy, Guan
Acknowledgements

Yejun, Yang Jie, Song liguo, Lv Kangbo, Leng Boxun, Duan Chengjun, Zhou Jiang, etc., for their enthusiastic help in my work and life. In addition to the friends in Helix, I would like to express my sincere thanks to the others in the Netherlands: Gu Bing, Li Ping, Zhang Shaoxian, Li Zhonggui, Zheng Yuhang, Liu Yan, Li Jing, Chen Jieyin and their partners, for sharing the delicious food and wonderful time with me during weekends. Remote support from my friends in Shanghai is also highly appreciated: Wang Zheng, Lin Lin, Zheng Liyi, Wu Zhuoqun, Pang Jingzhu, Li Yi, Nanie and Daisy, thank you for the online communications and your kind host during my holiday.

Last but not least, words fail me to express my appreciation to my parents, Mr. Xiao Peihua and Mrs. Jin Lanfang, for their love and support throughout my life.

最后感谢我的父母，是你们多年的养育成就了我这篇论文，是你们悉心的关爱包容了我所有优缺点，是你们无限的支持激励着我不断进步。爸爸妈妈，辛苦了！

Yan Xiao
肖艳
Yan Xiao was born on 23rd April 1981 in Zhangjiagang city, Jiangsu Province, China. After her graduation from Liangfeng high school in 1998, she started her undergraduate study at the Department of Chemistry and Chemical Engineering, Shanghai Jiao Tong University (SJTU), Shanghai, China. After obtaining the B.Sc. degree in Applied Chemistry in 2002, she continued her master study in the same department, where she carried out the research on “Crystallization and Melting Behavior of Polymers with Long Alkane Segments” under the supervision of Prof. dr. Deyue Yan and Prof. dr. Xinyuan Zhu. In 2005 she received her M.Sc. degree in Material Science. From April of the same year, she joined the Polymer Chemistry group in the Department of Chemical Engineering and Chemistry at Eindhoven University of Technology (TU/e) in the Netherlands as a PhD student under the supervision of Prof. dr. C. E. Koning and dr. A. Heise. Her PhD research was focused on Functional Polymers by Enzymatic Catalysis, the results of which have led to this thesis.
List of Publications


