Polyesters from natural macrolactones for biomedical applications

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Polyesters from Natural Macrolactones for Biomedical Applications

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 woensdag 1 december 2010 om 16.00 uur

door

Inge van der Meulen

geboren te Veldhoven
van der Meulen, I.

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Glossary

3T3 3-day transfer
4MeCL 4-Methylcaprolactone
AIBN 2,2’-Azobisisobutyronitrile
Am Ambrettolide
ANOVA Analysis of variance
ATR-FTIR Attenuated total reflection fourier transform infrared spectroscopy
β-β-Butyrolactone
BnOH Benzyl alcohol
cp Heat capacity
CALB Candida antarctica lipase B
ε-ε-Caprolactone
CNS Central nervous system
δ-Chemical shift
Δh or ΔH Melting enthalpy
DCP Dicumyl peroxide
DL 10-Decanolactone
DSC Differential scanning calorimetry
DMA Dynamic mechanical analysis
DMEM/NUT Dulbecco’s minimal essential medium/nutrients
DXO 1,5-Dioxepan-2-one
ε-break Strain at break
ε-yield Strain at yield point
E Young’s modulus
eROP Enzymatic ring opening polymerization
φ-Angle
f Hermans orientation factor
FDA Food and drug administration
FTIR Fourier transform infrared spectroscopy
γ-γ-Butyrolactone
g-Cosine of the angle
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Enzymatic ring opening polymerization has become a widely used technique in the synthesis of biomedical polyesters. Many different copolymers based on all kinds of lactones were made during the last decade. In this study copolyesters based on unsaturated macrolactones are made using this technique. Changing the nature and the ratio of the comonomers and using the unsaturation to cross-link the polymers, the properties of the obtained polyesters were modified with the aim to develop a materials platform consisting of polyesters, which can be tuned for the right properties in an easy way. The applications focused on are suture materials and nerve repair.

High molecular weight polypentadecalactone was synthesized via enzymatic ring opening polymerization using Novozym 435 as a catalyst. By scaling-up the process larger batches of polymer could be obtained. The mechanical and thermal properties of the products were determined. The obtained polymer was melt-spun into fibers, which were further elongated. Analysis of the fibers revealed differences in crystal orientation as a function of the processing and drawing conditions. Preliminary fiber tensile measurements confirm the highest strength for the fiber with the highest crystal orientation.

A library was made based on the macrolactones globalide and ambrettolide, which are the unsaturated analogues of \( \omega \)-pentadecalactone and 16-hexadecalactone, respectively. Polymerization was performed using Novozym 435 and all monomers could be polymerized to convenient molecular weights. Solubility of the unsaturated analogues is better in common solvents as compared to their saturated counterparts. Their crystallinity is still high, however melting and crystallization temperatures of the polymers obtained from ambrettolide and globalide are significantly lower than the corresponding values of their saturated analogues. All homopolymers were tested and proved to be non-cytotoxic. Hydrolytic as well as enzymatic degradation is very slow. By thermal and UV cross-linking via the main chain unsaturation, fully amorphous materials were obtained. However, degradation of these amorphous materials was still slow, what indicated that the high crystallinity is not the only property retarding the degradation.

To investigate the influence of hydrophobicity and crystallinity on the material properties of these polyesters, and especially the degradation properties, random copolymers were made using small lactones like 4-methyl caprolactone, 1,5-dioxapan-2-one and 2-oxo-12-crown-4-ether as comonomers. All polymers were readily made, although copolymers containing 2-oxo-12-crown-4-ether were more difficult to prepare.

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**Abbreviations:**

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<tr>
<td>TCB</td>
<td>1,2,4-Trichlorobenzene</td>
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<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
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<tr>
<td>THP</td>
<td>Tetrahydro-4H-pyran-4-one</td>
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<tr>
<td>TIPS</td>
<td>Temperature induced phase separation</td>
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<tr>
<td>UHMWPE</td>
<td>Ultra high molecular weight polyethylene</td>
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<tr>
<td>UL</td>
<td>11-Undecanolactone</td>
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<tr>
<td>VL</td>
<td>( \delta )-Valerolactone</td>
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<tr>
<td>( w_c )</td>
<td>Crystalinity value</td>
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<tr>
<td>WAXS</td>
<td>Wide angle X-ray spectroscopy</td>
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<td>w/w-%</td>
<td>Weight percentage</td>
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crown-4-ether only yielded low molecular weight products. All copolymers are proved to be non-cytotoxic, what makes these copolymers suitable for biomedical applications. By altering the amount of comonomer added to the reaction mixture, properties like hydrophilicity (by adding 1,5-dioxepan-2-one or 2-oxo-12-crown-4-ether) and crystallinity (by adding 4-methyl caprolactone) could be tuned. The incorporation of all monomers led to an increase in degradation rate of the polyesters, showing that both crystallinity and polarity are crucial for the degradation. The relation between the amount of comonomer added and the ability to form fully cross-linked polymers was investigated using a model system of ε-caprolactone and globalide. It was found that only a small percentage of the used monomers needs to contain an unsaturation to be able to obtain complete network formation. Moreover, only a part of all double bonds is cured, which leaves opportunities to incorporate biologically active components via e.g. thiol-ene chemistry or other addition reactions.

Next to utilizing enzymes as catalysts in the synthesis of polyesters, aluminum salen complexes with different phenolic substituents were explored for their catalytic activity towards polypentadecalactone synthesis. A clear influence of bulky substituents in the complex was observed, however the polymerization rates remained high. An unsubstituted aluminum salen complex was explored in the polymerization of lactones of different sizes. All lactones were polymerized to high conversions within a reasonable timescale. The smaller lactones polymerize fastest, followed by the large lactones. The middle-sized lactones react slowest, however still faster than ever reported before using a chemical catalyst different from ours.

After synthesis of this library of different copolyesters, permeable scaffolds were aimed for using different porogens. To control the porosity of the material different ratios of porogen/polymer were investigated. After cross-linking the porogen is easily washed out using water. The cytotoxicity of the cross-linked scaffolds is low.

In conclusion it can be stated that we were able to synthesize a complete library of novel biomedical polyesters based on unsaturated macrolactones which contains many different polymers suitable for different biomedical applications. The properties (degradation, strength etc.) of the products can easily be tuned to the specific needs for a specific biomedical application.
Alle materialen, dus ook plastics, die tegenwoordig gebruikt worden moeten aan strenge eisen voldoen. Dit geldt zeker voor biomedische materialen. Deze mogen bijvoorbeeld geen resten van stoffen bevatten die schadelijk zijn voor de gezondheid. Dit betekent ook dat de katalysatoren die gebruikt worden, incompatibel moeten zijn. Daarom wordt er in dit onderzoek gebruik gemaakt van een metaalvrij enzym om nieuwe polyesters te maken die geschikt zijn voor gebruik in medische toepassingen. Deze polymeren zijn gebaseerd op onverzadigde macrolactonen. Het doel van dit onderzoek is een groep nieuwe polyesters te ontwikkelen welke gemakkelijk te synthetiseren en af te stemmen zijn op de behoeften van de beoogde toepassing. De toepassingen die hier uitgezocht zijn, zijn hechtdraden en materialen voor het herstellen van gebroken zenuwen.

Hoog molecuul gewicht polypentadecalacton is verkregen via enzymatische ring opening polymerisatie. Novozym 435 is hier gebruikt als katalysator. Door het opschalen van de lab-schaal synthese konden grotere hoeveelheden polymer meer verkregen worden. De mechanische en thermische eigenschappen zijn bepaald en het polymer werd gesponnen vanuit de smelt, waarna de verkegen vezels verstrekt zijn. De oriëntatie van de kristallen in de vezels werd beïnvloed door de procescondities; hoe groter de oriëntatie, hoe sterker de vezel.

Naast polypentadecalacton zijn er polymeren gesynthetiseerd van de macrolactonen ambrettolide en globalide, welke de onverzadigde analogen zijn van respectievelijk ω-polypentadecalacton en 16-hexadecalacton. De oplosbaarheid van deze onverzadigde lactonen is veel beter dan die van de verzadigde versies. De kristalliniteit van alle verkregen polymeren is hoog, maar de smelt en kristallisatie temperaturen van de onverzadigde polymeren zijn aanzienlijk lager dan die van de verzadigde polyesters. De cytotoxiciteit van alle homopolymeren is laag. Ook de hydrolytische en enzymatische afbreekbaarheid is laag. Door het thermisch of door blootstelling aan UV licht vernetten van de onverzadigde bindingen in de ketens kon een compleet amorf materiaal verkregen worden. Deze afname van de kristalliniteit had echter geen invloed op de afbreekbaarheid, waaruit kon worden geconcludeerd dat de mate van kristalliniteit niet de enige parameter is die de afbraaksnelheid bepaalt.

Om de invloed van de kristalliniteit en hydrofobicitie verder te onderzoeken zijn er copolymeren van apolaire macrolactonen met verschillende kleine lactonen zoals 4-methyl caprolacton, 1,5-dioxapan-2-on en 2-oxo-12-kroon-4-ether gesynthetiseerd. Alle copolymeren zijn gemakkelijk verkregen, alhoewel de molecuulgewichten van copolymeren met 2-oxo-12-
kroon-4-ether laag bleven. Alle copolymeren zijn voorheen getest op hun toxiciteit en niet toxisch bevonden. Daarom zijn alle hier gemaakte copolymeren geschikt voor medische toepassingen. Door het aanpassen van de hoeveelheden comonomeer welke toegevoegd worden aan het reactiemengsel, kunnen eigenschappen als hydrofiliciteit (gebruik makend van 1,5-dioxapan-2-on en 2-oxo-12-kroon-4-ether) en kristalliniteit (door toevoegen van 4-methyl caprolacton) beïnvloed worden. Door het toevoegen van een van deze comonomeren, ongeacht welke, bleek de afbreekbaarheid toe te nemen. Ook is de invloed van de hoeveelheid toegevoegd comonomeer op de vorming van een totaal vernet materiaal bekeken. Maar een klein deel van de onverzadigde bindingen wordt gebruikt voor het vormen van het netwerk. De andere onverzadigingen kunnen wellicht gebruikt worden om biologisch actieve componenten in te bouwen in het materiaal.

Naast het gebruiken van een enzym als katalysator voor de synthese van deze polymeren is ook een aluminium salen complex onderzocht. Van salen-liganden met verschillende zijgroepen is de activiteit in de polymerisatie van $\omega$-pentadecalacton onderzocht. De invloed van de zijgroepen was duidelijk zichtbaar in de reactiesnelheden. Deze snelheden bleven echter wel hoog. Polymerisatie van lactonen met verschillende grootte is getest met het aluminium salen complex zonder enige zijgroepen. De kleine lactonen polymeriseren het snelst, de grote volgen en de middel-grote reageren het langzaamst. De reactiesnelheden van deze groep blijven echter wel hoger dan ooit gerapporteerd werd voor gebruik van een chemische katalysator.

Naast de synthese van deze nieuwe groep polyesters is de mogelijkheid tot het maken van poreuze materialen met behulp van verschillende hulpstoffen onderzocht. Door middel van het inmengen van verschillende hoeveelheden van deze hulpstoffen werd bekeken wat de invloed op de porositeit van het eindproduct was. Na het vernetten van de polyester zijn de hulpstoffen gemakkelijk weg te wassen met water.

In dit onderzoek is aangetoond dat een complete nieuwe groep biomedische polyesters gemaakt kan worden vanuit onverzadigde macrolactonen. Deze groep omvat veelzijdige materialen welke geschikt zijn voor uiteenlopende toepassingen. De materiaal eigenschappen (zoals sterkte en afbreekbaarheid) kunnen zo beïnvloed worden dat het product aan de eisen voldoet voor de beoogde toepassing.
All copolymers have been previously tested for their toxicity and found to be non-toxic. Therefore, all the copolymers made here are suitable for medical applications. By adjusting the amounts of comonomers added to the reaction mixture, properties such as hydrophilicity (using 1,5-dioxapan-2-one and 2-oxo-12-crown-4-ether) and crystallinity (by adding 4-methylcaprolactone) can be influenced. By adding one of these comonomers, regardless of which, it was found that the degradation increased. Also, the influence of the amount of added comonomer on the formation of a fully crosslinked material was examined. But a small part of the unsaturated bonds is used to form the network. The other unsaturated bonds can possibly be used to incorporate biologically active components into the material.

Aside from using an enzyme as a catalyst for the synthesis of these polymers, an aluminum chelate complex was also investigated. The activity in the polymerization of \( \omega \)-pentadecalactone was studied. The influence of the side groups was clearly visible in the reaction rates. However, these reaction rates remained high. Polymerization of lactones with different sizes was tested with the aluminum chelate complex without any side groups. The small lactones polymerize the fastest, the large ones follow, and the medium-sized ones react the slowest. The reaction rates of this group remain higher than ever reported for the use of a chemical catalyst. In addition to the synthesis of this new group of bio-medical polyesters, the possibility of making porous materials with the help of different additives was investigated. By mixing different amounts of these additives, it was looked at what the influence on the porosity of the end product was. After crosslinking the polyester, the additives are easily washed away with water.

In this research, it has been shown that a complete new group of biologically active polyesters can be made from unsaturated macrolactones. This group includes versatile materials that are suitable for various applications. The material properties (such as strength and degradation) can be influenced so that the product meets the requirements for the intended application.
1.1 Biomedical materials

Nowadays many polymers are used in the medical world. These polymers are used in various applications, like surgical material, drug delivery, bone replacement and tissue repair. Surgical material includes all materials applied in a hospital. For example wound dressings, bandages, band-aids, staples and needles belong to this group and a variety of materials are used in the medical world (plastics, metals, textiles, etc.). Another group of medical materials are the invasive materials, like sutures. There are two different types of sutures, i.e. degradable and non-degradable. The first are often used in surgery when internal wounds need suturing, to prevent a second surgery to remove the sutures. For this type of sutures normally polyester or polylactide fibers are used. However, polylactide fibers are under discussion due to the acidity of their degradation products (lactic acid), which can cause tissue inflammation.

Materials for drug delivery was and still is a field of intense research. Many polymers, polymer hybrids and polymer formulations have been investigated and tested. For drug delivery mostly dendritic structures and hyperbranched polymers are used. Their structure makes it possible for small molecules to penetrate the space in between the chains and drug molecules can be ‘released’ by diffusion or during degradation of the polymer. Sometimes the guest molecules are linked to the host by hydrogen bonding or other non-covalent interactions. Polysters are just one kind of the many different polymers that are used. One specific example is a dendritic structure based on succinic acid and glycerol.

In bone replacement there are two separate fields, bone repair and bone (or joint) replacement. For bone repair often a porous ceramic scaffold is used. To enhance the material strength the scaffolds can be coated with a polymer. The most widely used polymers for bone repair are polylactic acid (PLA), polyglycolide (PGL) and poly(ε-caprolactone) (PCL). During the healing process, the complete scaffold degradables and is replaced by new bone upon repair. For bone replacement other, non degradable polymers are used. For example ultra high molecular weight polyethylene (UHMWPE) is one of the most widely applied materials for artificial knee and hip joints. Besides the general demands for biomedical materials, e.g. non-toxicity, all applications have their own set of individual requirements. In this work the suitability of new polymers for nerve repair was investigated. For this application the polymer has to be processed into a porous scaffold. The work carried out in this thesis covers the whole chain of knowledge. i.e. from monomer and polymer synthesis, degradation tests, processing of the polymers into porous scaffolds up to the biomedical investigation of selected materials.
Since there is vast literature on every individual stage of this work, only a short and very selective introduction will be given in the following paragraphs. Nevertheless, to make materials suitable for the fabrication of scaffolds for tissue repair the following general requirements can be defined,\textsuperscript{12,13} which also guide the choice of materials and methods applied in this work:

1. The material should be biocompatible and degradation should be controlled.
2. The polymer should have the right surface chemistry for cell attachment, proliferation and differentiation.
3. An interconnected and permeable pore network should be present to promote nutrient and waste exchange.
4. The mechanical properties of the scaffold should compare with those of the site of implant.
5. The architecture of the material should promote formation of native anisotropic tissue and production of the material on a proper scale should be possible.

### 1.1.1 Nerve guide tubes

The human nervous system consists of two parts; a central (CNS) and a peripheral nervous system (PNS). Damage to the CNS is often thought to be permanent without possibility of repair. Contrarily, repair of damaged peripheral nerves is possible (Figure 1.1), although recovery is slow and complicated.

Peripheral nerves are involved in multiple body functions including movement, sensory function and pain. The nerve fibers embedded in the peripheral nerve are the actual components transmitting electrical signals which are fundamental to the communication between the CNS and the rest of the body. In general, two main types of nerve fibers exist: motoric nerve fibers (mainly innervating muscles, thus enabling movements) and sensory nerve fibers (mainly innervating skin, thus important for touch and/or pain sensations).

Peripheral nerves are composites of nerve fibers and connective tissue. More specifically, peripheral nerves consist of fascicles (bundles of nerve fibers which are embedded in the ‘endoneurial tube’) within an epineurial tube (connective tissue, Figure 1.1 B). Each fascicle contains endoneurium and is surrounded by perineurium (connective tissue, Figure 1.1 B). The nerve fibers in a fascicle are the extensions or processes of nerve cells (i.e. neurons) located either in the dorsal root ganglion, just outside of the vertebral column (in case of sensory and pain neurons) or in the spinal cord (in case of motor neurons). Importantly, the nerve fibers are...
surrounded by supporting cells called ‘neuroglia’. In the PNS the neuroglia are called Schwann cells (Figure 1.1 A, C). These Schwann cells form a fatty membrane around the nerve fibers, called a ‘myelin sheath’. As a result of this myelin sheath, a fast signal transduction along the length of the nerve fiber is warranted. However, not all nerve fibers are myelinated to the same extent. Typically, pain fibers remain unmyelinated, whereas sensory fibers for touch and motor fibers have a thick myelin sheath. The thickness of the myelin sheath around nerve fibers determines the speed of electrical signal conduction. Thus, myelinated nerves conduct signals somewhat faster, which makes the difference between touch sensation and pain: touch is felt immediately, whereas pain is perceived with a certain delay. In a peripheral nerve, a fascicle contains all sorts of nerve fibers, myelinated motor or (un)myelinated sensory nerves.

Figure 1.1 Schematic overview of a myelinated nerve (A), transverse section of a nerve trunk (B and C).

Upon damage of peripheral nerves, nerve fibers are disconnected. The proximal nerve fiber, which is still connected to the nerve cell itself, remains intact, while the disconnected distal part of the nerve fiber undergoes degeneration. Then, two processes take place simultaneously. Firstly, the nerve fibers present in the proximal nerve stump start to develop so-called growth-cones. A growth cone is an enlargement of the nerve terminal, which contains many small extentions with surface receptors for a wide variety of ligands associated with growth. Secondly, Schwann cells initiate a proliferation response to nerve injury and orientate themselves along the injured nerve in so-called ‘Bands-of-Büngner’. Now, regeneration of nerve fibers requires that the nerve fibers make contact with Schwann cells. Without such contact, regenerating nerve fibers are trapped within the scar tissue which has formed at the site of injury. This nerve fiber entrapment can cause spontaneous electrical discharges, which are considered to cause neuropathic pain (i.e. pain as a result of nerve injury or dysfunction). Nerve
fiber regeneration proceeds about 5 mm/day in large-diameter nerves and 2 mm/day in finer nerve branches.\textsuperscript{14} When the endoneurium is intact regeneration is faster.

For regeneration of peripheral nerves a stress- and strainless environment around the peripheral nerve fibers is required. Physiotherapy, which is applied in conditions of nerve injury as a method to improve the condition of muscles which are denervated by the injury, puts the injured peripheral nerve under stress. Implantation of a bridge between the two peripheral nerve stumps can largely prevent stress on the nerve and allows for a good environment to stimulate regeneration of injured nerve fibers. In clinical practice, autologous nerves from the upper arm or lower leg are most frequently used as bridging materials to repair injured peripheral nerves. The most widely used nerves are the nervus suralis (lower leg, approximately 40 cm in length), the nervus saphenus (lower leg, approximately 40 cm), the nervus sensoris (upper arm, 25 cm) or parts of the nervus cutaneus (upper arm, 10 – 27 cm). This clinical intervention implies that the patient needs two surgeries and that an extra wound is created (at the site of the donor nerve). An additional problem is that the diameter of donor nerves often does not match the one of the nerve which needs to be repaired. Therefore, donor nerves are cut and bundled to give the bridge the appropriate diameter. This also implies that the amount of donor tissue is highly limited and often insufficient for the repair demand.

A second option for peripheral nerve repair is the use of synthetic biocompatible polymers, e.g. aliphatic polyesters. The advantage of using synthetic materials is that these materials can be custom-designed in view of its requirements relating to the application. Properties like permeability, (bio)degradation and interaction with cells can be tuned. Polylactic acid (PLA) is often used in this respect, but the acidity relating to its metabolites limits its clinical use. Therefore we investigated whether polyesters made from macrolactones are suitable materials for this application. The presence of fewer ester bonds within the main chain of such polyesters (per unit of length) in comparison with for example polylactide, makes the degradation products less acidic. Moreover, these polymers are expected to have sufficient strength.

Vital requirements of nerve guide tube (NGT) in regard of peripheral nerve regeneration include the presence of [1] a tubular permeable structure having sufficient strength and flexibility to withstand the stresses the nerve is exposed to, [2] a filling of an inner matrix material that consists of an interconnected porous network and contains nutrients and other growth factors or even Schwann cells. The interconnected porous network enables nerve fibers to grow through the bridge. Both the tubular structure and the inner matrix of a NGT can be
made of the same material if this material fulfils all demands. Property requirements are degradability, biocompatibility, flexibility, strength (up to 7 MPa) and permeability.

1.2 Ring opening polymerization of lactones

Upon the synthesis of polyesters two polymerization techniques can be used: Ring opening polymerization (ROP) of lactones (Scheme 1.1 A) or condensation polymerization of hydroxyl-acids (or their derivatives, Scheme 1.1 B) or of dicarboxylic acids or the corresponding anhydrides and diols (Scheme 1.1 C). Commonly condensation polymerizations are acid- or metal- catalyzed and for some polyesters they are conducted on large industrial scale. One disadvantage of using this polymerization technique is that a condensation product is formed which has to be removed for obtaining high molecular weight material and makes molecular weight control difficult.

ROP reactions of lactones go back to 1903 when the first observations were made on the polymerization of δ-valerolactone.16 This first ROP started a century of research into ROP. Since the first lactones were polymerized, a whole library of monomers has been explored for ring opening polymerization, for example lactams, cyclic ethers and anhydrides.17 These monomers give rise to a whole range of polymers that can be designed according to specific demands. Nowadays ROP is possibly one of the most important reactions in the field of polymer chemistry. ROP is a type of chain-growth polymerization and therefore one of the three basic
mechanisms in polymer chemistry next to step-growth polymerization and coordination polymerization. Here ROP is applied to form polyesters from lactones. The reason for this choice is that ROP can be controlled to a high degree, what makes it ideal to tailor polymer properties.\(^{18}\) In ROP chemical catalysts can be used, however also enzymes are widely applied.

### 1.2.1 Chemical ring opening polymerization

Polyester synthesis using ROP is not straightforward. Many factors are influencing the course of the reaction, including ring size, substituents on the ring, type of initiator, catalyst, solvent, concentration, temperature and possible transesterification reactions (back biting, end-to-end biting, etc).\(^{19}\) Effective initiators are organometallic compounds like oxides, carboxylates or alkoxides\(^{20}\) and since recently organic catalysts (only for lactides).\(^{21,22}\) These initiators determine the mechanism of the reaction, which can be of the anionic, carbocationic or coordination-insertion type. In the synthesis of high molecular weight polyesters only anionic and coordination-insertion have been successful. The activated monomer mechanism is observed upon polymerization of lactones using rare-earth metal triflates.\(^{23}\) Scandium triflate is used to ring open lactones with molecular weights over 25 kg/mol. In anionic polymerizations, alkali metals or alkali metal oxides are effective initiators. Depending on the initiator and the monomer, the polymerization can be living.\(^{24}\) For coordination-insertion pathways various aluminum and tin alkoxides are widely used. One drawback of all used catalysts is the toxicity of the metals used. For the synthesis of polyesters for biomedical applications, the use of toxic chemical catalysts should thus be avoided. Other routes towards polymers were explored and the use of enzymes as catalysts is suggested as a non-toxic alternative.\(^ {25,26}\) Therefore, in this work synthesizing polyesters for biomedical applications is performed with enzymes as a catalyst.

### 1.2.2 Enzymatic ring opening polymerization

Since the discovery of lipase-catalyzed ring opening polymerizations of lactones in 1993 by two groups independently, i.e. Uyama, Kobayashi and coworkers\(^ {27}\) and the group of Knani,\(^ {28}\) this is by far the most studied enzymatic polymerization. Reaction parameters investigated are for example the enzyme origin,\(^ {29-33}\) concentration,\(^ {34}\) temperature,\(^ {35,36}\) organic solvent\(^ {29,34,37,38}\) and water content.\(^ {39,40}\) Noteworthy is that the reaction kinetics and the
achievable molecular weights of the polyesters obtained by enzymatic ROP both appeared to increase with the lactone ring-size. In metal-catalyzed ROP this is reversed due to the absence of ring strain in larger lactones, which makes these lactones start behaving like open chain esters. The *transoid* conformation results in a lower energy level of the ground state. This opens opportunities for the polymerization of macrolactones, which can be derived from natural sources. A prominent example is \( \omega \)-pentadecalactone (PDL), which belongs to the class of naturally occurring macrocyclic musks and is used in the fragrance industry. Its eco-friendliness led to an increased demand and the development of improved synthetic routes, which makes PDL commercially available in larger quantities. Poly(\( \omega \)-pentadecalactone) (PPDL) with significantly high molecular weight can only be obtained by lipase-catalyzed ROP. Molecular weights \((M_n)\) of up to 150 kg/mol and even 480 kg/mol were reported employing Novozym 435 and up to 200 kg/mol in miniemulsion with *Pseudomonas cepacia* lipase.

### 1.2.3 Ring opening polymerization of macrolactones

Larger lactones, ring size > 12, are usually polymerized using enzymes as catalysts. Examples exist of chemically-catalyzed polymerization, however, no high molecular weight products are obtained. The macrolactone studied most extensively is PDL. Its polyethylene-like properties make it an interesting material. Also many copolymers based on different sized lactones were synthesized via enzymatic ring opening polymerization (eROP). One example is a combination of a substituted smaller lactone and a larger lactone, for example \( \beta \)-butyrolactone and \( \omega \)-pentadecalactone. Due to the enantioselectivity of the enzyme, optically active polymers are obtained from a racemic starting mixture.

Many other copolymer systems based on a macrolactone and a smaller lactone can be found in literature. Copolymers of lactones synthesized via eROP usually result in random copolymers due to transesterification reactions. Enzymatic copolymerization of \( \varepsilon \)-caprolactone (CL) and PDL results in highly crystalline material (60 – 70 %). Due to the resemblance in crystal structure of the two homopolymers and due to the negligible effect the small ester groups have on the crystallization process, the random copolymers cocrystallize over the whole composition range. Upon copolymerization of \( \rho \)-dioxanone with PDL highly crystalline random copolymers are obtained. However, unlike in the copolymers of PDL and CL, here no cocrystallization is observed, the crystal lattice of either monomer host units of the other monomer.
End-functionalization of PPDL was shown by Martinelle et al.\textsuperscript{55,56} Two different routes were explored. The eROP of PDL was initiated by 6-mercapto-1-hexanol (Scheme 1.2). Addition of either \(\gamma\)-thiobutyrolactone or vinyl acrylate in a second stage of the polymerization resulted in the thiol-thiol and the thiol-acrylate, respectively. High conversions were obtained: For the thiol-thiol 97 m/m-% of the chains were thiol-initiated and 92 m/m-% were end-capped with thiol. In the synthesis of the thiol-acrylate 85 m/m-% of the chains contained a free thiol and over 95 m/m-% were acrylate end-capped.

\[ \text{Scheme 1.2 End-functionalization of PPDL initiated by 6-mercapto-1-hexanol. In the left route PPDL is reacted with } \gamma\text{-thiobutyrolactone resulting in a dithiol end-capped PPDL. The right route gives a thiol on one end of the chain and an acrylate function on the other side.} \]

Thiol-ene chemistry was applied to form network structures of the thiol-thiol end-functionalized PPDL. Upon addition of an ‘ene monomer’, (tri- or tetra-functional), a photoinitiator and UV irradiation, semicrystalline networks are formed.

Unsaturated block copolymers were obtained using a monohydroxyl-terminated polybutadiene.\textsuperscript{57} Different butadiene block lengths (2.6, 10 and 19 kg/mol) were used to initiate the eROP of PDL. After polymerization oligomers and homopolymers were separated by fractionation methods. This method provides a robust method for the synthesis of polybutadiene-polyester diblock copolymers.

\(\alpha\)-Methylenemacrolides were shown to undergo eROP utilizing \textit{Candida antarctica} Lipase (Scheme 1.3, left route).\textsuperscript{58} Polyster s with methacrylic methylene groups were obtained. These functionalities can be radically polymerized with 2,2’-azobisisobutyronitrile (AIBN) in a second step to result in a cross-linked polymer gel. In a second route, using an anionic or radical initiator a completely different polymer was obtained (Scheme 1.3, right route). This polymer
contains the lactone as a side-group. In a second step this lactone can be opened and a polyester can be grafted from the main chain.

![Scheme 1.3](image)

**Scheme 1.3** Two polymerization routes starting from an \(\alpha\)-methyleneacrolide. Ring opening polymerization results in a polyester and via vinyl polymerization a polymer with lactone side-groups is obtained.\(^{58}\)

### 1.3 Degradable polyesters

Degradation rates of polymers depend on multiple factors. The chemical structure of the polymer, its polarity and its degree of crystallinity have the largest influence. It was shown that degradation of polymers is decreased when the polymer has a higher molecular weight (> 50 kg/mol).\(^ {59}\) Other factors influencing the degradability of polyesters are the monomers used, comonomer composition, molecular geometry, orientation of crystals, hydrophilicity, surface area and the use of additives.\(^ {60}\) Among the degradable materials, aliphatic polyesters are widely used. The formed hydroxyl carboxylic acids are known to be metabolized.\(^ {61-63}\)

Degradation of polyesters takes place via hydrolysis of the ester functionalities, either via surface or bulk erosion. The hydrolysis rate is also influenced by pH, the presence of anions or cations and enzymes.\(^ {64}\) Hydrolytic degradation is believed to be the most dominant mechanism in synthetic aliphatic polyesters, however enzymatic activity promotes degradation. Enzymatic degradation is more pronounced in naturally occurring polysaccharides and poly(hydroxyl alkanoate)s.\(^ {53,65}\)

Hydrolysis is an autocatalytic process, since the formed carboxylic acids participate in the transition state. Water enters the amorphous domains in the polymer, which enhances hydrolysis. However, crystalline domains can also be affected.\(^ {66}\) In aqueous media degradation takes place in two stages. First water will diffuse into the amorphous regions. Afterwards, when the amorphous phase has almost been degraded, degradation proceeds from the edge to the center of the crystals.\(^ {67,68}\) The large amount of different factors that
Degradation behavior of different polyesters has been studied intensively. Polyglycolide was one of the first synthetic polymers used as biodegradable suture material.\textsuperscript{69} Completely degradable fibers with appropriate strength can be spun. Various copolymers were synthesized using glycolide (Figure 1.2 a).\textsuperscript{70} A second widely used monomer is lactide. This monomer contains two chiral centers and therefore there are three different stereoforms of this monomer. Polymers obtained from pure L,L-lactide or D,D-lactide (Figure 1.2 b, c) are semicrystalline. Starting from meso-lactide (Figure 1.2 d) or using a racemic mixture of L,L-lactide and D,D-lactide results in amorphous polymers.\textsuperscript{71} The racemic polymer is more prone to hydrolysis than poly(L-lactide) or poly(D-lactide), which can be explained by the difference in crystallinity. One already mentioned drawback of polyglycolide and polylactide is the relatively high acidity of their degradation products. Glycolic and lactic acid are small hydroxyl-acids which can cause inflammation due to the strong local drop in pH.

Poly(\(\varepsilon\)-caprolactone) is another degradable polyester. However, compared to polylactide and polyglycolide, this polymer has a long hydrolysis time.\textsuperscript{72} This can be altered by synthesizing copolymers with for example 1,5-dioxepan-2-one DXO (Figure 1.2 f).\textsuperscript{73-75} The homopolymer of DXO is shown to be degraded by microorganisms.\textsuperscript{76} Cross-linking decreases the enzymatic degradation rate.\textsuperscript{77} Unidirectional orientation slows down degradation of PCL films.\textsuperscript{78}
Another slowly degrading polymer is the naturally occurring poly(3-hydroxybutyrate) (PHB). It is produced by bacteria as an intracellular reserve of carbon energy. The biosynthesis of PHB is mostly performed using the bacterium *Alcaligenes eutrophus*.\textsuperscript{79-81} PHB is completely amorphous within the intact cells, but upon extraction the polymer crystallizes. The high crystallinity is the main reason for its slow degradation rate, in spite of its relatively high polarity. Copolymerizing with 3-hydroxyvalerate enhances degradation, however, the crystallinity of these copolymers is not significantly lower than that of the homopolymers.\textsuperscript{82,83} There is no unambiguous explanation for this phenomenon.

### 1.4 Outline of this thesis

The goal of the work described in this thesis was the synthesis, the characterization and the investigation of degradable polymers based on macrolactones for nerve guide applications. The requirements to achieve this goal, i.e. understanding of the enzymatic macrolactone polymerization, tuning of biodegradability, formation of porous materials and testing biocompatibility, dictated the approach of the work and is reflected in the thesis outline.

In the second chapter enzymatic ROP was applied to synthesize high molecular weight PPDL. The synthetic procedure was optimized on small scale and subsequently transferred to 30 g scale to yield sufficient high molecular weight material for compression molding and fiber spinning. Mechanical and thermal properties of the PPDL were determined and compared with literature data. The high molecular weight PPDL was melt-processed into fibers, which were further elongated to about 9 – 10 times their original length. Analysis of the fibers revealed differences in crystal orientation as a function of the processing conditions. The maximum obtained tensile strength was encouraging.

A series of polymers derived from macrolactones, namely \( \omega \)-pentadecalactone, 16-hexadecalactone and their unsaturated analogues ambrettolide and globalalide were investigated as potential biomaterials in the third chapter. By enzymatic ROP these monomers can conveniently be polymerized to high molecular weight polyesters. Properties like thermal behavior, biocompatibility and degradation were investigated for all highly crystalline materials. Different routes towards cross-linking of the main chain double bond in the unsaturated lactones were explored, in order to investigate its impact on crystallinity and degradability.

The fourth chapter discusses the possibilities to increase the biodegradation of the polymers made from unsaturated macrolactones by incorporating comonomers that are more
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hydrophilic or contain side-groups to distort the crystallinity of the materials. The obtained copolymers were tested on their biodegradability and ability to construct cross-linked materials with sufficient strength and stiffness for scaffold applications (in particular nerve guide tubes).

As a novel alternative to enzymatic ROP, an aluminum salen catalyst is synthesized and explored in the ROP of different sized lactones in the fifth chapter. The kinetics is investigated and compared to eROP.

The sixth chapter describes different techniques to obtain porous materials. Sugar and salt leaching are explored as well as lyophilization techniques. Not only isotropic materials are obtained, also anisotropic products can be made. The pore size and the porosity can be altered by tuning the concentration and size of the leaching agent.

To be able to get a view of the economical feasibility of the production of these materials a technology assessment is presented in chapter seven. Different aspects of the upscaling of this synthesis are addressed. The results of this work are critically reviewed and put in perspective.

1.5 References and notes

15. This figure was published in: Clinical Neuroanatomy and Neuroscience, M. J. Turlough Fitzgerald, G. Gruener, E. Mtui, 5th Ed. Copyright Saunders Elsevier 2007, p 113.
Chapter 1

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HIGH MOLECULAR WEIGHT POLY(ω-PENTADECALACTONE) FOR FIBER APPLICATIONS
HIGH MOLECULAR WEIGHT POLY(ω-PENTADECALACTONE) FOR FIBER APPLICATIONS

Chapter 2

2.1 Introduction

Under legislative, environmental and economic pressure the implementation of eco-friendly production methods and raw materials have received academic and industrial attention. However, novel materials face the challenge that they have to compete with current materials on performance as well as price. In particular polymers from bio-derived resources often have inferior physical properties when compared to synthetic performance materials, which limit their applicability in more demanding applications. Aliphatic polyesters, of which poly(hydroxyalkanoate) and poly(lactic acid) are prominent examples, form a particular class of renewable polymers. Increasing attention was recently also given to polymers derived from long chain \( \omega \)-hydroxy fatty acids.\(^1\) Primarily materials with various substituents along the polymer chain, obtained by polycondensation, have been reported. We are particularly interested in the corresponding unsubstituted polymers obtained by ring opening polymerization (ROP) of cyclic \( \omega \)-hydroxy fatty acids like \( \omega \)-pentadecalactone (PDL). PDL belongs to the class of naturally occurring macrocyclic musks and is used in the fragrance industry. Its eco-friendliness led to an increased demand and the development of improved synthetic routes, which makes PDL commercially available in larger quantities.\(^2\)

Poly(\( \omega \)-pentadecalactone) (PPDL) is a semi-crystalline polymer with a melting point around 95 °C and a glass transition temperature far below room temperature (-27 °C).\(^3\) The crystallization behavior and the crystal structure of PPDL reveal large similarities with polyethylene (PE).\(^6\) End-functionalized low molecular weight PPDL was recently applied for coating applications.\(^9\) While often referred to as a degradable PE, we have recently shown that PPDL is neither enzymatically nor hydrolytically degradable in buffer solutions resembling physiological conditions.\(^10\) Nevertheless, under harsher hydrolytic conditions as often found in the environment (e.g. soil) and in recycling processes, PPDL can be expected to degrade. Availability of PPDL of high molecular weight could open high performance application areas typically occupied by PE for example in tape and fiber products. The goal of our research is to develop a synthetic route, which gives access to larger quantities of high molecular weight PPDL (\( M_w > 100 \) kg/mol) and its application in fibers.

High molecular weight polyesters can only be realized by ring opening polymerization (ROP) of the corresponding macrocyclic lactones (Figure 2.1). Several groups have reported on the polymerization of PDL, via chemical as well as via biocatalytic routes.\(^11\) - \(^16\) It was observed that PDL and other macrolides behave differently from smaller lactones (e.g. \( \delta \)-valerolactone and \( \delta \)
High molecular weight poly(ω-pentadecalactone) for fiber applications

caprolactone) with respect to their polymerization behavior. Lebedev reported the chemical ROP using diethyl zinc as a catalyst but no details about the synthesis or the polymer product were given. Duda et al. used zinc octoate/butyl alcohol for the ROP and reported low activity of the catalyst towards PDL, resulting in low monomer conversion after long reaction times at elevated temperatures (26% after 7 days at 100 °C). In addition, the molecular weight of the obtained PPDL was low (~1.4 kg/mol). Feijen et al., who reported high monomer conversion and reasonable molecular weights ($M_n = 30$ kg/mol) by using yttrium isopropoxide as a catalyst. Up to date, the best results for a chemical ROP of PDL were obtained by Kowalczuk et al. via anionic polymerization using potassium alkoxides ($M_n$ up to 100 kg/mol). Significantly higher molecular weight PPDL was reported by enzymatic ROP using lipases. Employing Novozym 435 (Candida antarctica Lipase B immobilized on a macroporous resin), number average molecular weights ($M_n$) of up to 86 kg/mol (polydispersity index (PDI) = 2.37) were reported by Gross after precipitation in methanol. Even higher molecular weights were reported by the enzymatic polymerization of PDL in miniemulsion with Pseudomonas cepacia lipase, giving PPDL with $M_n$ of 200 kg/mol. The highest molecular weight reported to the best of our knowledge is an $M_w$ of 480 kg/mol by Gross et al. However, these reported molecular weights have to be interpreted with great care as they were measured by size exclusion chromatography (SEC) using chloroform as eluent. Our own analysis showed irreproducible molecular weights and often broad molecular weight distributions under the conditions reported by Taden et al. A closer look at the system revealed a large pressure built up during SEC-analysis of PPDL-samples above a molecular weight of ca. 10 kg/mol. Although apparently transparent polymer solutions were obtained in chloroform, this indicates that the polymer did not dissolve properly in chloroform at 25 °C even after a prolonged dilution time. This is not surprising since not only the solid state but also the solution properties are similar to PE. Only Palmans et al. reported on the poor solubility of PPDL in chloroform, after which they decided to use elevated temperature (80 °C) and ω-dichlorobenzene as an eluent for SEC analysis.

![Figure 2.1](image)

**Figure 2.1** Enzymatic synthesis of poly(ω-pentadecalactone) by ring opening polymerization of ω-pentadecalactone.
This prompted us to carefully re-examine the route to and characterization of high molecular weight PPDL on small scale (1 – 2 g of product) with the goal to push the molecular weight of PPDL as high as possible. The obtained information was then applied to produce high molecular weight PPDL on a larger scale (30 g of product), which allowed for the melt-spinning and investigation of PPDL fibers.

A prerequisite for mechanically strong fibers is a maximum of lateral interactions between the polymer chains in the fiber. In the case of PE this is achieved by the orientation of the chains in crystallites. The crystallinity and thus the mechanical strength can be further increased by a post-spinning drawing process, which aligns the polymer chains and crystallites. Similar to PE, the long linear aliphatic carbon chain of PPDL is responsible for its high crystallinity. It is thus reasonable to expect good fiber properties from this material similar to those of PE. Important material parameters in that respect are the molecular weight of the polymer, which should be as high as possible for maximum lateral chain interaction and for limiting the number of chain ends. Moreover, short plasticizing polymer chains should be absent as they have a negative influence on the desired mechanical properties of the bulk material.

2.2 Synthesis of high molecular weight poly(ω-pentadecalactone)

The approach that can be followed to obtain high molecular weight polymers in enzymatic ROP is similar to the approach that is followed in chain growth polymerization by using a very high monomer to initiator ratio. It has to be noted, though, that enzymatic ROP is not a controlled polymerization and inter-chain and intra-chain transesterification reactions occur throughout the polymerization process and become dominant at high monomer conversion. The reason is that the applied enzymes (usually lipases) are transesterification catalysts. In this approach, water, which is intrinsically present in the reaction medium, acts as a very efficient nucleophile (initiator and transfer agent). We followed an earlier reported optimized drying protocol to minimize the water concentration in the solvent, monomer and the enzyme but leaving traces of water for the enzyme to retain its activity.26,36 This drying protocol was applied to all experiments to ensure the critical water concentration to be similar within experimental errors.

Initially the enzymatic ROP of PDL to high molecular weight was performed on a small scale (1 - 2 g of product) at 70 °C in order to gain information on polymerization characteristics and product analysis. Immediately noticeable was the high viscosity of the polymerization mixture at
longer reaction times due to the high molecular weight and crystallinity of PPDL. The impact this has on up-scaling will be discussed later but even on small scale this causes difficulties as representative sampling from the reaction mixture proved impossible. Therefore, parallel reactions were performed and stopped at various times so as to obtain insight into the rate of polymerization and the development of molecular weight during the reaction. Special care was taken to ensure that all reactions were performed under the same conditions, since one has to be aware that small variations in reaction composition (like water concentration) can have an effect on the obtained results. Figure 2.2 depicts the monomer conversion of a typical polymerization reaction series.

Noticeable is that the reaction is relatively slow in comparison to literature reports, reaching only about 40 % conversion within the first hour. This suggests a low initial concentration of nucleophiles and a less active enzyme conformation due to the absence of water in the reaction medium due to the successful drying process of the reaction components. After a reaction time of about 1440 min (24 h) approximately complete monomer conversion was reached.

The molecular weights of all reaction samples were initially determined by SEC in chloroform as suggested in the literature. However, due to the large pressure build-up and the
irreproducible results in chloroform, all SEC analyses in this study were performed in 1,2,4-
trichlorobenzene (TCB) at 160 °C, i.e., under common conditions for polyolefins. Inspection of
Figure 2.3 reveals an almost linear increase of the molecular weight ($M_w$ is reported here as this
is common in fiber applications), reaching a maximum of about 305 kg/mol (polystyrene
standards) at 95 % conversion. When the reactions were continued beyond that point a slight
drop in the molecular weight was observed. We speculate that this is due to enzyme catalyzed
chain scission, which is becoming more frequent at strongly reduced monomer concentration.
Due to the high viscosity of the reaction medium and the inefficient agitation at this stage
water could locally result in enzyme-catalyzed ester hydrolysis and cause significant chain
degradation. This is also supported by an increase of the PDI to about 3 at high monomer
conversion, while the PDI of all other samples is between 2 and 2.5. The PPDL molecular
weights obtained in this study are among the highest reported so far. However quantitative
comparison with literature data is not possible since in previous reports SEC analysis was
performed in chloroform. For the up-scaling reaction it can be concluded that the
polymerization should be stopped at a maximum of 95 % monomer conversion in order to
produce polymer with a maximum molecular weight.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.3.png}
\caption{Weight average molecular weight ($M_w$) and polydispersity index (PDI) of poly($\omega$-pentadecalactone)
as a function of monomer conversion for the small-scale reaction (1 – 2 g). Data were obtained from SEC in
1,2,4-trichlorobenzene at 160 °C (polystyrene standards). Dashed lines are added to guide the eye.}
\end{figure}

In order to obtain sufficient polymer for fiber spinning, the enzymatic polymerization was
then conducted on larger scale. To obtain a high molecular weight polymer with a relatively
narrow molecular weight distribution (PDI ~ 2.0), mass transport limitations, both in the enzyme particle (internal) as well as in an outer stagnant film (external), should be avoided. Two approaches can be followed to achieve this: [1] decreasing the particle size and [2] ensure good mixing. Intra-particle mass transport limitation depends strongly on the size of the catalyst particle, as the molar flux of a compound in a catalyst particle is inversely correlated with the size of the particle. The average particle size of Novozym 435 is ~ 400 µm (varying from 100 – 1000 µm), with the enzyme located in the outer shell of the slightly cross-linked PMMA-divinyl benzene particles (~ 80 µm). Therefore, it is not possible to reduce the (effective) particle size without grinding the particles. In this study, this has not been performed, as it was believed that this reduces the enzyme activity.

A Teflon overhead anchor stirrer was used to ensure good agitation and mixing throughout the entire reaction even at high conversion (high viscosity). Moreover, since the obtained PPDL was difficult to dissolve, a relatively large reactor volume (250 – 300 mL) was used for the reaction. This allowed for the addition of extra solvent after the reaction, so that the polymer and enzyme could subsequently be separated by filtration. In addition, a double-walled glass reactor vessel connected to a thermostatic oil bath was chosen for heating. An enzyme to monomer ratio of 1:20 (w/w-%) and an equal monomer to solvent (toluene) weight ratio were used. The total reaction volume was around 70 mL. In all polymerizations nearly complete monomer conversion was obtained after ~ 70 hours of reaction as determined by 1H-NMR spectroscopy. Table 2.1 gives two typical examples of results obtained from the large scale ROP of PDL (PPDL-2 – PPDL-4). Samples PPDL-3 and PPDL-4 were obtained under identical conditions, what confirms the reproducibility of the synthesis.

<table>
<thead>
<tr>
<th>Entry</th>
<th>$T_r$ [°C]</th>
<th>$t_r$ [h]</th>
<th>$M_n$ [g/mol]</th>
<th>$M_w$ [g/mol]</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPDL-1</td>
<td>70</td>
<td>24</td>
<td>143 000</td>
<td>305 000</td>
<td>2.1</td>
</tr>
<tr>
<td>PPDL-2</td>
<td>80</td>
<td>70</td>
<td>83 000</td>
<td>136 000</td>
<td>1.9</td>
</tr>
<tr>
<td>PPDL-3</td>
<td>85</td>
<td>70</td>
<td>80 000</td>
<td>143 000</td>
<td>2.0</td>
</tr>
<tr>
<td>PPDL-4</td>
<td>85</td>
<td>70</td>
<td>77 000</td>
<td>143 000</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*All SEC data were obtained in 1,2,4-trichlorobenzene at 160 °C (polystyrene standards). bSmall scale reaction, 10 w/w-% Novozym. cMolecular sieves added to reaction; crystallization of polymer at the stirrer during polymerization, 10 w/w-% Novozym. dMolecular sieves added to reaction; no crystallization of polymer during polymerization, 1 w/w-% Novozym.
Initially all large-scale polymerizations were conducted at 70 °C similar to the small-scale reaction. However, polymer crystallization was observed on the stirrer shaft and only low molecular weight polymers were isolated. Therefore, the reaction temperature was increased to 80 °C and molecular sieves were added to the reaction mixture in order to further reduce the participation of water in the reaction (PPDL-2, Table 2.1). While crystallization was still observed at this temperature, a sharp increase in molecular weight was noted ($M_w = 136$ kg/mol). For PPDL-3, only 1 w/w-% of Novozym 435 was used with respect to monomer in addition to the molecular sieves. Moreover, the reaction temperature was further increased to 85 °C and thus crystallization was avoided. The reaction yielded white polymer with a weight average molecular weight of 143 kg/mol after precipitation. The SEC traces of all polymers were monomodal (Figure 2.4). Noticeable is that the highest molecular weight obtained in the large-scale reactions is only half of the molecular weight of the small-scale reaction (PPDL-1, Table 2.1). Although the same drying protocol was followed as in the small-scale reaction, the larger reaction volume and higher temperature present more challenges for efficient drying. Therefore it is reasonable to assume that the lower molecular weight as compared to the small-scale reactions is thus due to a higher water concentration. By further optimization and specialized equipment it should be possible to further increase the PPDL molecular weight even on large scale.

![SEC traces of polymer obtained from small-scale reaction at 98 % monomer conversion (dashed line PPDL-1; $M_w = 305$ kg/mol) and up-scaling reaction (solid line; $M_w = 143$ kg/mol; PPDL-3, Table 2.1). Samples measured in TCB at 160 °C.](image)
Chapter 2

Initially all large-scale polymerizations were conducted at 70 °C similar to the small-scale reaction. However, polymer crystallization was observed on the stirrer shaft and only low molecular weight polymers were isolated. Therefore, the reaction temperature was increased to 80 °C and molecular sieves were added to the reaction mixture in order to further reduce the participation of water in the reaction (PPDL-2, Table 2.1). While crystallization was still observed at this temperature, a sharp increase in molecular weight was noted ($M_w = 136 \text{ kg/mol}$). For PPDL-3, only 1 w/w-% of Novozym 435 was used with respect to monomer in addition to the molecular sieves. Moreover, the reaction temperature was further increased to 85 °C and thus crystallization was avoided. The reaction yielded white polymer with a weight average molecular weight of 143 kg/mol after precipitation. The SEC traces of all polymers were monomodal (Figure 2.4). Noticeable is that the highest molecular weight obtained in the large-scale reactions is only half of the molecular weight of the small-scale reaction (PPDL-1, Table 2.1). Although the same drying protocol was followed as in the small-scale reaction, the larger reaction volume and higher temperature present more challenges for efficient drying. Therefore it is reasonable to assume that the lower molecular weight as compared to the small-scale reactions is thus due to a higher water concentration. By further optimization and specialized equipment it should be possible to further increase the PPDL molecular weight even on large scale.

**2.3 Properties of high molecular weight poly($\omega$-pentadecalactone)**

The differential scanning calorimetry (DSC) $c_p(T)$ based melting point (95 °C) and the degree of crystallinity of 67 % (Table 2.4 and Figure 2.7) of the high molecular weight PPDL-4 (Table 2.2) are in agreement with reported literature values. From dynamic mechanical analysis (DMA) a glass transition temperature ($T_g$) of -25 °C was determined. Compression molded films for tensile testing were prepared using a press by heating the material to 130 °C for 20 minutes at a pressure of ~ 150 bar. The films were rapidly quenched to room temperature with water after which dumbbell shaped objects were perforated (approx. $16 \times 4.9 \times 1.2 \text{ mm}$).

<table>
<thead>
<tr>
<th>Sample</th>
<th>$M_w$ [kg/mol]</th>
<th>PDI [-]</th>
<th>$E$ [MPa]</th>
<th>$\sigma_{\text{break}}$ [MPa]</th>
<th>$\sigma_{\text{yield}}$ [MPa]</th>
<th>$\varepsilon_{\text{yield}}$ [%]</th>
<th>$\varepsilon_{\text{break}}$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Literature$^a$</td>
<td>129$^a$</td>
<td>2.0$^a$</td>
<td>370</td>
<td>-</td>
<td>14.5</td>
<td>12</td>
<td>100-200</td>
</tr>
<tr>
<td>This study</td>
<td>143$^b$</td>
<td>2.0$^b$</td>
<td>420</td>
<td>38</td>
<td>17.5</td>
<td>15</td>
<td>$&gt;$1200</td>
</tr>
</tbody>
</table>

$^a$Determined using SEC in chloroform at 25 °C. $^b$Determined using HT-SEC in 1,2,4-trichlorobenzene at 160 °C; both samples were calibrated on polystyrene standards.

![Stress-strain behavior of dumbbells obtained from a compression molded film from PPDL-4 (Table 2.1).](Image)

---

*High molecular weight poly($\omega$-pentadecalactone) for fiber applications*
Mechanical properties of PPDL have hardly been investigated. To the best of our knowledge, only Scandola et al. have reported very briefly on these properties. In Table 2.2, the tensile properties are collected as they were observed in both the study of Scandola et al. and in the present study. The tensile modulus ($E$) is around 420 MPa for the compression molded films, which is in the same order as reported in literature. The stress at break ($\sigma_{\text{break}}$), which had not been reported before, was observed to be as high as 38 MPa. Typically, the strain at break of the PPDL synthesized in this study, being $\varepsilon_{\text{break}} > 1200\%$ (Figure 2.5), is much higher than the previously reported value ($\varepsilon_{\text{break}} = 100 - 200\%$). This difference could be an effect of the higher molecular weight of our samples, as low molecular weight fractions act as plasticizer and have a deleterious effect on the elongation at break. However, it is difficult to draw a final conclusion since different methods were used to determine the molecular weight. On the other hand, we also observed that already small defects (air bubbles, etc.) in the PPDL-film could cause a decrease of the elongation at break.

### 2.4 Poly(âpentadecalactone) fibers

PPDL fibers were first produced by melt-spinning through a nozzle but this method resulted in very inhomogeneous fibers. Better results were obtained by using Ram-extrusion following the principle of a melt indexer in which a polymer is molten in a thermostated chamber (slightly) above the melting temperature. Using a certain load on top of the chamber, the molten polymer is pushed through a nozzle (1.05 mm) and collected on a godet roll after quenching in a water bath (Figure 2.6).
The thickness of the PPDL fiber was varied by changing the mass of the weight cell (extrusion speed), temperature (110 and 120 °C), and the winding speed (1 – 10 m/min) (Table 2.3). The PPDL fibers were further elongated over a hot plate to about 9 – 10 times their original length.

<table>
<thead>
<tr>
<th>Fiber code</th>
<th>PPDL&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Processing conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1-1</td>
<td>3</td>
<td>Melt pushed trough nozzle, stretched over hotplate</td>
</tr>
<tr>
<td>F2-1</td>
<td>4</td>
<td>Ram-extrusion: ( T = 110 \ \degree \mathrm{C} ); ( W = 15 \ \mathrm{kg} ); ( WS = 0 )</td>
</tr>
<tr>
<td>F2-2</td>
<td>4</td>
<td>Ram-extrusion: ( T = 110 \ \degree \mathrm{C} ); ( W = 15 \ \mathrm{kg} ); ( WS = 1 \ \mathrm{m/min} ).</td>
</tr>
<tr>
<td>F2-3</td>
<td>4</td>
<td>Ram-extrusion: ( T = 120 \ \degree \mathrm{C} ); ( W = 17 \ \mathrm{kg} ); ( WS = 1 \ \mathrm{m/min} ).</td>
</tr>
<tr>
<td>F2-4</td>
<td>4</td>
<td>Ram-extrusion: ( T = 120 \ \degree \mathrm{C} ); ( W = 17 \ \mathrm{kg} ); ( WS = 10 \ \mathrm{m/min} ).</td>
</tr>
</tbody>
</table>

<sup>a</sup>Numbers refer to entries in Table 2.1.

The thermal behavior and crystallinity of the powders as synthesized and the resulting fibers were obtained via specific heat capacity, \( c_p(T) \), measurements using differential scanning calorimetry. Typically, 5 mg of sample was loaded in a standard aluminum solid state pan, except for sample F2-4 where only 1 mg was used. This particular fiber sample was very thin and could not easily be compacted into a DSC pan. As can be expected, the lower sample mass resulted in somewhat sharper melting and crystallization transitions. From the measured heat flow the corresponding heat flow of an empty pan was subtracted and after dividing by the sample mass and scanning rate, \( c_p(T) \) data were obtained \((\mathrm{Jg}^{-1}\mathrm{K}^{-1})\), which in turn were used to calculate the sample weight fraction crystallinity, \( w_c(T) \), as a function of temperature according to:

\[
w_c(T) = \frac{\Delta h(T)}{\Delta h_{ref}(T)}
\]

with \( \Delta h_{ref}(T) \), the temperature dependent reference melting enthalpy for PPDL as recommended by the ATHAS database:\(^{31}\) \( \Delta h_{ref}(T) = 224.99329 + T*0.48866 - T^2*0.00104 \), with \( T \) in °C and:

\[
\Delta h(T) = \int_{T}^{110^\circ\mathrm{C}} \left[ c_p(T) - c_{pa}(T) \right]dT
\]
Chapter 2

with \( c_{pa}(T) \) the heat capacity for fully amorphous PPDL obtained via a linear regression and extrapolation of the high temperature melt \( c_p(T) \) data between 110 and 160 °C.\(^{32}\)

Figure 2.7 illustrates the evolution of \( c_p(T) \) and \( \omega_c(T) \) during a heat – cool – heat run at 10 °C/min for sample PPDL-4, representing an original powder after synthesis, and F2-3 and F1-1, representing typical fiber materials.

![Graphs A, B, C showing the evolution of \( c_p(T) \) and \( \omega_c(T) \).](image)

**Figure 2.7** Evolution of \( c_p(T) \) and \( \omega_c(T) \) during a heat – cool – heat run at 10 °C/min for three representative samples, A: PPDL-4, B: F2-3, C: F1-1.
**High molecular weight poly(ω-pentadecalactone) for fiber applications**

Table 2.4 gives an overview of all thermal and structural properties of the obtained fibers and the synthesized powders. The crystallinity values at room temperature prior to first ($w_{c1}(25\ °C)$) and second heating ($w_{c2}(25\ °C)$) are listed for all samples investigated together with the crystallization ($T_c$) and melting peak temperatures during first ($T_{m1}$) and second heating ($T_{m2}$). Clearly, the crystallinity of the powders as synthesized is higher than that of the melt-processed fibers. The crystallinity of all fibers is comparable (0.54 on average) and does not alter after a heat – cool cycle. The melting points, too, do not seem to depend on the fiber processing protocol and do not shift after a heat – cool cycle.

**Table 2.4** Overview of thermal and structural properties of the poly(ω-pentadecalactone) fibers (for processing conditions and sample codes see Table 2.3), including those of the starting powders entry PPDL-4 and PPDL-3 (Table 2.1): $T_{m1}$: Melting temperature during first heating run; $T_{m2}$: Melting temperature during second heating run; $w_{c1}$: crystallinity values prior to first heating; $w_{c2}$: crystallinity values prior to second heating; $f$: crystal orientation function.

<table>
<thead>
<tr>
<th>Entry</th>
<th>$T_{m1}$</th>
<th>$T_c$</th>
<th>$T_{m2}$</th>
<th>$w_{c1}(25\ °C)$</th>
<th>$w_{c2}(25\ °C)$</th>
<th>$f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPDL-4</td>
<td>95</td>
<td>79</td>
<td>96</td>
<td>0.68</td>
<td>0.51</td>
<td>0</td>
</tr>
<tr>
<td>F2-1</td>
<td>96</td>
<td>79</td>
<td>95</td>
<td>0.56</td>
<td>0.54</td>
<td>0.12</td>
</tr>
<tr>
<td>F2-2</td>
<td>95</td>
<td>80</td>
<td>95</td>
<td>0.52</td>
<td>0.54</td>
<td>0.39</td>
</tr>
<tr>
<td>F2-3</td>
<td>94</td>
<td>80</td>
<td>94</td>
<td>0.53</td>
<td>0.54</td>
<td>0.26</td>
</tr>
<tr>
<td>F2-4</td>
<td>94</td>
<td>80</td>
<td>93</td>
<td>0.54</td>
<td>0.55</td>
<td>0.85</td>
</tr>
<tr>
<td>PPDL-3</td>
<td>96</td>
<td>80</td>
<td>96</td>
<td>0.62</td>
<td>0.53</td>
<td>0</td>
</tr>
<tr>
<td>F1-1</td>
<td>95</td>
<td>81</td>
<td>95</td>
<td>0.53</td>
<td>0.52</td>
<td>0.48</td>
</tr>
</tbody>
</table>

**2.5 Poly(ω-pentadecalactone) crystal molecular orientation**

Single fibers were selected and mounted perpendicular to an X-ray beam (CuK$_\alpha$ radiation, 500 μm across) for the collection of 2D X-ray fiber diffraction patterns at room temperature in view of retrieving information on the polymer chain orientation within the fiber crystals as a function of the elongation. According to the literature, the orientation of a crystallographic plane can be extracted from a radial scan of the appropriate diffraction peak using equation 3.33

$$f_{hkl} = \frac{1}{2} (3 < \cos^2 \phi_{hkl} > - 1)$$

(3)
with:
\[
< \cos^2 \phi_{hkl} > = \frac{\int_0^\pi I(\phi) \sin \phi \cos^2 \phi \, d\phi}{\int_0^\pi I(\phi) \sin \phi \, d\phi}
\]
(4)

where \(f_{hkl}\) is the Hermans orientation value of the normal to the \(hkl\) plane and \(I(\phi)\) is the intensity measured at an angle \(\phi\) in an azimuthal scan. This gives the orientation of a certain crystallographic plane, which is not necessarily the same as the orientation of the chain axis. It was shown by Wilchinsky, however, that in the case of fiber symmetry, this \(f\) value can be found via:

\[
< \cos^2 \phi_c > = \frac{1 - g^2 - 2 < \cos^2 \phi_{hkl} >}{1 - 3g^2}
\]
(5)

with \(\phi_c\) being the angle between the crystallographic \(c\)-axis and the stretch direction, and \(g\) being the cosine of the angle between the normal to the \(hkl\) plane and the crystallographic \(c\)-axis. Gazzano et al. reported a pseudo orthorhombic crystal structure for PPDL, which results in \(g = 0\) when considering an \(hk0\) plane. In this case the Hermans orientation value \(f\) for the molecular orientation within the crystals is given by:

\[
f = \frac{1}{2}(3 < \cos^2 \phi_c > - 1) = 1 - 3 < \cos^2 \phi_{hk0} >
\]
(6)

Equation 6 allowed us to obtain the orientation value for PPDL simply by making an azimuthal scan through one single \(hk0\) reflection. The strongest equatorial reflection observed in our diffraction experiments is the \(110\) reflection. After an appropriate correction for the detector dark current and the distortion associated with the use of a flat detector, azimuthal scans were made through the \(110\) reflection.

The scattering of amorphous PPDL at the position of the \(110\) reflection was subtracted by linearly interpolating the amorphous contribution found at 1.4°2\(\theta\) above and below the \(110\) scattering angle (2\(\theta_{110}\)) at all \(\phi\) values. At 2\(\theta_{110} + 1.4\) and 2\(\theta_{110} - 1.4\), only amorphous material contributes to the scattering pattern. The scattering angle at 2\(\theta_{110} + 1.4\) lays in between the
PPDL 110 and 200 reflection as illustrated in Figure 2.8, where for the isotropic PPDL powder the scattered intensity is azimuthally averaged (with the beam stop and its arm being masked) using the software ConeX1 developed by Gommes and Goderis. The corrected azimuthal 110 intensities were fitted to the sum of a constant value and a Gaussian. The fitted data were used as input for equation 6. Processing of the data was performed with homemade scripts running under V for Windows (version 3.5b, Digital Optics Ltd.).

Figure 2.8 Corrected 2D scattering pattern of PPDL powder using a logarithmic gray scale for the intensity (# detector counts) (left) and corresponding azimuthally averaged 1D scattering pattern with the Miller indices at the most important reflections (right).

Figure 2.9 Corrected 2D scattering pattern of three representative fiber samples using the same logarithmic gray scale as in Figure 2.8. The fiber axis lays approximately parallel to the beam stop axis.
In Figure 2.9, the 2D scattering patterns of three representative fiber samples are illustrated. The anisotropy in the scattering pattern is most obvious in the pattern of sample F2-4, where the 110 arcs are in equatorial position with respect to the fiber axis. The other features in this particular scattering pattern are rather weak because the fiber was extremely thin. The arcs of the (small angle) 001 reflection are better visible in the other scattering patterns and are positioned meridionally, as expected.

Figure 2.10 displays the fits through the corrected 110 reflections as a function of the azimuthal angle \( \phi \), which is defined zero in the direction of the fiber axis. The integral of the displayed intensity is normalized as to have an enclosed area in the \( \phi \) range between 0 and 180° equal to 1 for easy comparison. The corresponding values for the crystal orientation function \( f \) (equation 5) are listed in Table 2.4. A zero value is obtained when the crystals are oriented randomly with respect to the fiber direction and a value of 1 corresponds to a perfect alignment with respect to the fiber axis. The highest degree of orientation was reached for the sample that experienced the highest winding speed (Table 2.3). Although the crystals and hence the molecules in the crystals are partially oriented with respect to the fiber axis, it seems that the majority of the amorphous material in between the crystallites is randomly oriented. Azimuthal scans through \( 2\theta \) values where only amorphous material contributes to the scattering pattern (see experimental section) do not reveal any anisotropy.

![Figure 2.10](image-url)  
**Figure 2.10** Fits through the azimuthal intensity distribution of the 110 reflections of the different investigated samples (for sample codes see Table 2.3).
2.6 Poly(ω-pentadecalactone) fiber mechanical properties

Unfortunately it was not possible to monitor the tensile properties for the entire range of elongated fibers reported in Table 2.4. Since some fibers proved to be too thin to be measured, preliminary tensile test were conducted on selected PPDL fibers, i.e. F1-1, F2-2 and F2-1. As the crystallinity and the melting points of all fibers are identical, only the degree of crystal (and thus molecular) orientation can explain the differences in mechanical behavior. Figure 2.11 clearly shows that there is a significant increase of the fiber strength with the degree of crystal orientation. A maximum value of the fiber strength of 0.74 GPa is reached for F1-1 with an orientation factor of 0.48. Furthermore, the strength of the fibers is significantly higher than that of the isotropic films (Table 2.2), which confirms that the elongation and orientation of the PPDL crystals and thus the molecules in the fiber during and after the spinning process is a crucial factor. On the other hand, a decrease of the elongation at break with increasing degree of orientation is evident due to the fact that a maximum orientation in these fibers has been reached during processing, not or hardly allowing for any further elongation in a tensile experiment.

![Graph showing fiber tensile strength and elongation at break as a function of degree of crystal orientation](image-url)

**Figure 2.11** Fiber tensile strength and elongation at break of three poly(ω-pentadecalactone) fibers as a function of their degree of crystal orientation. Dashed lines are added to guide the eye.


Chapter 2

2.7 Conclusions

PPDL with the highest molecular weight reported up to date was synthesized. Optimization of the SEC analysis revealed that reliable molecular weight data could only be obtained by high temperature SEC in 1,2,4-trichlorobenzene similar to polyethylene. By up-scaling of the polymerization larger amounts of PPDL were produced. Despite preliminary optimization of the reaction conditions the molecular weights obtained in the up-scaling process were lower than those obtained on smaller scale. This was probably due to the higher water amounts introduced in the reaction mixture. Further optimization and the use of specialized equipment are expected to circumvent these issues.

Mechanical and thermal properties of the non-oriented, high molecular weight PPDL were determined and are largely in agreement with literature data. The high molecular weight PPDL was melt-processed into fibers, which were further elongated. Analysis of the fibers revealed differences in crystal orientation as a function of the processing conditions. Preliminary fiber tensile measurements confirm a high strength of up to 0.74 GPa for the fiber with the highest crystal orientation.

2.8 Experimentals

2.8.1 Materials

All chemicals were purchased from Aldrich, stored over molecular sieves and used without further purification unless otherwise noted. Toluene (Biosolve, AR-grade) was dried over alumina and stored over molecular sieves. Novozym 435 was obtained from Novozymes A/S and stored over phosphorous pentoxide in a desiccator. Molecular sieves (3 Å) were dried in an oven at 420 °C prior to use. Para-oxon was dissolved in toluene before use as enzyme-inhibitor to instantly stop the polymerization reaction.

2.8.2 Methods

Size exclusion chromatography (SEC) was performed on a Polymer Laboratories PLXT-20 Rapid GPC Polymer Analysis System (including pump, refractive index detector and viscosity
High molecular weight poly(ω-pentadecalactone) for fiber applications

detector) at 160 °C with 3 PLgel Olexis (300 × 7.5 mm, Polymer Laboratories) columns in series. 1,2,4-Trichlorobenzene was used as eluent at a flow rate of 1.0 mL/min. The molecular weights were calculated against polystyrene standards (Polymer Laboratories, $M_p = 580$ up to $M_p = 7.1 \times 10^6$ g/mol). A Polymer Laboratories PL XT-220 robotic sample handling system was used as autosampler.

DSC thermograms were collected by a Perkin-Elmer Pyris-1 Differential Scanning Calorimeter with the block surrounding the measuring unit thermostated at -10 °C and the measuring unit being flushed with dry nitrogen. Calibration was done at 10 °C/min with benzophenone and Indium for the temperature and with Indium for the enthalpy. The thermal profile was composed of a heating-cooling-heating sequence between 20 and 170 °C at 10 °C/min with 10 min waiting each time at 20 or 170 °C.

A SMART 6000 diffractometer was used to measure X-ray scattering patterns, equipped with 2D CCD detector. The sample-to-detector distance was 45 mm and an irradiation time of 15 min/image was used. Processing of the data was performed with homemade scripts running under V for Windows (version 3.5b, Digital Optics Ltd.).

Dynamic mechanical analysis (DMA) was performed on a TA DMA Q800 V5.1 with dual cantilever clamp. Data was acquired using TA Universal Analysis. Compression molded bars of approx. $14.4 \times 5.4 \times 1.2$ mm size were prepared and clamped in. A temperature profile from -100 °C to 95 °C with a heating rate of 1 °C/min and a frequency sweep of 1 Hz was applied. TA Universal Analysis v4.1D software was used for data acquisition.

Tensile tests were performed on a Zwick tensile meter with a 10 kN load cell with a tensile speed of 10 mm/min. Data acquisition was performed with TestXpert V8.1 software.

RAM extrusion was performed on a Tinius Olsen MP993 Extrusion Plastometer (Melt Indexer) and a godet-role melt flow indexer with a 1.05 mm dye. Elongation of these fibers was applied at 90 °C over a hot plate. After reaching the maximum of 6 – 8 times the original length, the elongation was continued at 103 °C to about 9 – 10 times the original length.

Determination of filament mechanical properties were carried out on a semi-automatic, microprocessor controlled tensile tester (the Favimat from Textechno Herbert Stein GmbH & Co. KG, Mönchengladbach, Germany), which works according to the principle of constant rate of extension (ISO 5079). The Favimat tester was equipped with a 1200 cN balance. Gauge length was 50 mm and rate of extension was 25 mm/min. Filament linear density (i.e. mass/length) was determined by weighing of the filaments on a microbalance. Tensile strengths in cN/dtex were converted to GPa assuming a density of PPDL of 1 g/cm³.
2.8.3 Synthesis

**Small scale enzymatic synthesis of high molecular weight PPDL (1-2 g scale)**

In a typical enzymatic polymerization, Novozym 435 (10 w/w-% to monomer, 0.25 g) was dried in a 10 mL flask with molecular sieves (3 Å) and a magnetic stirring bar under vacuum at 50 °C overnight. After drying, the flask was removed from the oven under nitrogen atmosphere and closed with a septum. The flask was then heated to 70 °C in an oil bath and a stock solution of $\alpha$-pentadecalactone (2.5 g, 10.41 mmol) in toluene (5.02 g, 54.49 mmol) was added to the enzyme through the septum. The reaction was terminated after 24 hours by the addition of para-oxon. After dilution with hot xylene the immobilized enzyme and molecular sieves were filtered off and the polymer was recovered after solvent evaporation. Yield: 1.9 g (79 %).

**Up-scaled enzymatic synthesis of high molecular weight PPDL (30 g)**

For this reaction a larger setup was used, consisting of a double-wall glass vessel (300 mL). First, Novozym 435 (1 w/w-% to monomer 0.30 g) was dried in a glass vial at 50 °C under vacuum for 16 hours according to a literature procedure. The reaction vessel was dried in an oven at 150 °C together with molecular sieves. The vessel was removed from the oven and after adding the enzyme, it was equipped with a metal overhead stirrer and closed under argon atmosphere. Then the vessel was placed in an oil bath at 85 °C and a stock solution of PDL (33.69 g) in toluene (29.60 g) were added using a preheated glass syringe and needle (stirrer-speed: 30 – 50 rpm). After 72 hours, a highly viscous slurry of enzyme and PPDL in toluene was obtained and the polymerization was stopped by adding a mixture of para-oxon and toluene (35.5 mg in 15 g of toluene) in double excess to the actual amount of enzyme as inhibitor, followed by 150 mL of preheated $p$-xylene (100 °C) to dissolve the polymer. The slurry was stirred for 1 hour and subsequently the enzyme was carefully filtered off over a Büchner-funnel using an additional amount of preheated $p$-xylene. $p$-Xylene was partially removed by evaporation (until ~ 400 mL was left) and then methanol (200 mL) was added at 60 °C to neutralize the para-oxon. By further reducing the temperature to 20 °C the polymer precipitated and was filtered over a Buchner-funnel. Finally, the polymer was dried under vacuum overnight at 50 °C. Yield: 28.65 g (85 %).
2.9 References

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EROP OF MACROLACTONES RESULTING IN HOMOPOLYMERS

This chapter has been published: I. van der Meulen, M. de Geus, H. Antheunis, R. Deumens, E. A. J. Joosten, C. E. Koning, and A. Heise, Biomacromolecules, 2008, 9, 3404–3410.
3.1 Introduction

Polymers claim an ever increasing segment of the market of biomedical materials.\textsuperscript{1,2} The diversity in polymer synthesis and properties allows for the tailor-design of materials for a wide range of biomedical applications. In order to qualify for these applications high entry barriers in terms of product safety and properties have to be passed. Biocompatibility can be regarded as the primary requirement that all materials have to meet for applications in the human body. A further division of material can be made on biodegradability, i.e., into biodegradable and non-biodegradable materials. While certain applications require the ability of the polymer to degrade \textit{in vivo} at a certain rate, for example in drug delivery devices or scaffolds, other materials need to be non-degradable such as in permanent implants (e.g. hip-joints). Accordingly, the applied materials differ significantly in their chemical structure and properties. For example, for non-degradable implants mainly ultra high molecular weight polyethylene (UHMWPE), is applied as it combines biological inertness with mechanical strength.\textsuperscript{3} The latter is a result of the high crystallinity and chain length of UHMWPE. Since the lifetime of implants in the body is relatively high, improvement of wear resistance becomes one of the main issues associated with these materials.\textsuperscript{4} Post-processing cross-linking by radiation\textsuperscript{5} and the incorporation of metallic nanoparticles\textsuperscript{6} have shown some success to reduce wear.

Typical degradable materials are aliphatic polyesters such as poly($\varepsilon$-caprolactone), polylactides, polyglycolides and copolymers thereof.\textsuperscript{7,8} While the mechanism of the degradation can vary depending on the environment, it is the labile ester bond that gives rise to the polymer degradation. Various aspects of biodegradable polymers ranging from synthesis to applications have been studied and reviewed extensively.\textsuperscript{9-11}

An interesting class of materials that seems to combine the advantages of both worlds is derived from macrocyclic lactones (macrolactones). For example, poly($\omega$-pentadecalactone) (PPDL) is an aliphatic polyester resembling the mechanical properties of low density PE (LDPE).\textsuperscript{12} It owes its PE-like properties to the high crystallinity of the methylene units, giving it a melting point ($T_m$) around 95 °C and a glass transition temperature ($T_g$) of -27 °C (LDPE: $T_m$ = 97 – 117 °C; $T_g$ = -25 °C)\textsuperscript{13}. Several authors suggested potential biodegradation due to the presence of ester bonds in the polymer main chain, although this has never been investigated to the best of our knowlegde.\textsuperscript{14-16}

Synthetic polymers from macrolactones such as PPDL, are readily accessible by enzymatic ring opening polymerization (ROP).\textsuperscript{17-19} Comparative studies have shown that the enzymatic
ROP of \(\omega\)-pentadecalactone (PDL, 16-membered lactone) catalyzed by *Candida antarctica* Lipase B (CALB immobilized on macroporous resin: Novozym 435) proceeds much faster and to higher molecular weights than with chemical catalysts.\(^{15}\) Furthermore, the enzymatic reaction proceeds under mild conditions and yields materials free of any metal contamination, which can be considered a great advantage for biomedical applications in view of the toxicity of many metal containing catalysts.\(^{20}\)

![Figure 3.1](image) Polymers obtained by enzymatic ring opening polymerization of macrolactones. Globalide (G1) is a mixture of two constitutional isomers with the double bond at the 11 or 12 position (indicated by the dashed line).

The interesting structural features and properties of polymers derived from macrolactones make it worthwhile to further investigate their potential as biomaterials. The goal of this research is to position these polymers among other biomaterials in order to identify possible applications. Initially we investigated the biocompatibility and biodegradation of PPDL, as the synthesis and the physical properties of this polymer have already been reported.\(^{18}\) Furthermore, the introduction of functionality is aimed for, which allows for the manipulation of the polymer properties in the solid state by post-modification, e.g. cross-linking. For that purpose the macrolactones globalide (G1) and ambrettolide (Am) are selected. Both monomers are used in the fragrance industry for their musky odor and their quality to loose scent slowly.\(^{21-23}\) Globalide (11/12-pentadecen-15-olide) is an unsaturated 16-membered lactone that contains one unsaturated double bond.\(^{24}\) It is a mixture of two different constitutional isomers with the double bond at the 11 or 12 position (Figure 3.1). Ambrettolide (oxacycloheptadec-10-
en-2-one), on the other hand, is a well-defined unsaturated 17-membered lactone. Poly(16-hexadecalactone) (PHDL), the saturated 17-membered lactone, completes the library of these macrolactones.

### 3.2 Polymer synthesis and characterization

![Graph showing monomer conversion over time for enzymatic ring opening polymerization of macrolactones.](image)

**Figure 3.2** Comparison of the monomer conversion over time for enzymatic ring opening polymerization of macrolactones using water as an initiator, $T = 60 \, ^\circ\text{C}$, 2 M in toluene, 5 w/w-% Novozym: • globalide, ▲ ambrettolide, ♦ 16-hexadecalactone, ■ $\omega$-pentadecalactone.

With the exception of PDL and HDL, the enzymatic polymerization of the proposed macrolactones has not yet been investigated. In order to compare the reactivity of the unsaturated lactones with that of $\omega$-pentadecalactone in the enzymatic ROP, a kinetic study was performed. All polymerizations were carried out at 60 °C at a concentration of 2 M in toluene using the water present in the reaction mixture as the initiator. Since the concentration of water has a very large influence on the kinetics of the polymerization all reactants were dried. While the water concentration is difficult to determine accurately, Karl-Fischer coulometry confirmed a concentration < 0.06 mg/g at the start of every reaction. Figure 3.2 shows that the reaction rates of PDL, HDL and Am are comparable under these conditions. The polymerization of Gl seems somewhat faster but this is probably due to variation in the water concentration and thus within the error of the experiment. In all polymerizations an almost quantitative monomer conversion was reached after three hours. These experiments suggest that the polymerization behavior of the investigated macrolactones is comparable. In an earlier report by Palmans et al. the enzymatic ROP of macrolactones was described by Michaelis-
Menten kinetics.\textsuperscript{25} It is reasonable to assume that Michaelis-Menten based kinetics also apply to the macrolactones Gl and Am, respectively.

For the molecular and physical characterization, preparative amounts of homopolymers were synthesized by enzymatic ROP of the respective monomers in solution. All polymers were obtained as white powders after precipitation in methanol and characterized by NMR, DSC, SEC and WAXS (Table 3.1). Significant differences in solubility were observed between the unsaturated polymers (PGl and PAm) and, PPDL and PHDL, respectively. While the latter are hardly soluble in any organic solvent, PAm and PGl dissolve readily. This has consequences for the molecular weight analysis by standard SEC in solvents like THF or CHCl\textsubscript{3}. Previously a large pressure build-up was experienced and irreproducible results were obtained when SEC of PPDL was conducted in CHCl\textsubscript{3}. Polymers containing PPDL and PHDL therefore have to be measured in 1,2,4-trichlorobenzene (TCB) at 145 °C as it is common for polyolefins, while the good solubility of PAm and PGl allows measurements under standard conditions in THF. This should be kept in mind when comparing molecular weights given in Table 3.1. PAm and PGl SEC-samples were measured under the same conditions as PPDL and PHDL (160 °C in TCB), only very low molecular weights were obtained and the samples colored brown. Therefore it is assumed that under these conditions, PAm and PGl samples degrade. Generally it can be concluded that the molecular weights of all polymers are high enough so as to exclude molecular weight effects on thermal properties.

| Table 3.1 Properties of polyesters obtained via enzymatic ROP of macrolactones. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Polyester       | \( M_n \)      | PDI             | \( T_{m1} \)    | \( T_{m2} \)    | \( T_c \)      | \( \Delta H_1 \) | \( \Delta H_2 \) | Crystallinity   |
| Gl              | 24 000\textsuperscript{a} | 1.9             | 48.7            | 46.2            | 29.8            | 76.7            | 77.4            | 60              |
| PAm             | 24 200\textsuperscript{a} | 1.9             | 58.8            | 54.9            | 37.7            | 113.2           | 88.4            | 62              |
| PPDL            | 45 800\textsuperscript{b} | 2.0             | 97.6            | 95.9            | 78.2            | 158.8\textsuperscript{f} | 125.6           | 68              |
| PHDL            | 18 500\textsuperscript{b} | 1.9             | 96.2            | 92.4            | 76.6            | 156.2           | 145.5           | 74              |

\textsuperscript{a}Determined from the first DSC heating run. \textsuperscript{b}Determined from the second DSC heating run. \textsuperscript{c}Determined by WAXS and DSC. \textsuperscript{d}Determined by SEC in THF at 40 °C (polystyrene standards). \textsuperscript{e}Determined by SEC in TCB at 145 °C (polystyrene standards). \textsuperscript{f}\( \Delta H_m^0 = 233 \text{ J/g} \)\textsuperscript{26}

Thermal analysis of all polymers was conducted by DSC. In agreement with the literature, a melting point (\( T_m \)) of 95.9 °C was obtained for PPDL.\textsuperscript{23} The melting point of PHDL (92.4 °C) is quite similar to that of PPDL due to the structural similarity of the polymers. The influence of the double bonds in the polymer main chain on the melting point can clearly be seen from the
DSC thermograms shown in Figure 3.3. PAm has a melting point of 54.9 °C, which is 38 °C lower than the melting point of its saturated analogue PHDL. Similarly, the melting point of PPDL drops by 50 °C to 46.2 °C for its unsaturated analogue PGl. The larger difference in comparison with the Pam/PHDL couple can be explained by the fact that the Gl is a mixture of different constitutional isomers, thereby inducing even more irregularities in the polymer chain and crystals of the polymer.

![DSC melting curves](image)

**Figure 3.3** DSC melting curves (second heating run) of polymers from macrolactones.

For the same reason one can also expect that the crystallinity of both PGl and PAm is lowered by the presence of the double bond, which causes more difficult packing of the chains into a crystal. To verify these assumptions, powder-WAXS was performed on all polymers. The measurements confirmed that all polymers are semi-crystalline. The degree of crystallinity in the material was estimated from the ratio of the intensity of the peaks corresponding to the crystalline phase and the total intensity of all peaks. PHDL displayed the highest crystallinity of 74 %, followed by PPDL (68 %). The unsaturated polyesters PAm (62 %) and PGl (60 %) exhibited the lowest crystallinity (Table 3.1).

The WAXS diffractogram and the crystal structure of PPDL were already elucidated in the literature. The WAXS diffractogram of the PPDL obtained in this study is in full agreement with the published data, showing strong reflections at 2θ = 21.4° and 23.8°. As can be seen from Figure 3.4, the crystal structures of the other investigated polymers qualitatively show reflections in the same regions, which suggest a similar crystal structure. For comparison, the positions of the peak maxima of the orthorhombic structure of PE are indicated in Figure 3.4 (dashed lines). This structure is clearly also the dominating crystal structure of all polymers.
Interestingly, in particular PAm reveals a second reflection pattern, which suggests the coexistence of a second crystal structure. While the peak maxima are close to those of monoclinic PE (also indicated in Figure 3.4), a conclusive structural assignment of the crystal structure would require an in depth study, which is outside the scope of this investigation.

![Figure 3.4 WAXS diffractograms of the polymers from macrolactones.](image)

### 3.3 Biocompatibility

The biocompatibility of all polymers was tested using a standard cytotoxicity assay (i.e. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay). In brief, the metabolic activity of a 3T3 (3-day transfer, inoculum $3 \times 10^5$ cells) mouse fibroblast cell line was tested under several conditions. First, 3T3 cells were grown for 96 h in the presence of standard cell culture medium (DMEM/NUT (Dulbecco’s minimal essential medium/nutrients) mix F12 with glutamax-1 supplemented with 10 % inactivated fetal calf serum and antibiotics). This serves as a positive control as cells readily grow under these conditions. Second, 3T3 cells were grown for 96 h in the presence of standard cell culture medium which had been conditioned by latex (0.1 g/mL) for 48 h, which serves as a negative control as this latex is highly toxic to cells. Hence, the metabolic activity in the presence of latex will be strongly diminished. Third, 3T3 cells were grown for 96 h in the presence of cell culture medium which had been conditioned by either PPDL, PAm or PGl (undiluted 0.1 g/mL, 1:1 diluted, and 1:3 diluted) for 48 h.
Figure 3.5 Cytotoxicity test of PPDL (left: 10K cells; right: 5K cells) at three concentrations. **, p < 0.01 (as compared to medium); *, p < 0.05 (as compared to medium); O.D., optical density blue formazan crystals produced in the cell metabolism and measured spectroscopically in duplo at $\lambda = 570$ nm.

Figure 3.6 Cytotoxicity of PGI and PAm (5K cells) at three concentrations **, p < 0.01 (as compared to medium); *, p < 0.05 (as compared to medium); O.D., optical density blue formazan crystals produced in the cell metabolism and measured spectroscopically in duplo at $\lambda = 570$ nm.

The data collected in Figure 3.5 indicate that the latex extracts are highly toxic to the cells for both plating densities as reflected by strongly decreased metabolic cell activity. On the other hand, PPDL is not toxic to the cells for the high plating density (i.e. 10K cells per well) and there is negligible toxicity for the low plating density (i.e. 5K cells per well). The cytotoxicity of PAm and PGI was only measured with 5K cells per well (Figure 3.6). Again, the latex extracts revealed high toxicity to the cells, while statistically PAm and PGI are not toxic to the cells. These experiments confirm for the first time that there is no detectable toxicity of the PPDL, PAm or PGI to 3T3 cells as measured by the MTT assay. Of note, the 3T3 cell line is an often used cell line in these assays for its sensitivity to toxic substances.
3.4 Hydrolytic and enzymatic degradation

Property analysis confirmed the similarity of the polymers synthesized from macrolactones with HDPE. A significant structural difference is the presence of ester bonds in the polymer main chain, which gave rise to the suggestion that these materials might be biodegradable.\textsuperscript{14-16} However, this has never been experimentally confirmed, which prompted us to conduct a systematic long term study of the degradation behavior of these polymers. For the degradation experiments PPDL was extruded into a 1 mm thick strand which was cut into pieces of 10 cm. Hydrolytic degradation of these strands was studied by placing individual samples in 10 mL PBS (Phosphate Buffered Saline).

![Graph 1: Hydrolytic degradation of poly(\(\omega\)-pentadecalactone) strand (diameter 1 mm, length 10 cm) (\(M_n = 12.4\) kg/mol, determined by \(^1\)H-NMR) in PBS at 37 °C. Cumulative mass loss and molecular weight, \(M_n\), as a function of degradation time.](image1)

**Figure 3.7** Hydrolytic degradation of poly(\(\omega\)-pentadecalactone) strand (diameter 1 mm, length 10 cm) (\(M_n = 12.4\) kg/mol, determined by \(^1\)H-NMR) in PBS at 37 °C. Cumulative mass loss and molecular weight, \(M_n\), as a function of degradation time.

![Graph 2: Enzymatic degradation of polymers from macrolactones in PBS at 37 °C in the presence of Pseudomonas cepacia lipase. Cumulative mass loss as a function of degradation time.](image2)

**Figure 3.8** Enzymatic degradation of polymers from macrolactones in PBS at 37 °C in the presence of Pseudomonas cepacia lipase. Cumulative mass loss as a function of degradation time. • Polambrettolide film (\(M_n = 6.7\) kg/mol, determined by SEC), ■ Polyglobalide film (\(M_n = 8.3\) kg/mol, determined by SEC), ▲ Poly(\(\omega\)-pentadecalactone) strand (\(M_n = 12.2\) kg/mol, determined by \(^1\)H-NMR).
Over a period of two years neither mass loss nor a reduction of the molecular weight of the polymer was detected (Figure 3.7 shows the data for 375 days). Moreover, the crystallinity of the samples did not change over the time period of the degradation experiments.

Additionally, the enzymatic degradation of PPDL strands by Lipase PS from *Pseudomonas cepacia* in PBS was followed, showing no detectable mass loss over 100 days (Figure 3.8). Similar experiments were conducted with 1 mm thick solution-cast films of PGI and PAm. Over a period of hundred days the mass loss, crystallinity and molecular weight were monitored. With the exception of an initial mass loss, probably due the removal of low molecular weight materials, no significant detectable changes in either of the parameters were detected. It is reasonable to make a comparison to the semi-crystalline PCL for which biodegradation occurs in stages. The main degradation takes place in amorphous regions of polymer followed by a very slow degradation of the crystalline regimes. While for the semi-crystalline polymers investigated in this study a similar process was expected, the results show that not even the amorphous regions of the polymer degrade under the applied conditions. This suggests that the main reason for the hydrolytic stability of the polymers is their hydrophobicity, which prevents any water from penetration into the materials to initiate degradation. It has to be noted that the mechanical properties of the polymer samples were not monitored during the degradation experiments. A change of mechanical properties due to degradation is often observed before detection of any polymer weight loss. However, based on the long time the degradation experiments were conducted and the absence of any changes of both molecular weight and weight loss, it seems reasonable to assume that mechanical properties remain unchanged as well.

### 3.5 Cross-linking

Cross-linking of polymers is often applied to improve the thermal and mechanical properties of the materials, for example wear and creep resistance of PE. The presence of the main chain double bond in PAm and PGI suggests that both polymers can be cross-linked in the liquid or solid state. Usually aliphatic main chain double bonds are difficult to cross-link due to their unreactive character. However, two pathways were found to cure these main chain double bonds.

Dicumyl peroxide (DCP) is known to be a very reactive thermal curing agent and has been used to cross-link the main chain double bond in polyricinoleate. In order to investigate
whether cross-linking can be achieved with the unsaturated polyesters and how cross-linking would affect the polymer properties, DCP was mixed and ground with PAm powder. This mixture was put into a mold and was processed at 170 °C at 100 bar for 30 minutes. First evidence for the successful conversion of the double bonds was obtained from the FTIR spectra as evident from the decrease in the absorption signal at 1650 cm\(^{-1}\), corresponding to the C=C stretching vibration, see Figure 3.9.

Interestingly, the obtained material was almost completely transparent while the starting non-cured polymer was white and non-transparent due to the crystalline structure (Figure 3.10). This indicates the formation of a completely amorphous material resulting from the interference of the cross-links with the formation of the crystallites. This was further confirmed by the complete disappearance of the crystallization and melting peak in the DSC thermogram (Figure 3.11).

![Figure 3.9](image_url) **Figure 3.9** IR-spectrum of polyambrettolide (solid line) and cross-linked polyambrettolide (dashed line). Double bond conversion due to cross-linking is evident from the decrease of the C=C stretching band at 1650 cm\(^{-1}\).

![Figure 3.10](image_url) **Figure 3.10** PAm plate before curing (top) and after curing (bottom) with dicumyl peroxide.
Moreover, the material proved to be completely insoluble upon cross-linking. To calculate the gel-fraction of the cured material, the cross-linked polymer was washed with methanol for at least three hours to wash out the non-decomposed DCP and its decomposition by-products. After drying the cross-linked polymer was swollen in toluene to dissolve the uncross-linked fraction. The gel fraction of the cross-linked polyambrettolide was 97 %, as determined from the weight percentage of the undissolved fraction.

In a second cross-linking route, thiol-ene chemistry was used. The polymer was molten and mixed with a dithiol (ethylene glycol bis(3-mercaptopropionate)). It is known that the reaction between a thiol and an unsaturated carbon-carbon bond is very fast. Upon addition of a UV-initiator (4-hydroxybenzophenone) and when exposed to UV light cross-linking takes place. No elevated temperatures are needed for curing and no byproducts are formed. Unreacted dithiol can be washed out after curing.

The absorbance spectrum of 4-hydroxybenzophenone shows a maximum around 300 nm. However, upon increasing the concentration, other wavelengths are sufficient to obtain cross-linking. In the absence of di-thiol or UV-initiator, no cross-linking takes place. To follow the reaction an IR kinetic study was performed (Figure 3.12). The intensity of the trans C=O bond (970 cm⁻¹) was followed over time. The decrease in intensity of this band as a function of time is shown in Figure 3.12. The initial increase in signal is probably caused by drying of the system. The materials obtained are completely amorphous and transparent and are not soluble in the solvents in which the uncured polymer is soluble.
Moreover, the material proved to be completely insoluble upon cross-linking. To calculate the gel-fraction of the cured material, the cross-linked polymer was washed with methanol for at least three hours to wash out the non-decomposed DCP and its decomposition by-products. After drying the cross-linked polymer was swollen in toluene to dissolve the uncross-linked fraction. The gel fraction of the cross-linked polyamidobrettolide was 97 %, as determined from the weight percentage of the undissolved fraction.

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The materials obtained are completely amorphous and transparent and are not soluble in the solvents in which the uncured polymer is soluble.

Different ratios of polymer/dithiol were mixed together with 5 w/w-% UV-initiator and cured with UV light (Table 3.2). The curing was followed over time using IR spectroscopy. The results showed that after 15 minutes of disposal to UV light, all kinetic curves leveled off and no further decrease in intensity was observed. Depending on the ratio monomer/dithiol, the density was higher or lower. The molar ratio of polymer/dithiol of 2/1 was found to be the optimum for this constant initiator concentration. This can be seen in the results as the amount of reacted double bonds is the highest at that ratio. Without initiator, no network was formed upon UV radiation for 0.5 h.

Table 3.2 Results of thiol-ene cross-linking of polyamidobrettolide

<table>
<thead>
<tr>
<th>Polymer [μmol]</th>
<th>Dithiol [μmol]</th>
<th>Initiator [μmol]</th>
<th>Reacted double bonds [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.1</td>
<td>21.9</td>
<td>0.1</td>
<td>11.3</td>
</tr>
<tr>
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<td>41.5</td>
<td>0.1</td>
<td>19.9</td>
</tr>
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<td>30.7</td>
<td>7.6</td>
<td>0.1</td>
<td>11.5</td>
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<td>17.4</td>
<td>8.6</td>
<td>-</td>
<td>0</td>
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<td>9.9</td>
<td>0.1</td>
<td>42.3</td>
</tr>
<tr>
<td>19.8</td>
<td>-</td>
<td>0.1</td>
<td>0</td>
</tr>
</tbody>
</table>
3.6 Conclusions

Polymers from macrolactones are a class of materials with promising thermal and mechanical properties which might be suitable for applications as biomaterials, especially if metal-free polymerization catalysts are used. We systematically investigated a series of polymers derived from ω-pentadecalactone, 16-hexadecalactone and their unsaturated analogues ambrettolide and globalide. By enzymatic polymerization these monomers can conveniently be polymerized to high molecular weight aliphatic polyesters. The polymers are highly crystalline with lower melting points for the unsaturated polymers. All polymers are non-toxic as measured by an MTT assay for metabolic cell activity of a 3T3 mouse fibroblast cell line. Degradation studies show no hydrolytic or enzymatic degradability of the polymers, which we ascribe to the high crystallinity and hydrophobicity of the materials. The unsaturated polymers were successfully cross-linked in the melt yielding completely amorphous materials. The influence of the cross-linking and the resulting absence of crystalline domains, on the polymer degradation is currently under investigation. While the amorphous character of this material should result in an increasing degradation rate, the cross-linking could cause the opposite effect.

Applications for the non-cross-linked, semi-crystalline homopolymers have therefore to be identified among non-degradable biomaterials. The ability to easily cross-link materials like PGI and PAm could offer advantages over commonly used PE, and might result partially degradable materials.

3.7 Experimental

3.7.1 Materials

ω-Pentadecalactone and 16-hexadecalactone were purchased from Aldrich and used as received. Mesitylene was purchased from Aldrich and stored over 4 Å molecular sieves. Novozym 435 (Candida antarctica Lipase B immobilized on cross-linked polyacrylate beads) was purchased from Novozymes A/S and dried following a literature procedure. Globalide and ambrettolide were kind gifts of Symrise. Toluene was dried over aluminum oxide and stored over molecular sieves. All other solvents used were purchased from Biosolve and used without
Polymers from macrolactones are a class of materials with promising thermal and mechanical properties which might be suitable for applications as biomaterials, especially if metal-free polymerization catalysts are used. We systematically investigated a series of polymers derived from \( \omega \)-pentadecalactone, 16-hexadecalactone and their unsaturated analogues ambrettolide and globalide. By enzymatic polymerization these monomers can conveniently be polymerized to high molecular weight aliphatic polyesters. The polymers are highly crystalline with lower melting points for the unsaturated polymers. All polymers are non-toxic as measured by an MTT assay for metabolic cell activity of a 3T3 mouse fibroblast cell line. Degradation studies show no hydrolytic or enzymatic degrad ability of the polymers, which we ascribe to the high crystallinity and hydrophobicity of the materials. The unsaturated polymers were successfully cross-linked in the melt yielding completely amorphous materials. The influence of the cross-linking and the resulting absence of crystalline domains, on the polymer degradation is currently under investigation. While the amorphous character of this material should result in an increasing degradation rate, the cross-linking could cause the opposite effect. Applications for the non-cross-linked, semi-crystalline homopolymers have therefore to be identified among non-degradable biomaterials. The ability to easily cross-link materials like PGl and PAm could offer advantages over commonly used PE, and might result partially degradable materials.

### 3.7.1 Materials

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### 3.7.2 Methods

The water content of all reaction mixtures was measured with Karl-Fischer Coulometry using a Mettler Toledo DL32 Coulometer and Apura CombiCoulomat fritless (Merck) as electrolyte. Gas Chromatography was performed on a Hewlett Packard 5890 series II Chromatograph with autosampler using THF as a solvent. Size Exclusion Chromatography (SEC) samples containing PPDL and PHDL were prepared in 1,2,4-trichlorobenzene and measured on a Polymer Labs PLXT-20 Rapid GPC Polymer Analysis System at 145 °C with PLgel Olexis (300 × 7.5 mm, Polymer Labs) columns using 1,2,4-trichlorobenzene as an eluent at a flow rate of 1.0 mL/min. A Polymer Labs PLXT-R Robotic Sample Handling System was used as autosampler. SEC on all other samples was performed on a Waters Model 510 pump and Waters 712 WISP, using PL-gel mix D columns (300 × 7.5 mm, Polymer Labs) at 40 °C. THF was used as an eluent at a flow rate of 1.0 mL/min. All samples were diluted to 1.0 mg/mL and filtered using 0.2 μm syringe filters. Molecular weights of all polymers were calculated based on polystyrene standards. \(^1\)H and \(^13\)C-NMR spectroscopy was performed on a VARIAN Mercury 400 MHz NMR in CDCl\(_3\). Data was acquired using VNMR software. Chemical shifts are reported in ppm relative to tetramethylsilane. Differential Scanning Calorimetry (DSC) was performed on a TA Q100 DSC. Approximately 5 mg of dried polymer was weighed into aluminum hermetic pans. Temperature profiles from -50 °C up to 130 °C with a heating and cooling rate of 10 °C/min were applied. TA Universal Analysis software was used for data acquisition. The crystallinity of the material was determined using \( \Delta H_m \) from the first heating run. WAXS patterns were recorded in the reflection mode at room temperature using a Rigaku powder diffractometer with CuK\( \alpha \) radiation. The scans were obtained using a continuous mode at 2 °C/min from 10 °C to 60 °C. IR measurements were performed on a PerkinElmer SpectrumOne FT-IR Spectrometer, using a universal ATR sampling accessory. Kinetic attenuated total reflection Fourier Transform InfraRed (ATR-FTIR) spectroscopy measurements were performed on a Bio-Rad Excalibur FTS3000MX infrared spectrometer using the golden gate setup. 32 scans were recorded per spectrum with a resolution of 4 cm\(^{-1}\). Data was acquired using Varian Resolutions Pro. Curing measurements were performed under nitrogen atmosphere using a Driel Spectral Luminator.
Chapter 3

with a wavelength of 365 nm. UV curing was performed using a Philips HPR-125 mercury discharge lamp with an output of 125 W/ 1.15 A.

3.7.3 General procedure for enzymatic ring opening polymerizations

A stock solution of monomer (4 mmol) and dried toluene (2.00 g) was prepared and dried overnight at 40 °C in the presence of 4 Å molecular sieves. Novozym 435 (44 mg) was dried in the reaction flask over molsieves at 40 °C overnight in a vacuum oven. After drying, the oven was opened under nitrogen flow. The flask containing Novozym 435 was placed in an oil bath at 60 °C and the stock solution was added. The amount of water in the reaction mixture was measured at the beginning of the reaction using Karl-Fischer Coulometry. After four hours the high viscosity of the mixture prevented proper stirring. Dichloromethane was added to the reaction mixture to dissolve the product and inhibit the enzyme. After filtering off the enzyme, the filtrate was precipitated in cold methanol. The polymer was filtered off and dried at room temperature in vacuum. Typical yields after precipitation were 70 %. In the kinetic evaluations of the different homopolymers mesitylene was added (25 w/w-%) to the stock solution as an internal standard. At regular time intervals samples were taken and the conversion was determined using GC.

Poly(ω-pentadecalactone): $^1$H-NMR: δ (ppm) = 4.04 (t, CH$_2$O(C=O)), 2.28 (t, CH$_2$(C=O)O), 1.64 – 1.57, 1.28 – 1.25 (m, CH$_2$).

Poly(16-hexadecalactone): $^1$H-NMR: δ (ppm) = 4.05 (t, CH$_2$O(C=O)), 2.28 (t, CH$_2$(C=O)O), 1.62 – 1.57, 1.27 – 1.24 (m, CH$_2$).

Polyglobalide: $^1$H-NMR: δ (ppm) = 5.53 – 5.29 (m, CH=CH), 4.06 – 4.03 (m, CH$_2$O(C=O)), 2.30 – 2.26 (m, CH$_2$(C=O)O), 2.12 – 1.92, 1.71 – 1.59, 1.25 (m, CH$_2$).

Polyambrettolide: $^1$H-NMR: δ (ppm) = 5.38 – 5.36 (m, CH=CH), 4.06 – 4.03 (t, CH$_2$O(C=O)), 2.30 – 2.26 (CH$_2$(C=O)O), 1.96, 1.63 – 1.57, 1.34 – 1.29 (m, CH$_2$).

3.7.4 Biocompatibility tests

Extracts of the polymers (PPDL, PGI and PAm) were made by incubating them (0.1 g/mL) for 48 h in cell culture medium (DMEM/NUT mix F12 with glutamax-1 supplemented with 10 % inactivated fetal calf serum and 100 U/mL penicillin and 100 μg/mL streptomycin) at 37 °C/5 %
CO₂. These extracts were added to cultures of a mouse fibroblast 3T3 cell line, which had been plated 18 h before. Cell cultures were subsequently maintained for 4 days after which an MTT assay was performed to assess the metabolic activity of the cell cultures. Next to the biomaterial extracts, which were used undiluted, 1:1 diluted, and 1:3 diluted, normal cell culture medium (as a positive control for ‘normal’ metabolic activity of cells) and latex extract ((48 h incubation of 0.1 g latex per mL culture medium), which served as a negative control for strongly decreased metabolic activity in a toxic environment) were used in these cytotoxicity experiments. In addition, all tests were performed on two different plating densities of cells (10K and 5K 3T3 cells per well in a 96-wells plate).

**MTT assay**

The cell cultures were incubated with 0.5 mg/mL MTT for 90 minutes. Endogenous enzymes in the cells metabolize MTT thereby producing blue formazan crystals, which can be measured by spectrometry using a wavelength of 570 nm. The values regarding this test are calculated as the optical density of the tested cell culture minus the optical density of a well with cell culture medium but no cells (background). Every condition (3T3 cell culture with respective conditions; cell culture medium only or extracts of undiluted polymer, 1:1 diluted polymer, or 1:3 diluted polymer) was tested two-fold (in duplo). The metabolic activity of the cell cultures under the various conditions was statistically compared using a one-way analysis of variance (ANOVA) with Bonferroni post-hoc correction.

**3.7.5 Degradation of polymers in a pH buffer**

Polyglobalide and polyambrettolide were solution cast from toluene into a 1 mm thick film. 100 mg of polymer film was added to 10 mL of 0.1 M PBS (pH = 7.4) containing 1 mg Lipase PS per sample. For the degradation studies of poly(ω-pentadecalactone) extruded strands (diameter: 1 mm, length: 10 cm) were added to the PBS solution. At regular time intervals the buffer of one sample was removed and the polymer was dried overnight. The weight loss, molecular weight and crystallinity were measured.
3.8 References

eROP of macrolactones resulting in homopolymers

COPOLYMERS WITH HYDROPHILIC MONOMERS
This chapter has been submitted to Biomacromolecules: ‘Copolymers from Unsaturated Macrolactones–Towards the Design of Cross-linked Biodegradable Polyesters’ Inge van der Meulen, Yingyuan Li, Ronald Deumens, Elbert A.J. Joosten, Cor E. Koning, Andreas Heise
Polyesters are widely used in different branches of industry, from packaging to biomedical materials as they combine excellent properties with synthetic versatility.\(^1,^2\) A relatively new class of polyesters is derived from cyclic \(\omega\)-hydroxy fatty acids. Compared to the very common lactones such as \(\varepsilon\)-caprolactone, the polymerization of these so-called macrolactones to high molecular weight materials is challenging and has been discussed in Chapter 2. From the materials perspective, the interest in polyesters derived from macrolactones stems from the excellent mechanical properties of the polymers (Chapter 2) and the fact that degradation products should be harmless, as they resemble fatty acid derivatives. Unfortunately, homopolymers of the macrolactones investigated by us are non-degradable under physiological conditions due to their semi-crystalline morphology and high hydrophobicity (Chapter 3). We hypothesized that both can be overcome by the introduction of more hydrophilic comonomers. At the same time, the expected transition from a semi-crystalline to an amorphous material by copolymerization requires a post-synthesis cross-linking step in order to be able to process the polymers into stable shapes for biomedical applications. Cross-linked polyesters are usually made with \(\alpha,\omega\)-functionalized macromonomers of the polyester used.\(^3^\text{--}^5\) For example this can be done by using acrylates as end groups which can be polymerized via radical polymerization.\(^6\) The formed network contains a polyacrylate with polyester cross-links. One drawback of this system is that the formed cross-linked polyester is not completely degradable. Only the polyester cross-links can be degraded, while the acrylate polymers in between cannot (Figure 4.1 A).

\[\text{Figure 4.1 Degradation of different cross-linked polyester networks. A: Only cross-links are degradable, polymer backbone not. B: Complete degradable network, cross-links as well as the backbone are degradable.}\]

An alternative to radical polymerization for the cross-linking is thiol-ene chemistry, using macromonomers end-functionalized with thiols or acrylates.\(^7\) These groups can react with each
Polyesters are widely used in different branches of industry, from packaging to biomedical materials as they combine excellent properties with synthetic versatility. A relatively new class of polyesters is derived from cyclic $\omega$-hydroxy fatty acids. Compared to the very common lactones such as $\varepsilon$-caprolactone, the polymerization of these so-called macrolactones to high molecular weight materials is challenging and has been discussed in Chapter 2. From the materials perspective, the interest in polyesters derived from macrolactones stems from the excellent mechanical properties of the polymers (Chapter 2) and the fact that degradation products should be harmless, as they resemble fatty acid derivatives. Unfortunately, homopolymers of the macrolactones investigated by us are non-degradable under physiological conditions due to their semi-crystalline morphology and high hydrophobicity (Chapter 3). We hypothesized that both can be overcome by the introduction of more hydrophilic comonomers. At the same time, the expected transition from a semi-crystalline to an amorphous material by copolymerization requires a post-synthesis cross-linking step in order to be able to process the polymers into stable shapes for biomedical applications. Cross-linked polyesters are usually made with $\alpha$, $\omega$-functionalized macromonomers of the polyester used. For example this can be done by using acrylates as end groups which can be polymerized via radical polymerization. The formed network contains a polyacrylate with polyester cross-links. One drawback of this system is that the formed cross-linked polyester is not completely degradable. Only the polyester cross-links can be degraded, while the acrylate polymers in between cannot (Figure 4.1 A).

Fig 4.1 Degradation of different cross-linked polyester networks. A: Only cross-links are degradable, polymer backbone not. B: Complete degradable network, cross-links as well as the backbone are degradable.

An alternative to radical polymerization for the cross-linking is thiol-ene chemistry, using macromonomers end-functionalized with thiols or acrylates. These groups can react with each other, resulting in the formation of thioethers. However, the same holds for this system as for the acrylate system, namely the formed polythioether is not degradable. Another major drawback of these systems is that the synthesis of these polymers is very laborious. First a macromonomer has to be synthesized. Secondly the end groups have to be introduced and only in the third step the cross-linked polyester is formed.

Direct lateral cross-linking of polyesters would provide a clear advantage since non-degradable residues could be avoided (Figure 4.1 B). This requires lactones with lateral cross-linkable functionalities, which are inert in the ring opening polymerization step. Generally, the synthetic routes of substituted lactones have been described in the literature but most of them require tedious monomer synthesis. Chlorine-substituted lactones could be synthesized via Baeyer Villiger oxidation of the corresponding ketone. Polymerization results in a polyester with a chlorine function which can be reacted to an azide. Huisgen’s cycloaddition with an alkyne results in cross-linked networks.

Synthesizing block-copolymers with polyesters can lead to cross-linked structures. Stenzel et al. showed that utilizing a difunctional initiator, block-copolymers of PLA and PNIPAAm could be synthesized. Via chain extension of the PNIPAAm with hexamethylene diacrylate cross-linked vesicles were obtained.

Another route towards such networks is the use of monomers with a cross-linkable pendant group. This can be for example an amine or a mercapto group. Matsumura et al. showed the formation of a cross-linked polyester network after enzymatic condensation polymerization of hexane-1,6-diol with dimethyl-2-mercaptosuccinate. A network with disulfide bridges as cross-links is formed upon oxidation of the pendant thiols, what makes the properties of these materials susceptible towards temperature and pH changes.

A main chain unsaturation provides another opportunity for cross-linking. This was shown by Jérôme et al. by polymerizing 6,7-dihydro-2-(5H)-oxepinone. We have shown that homopolymers from unsaturated macrolactones can be cross-linked by radical and thiol-ene reactions (Chapter 3). In this chapter we provide an easy route towards cross-linked polyesters based on an unsaturated macrolactone as a monomer. Polymerization of these monomers is straightforward by using enzymatic ring opening polymerization. Here, the copolymerization with smaller biocompatible comonomers and their influence on the polymer properties is investigated (Figure 4.2). It is shown that cross-linking of the polymer afterwards can be performed using dicumyl peroxide as a thermal cross-linker or using a dithiol in combination with a UV-initiator. The degradation behavior of these copolymers is investigated and it will be
shown that via this route completely degradable cross-linked polyesters can be obtained in a simple way.

Figure 4.2 Monomers used for enzymatic ring opening polymerization of lactones. Globalide (Gl) is a mixture of two constitutional isomers with the double bond at the 11 or 12 position (indicated by the dashed line).

4.2 Polymer synthesis and characterization

The enzymatic polymerization of all individual monomers was already investigated by us and by others.\textsuperscript{17-19} Copolymers with \( \omega \)-pentadecalactone, the saturated analogue of globalide, and these smaller lactones are described in literature,\textsuperscript{20,21} but copolymers were never made with these unsaturated macrolactones. All polymerizations were carried out in the same manner using Novozym 435 as a catalyst. For the molecular and physical characterization, preparative amounts of the polymers were synthesized by enzymatic ROP of the respective monomers in solution. Water present in the enzyme was used as initiator. Precipitation of the polymers containing 2-oxo-12-crown-4-ether (OC) was troublesome in methanol and therefore these copolymers were precipitated in hexane. Typical yields were between 80 – 90 % after precipitation. Copolymers containing more than 50 % ambrettolide or globalide were obtained as white powders, copolymers containing less than 50 % of one of these monomers were obtained as oils. Due to the different reactivity ratios of the monomers all polymerizations were carried out over at least 24 hours to ensure incorporation of both monomers and complete randomization of the polymer. All polymers were characterized by NMR, DSC and SEC, and the results are summarized in Table 4.1. Copolymers based on globalide and DXO were synthesized in different Gl/DXO ratios ranging from 91/09 to 28/72 (entries 1 – 4, Table 4.1) and in all polymers the monomer ratio found in the polymer corresponds to the feed ratio. Relatively consistent molecular weights were obtained, with the exception of 44 kg/mol for P(Gl-co-DXO) (50/50). The variation in the obtained molecular weights is probably due to varying amounts of
water in the polymerization mixture. While a drying protocol was applied, the absolute amount of water in the mixture is more difficult to control with the introduction of hydrophilic monomers. Polydispersities are all around 2, which is expected for eROP because lipases are transesterification catalysts.

Table 4.1 Properties of polyesters obtained via enzymatic ROP of macrolactones. Names of the polymers are based on the abbreviations given in Figure 4.2. Reaction conditions: \( T = 60 \, ^\circ\text{C}, t = 24 \, \text{h}, \text{toluene is used as a solvent.} \)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Polymer</th>
<th>Feed ratio [m/m-%]</th>
<th>Ratio (NMR) [m/m-%]</th>
<th>( M_n^a ) [g/mol]</th>
<th>PDI(^a)</th>
<th>( T_m^b ) [°C]</th>
<th>( T_c^c ) [°C]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P(Gl-co-DXO)</td>
<td>91/09</td>
<td>91/09</td>
<td>11 000</td>
<td>2.5</td>
<td>42.4</td>
<td>26.8</td>
</tr>
<tr>
<td>2</td>
<td>P(Gl-co-DXO)</td>
<td>73/27</td>
<td>71/29</td>
<td>13 000</td>
<td>1.8</td>
<td>35.3</td>
<td>17.9</td>
</tr>
<tr>
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<td>55/45</td>
<td>44 000</td>
<td>1.8</td>
<td>26.6</td>
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</tr>
<tr>
<td>4</td>
<td>P(Gl-co-DXO)</td>
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<td>22 000</td>
<td>2.0</td>
<td>0.3</td>
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<tr>
<td>5</td>
<td>P(Gl-co-4MeCL)</td>
<td>89/11</td>
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<td>6</td>
<td>P(Gl-co-4MeCL)</td>
<td>73/27</td>
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<td>2.0</td>
<td>36.5</td>
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<td>33/67</td>
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<tr>
<td>9</td>
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<td>-</td>
<td>1 000</td>
<td>1.57</td>
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</table>

\(^a\) Determined by SEC in THF at 40 \, ^\circ\text{C} (polystyrene standards). \(^b\) Determined from the second DSC heating run. \(^c\) Determined from the first cooling run.

P(Gl-co-4MeCL) was prepared using the same ratios as the P(Gl-co-DXO) set. Here, the incorporation ratio was also in agreement with the feed ratio. All molecular weights obtained were around 17 kg/mol, with the exception of P(Gl-co-4MeCL), for which only a mass of 6 kg/mol could be obtained.

Despite the same polymerization procedure, the molecular weights of OC-containing copolymers were significantly lower than those of the other copolymers. One probable cause
can be the hydrophilicity of OC. It is more difficult to dry the reactants which causes a higher water amount in the reaction mixture and consequently a lower molecular weight.

![Diagram of polymer structure](image)

**Figure 4.3** $^{13}$C-NMR spectrum of 3. Left: Complete spectrum. Right: $\text{RCH}_2\text{C}=\text{OOR}$ region, peaks are assigned using the diad codes in Table 4.2.

The randomness of all copolymers was derived from $^{13}$C NMR integrals (Figure 4.3, Table 4.2). The calculated diad content of A-B copolymers was compared with the theoretical diad contents assuming a statistic random distribution. The signal from the carbon adjacent to the oxygen of the ester function (RC=OOCR) at 70 – 60 ppm was used to calculate the diad ratios for 4MeCL copolymers. Signals from GI repeating units (AA, 34.1 ppm) can be found further upfield in the spectrum than signals from 4-methyl caprolactone repeating units (BB, 35.0 ppm). AB (34.3 ppm) and BA (34.8 ppm) diads can be found in between the AA and BB peaks. With DXO containing copolymers the ether carbon signal of this monomer interferes with the RC=OOCR signals. Therefore the diads for these polymers were calculated using the signal from the carbon next to the carbonyl (RCH$_2$C=OOR) between 33 – 36 ppm (Figure 4.3).

Next to comparing the obtained integrals to the statistically expected values, a degree of randomness (R) can be calculated.$^{23,24}$ The following set of equations was used:

$$P_{A/B} = P_{AB} + P_{BA}$$

(1)
Copolymers with hydrophilic monomers

\[ P_{AX} = \frac{P_{A/B}}{2} + P_{AA} \]  
\[ P_{BX} = \frac{P_{A/B}}{2} + P_{BB} \]  
\[ R = \frac{P_{A/B}}{2P_{AX}P_{BX}} \]

Here, \( P_{A/B} \), \( P_{BA} \), \( P_{AA} \) and \( P_{BB} \) denote the integral values of the AB, BA, AA and BB diads, respectively. \( P_{A/B} \) denotes the total integral of the mixed diads (AB and BA) and \( P_{AX} \) the integral value of AX where X can be either A or B. \( P_{BX} \) is the same as \( P_{AX} \) but then for monomer B. \( R \) is the degree of randomness which is given in Table 4.2.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Composition</th>
<th>( \frac{A}{B} ) [mole %]</th>
<th>( AA^b ) [-]</th>
<th>( AB^b ) [-]</th>
<th>( BA^b ) [-]</th>
<th>( BB^b ) [-]</th>
<th>( R_{total} ) [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>91/09</td>
<td>0.81 (0.828)</td>
<td>0.12 (0.082)</td>
<td>0.06 (0.082)</td>
<td>0.01 (0.008)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>71/29</td>
<td>0.61 (0.504)</td>
<td>0.17 (0.206)</td>
<td>0.15 (0.206)</td>
<td>0.07 (0.084)</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>55/45</td>
<td>0.32 (0.302)</td>
<td>0.26 (0.248)</td>
<td>0.23 (0.248)</td>
<td>0.19 (0.202)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>29/71</td>
<td>0.14 (0.084)</td>
<td>0.19 (0.206)</td>
<td>0.22 (0.206)</td>
<td>0.45 (0.504)</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>89/11</td>
<td>0.51 (0.792)</td>
<td>0.40 (0.098)</td>
<td>0.07 (0.098)</td>
<td>0.02 (0.012)</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>75/25</td>
<td>0.40 (0.562)</td>
<td>0.32 (0.188)</td>
<td>0.19 (0.188)</td>
<td>0.09 (0.062)</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>53/47</td>
<td>0.28 (0.281)</td>
<td>0.29 (0.249)</td>
<td>0.23 (0.249)</td>
<td>0.20 (0.221)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>71/29</td>
<td>0.58 (0.504)</td>
<td>0.19 (0.206)</td>
<td>0.16 (0.206)</td>
<td>0.07 (0.084)</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>37/63</td>
<td>0.11 (0.137)</td>
<td>0.18 (0.233)</td>
<td>0.34 (0.233)</td>
<td>0.37 (0.397)</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>27/73</td>
<td>0.12 (0.073)</td>
<td>0.16 (0.197)</td>
<td>0.30 (0.197)</td>
<td>0.42 (0.533)</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Determined with \(^1\)H-NMR. \(^b\)Determined with \(^13\)C-NMR. Numbers in parenthesis are calculated results for a statistically random distribution.

Noticeable is that the diad ratios calculated from \(^13\)C NMR integration correspond very well with the theoretical values for a statistically random distribution (Table 4.2). Moreover, the degree of randomness \( R \) is between 0.9 and 1.3, which is reasonably close to that expected for fully random copolyesters, for which the distribution of the two different monomer units should obey Bernoullian statistics and \( R \) should be 1 (\( R < 1 \) for blocky structures, \( R = 0 \) for
homopolymers, R = 2 for alternating copolymers and R > 1 indicates a shorter sequence length). This confirms, that despite the potentially different reactivity ratios of the monomers, all obtained copolymers are randomly distributed due to the rapid transesterification by Novozym 435.

4.3 Thermal properties

In Table 4.1 all melting points of the copolymers with different compositions are listed. Homopolymers of DXO, 4MeCL and OC are amorphous and no melting point could be observed. All copolymers that are not completely amorphous show a single crystallization and melting process. Due to the amorphous character of polyesters based on the comonomers DXO, 4MeCL and OC, the melting enthalpies of the copolymers decrease upon increasing the amount of these comonomers (Figure 4.4).

The difference between $T_m$ and $T_c$ is very small for all polymers (ca. 20 °C) and in the thermograms of the cooling run (Figure 4.4, right) sharp peaks are observed. This shows that crystallization in these materials is fast.

All glass transition temperatures are far below 0 °C. The $T_g$ of PCL, a less flexible material, is known to be -60 °C, all copolymers have a $T_g < -60$ °C. However, these could not be measured due to the limit of the DSC (-60 °C).

A trend can be observed in the melting (ranging from 42.4 to 0.3 °C) and crystallization temperatures of P(Gl-co-DXO) with different compositions, namely the higher the amount of
DXO incorporated, the lower the melting and crystallization temperature (Figure 4.4). Explanation can be found in the fact that the homopolymer of DXO is completely amorphous, and incorporation of this monomer will lower the melting and crystallization temperature.\textsuperscript{25}

Copolymer 8 is the only in this set which is obtained as a completely amorphous copolymer. No melting and crystallization temperatures could be observed. Moreover, no glass transition could be observed either, which is probably due to the limit of the DSC (-60 °C). All other Gl-4MeCL copolymers show melting transitions and here the same trend is observed as for the DXO copolymers, namely the incorporation of 4MeCL results in lowering of the melting and crystallization temperature. The influence of incorporation of OC on the melting temperature is the same as for the other comonomers, DXO and 4MeCL.

### 4.4 Cross-linking

Two different ways of preparing polyester networks were explored. The first uses a thermal cross-linker, dicumyl peroxide (Figure 4.5, route A). Upon mixing the polymer melt with this curing agent and curing at elevated temperatures completely cross-linked networks can be obtained (Chapter 3). In the second method UV-curing was applied using ethylene glycol bis(3-mercaptopropionate) as a cross-linker and 4-hydroxybenzophenone as a UV-initiator (Figure 4.5, route B). Exposure to UV light results in network formation.

\[ \text{unsaturated polyester} + \text{heat} \rightarrow \]

\[ \text{unsaturated polyester} + \text{UV} \rightarrow \]

**Figure 4.5** Two different routes towards biodegradable polyester networks. A: Thermal cross-linking with dicumyl peroxide. Cross-links are non-degradable. B: UV cross-linking with a dithiol and a UV-initiator. Cross-links are degradable.
4.4.1 Curing using dicumylperoxide

In order to determine the lowest amount of cross-linkable comonomer needed to obtain a fully cross-linked material, a series of copolymers was made with globalide and ε-caprolactone (Table 4.3). This set of copolymers was chosen as a model system because of the sufficient availability of ε-caprolactone. These copolymers show the same polymerization behavior and trends as the other copolymer sets reported earlier in this chapter. All polymers were obtained as random copolymers as evident from diad analysis. Moreover, the molecular weights are representative for the ones obtained with the other copolymer systems (Table 4.1). The amount of globalide was varied between 10 and 50 m/m-%. For the cross-linking, all materials were melted, mixed with dicumylperoxide in the melt and cured at 150 °C for 30 min. Afterwards the cured materials were cooled to room temperature.

The amount of cross-links in the material was estimated with IR-spectroscopy. The signal of the trans unsaturated carbon-carbon bond around 970 cm⁻¹ decreased upon curing and was used to monitor the consumption of double bonds in the process. A clear trend can be seen in the percentage of cross-linked monomer residues in the polymer. The lower the amount of residual cross-linkable comonomer, the higher the percentage of reacted double bonds (58 % for P(Gl-co-CL) (10/90) to 13 % for P(Gl-co-CL) (40/60)). This is probably due to the formation of the network itself. As soon as there are some bonds cross-linked, the peroxide is no longer able to penetrate the material and cross-linking stops. When the amount of Gl decreases, the network becomes less dense and this facilitates a continuous and more complete cross-linking of the unsaturated bonds in the network.

The gel content of the cross-linked polymers was determined with soxhlet extraction in chloroform. In Table 4.3 it can be seen that 10 m/m-% of cross-linkable monomer is sufficient to obtain a fully cross-linked material. This means that at least 90 % of any monomer can be incorporated without losing the advantages of a cross-linked system, the advantages being the possibility to make completely amorphous and easily degradable materials with adjustable modulus by changing the amount of cross-linked double bonds. In literature it was described that monomers like DXO could only be incorporated up to 20 m/m-% in non-cured systems, due to the unfavorable influence on the melting point. However, incorporation of a higher amount of comonomer is desirable as it can enhance the hydrophilicity and thus degradation properties of materials significantly. By cross-linking the material can be tuned to the desired
properties without taking the melting point and crystallinity into account. This opens various possibilities for degradable biocompatible polymers.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Feed Gl/CL</th>
<th>Ratio Gl/CL</th>
<th>(M_n) (^b)</th>
<th>PDI (^b)</th>
<th>Reacted C=(C) (^d)</th>
<th>Gel content (^d)</th>
<th>Reacted C=(C) (^e)</th>
<th>Gel content (^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>50/50</td>
<td>47/53</td>
<td>20 000</td>
<td>3.3</td>
<td>n.d.</td>
<td>99.2</td>
<td>23</td>
<td>89</td>
</tr>
<tr>
<td>18</td>
<td>40/60</td>
<td>40/60</td>
<td>21 000</td>
<td>3.0</td>
<td>13</td>
<td>96.8</td>
<td>37</td>
<td>88</td>
</tr>
<tr>
<td>19</td>
<td>30/70</td>
<td>32/68</td>
<td>23 000</td>
<td>2.4</td>
<td>16</td>
<td>97.5</td>
<td>43</td>
<td>82</td>
</tr>
<tr>
<td>20</td>
<td>20/80</td>
<td>22/78</td>
<td>18 000</td>
<td>2.8</td>
<td>33</td>
<td>96.2</td>
<td>25</td>
<td>79</td>
</tr>
<tr>
<td>21</td>
<td>10/90</td>
<td>10/90</td>
<td>16 000</td>
<td>2.4</td>
<td>58</td>
<td>94.5</td>
<td>11</td>
<td>33</td>
</tr>
</tbody>
</table>

\(^a\) Determined with \(^1\)H-NMR, \(^b\) Determined by SEC in tetrahydrofuran, \(^c\) Determined by IR, \(^d\) Cross-linked using thermal methods, \(^e\) Cross-linked using thiol-ene chemistry.

### 4.4.2 Cross-linking by thiol-ene chemistry

Upon curing of copolymers utilizing a dithiol and a UV initiator, different results were obtained in comparison with the thermal cross-linker. Cured films containing \(\geq 20\) m/m-% cross-linkable monomer were obtained as transparent films (Figure 4.6), only entry 21 was obtained as an opaque film. The amount of reacted C=\(C\) bonds was determined by IR spectroscopy. Using this curing system, no trend could be observed in the relation of cross-link density and amount of cross-linkable monomer. In contrast to the thermal curing, where the double bonds are connected directly, using the thiol-ene reaction a flexible linker is placed between the double bonds. This creates a more open network where it is still possible for the dithiols to reach the unreacted double bonds. Noticeable was also that all reactions were finished within twenty minutes. The gel content of all copolymers was lower than when using the thermal cross-link system. However, the polymer was mixed with dithiol in a 2/1 mole ratio. Since only a percentage of the added dithiol is used in the cross-linking (with a maximum of 43 m/m-% in entry 19, Table 4.3), the unreacted dithiol was still present as a small molecule in the cured film. These small molecules are washed out easily upon extraction. Taking this into account during calculation of the gel content (weight after extraction divided by the weight before extraction), a much higher gel content has been calculated. Only curing of entry 21 did not
result in complete network formation. Upon addition of low amounts of cross-linkable monomer, the crystallinity of the cured material can be tuned to a certain extend.

**Figure 4.6** Transparent film of $P(Glu-co-CL)$ (30/70) obtained via thiol-ene cross-linking using UV radiation for 15 min.

## 4.5 Enzymatic degradation

In previous work the degradation of cured homopolymers of PAm and PGI was studied.\(^{27}\) It was concluded that no degradation could take place due to the highly hydrophobic character of the polymers. It was hypothesized that upon the incorporation of comonomers like 4MeCL, DXO or OC, the hydrophobicity is lowered, thereby enhancing degradation. Degradation studies were performed on $P(Glu-co-4MeCL)$ and a range of copolymers containing Am and DXO. In a preliminary experiment the influence of the comonomer was investigated using $P(Am-co-DXO)$ (25/75) and $P(Glu-co-4MeCL)$ (53/47) (Figure 4.7, left). Both polymers were cross-linked with dicumyl peroxide and compression molded. The amount of cross-linked double bonds was for all polymers ca. 15 m/m-%. As can be seen in the left graph in Figure 4.7, over a period of 10 weeks significant degradation occurs in both copolymers.

Degradation of $P(Am-co-DXO)$ (25/75) reaches more than 90 w/w-% after 100 days. The material shows a linear degradation curve. It can be concluded that this polymer will degrade completely. $P(Glu-co-4MeCL)$ (53/47) degrades slower and after 130 days only 30 w/w-% of the material has been degraded. The difference in degradation rate can be explained by the higher hydrophilicity of the Am-DXO copolymer caused by the incorporation of DXO repeating units.

A second experiment was performed to investigate the reproducibility of the degradation tests. Therefore, all experiments were performed in triplo. This set contains three copolymers of $P(Am-co-DXO)$ of different comonomer ratio. These polymers were cross-linked using dicumyl peroxide in an oven. The obtained films were cut into pieces of around 1 cm\(^2\) (~ 100 mg) and placed in test tubes with 4 mL PBS buffer. The tubes were placed in a shaker at 37 °C.
Degradation was followed during a period of ten weeks (Figure 4.7). Every week the medium and enzyme were replaced. Only the mass loss of the samples could be measured. No molecular weights could be determined due to cross-linking of the polymers.

In the right graph in Figure 4.7 the degradation curves of the P(Am-co-DXO) copolymers are depicted. The degradation of these materials is slower than that of the polymers for which the degradation is depicted in the left graph. However, it needs to be noted that the test samples were obtained in a slightly modified process as they were cross-linked at atmospheric pressure instead of 150 bar. This may have an influence on the mobility of the chains. The results clearly show, that the degradation can be tuned by altering the ratio of the monomers. By incorporating more degradable monomer, the degradation proceeds faster. Upon incorporating 75 % of DXO, mass loss is observed from the start of the degradation experiment. This can be due to the formation of DXO rich polymer chains which can dissolve in the degradation medium. The chains that remain in the sample are richer in ambrettolide residues what causes the slower degradation rate.

### 4.6 Conclusions

Biodegradable random copolyesters were made via eROP using macrolactones and various comonomers. All copolymers were easily obtained, although the molecular weight of 2-oxo-12-
crown-4-ether copolymers stayed low. The comonomers used and all macrolactones are known non-cytotoxic monomers, what makes these materials suitable for biomedical applications. All copolyesters made show degradation, however, incorporating more hydrophilic comonomers like 1,5-dioxepan-2-one enhances degradation more than incorporating sterically hindered and less polar comonomers like 4-methyl caprolactone.

The randomness of the copolymers was investigated with $^{13}$C-NMR. The values found for the intensity of the different signals are close to calculated values for a statistically random distribution of the comonomer residues.

Thermal investigation shows that the melting point is lowered upon incorporating a comonomer and that the extend of decrease was dependent on the comonomer ratio. However, due to the presence of the main chain double bond of the unsaturated macrolactones, all copolymers could be cross-linked, which resulted in completely amorphous insoluble networks. Even upon incorporating 90 m/m-% of comonomer a gel content of 95 w/w-% was obtained. This means that less than 10 m/m-% of the main chain double bond is needed to form a complete network, what leaves the other 90 m/m-% of the material for polar comonomers facilitating the biodegradation. Another option is to incorporate more than 10 m/m-% unsaturated macrolactone and use part of the excess double bond as a functionality to attach bio-active pendant groups.

4.7 Experimental

4.7.1 Materials

Novozym 435 (Candida antarctica Lipase B immobilized on cross-linked polyacrylate beads) was purchased from Novozymes A/S and dried following a literature procedure. Globalide and ambrettolide were kind gifts of Symrise. Toluene was dried over aluminum oxide and stored over molecular sieves. All other solvents used were purchased from Biosolve and used without further purification. DXO, 4MeCL and OC were synthesized following literature procedures. Dicumyl peroxide was purchased from Aldrich, 4-hydroxybenzophenone from Fluka and ethylene glycol bis(3-mercaptopropionate) was from Wako.
4.7.2 Methods

The water content of all reaction mixtures was measured with Karl-Fischer Coulometry using a Mettler Toledo DL32 Coulometer and Apura CombiCoulomat fritless (Merck) as electrolyte. Size exclusion chromatography (SEC) was performed on a Waters Alliance system equipped with a Waters 2695 separation module, a Waters 2414 refractive index detector (40 °C), a Waters 2487 dual absorbance detector and a Polymer Laboratories PLgel guard column followed by 2 PLgel 5mm Mixed-C columns in series at 40°C. Tetrahydrofuran (THF, Biosolve), stabilized with BHT, was used as eluent at a flow rate of 1 mL/min. The molecular weights were calculated against polystyrene standards (Polymer Laboratories, $M_p = 580$ Da up to $M_p = 7.1 \times 10^6$ Da). All samples were filtered through a 0.2 µm PTFE filter (13 mm, PP housing, Alltech) before analysis. $^1$H and $^{13}$C-NMR spectroscopy was performed on a VARIAN Mercury 400 MHz NMR in CDCl$_3$. Data was acquired using VNMR software. Chemical shifts are reported in ppm relative to tetramethylsilane. Differential Scanning Calorimetry (DSC) was performed on a TA Q100 DSC. Approximately 5 mg of dried polymer was weighed into aluminum hermetic pans. Temperature profiles from -50 °C up to 130 °C with a heating and cooling rate of 10 °C/min were applied. TA Universal Analysis software was used for data acquisition. Melting and crystallization temperatures were determined from the second heating run and the first cooling from the melt respectively. IR measurements were performed on a PerkinElmer SpectrumOne FT-IR Spectrometer, using a universal ATR sampling accessory. Kinetic attenuated total reflection Fourier Transform InfraRed (ATR-FTIR) spectroscopy measurements were performed on a Bio-Rad Excalibur FTS3000MX infrared spectrometer using the golden gate setup. 32 scans were recorded per spectrum with a resolution of 4 cm$^{-1}$. Data was acquired using Varian Resolutions Pro. UV-curing measurements were performed under nitrogen atmosphere using a Driel Spectral Luminator with a wavelength of 365 nm. UV curing was performed using a Philips HPR-125 mercury discharge lamp with an output of 125 W/ 1.15 A.

4.7.3 General procedure for enzymatic ring opening polymerizations

A stock solution of selected monomers (1.08 g) and dried toluene (0.90 g) was prepared and dried overnight at 40 °C in the presence of 4 Å molecular sieves. Novozym 435 (40 mg) was dried in the reaction flask over molsieves at 40 °C overnight in a vacuum oven. After drying, the oven was opened under nitrogen flow. The flask containing Novozym 435 was placed in an oil
Chapter 4

bath at 60 °C and the stock solution was added. The amount of water in the reaction mixture was measured at the beginning of the reaction using Karl-Fischer Coulometry. The water content of all reactions was below 0.07 mg water per gram of reaction mixture. After several hours the viscosity of the mixture prevented proper stirring. After 24 hrs dichloromethane was added to the reaction mixture to redissolve the product. After filtering off the enzyme, the filtrate was precipitated in cold methanol (or hexane in case of OC copolymers). The polymer was dried at room temperature in vacuum. Typical yields after precipitation were 70 %.

**Polyglobalide:** 1H-NMR: δ (ppm) = 5.53 – 5.29 (m, 2H, C\(\text{H}=\text{CH}\)), 4.06 – 4.03 (m, 2H, C=OOC\(\text{H}_2\)), 2.30 – 2.26 (m, 2H, C\(\text{H}_2\))C=O), 2.12 – 1.92, 1.71 – 1.59, 1.25 (m, 22H, C\(\text{H}_2\)). 13C-NMR: δ (ppm) = 173.8 (C=O), 133.5, 132.9, 131.5, 131.0, 128.6, 128.1, 125.0 (CH=CH), 63.9, 63.7 (C=OOC\(\text{H}_2\)), 34.4 (CH\(\text{H}_2\)=O), 32.6, 32.5 (CH\(\text{H}_2\)=CH=CH\(\text{H}_2\)), 32.0 (C=OOC\(\text{H}_2\)), 29.6 - 25.0 (CH\(\text{H}_2\)).

**Polyambrettolide:** 1H-NMR: δ (ppm) = 5.38 – 5.36 (m, 2H, C\(\text{H}=\text{CH}\)), 4.06 – 4.03 (t, 2H, C=OOC\(\text{H}_2\)), 2.30 – 2.26 (m, 2H, C\(\text{H}_2\))C=O), 1.96, 1.63 – 1.57, 1.34 – 1.29 (m, 24H, CH\(\text{H}_2\)). 13C-NMR: δ (ppm) = 173.8 (C=O), 131.0, 130.5 (CH=CH), 64.2 (C=OOC\(\text{H}_2\)), 34.8 (CH\(\text{H}_2\)=C=O), 31.7 (CH\(\text{H}_2\)=CH=CH\(\text{H}_2\)), 31.3 (C=OOC\(\text{H}_2\)), 29.5 – 24.9 (CH\(\text{H}_2\)).

**Poly(1,5-dioxepan-2-one):** 1H-NMR: δ (ppm) = 4.30 – 4.28 (t, 2H, C=OOC\(\text{H}_2\)), 3.66 – 3.64 (m, 2H, C\(\text{H}_2\))C=O), 2.56 – 2.54 (m, 2H, C\(\text{H}_2\))C=O). 13C-NMR: δ (ppm) = 171.3 (C=O), 68.7 (C=OOC\(\text{H}_2\)), 66.4 (OCH\(\text{H}_2\)=CH\(\text{H}_2\)=C=O), 63.4 (C=OOC\(\text{H}_2\)), 34.8 (CH\(\text{H}_2\)=C=O).

**Poly(4-methylcaprolactone):** 1H-NMR: δ (ppm) = 4.13 – 4.06 (m, 2H, C=OOC\(\text{H}_2\)), 2.34 – 2.27 (m, 2H, C\(\text{H}_2\))C=O), 1.73 – 1.41 (m, 6H, CH\(\text{H}_2\), CH\(\text{H}_2\), CH\(\text{H}_3\)).

**Poly(2-oxo-12-crown-4-ether):** 1H-NMR: δ (ppm) = 4.24 (m, 2H, C=OOC\(\text{H}_2\)), 4.12 (s, 2H, C\(\text{H}_2\)=C=O), 3.70 – 3.55 (m, 10H, CH\(\text{H}_2\)).

**Poly(globalide-co-1,5-dioxepan-2-one) (55/45):** 1H-NMR: δ (ppm) = 5.51 – 5.28 (m, C=CH), 4.28 – 4.00 (m, C=OOC\(\text{H}_2\)), 3.68 – 3.63 (m, C\(\text{H}_2\))C=O), 2.55 – 2.23 (m, C\(\text{H}_2\))C=O), 2.11 – 1.20 (m, C\(\text{H}_2\)). 13C-NMR: δ (ppm) = 174.0 (C=O, Gl), 171.4 (C=O, DXO), 133.7, 133.5, 131.6, 131.5, 128.6, 128.1, 125.0, 124.8 (CH=CH), 68.9 (C=OOC\(\text{H}_2\), DXO), 66.6 (OCH\(\text{H}_2\)=CH\(\text{H}_2\)=C=O) 64.3 – 63.2 (C=OOC\(\text{H}_2\), Gl), 35.0, 34.8, 34.4, 34.1 (CH\(\text{H}_2\)=C=O, DXO-DXO, DXO-Gl, Gl-DXO, Gl-Gl), 32.6, 32.5 (CH\(\text{H}_2\)=CH=CH\(\text{H}_2\)), 32.0 (C=OOC\(\text{H}_2\)), 29.5 - 24.9 (CH\(\text{H}_2\)).

**Poly(globalide-co-4-methylcaprolactone) (53/47):** 1H-NMR: δ (ppm) = 5.55 – 5.30 (m, CH=CH), 4.15 – 4.02 (m, C=OOC\(\text{H}_2\)), 2.35 – 2.25 (m, CH\(\text{H}_2\)=C=O), 2.13 – 1.22 (m, CH\(\text{H}_2\), CH\(\text{H}_2\), CH\(\text{H}_3\)). 13C-NMR: δ (ppm) = 173.9, 173.7 (C=O), 133.6, 133.5, 131.5, 131.1, 129.0, 128.1, 125.0 (CH=CH), 63.9,
Copolymers with hydrophilic monomers

63.7, 62.6, 62.4 (C=OOCH₂, 4MeCl-4MeCl, 4MeCl-Gl, Gl-4MeCl, Gl-Gl), 35.2, 34.3 (CH₂C=OO), 32.6, 32.5 (CH₂-CH=CH-CH₂), 32.0 (C=OOCH₂-CH₂), 29.6 - 23.5 (CH₂, CH), 19.0 (CH₃).

Poly(ambrettolide-co-2-oxo-12-crown-4-ether)) (37/63): ¹H-NMR: δ (ppm) = 5.39 – 5.36 (m, CH=CH), 4.25 – 4.01 (m, C=OOCH₂, CH₂C=OO from OC), 3.71 – 3.54 (m, CH₂O), 2.32 – 2.25 (m, CH₂C=OO Am), 1.93 – 1.32 (m, CH₃).

¹³C-NMR: δ (ppm) = 173.9, 173.7 (C=O), 130.5, 130.1 (CH=CH), 75.6 (CH₂C=OO), 71.1, 69.9, 68.6 (COCC) 65.7 – 63.3 (C=OOCH₂, COCC), 34.4, 34.2, 32.5, 32.4 (CH₂C=OO, OC-OC, OC-Am, Am-OC, Am-Am), 29.6 – 24.8 (CH₂).

4.7.4 Degradation of polymers in a pH buffer

All polymers were compression molded (150 bar, 30 min) into a 1 mm thick film and thermally cross-linked using dicumyl peroxide.³¹ Ca.100 mg of polymer film was added to 4 mL of a 0.1 M PBS buffer (pH = 7.4) containing 0.1 mg Pseudomonas cepacia Lipase (Lipase PS) per mL PBS buffer. The samples were placed in a shaking machine at 37 °C. Every week this buffer was replaced and new enzyme was added. At regular time intervals the buffer of one sample was removed and the polymer was dried overnight. The weight loss was determined.

4.8 References

Chapter 4

CATALYTIC RING OPENING POLYMERIZATION OF LACTONES

Parts of this chapter has been submitted to Angewandte Chemie, International Edition: ‘Size does not matter: A Highly Efficient Catalyst for the Ring Opening Polymerization of Macrolactones without Ring-Strain’ Inge van der Meulen, Saskia Huijser, Erik Gubbels, Rob Duchateau, Andreas Heise.
Chapter 5

5.1 Introduction

Ring opening polymerization (ROP) of cyclic esters is widely used for the synthesis of aliphatic AB-type polyesters. Particularly successful is metal-mediated ROP as it allows for the control of the polymer molecular weight, molecular weight distribution and end-groups by the use of a nucleophilic initiator. Among the metals applied, aluminum, zinc and tin are most prevalent.\(^1\) It is commonly agreed that the driving force behind the ROP of lactones is the release of ring-strain in the transition from the cyclic ester to the polyester chain or, in thermodynamic terms, by the negative change of enthalpy. Consequently, as the ring-strain decreases with increasing lactone size so does the reactivity in metal-mediated ROP. Experimentally this was shown by Duda in a comparative study of the ROP of various size lactones using zinc octoate/butyl alcohol as a catalyst/initiator.\(^2\) While the relative rates of polymerization were found to be 2 500 and 330 for the six-membered (\(\omega\)valerolactone) and seven-membered (\(\omega\)-caprolactone) lactones, respectively, the reaction rates of the 12 – 17 membered lactones were only around 1. Consequently, only a few examples of metal-catalyzed ROP of macrolactones like \(\omega\)-pentadecalactone (PDL) can be found in literature, which report only low yields and low molecular weights.\(^3\)\(^4\) The best results were obtained using yttrium tris(isopropoxide) leading to high conversions and reasonable molecular weights of up to an absolute \(M_n\) of 30 kg/mol.\(^5\)

The situation is inverted for the lipase-catalyzed ROP.\(^2\)\(^6\) Lipases like Candida antarctica Lipase B (CALB) are highly active in the ROP of lactones and show exceptionally high polymerization rates for macrolactones.\(^7\)\(^8\) The reactivity of lactones in this process is no longer governed by the high ring-strain of small lactones (cisoid ester bonds) but by the preference of the lipase for transoid ester bond conformation present in large ring lactones.\(^9\) Macrolactones can thus easily be polymerized by CALB and poly(\(\omega\)-pentadecalactone) (PPDL) with molecular weights (\(M_n\)) up to 150 kg/mol have been reported.\(^10\)\(^11\) It is thus commonly accepted (and repeatedly stated) that efficient polymerization of macrolactones to high molecular weight polyesters is only possible by enzymatic catalysis.

In this chapter a highly efficient metal-mediated ROP of a macrolactone, namely \(\omega\)-pentadecalactone was investigated. Moreover, a kinetic study of different sized lactones has been performed to elucidate the relation between ring size and polymerizability by metal-
mediated ROP. The obtained results are unprecedented in that a very high catalytic efficiency was found and high molecular weights were obtained in this process.

5.2 Mechanistic aspects of chemical ring opening polymerization

The catalyst used in this study is an aluminum salen complex (Scheme 5.2). These catalysts are widely used in the synthesis\(^\text{12}\) and the polymerization\(^\text{13}\) of cyclic carbonates. The aluminum alkoxide catalyzed ring opening polymerization of lactones is believed to obey the coordination-insertion mechanism (Scheme 5.1).\(^\text{14,15}\)

In a first step the monomer is coordinated to the metal alkoxide and the intermediate is formed. This coordination of the exocyclic oxygen results in a more polarized carbonyl which makes this carbon more prone to undergo nucleophilic attack.\(^\text{14}\) Subsequently, in the insertion step, the acyl-oxygen and the M-alkoxide bond are cleaved. This released alkoxide becomes the end-group of the growing polymer chain.

Propagation of the polymerization follows the same route as the initiation step. Transesterification reactions (inter- and intra molecular) can occur, however, these are often only observed upon reaching high monomer conversion and when high temperatures are applied. These side reactions cause broadening of the molecular weight distribution.

In the kinetics three different reactions are considered in this mechanism; initiation, propagation and chain transfer (when chain transfer agents such as alcohols are applied).\(^\text{16}\) Aggregation of the alkoxide initiator should be taken into consideration, since the activity towards polymerization of these aggregates is significantly lower than the activity of the non-aggregated catalyst. A chain is temporarily terminated when these metal complexes aggregate.
There is an equilibrium between this aggregated state and the active monomeric state (Equation 1):\textsuperscript{17}

$$\left( P_{n}^{*}\right)_{m} \rightleftharpoons mP_{n}^{*}$$  \hspace{1cm} (1)

$(P_n^*)_m$ depicts m units of aggregated catalyst and $P_n^*$ the non-aggregated form. This equilibrium influences the polymerization behavior significantly. Normally the consumption of monomer in a constant volume can be described as: \textsuperscript{2,17,18}

$$r_p = - \frac{d[M]}{dt} = k_{app} [M]$$  \hspace{1cm} (2)

$$\int_{0}^{M} \frac{d[M]}{[M]} = \int_{0}^{t} k_{app} dt$$  \hspace{1cm} (3)

$$\ln[M]_0 - \ln[M] = k_{app} t$$  \hspace{1cm} (4)

$$\ln \frac{[M]_0}{[M]} = k_{app} t$$  \hspace{1cm} (5)

Where $r_p$ denotes the reaction rate of the formation of polymer, $[M]$ the concentration monomer, $t$ the time and $k_{app}$ the apparent reaction rate constant. Equation 5 is the integrated form of equation 2 (via Eq. 3 and 4) and will be used to calculate $k_{app}$ from the experimental data. When the plot of the logarithm of the relative concentration against the time gives a linear relation, the number of growing chains is constant in time. This implies that no irreversible termination occurs, indicating that the polymerization is living. To calculate the reaction rate constant ($k_p$) from $k_{app}$ the aggregation of the catalyst should be taken into account (Equation 6).\textsuperscript{2,17}
There is an equilibrium between this aggregated state and the active monomeric state (Equation 1):

\[
P_{n}^{*} \overset{\text{m}}{\longleftrightarrow} \text{P} \quad (1)
\]

\(P_{n}^{*}\) depicts \(m\) units of a ggregated catalyst and \(P_{n}^{*}\) the non-aggregated form. This equilibrium influences the polymerization behavior significantly. Normally the consumption of monomer in a constant volume can be described as: 2,17,18

\[
[p]_{app} \frac{dM}{dt} = -k_{app}M \quad (2)
\]

\[
\int \int \ln[p]_{app} - \ln[p]_{0} = \int \ln[k_{app}] = -k_{app}M \quad (3)
\]

\[
\ln[p]_{app} - \ln[p]_{0} = \int k_{app} \quad (4)
\]

\[
k_{p} = \frac{k_{app}}{[P_{n}^{*}]} \quad (6)
\]

The ratio between the free and aggregated catalyst is difficult to determine. Therefore the assumption is made that at the applied reaction temperature (100 °C) no aggregation of the metal alkoxide initiator takes place.

### 5.3 Chemical ring opening polymerization of \(\alpha\)-pentadecalactone

The catalytic system used in the ROP of PDL is a combination of aluminum salen complex 1 and benzylalcohol (BnOH) (Scheme 5.2). The active species (the aluminum alkoxide) in this reaction is assumed to be formed instantaneously at the start of the polymerization. This assumption has been verified by NMR experiments. The efficiency of the catalyst has been tested in the bulk polymerization of PDL in the presence of BnOH as an initiator at a 1:1 ratio at 100 °C with increasing monomer:catalyst ratios (50 – 500). A constant reaction time of 4 h was applied after which the reaction was stopped and the polymer was analyzed.

![Scheme 5.2 Polymerization of \(\alpha\)-pentadecalactone using an aluminum salen complex.](image)

The high efficiency of the catalyst in the polymerization of PDL was immediately evident from the fast reaction. The viscosity of the reaction medium increased rapidly within minutes and after approximately 20 minutes agitation stopped. Even though a rapid viscosity increase with conversion is known from enzymatic PPDL synthesis, for a metal catalyst such fast polymerization kinetics for the ROP of PDL is remarkable.

For the lower ratios of monomer to 1, \(^1\)H-NMR spectroscopy (Table 5.1, entry 1 and 2) showed an almost quantitative monomer conversion within the applied reaction time of 1 hour. When the ratio was increased the monomer conversion leveled off between 70 and 74 % (Table 5.1, entry 3 and 4), most likely due to diffusion limitations caused by the high viscosity of the
reaction mixture. It should be noted that it is likely that monomer conversions for the higher molecular weight PPDL are underestimated due to the low solubility of PPDL in deuterated chloroform.

Table 5.1 Results of the ROP catalyzed by 1 in the presence of an equimolar amount of BnOH using various monomer to catalyst ratios (M:C). T = 100 °C, t = 4 h.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Monomer</th>
<th>Solvent</th>
<th>M:C</th>
<th>$M_n$calc. [g/mol]$^a$</th>
<th>$M_n$ [g/mol]$^b$</th>
<th>PDI</th>
<th>Conversion [%]$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PDL</td>
<td>-</td>
<td>44</td>
<td>11 000</td>
<td>24 000</td>
<td>2.8</td>
<td>&gt; 99</td>
</tr>
<tr>
<td>2</td>
<td>PDL</td>
<td>-</td>
<td>110</td>
<td>26 000</td>
<td>41 000</td>
<td>2.6</td>
<td>&gt; 99</td>
</tr>
<tr>
<td>3</td>
<td>PDL</td>
<td>-</td>
<td>212</td>
<td>38 000</td>
<td>99 000</td>
<td>2.1</td>
<td>74</td>
</tr>
<tr>
<td>4</td>
<td>PDL</td>
<td>-</td>
<td>424</td>
<td>71 000</td>
<td>118 000</td>
<td>2.5</td>
<td>70</td>
</tr>
<tr>
<td>5</td>
<td>PDL</td>
<td>toluene</td>
<td>109</td>
<td>26 000</td>
<td>33 000</td>
<td>2.5</td>
<td>&gt; 99</td>
</tr>
<tr>
<td>6</td>
<td>PDL</td>
<td>toluene</td>
<td>213</td>
<td>49 000</td>
<td>100 000</td>
<td>2.4</td>
<td>95</td>
</tr>
<tr>
<td>7</td>
<td>PDL</td>
<td>toluene</td>
<td>427</td>
<td>58 000</td>
<td>155 000</td>
<td>2.0</td>
<td>57</td>
</tr>
<tr>
<td>8</td>
<td>CL</td>
<td>toluene</td>
<td>520</td>
<td>59 000</td>
<td>36 000</td>
<td>1.5</td>
<td>&gt; 99</td>
</tr>
</tbody>
</table>

$^a$[Monomer]/[catalyst] $\times$ conversion $\times$ $M_n$(monomer). $^b$Determined by HT-SEC in TCB at 160 °C. $^c$Determined by $^1$H-NMR in CDCl$_3$ by comparison of the methylene peak adjacent to the ester group of the monomer ($\delta$ = 4.14 ppm) and the polymer ($\delta$ = 4.04 ppm).

The molecular weight of the polymers was analyzed by size exclusion chromatography (SEC). Due to its high crystallinity and hydrophobicity (polyethylene-like) PPDL has a very low solubility in common SEC solvents. Therefore the polymers were analyzed by high temperature SEC (160 °C) in 1,2,4-trichlorobenzene (TCB) (PS calibration). A clear shift of the SEC traces to higher molecular weight with an increasing monomer to catalyst ratio can be observed in Figure 5.1.

The measured number average molecular weights range from 24 kg/mol for the monomer to catalyst ratio of 44, to 118 kg/mol for the ratio of 424, respectively. Noticeable is that the molecular weight increases almost linearly with an increasing monomer to initiator ratio. However, without additional insights into the polymerization mechanism, any further discussion as to the control of the polymerization remains speculative. The polydispersity indices (PDI) of the obtained PPDLs are ranging from 2.1 to 2.8, which supports the expectation that the aluminum salen complex is a single-site catalyst. It also suggests the presence of transesterification reactions. Due to the high reaction rate, it is unclear at the moment whether these chain transfer reactions happen during the polymerization or at a later stage of the
reaction, i.e. at high monomer conversion when the chain mobility is reduced and less monomer is available for further chain extensions.

**Figure 5.1** High temperature SEC results of PPDL prepared by bulk polymerization with increasing monomer to catalyst ratio. The numbers refer to entries in Table 5.1.

In order to circumvent mass transport limitations a series of polymerizations has been carried out in solution (toluene) at monomer to catalyst ratios similar to the bulk reactions. While no complete gelation of the reaction medium was observed in these reactions, the viscosity again increased rapidly. The obtained molecular weights were even higher than for the bulk reactions at similar conversions. At the highest monomer to initiator ratio of 427 (Table 5.1, entry 7) an \( M_n \) of 155 kg/mol was obtained. Preliminary results of the ROP of smaller lactones such as \( \varepsilon \)-caprolactone, which produced a polymer with an \( M_n \) of 36 kg/mol under similar conditions, suggest that 1 might be universally applicable for the ROP of lactones irrespective of ring-size (Table 5.1, entry 8). Using aluminum trisisopropoxide or \( \text{Y[N(SiMe}_3)_2]_3 \) in the polymerization of PDL under the same conditions resulted in the formation of only low molecular weight products.

The molecular weights of PPDL obtained with 1 are unprecedented for the metal-catalyzed ROP of macrolactones and match the highest molecular weights reported by enzymatic polymerization. Obviously, these findings offer tremendous opportunities for the development of novel polymeric materials, as macrolactones are naturally occurring monomers and have already been investigated as biomaterials and for high-strength fiber applications.\(^{11,19}\)
Chapter 5

5.3.1. Influence of the catalytic structure

In addition to the polymerizations using complex 1 in combination with benzylalcohol as the catalytic system, other salen complexes were explored (Figure 5.2). A bulky substituent was placed on the phenyl ring to investigate the influence of steric hindrance at the catalyst on the polymerization rate. Secondly, complexes were pretreated with BnOH to form the active benzyloxy complex before the polymerization to investigate the instantaneous formation of the active species.

As can be observed in Figure 5.3, polymerization using complex 1 or 2 (Figure 5.2) resulted in complete conversion. The highest conversion reached using complex 4 was 87 % and 93 % using 2 (Table 5.2). To reach higher conversions using complex 4 longer reaction times are needed. An interesting feature of the polymerization using complex 4 was the induction period observed. Solubility problems normally cause such behavior, however, complex 4 was completely soluble in the reaction mixture. An explanation for this observation could not be found.

The relation between time and the logarithm of the relative concentration is linear for all complexes, implying a constant consumption of monomer and therefore first order kinetics (Figure 5.3, right). \( k_{app} \) was calculated from these results using Equation 3 (Table 5.2). Upon polymerization of PDL utilizing complex 2 with benzylalcohol as a co-initiator a slower reaction was observed in comparison with the use of 1, namely 0.01 min\(^{-1}\) versus 0.2 min\(^{-1}\) (Table 5.2, Figure 5.3). Complex 2 has four tertiary butyl groups on the phenyl rings of the salen ligand (Figure 5.2), which induces steric hindrance around the aluminum core.\(^{20}\) Theoretically this increases the energy barrier for the monomers to approach the core and therefore decreases the rate of the reaction substantially (Table 5.2). The difference between using complex 1 or 3
is small, which confirms the assumption that the active species is formed instantaneously. This confirms that in the polymerization of PDL the coordination-insertion mechanism is indeed followed. However, due to poor solubility only a small part of complex 3 was active in the polymerization, causing the relatively high molecular weight of the product.

![Graphs showing conversion and logarithm concentration over time](image)

**Figure 5.3** Conversion (left) and the logarithm of the relative concentration (right) vs. time plots of the polymerization of α-pentadecalactone using different salen complexes, [complex] = 0.16 M, [lactone]₀ = 1.5 M, T = 100 °C, t = 4 h. ● = 1, ▲ = 2, ♦ = 3, ■ = 4.

**Table 5.2** Results of the chemical ring opening polymerization of α-pentadecalactone using different complexes, [complex] = 0.16 M, [lactone]₀ = 1.5 M, T = 100 °C.

<table>
<thead>
<tr>
<th>Complex</th>
<th>(k_{\text{app}}) [min⁻¹]</th>
<th>(M_n) calc. [g/mol]</th>
<th>(M_n) (NMR) [g/mol]</th>
<th>(M_n) (SEC) [g/mol]</th>
<th>PDI</th>
<th>Conversion [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1c</td>
<td>0.20 ± 0.02</td>
<td>2700</td>
<td>3 900</td>
<td>8 100</td>
<td>1.7</td>
<td>98</td>
</tr>
<tr>
<td>2c</td>
<td>(10 ± 1)·10⁻³</td>
<td>2300</td>
<td>3 000</td>
<td>8 400</td>
<td>1.5</td>
<td>93</td>
</tr>
<tr>
<td>3d, e</td>
<td>(140 ± 6)·10⁻³</td>
<td>2700</td>
<td>8 000</td>
<td>17 000</td>
<td>1.6</td>
<td>99</td>
</tr>
<tr>
<td>4e</td>
<td>(89 ± 2)·10⁻⁴</td>
<td>2300</td>
<td>3 500</td>
<td>10 000</td>
<td>1.6</td>
<td>87</td>
</tr>
</tbody>
</table>

a/\([\text{Monomer}]/[\text{catalyst}] \times \text{conversion} \times M_n(\text{monomer})\); b/Determined by \(^1\text{H-NMR in CDCl}_3\) by comparison of the methylene peak adjacent to the ester group of the monomer and the polymer. c/\([\text{BnOH}]_0 = 0.16\ M\); d/Poor solubility of 3; e/\([\text{BnOH}]_0 = 0\ M\).

From complex 2 also a benzyl alkoxy derivative was prepared, complex 4. Complex 4 has a higher solubility in toluene than 2 and even at room temperature this complex completely dissolved. This has been observed earlier while comparing the solubility of complexes 1 and 2. It seems that the benzyloxy derivatives have a better solubility in toluene than their alkyl analogues. Complex 4 showed low polymerization rates due to the steric hindrance in this...
complex. Comparing polymerization using complex 2 and 4, it can be observed that the reaction rates are the same.

All plots showing the development of $M_n$ with conversion (Figure 5.4) follow approximately the same trend, with a slow start (except 2). This means that not all active centers have initiated polymer chains. Up to 80 – 90 % conversion all curves tend to follow the ideal living behavior, which results in a linear relationship between $M_n$ and conversion. After 80 – 90 %, chain coupling starts to occur and the molecular weight increases very rapidly. Larger cyclic structures which were formed at earlier stages also may start participating in the polymerization. This deviation from the ideal curve was not observed in reactions using complex 2 or 4, since total conversion did not exceed 80 %.

![Figure 5.4 $M_n$ versus the conversion for PDL polymerized with different complexes, ● 1, ▲ 2, ● 3, ■ 4.]

### 5.4 Kinetics of the ring opening polymerization of various lactones

To study the influence of the ring size of the lactone polymerized, a kinetic study with various ring sizes was performed. All polymerizations were done under the same conditions (100 °C under inert atmosphere) and the same catalyst: initiator: monomer ratio of 1:1:100 was applied. All polymerizations were carried out using complex 1 with BnOH as the catalytic system and toluene as solvent. The polymerizations were performed in a carrousel reactor using 5 mL crimp cap vials. All reactions were run for 4 hours, except the polymerization of γ-valerolactone, which was followed over 96 h. Due to the viscosity of most of the reaction mixtures, the samples were drawn form different vials. This can potentially cause an error in the
concentration of all reactants. Since the amount of catalyst used is very small, the errors can be quite large. Therefore $k_{\text{app}}$ is given in Table 5.3 and no values for $k_p$ are reported.

All results are summarized in Table 5.3. Due to low solubility of the polymers based on larger lactones in THF, all SEC measurements were performed in TCB at 160 °C. The complex is virtually unreactive towards polymerization of $\beta$-butyrolactone ($\beta$-BL). The conversion did not exceed 3 % and only low molecular weight products ($M_n = 850 \text{ g/mol}$) were obtained. As expected based on the thermodynamic stability of the 5-membered ring, no polymerization of $\gamma$-butyrolactone ($\gamma$-BL) was observed after 96 h.\textsuperscript{21}

### Table 5.3 Characterization results of all different lactones. ([Complex]$ = 15 \text{ mM}$, [Lactone]$ = 1.5 \text{ M}$, $T = 100 ^{\circ}\text{C}$, $\beta$-BL = $\beta$-butyrolactone, $\gamma$-BL = $\gamma$-butyrolactone, VL = $\delta$-valerolactone, CL = $\epsilon$-caprolactone, DL = 10-decanolactone, UL = 11-undecanolactone, PDL = $\omega$-pentadecalactone, HDL = 16-hexadecalactone.

| Monomer | Ring size | $k_{\text{app}}$ [min$^{-1}$] | $M_n$ calc. [g/mol]$^a$ | $M_n$ [g/mol]$^b$ | PDI | Conversion [%]$^c$
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$-BL</td>
<td>4</td>
<td>-</td>
<td>8 600</td>
<td>850</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>$\gamma$-BL</td>
<td>5</td>
<td>-</td>
<td>8 600</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VL</td>
<td>6</td>
<td>0.16 ± 0.01</td>
<td>10 000</td>
<td>10 000</td>
<td>2.1</td>
<td>96</td>
</tr>
<tr>
<td>CL</td>
<td>7</td>
<td>0.25 ± 0.03</td>
<td>11 400</td>
<td>13 000</td>
<td>2.3</td>
<td>&gt; 99</td>
</tr>
<tr>
<td>DL</td>
<td>11</td>
<td>(30 ± 4)×10$^{-3}$</td>
<td>17 000</td>
<td>24 000</td>
<td>1.7</td>
<td>84</td>
</tr>
<tr>
<td>UL</td>
<td>12</td>
<td>(10 ± 2)×10$^{-3}$</td>
<td>18 400</td>
<td>27 000</td>
<td>1.6</td>
<td>91</td>
</tr>
<tr>
<td>PDL</td>
<td>16</td>
<td>(30 ± 2)×10$^{-3}$</td>
<td>24 000</td>
<td>36 000</td>
<td>1.6</td>
<td>90</td>
</tr>
<tr>
<td>HDL</td>
<td>17</td>
<td>(40 ± 5)×10$^{-3}$</td>
<td>25 600</td>
<td>40 000</td>
<td>1.8</td>
<td>98</td>
</tr>
</tbody>
</table>

$^a$[monomer]/[catalyst] × conversion × $M_w$(monomer). $^b$Measured by SEC in 1,2,4-trichlorobenzene at 160 °C. $^c$Determined by $^1$H-NMR in CDCl$_3$ by comparison of the methylene peak adjacent to the ester group of the monomer ($\delta = 4.14$ ppm) and the polymer ($\delta = 4.04$ ppm).

The smaller sized lactones, $\delta$-valerolactone (VL) and $\epsilon$-caprolactone (CL), have higher rate constants compared to the larger lactones (Table 5.3 and Figure 5.5), which can be explained by either the high ring strain\textsuperscript{22} or by the energetically high cisoid conformation in the 6- and 7-membered lactones or both.\textsuperscript{22} However, the rate difference with macrolactones is not as large as using the zinc 2-ethylhexanoate/butyl alcohol system shown by Kobayashi et al.\textsuperscript{2} Molecular weights obtained are in good agreement with the calculated values (Table 5.3). Polydispersities of 2.1 and 2.3, respectively, are quite broad for a living system, which is probably due to transesterification reactions near complete conversion.
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10-Decanolactone (DL) and 11-undecanolactone (UL), the middle sized lactones show the lowest polymerization rates of all polymerizable lactones, namely 0.03 and 0.01 min\(^{-1}\), respectively. It has been known that no ring strain and therefore no driving force is present in these larger lactones.\(^{22}\) The same trend was observed in the hydrolysis rate of these lactones, these middle-sized lactones hydrolyze slower than the rest of the lactones.\(^{23-25}\) Although overestimation is known for these polymers when measured to polystyrene standards, remarkably high molecular weights are obtained. Polydispersities are somewhat lower than the ones obtained for the smaller lactones, which is probably due to the lower conversions.

The larger lactones show a somewhat higher polymerization rate than the middle sized lactones. Despite the high conversions obtained within an hour (98 % for HDL) the polydispersities are lower than 2.

The development of the molecular weight of the formed polymer was followed in time (Figure 5.5). For all polymerizations the molecular weight increased rapidly at the beginning of the reaction and only minor increase was observed when the conversion is constant. Polydispersities of all lactones increased upon increase in conversion. Two reasons can be found for this, [1] not all chains are initiated at the start of the reaction. [2] Side reactions such as transesterification can occur.

![Figure 5.5](image)

**Figure 5.5** Left: Evolution of molecular weight with polymerization time of all different lactones. Right: Kinetic plots of the relative monomer concentration versus time, ■ = VL, ○ = CL, ▲ = DL, ▼ = UL, □ = PDL, ▶ = HDL

As can be seen in Figure 5.5, the relation between the logarithm of the relative concentration of the monomer and time are linear up to 20 min, what means during the start of the reaction, it is first order in monomer. Deviation from this linear curve is observed for VL and CL. This can be explained by the fact that almost full conversion was reached rapidly and
agitation of the reaction mixture was troublesome afterwards. Inhomogeneity of the samples was observed for all polymers due to the increase in viscosity of the reaction mixture. All polymerizations were done at a monomer concentration of 1.5 M, what can cause increase in viscosity upon reaching full conversion. Therefore the reaction rate constants reported in Table 5.3 have been calculated from data up to 20 minutes of reaction time. Polydispersities of all reactions increased during all polymerizations, starting from ~ 1.4 to ~ 2.0, which indicates transesterification during reaction.

When comparing all lactones, the same trends can be observed as in the zinc 2-ethylhexanoate/butyl alcohol system and other catalytic systems. The middle-sized lactones are the slowest, the macrolactones follow and the smaller lactones polymerize the fastest. When using ethylhexanoate and butyl alcohol, VL polymerizes fastest. The slowest lactones are PDL and UL, which polymerize a factor 2 500 slower than VL. The differences in polymerization rate constants using the salen complex are not as pronounced as with the ethylhexanoate and butyl alcohol system. The difference between the slowest (UL) and the fastest (CL) is only a factor 25. The salen complex polymerized all tested lactones to a reasonable extent on a short timescale. That makes the catalytic system used in this study widely applicable in ring opening polymerization of lactones.

To obtain information about the end groups and the possible formation of cyclic structures polymerizations up to low molecular weight (catalyst:initiator:monomer ratio: 1:1:10) were performed. Samples were analyzed by MALDI-tof-MS.

![Figure 5.6 MALDI-tof-MS spectrum of UL. Left: spectrum of UL. Right: zoomed in on mass 1300. 1 represents the distribution of the linear structures and 2 the distribution of the cyclic structures.](image)
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In Figure 5.6 on the left, the distribution of the linear polymer of 11-undecanolactone (M = 184) \((N \times 184 + 1 + 107 + 39)\) can be seen. A hydroxyl (M = 1) and benzyl alkoxy end group (M = 107) are present in these chains and potassium (M = 39) is the counter ion. On the low mass side of the spectrum in Figure 5.6 a decreasing Schultz-Flory type distribution of the cyclic oligomer can be observed (see also 2 in Figure 5.6 right). These are the only two distributions seen in MALDI-tof-MS spectra of all monomers. These cyclics were found up to masses of around 2500 – 3000 g/mol. Not all polymers show a Gaussian shaped distribution, due to the ionization difficulties for higher molecular weight polyesters. Acid end groups were not observed. Although it is known that these molecules are difficult to detect with MALDI-tof-MS, minor peaks would be expected.\(^{26}\) This implies that water initiation is only taking place in very low amounts.

5.5 Conclusions

Aluminum salen complexes with different phenolic substituents were synthesized and their activity towards \(\omega\)-pentadecalactone polymerization was screened. The influence of the bulky side groups is clearly visible in the reaction rate, which lowers a factor ten when tert-butyl groups are introduced on the salen ligand. However, still high conversion is obtained within 4 hours.

The aluminum salen complex based on the unsubstituted salen ligand was screened for its activity in ring opening polymerization of various sized lactones. In combination with benzyl alcohol this complex provides a single-site catalyst system that is widely applicable in ring opening polymerizations of lactones of ring sizes > 5. It catalyzes polymerizations to high conversions on a reasonable timescale. However, the smaller lactones are polymerized fastest, followed by the macrolactones. Although the middle-sized lactones show the lowest polymerization rates as expected, the observed rates are unprecedented and exceed any catalytic or enzymatic system known to date. Due to the broad substrate range that is polymerizable with this aluminum salen complex a large platform of different copolymers based on lactones can be synthesized rapidly and to high conversions.\(^{2,6}\) The livingness of the system opens opportunities for producing block copolymers and other architectures, however, transesterification reactions take place.
5.6 Experimentalss

5.6.1 Materials

\(\gamma\)-Butyrolactone, \(\beta\)-butyrolactone, \(\omega\)-pentadecalactone, 16-hexadecalactone, \(\varepsilon\) caprolactone, \(\delta\)-valerolactone, 11-undecanolactone, mesitylene and benzylalcohol were purchased from Aldrich. 10-Decanolactone was synthesized following a literature procedure. All monomers, mesitylene and benzylalcohol were distilled before use. Toluene and 1,2,4-trichlorobenzene were purchased from Biosolve. Toluene was dried over an alumina column prior to use. The aluminum salen complexes were synthesized following literature procedures.

5.6.2 Methods

\(^1\)H and \(^{13}\)C NMR spectroscopy was performed on a Varian Mercury 400 MHz NMR in CDCl₃. Data was acquired using VNMR software. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS). Low molecular weight size exclusion chromatography (LMW-SEC) was performed on a Waters Alliance system equipped with a Waters 2695 separation module, a Waters 2414 refractive index detector (40 °C), a Waters 2486 UV detector and a Polymer Laboratories PLgel guard column (5 mm particles) 50 × 7.5 mm, followed by 2 PLgel 5 mm Mixed-D columns in series at 40 °C. Size Exclusion Chromatography (SEC) was measured on a Waters Alliance system equipped with a Waters 2695 separation module, a Waters 2414 refractive index detector (40 °C), a Waters 2487 dual absorbance detector and a PSS SDV 5 m guard column followed by 2 PSS SDV linearXL columns in series of 5 m (8 × 300) at 40 °C. Tetrahydrofuran (THF, Biosolve), stabilized with BHT, was used as eluent for LMW-SEC and SEC at a flow rate of 1 mL/min. The molecular weights were calculated with respect to polystyrene standards (Polymer Laboratories, \(M_p = 580\) up to \(M_p = 7.1 \times 10^6\) g/mol). Before analysis was performed, the samples were filtered through a 0.2 μm PTFE filter (13 mm, PP housing, Alltech). High temperature Size Exclusion Chromatography (HT-SEC) was performed on a Polymer Laboratories PLXT-20 Rapid GPC Polymer Analysis System (including pump, refractive index detector and viscosity detector) at 160 °C with 3 PLgel Olexis (300 × 7.5 mm, Polymer Laboratories) columns in series. 1,2,4-Trichlorobenzene was used as eluent at a flow rate of 1 mL/min. The molecular weights were calculated with respect to polystyrene standards (Polymer
Laboratories, $M_p = 580$ up to $M_p = 7.1 \times 10^6$ g/mol). A Polymer Laboratories PL XT-220 robotic sample handling system was used as autosampler. MALDI-tof-MS was performed on a PerSeptive Biosystem Voyager-DE STR Biospectrometry-Workstation in positive reflector mode. An acceleration voltage of 20000 V, a grid of 63.2 % and a delay time of 320 ns and 1000 shots per spectrum were used. Trans-2-[3-(4-tert-butylphenyl)-2-methyl-propenylinden]-malononitril ≥ 99 % from Fluka was used as a matrix and potassium trifluoracetate was used as a salt in a ratio salt:matrix:sample of 1:4:4. For sample preparation all PPDL samples were dissolved in HFIP and all other samples in THF.

### 5.6.3 Chemical ring opening polymerization of \(\omega\)-pentadecalactone

\(\omega\)-Pentadecalactone (1.0 g; 4.2 mmol), aluminum salen catalyst and benzylalcohol (co-initiator) were added to a vial under nitrogen atmosphere. Benzyl alcohol was only added when complex 1 or 3 were used. The molar ratio of benzyl alcohol to the catalyst was kept constant at 1:1, while the monomer to initiator ratio was varied from 44 to 520. The vial was then closed and stirred at 100 °C for 4 h. For the reactions in solution, toluene (2 mL) was added to the polymerizations prior to heating. After the reaction the mixture was cooled in an ice bath and the solvent was evaporated. The products were analyzed without further precipitation.

### 5.6.4 Kinetic experiments

Monomer (1 mmol) and aluminum salen catalyst (10 \(\mu\)mol) were added in a 5 mL crimp cap vial in a glovebox under \(N_2\) atmosphere. Eight samples were made per polymerization reaction. The samples were taken out of the glovebox and 0.25 mL stock solution containing BnOH in a concentration of 40 \(\mu\)mol/mL in toluene was added. A ratio monomer:catalyst:BnOH of 100:1:1 was obtained and a monomer concentration of 4 mol/L. The vials were put in a carrousel reactor preheated to 100 °C and the samples were quenched with 4 mL of cold methanol at predetermined times. The samples were dried to the air at room temperature prior to analysis. All samples were analyzed with GC, HT-SEC and \(^1\)H-NMR.

**Poly(\(\delta\)-valerolactone):** \(^1\)H-NMR (CDCl\(_3\)): \(\delta\) (ppm) = 7.38 (s, 5H, Ph-\(\text{H}\)), 5.08 (s, 2H, Ph-CH\(_2\)-O), 4.07 (t, 2H, CH\(_2\)-O), 2.34 (t, 2H, CH\(_2\)C=O), 1.62 (m, 4H, CH\(_2\)-CH\(_2\)-O).
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5.6.3 Chemical ring opening polymerization of ω-pentadecalactone (1.0 g; 4.2 mmol), aluminum salen catalyst and benzylalcohol (co-
or 3 were used. The molar ratio of benzyl alcohol to the catalyst was kept constant at 1:1, while the monomer to initiato r ratio was varied from 44 to 520. The vial was then closed and stirred at 100 °C for 4 h. For the reactions in solution, toluene (2 mL) was added to the polymerizations prior to heating. After the reaction the mixture was cooled in an ice bath and the solvent was evaporated. The products were analyzed without further added to analysis. All samples were analyzed with GC, HT-SEC and 1H-NMR.

5.6.4 Kinetic experiments

Precipitation of 1 initiator) were added to a vial under nitrogen atmosphere. Benzyl alcohol was only added when complex 1

The samples were taken out of the glovebox and 0.25 mL stock solution containing 0.25 mL methanol at predetermined times. The samples were dried to the air at room temperature prior carrousel reactor preheated to 100 °C and the samples were quenched with 4 mL of cold methanol from Fluka was used as a matrix and potassium trifluoracetate was used as a salt in a ratio of 100:1:1 was obtained and a monomer concentration of 4 mol/L. The vials were put in a catalyst:matrix:sample of 1:4:4. For sample preparation all PPDL samples were dissolved in HFIP and methanol.

All other samples in THF.

Poly(ω-pentadecalactone): 1H-NMR (CDCl3): δ (ppm) = 7.35 (s, 5H, Ph- H), 5.10 (s, 2H, Ph-CH2-O), 4.06 (t, 2H, CH2-O), 2.28 (t, 2H, CH2-C=O), 1.60 (m, 4H, CH2-CH2-O), 1.27 (m, 12H, CH2-CH2).

Poly(10-decanolactone): 1H-NMR (CDCl3): δ (ppm) = 7.38 (s, 5H, Ph- H), 5.08 (s, 2H, Ph-CH2-O), 4.04 (t, 2H, CH2-O), 2.31 (t, 2H, CH2C=O), 1.66 (m, 4H, CH2-CH2-O), 1.37 (m, 2H, CH2-CH2).

Poly(16-hexadecalactone): 1H-NMR (CDCl3): δ (ppm) = 7.34 (s, 5H, Ph-H), 5.09 (s, 2H, Ph-CH2-O), 4.05 (t, 2H, CH2-O), 2.28 (t, 2H, CH2C=O), 1.60 (m, 4H, CH2-CH2-O), 1.23 (m, 22H, CH2-CH2).

5.7 References

Chapter 5

POROUS MATERIALS
6.1 Introduction

In order to make a polymer scaffold suitable for nerve repair, the material should contain an interconnected network of pores. In the ideal case, an axon should be able to grow from one end of the tube to the other without being blocked by scaffold material (Figure 6.1).

![Figure 6.1 Regrowth of the nerve supported by a nerve conduit. The nerve end can grow through the scaffold from one end to the other.](image)

The pore size should be in between 20 – 80 μm. Such a scaffold can be realized in different ways: a random porous scaffold can be formed, a template can be used or porous channels can be made through the material. In this chapter the fabrication of random porous scaffolds by leaching techniques will be discussed. Biocompatibility is tested and the degradation of these scaffolds is followed over time and compared to the solid cross-linked materials. Here we focus on a nerve repair application, however, these porous scaffolds can be used in many different tissue engineering applications.

6.2 Manufacturing of porous materials

Several techniques are currently employed to manufacture porous scaffolds. These include among others, particulate leaching, exploring gases as porogens, temperature induced phase separation (TIPS), fiber spinning and templating.

Particulate leaching is a very straightforward technique based on differences in solubility of the components used. Salt and sugar are two of the widest used porogens in particulate leaching. The polymer solution is mixed with the porogen after which the mixture is lyophilized.
In order to make a polymer scaffold suitable for nerve repair, the material should contain an interconnected network of pores. In the ideal case, an axon should be able to grow from one end of the tube to the other without being blocked by scaffold material (Figure 6.1).

The pore size should be between 20 – 80 μm. Such a scaffold can be realized in different ways: a random porous scaffold can be formed, a template can be used or porous channels can be made through the material. In this chapter, the fabrication of random porous scaffolds by leaching techniques will be discussed. Biocompatibility is tested and the degradation of these scaffolds is followed over time and compared to the solid cross-linked materials. Here we focus on a nerve repair application, however, these porous scaffolds can be used in many different tissue engineering applications.

Several techniques are currently employed to manufacture porous scaffolds. These include, among others, particulate leaching, exploring gases as porogens, temperature induced phase separation (TIPS), fiber spinning and templating. Particulate leaching is a very straightforward technique based on differences in solubility of the components used. Salt and sugar are two of the most widely used porogens in particulate leaching. The polymer solution is mixed with the porogen after which the mixture is lyophilized. Afterwards, the porogen is leached out by a good solvent for the porogen and a non-solvent for the polymeric scaffold. By altering the crystal size and the porogen weight fraction, the porous structure can be designed. Not only solids can be utilized as particulate, also other polymers can be used, such as PEG for example in a system where the polymer matrix is not soluble in water. Moreover, gasses can be used as porogen. Supercritical carbon dioxide (scCO₂) is explored in the fabrication of porous scaffolds. In that case, a polymer sample is exposed to high pressure gas to saturate the sample. Afterwards, the gas pressure is slowly decreased causing nucleation and pore formation in the sample. One drawback is that the pore size is inhomogeneous. Scandola et al. explored foaming of semi-crystalline polymers. Poly(ω-pentadecalactone-co-ε-caprolactone) (69/31) foams were obtained with porosities up to 72%.

Freeze drying is one of the most widely used TIPS processes. Phase separation occurs when a homogeneous polymer solution is quenched. Depending on whether the temperature of quenching (T𝑞) is above or below the critical temperature (T𝑐), solid-liquid (T𝑞 > T𝑐) or liquid-liquid (T𝑞 < T𝑐) phase separation occurs. The phase separation mechanism has a direct effect on the morphology of the scaffold.

Spinning of fibers to manufacture woven or knitted three-dimensional meshes creates a large surface area for cell attachment. The highly porous structure makes rapid diffusion of nutrients and waste possible. Melt-, solution- or electro-spinning are used as casting techniques.

Different patterning methods are an alternative approach towards scaffolds. This technique provides very high control over the 3D structure of the scaffold. Some examples are layer-by-layer microfluidic patterning, 3D printing or microsyringe deposition. In this investigation, particulate leaching is used to create porous structures.

### 6.3 Results

#### 6.3.1 Synthesis and characterization

Sugar, poly(ethylene glycol) (PEG) and different Pluronics® were used as porogen to make porous structures. Sugar was ground to a crystal size of > 125 μm. Various concentrations of sugar were added to polyambrettolide, ranging from 1 to 10 grams of sugar per gram of
polymer. The porogen was added to a concentrated unsaturated polyester solution in tetrahydrofuran. Adding more than 10 grams of sugar per gram of polymer resulted in disintegration of the scaffold.

Figure 6.1 Scanning electron microscopy pictures from porous polyambrettolide networks made in different manners. Top left: 1 w/w-% of sugar; top right: 10 w/w-% of sugar; bottom left: 0.5 w/w-% of PEG 35 kg/mol; bottom right: 0.5 w/w-% PEG 600 kg/mol.

All samples were UV-cured in the presence of a dithiol and 4-hydroxybenzophenone as an initiator. Due to the opaque character of the mixture, curing was performed using longer exposure times. The pore size and pore size distribution were analyzed with scanning electron microscopy (SEM). When water was added, swelling of the scaffolds was observed. The best results were obtained by adding a tenfold of sugar with respect to PAm (Figure 6.2, top right).
This provided the most open structure. However, interconnection of the pores is not easy to determine. Moreover, the pore size of these pores is difficult to determine due to the irregular shape of the pores.

Particulate leaching using PEG of different molecular weights (35 kg/mol and 600 kg/mol) using different ratios of porogen/polymer (ranging from 0.5 – 8) resulted in molecular mixing of the porogen with the polymer. Micropores instead of the desired macropores were formed (Figure 6.2, bottom left).

To obtain phase separation block copolymers based on poly(ethylene oxide) and poly(propylene oxide) (Pluronic®) with different masses were used. Three different types of Pluronic® were used, RPE 1740, RPE 2525 and PE 10100. The RPE types are PPO-PEO-PPO block-copolymers. The PE type is a PEO-PPO-PEO block-copolymer. Only RPE 1740 is water soluble, however, all can be dissolved in ethanol. Therefore ethanol was used as a solvent to leach out the block-copolymers. However, no porous structures were obtained. Instead, fast phase separation was observed after mixing.

![Figure 6.3 SEM results of porous scaffolds from P(Am-co-DXO) (50/50) prepared by sugar leaching (porogen = 10 w/w-%).](image)

After determination of the best leaching method, using sugar as porogen, degradable scaffolds were prepared with P(Am-co-DXO) (50/50). These scaffolds were prepared in the same way as for the homopolymer. Analyzing the scaffold with SEM revealed the open structure of the scaffold (Figure 6.3). In the left micrograph in Figure 6.3 it can be observed that the top part of the scaffold has a more dense structure than the inside, what would be ideal for
nerve guidance channels. The enlargement (Figure 6.3, right) shows that a continuous porous network is formed in the interior of the material.

6.3.2 Biocompatibility

During construction of porous scaffolds a dithiol and a UV initiator were used to cross-link the material. After curing the structures were put in THF for 4 hours to leach out the unreacted dithiol and residual initiator. Afterwards the materials were put in water during several days to leach out the porogen. However, it cannot be excluded that some unreacted agents remain in the material, which could have an influence on the biocompatibility. Therefore cytotoxicity tests were performed on the porous materials and on the cross-linked homopolymer. These tests were performed in the same manner as with the uncured polymers (see Chapter 3). The data in Figure 6.4 indicate that the latex extracts are highly toxic to the cells as reflected by strongly decreased metabolic cell activity. These values are a read-out of the metabolism measured against a blanco (medium only). The cells in culture medium show an optical density of 1.2, which is a reference value for metabolism under non-toxic conditions. Unfortunately both materials tested (the porous copolymer and the cured PAm) show significant reductions in metabolism of 3T3 cell cultures as measured by the MTT assay. This is evidenced by a
significantly lower optical density when comparing with the reference value. The porous scaffold was found to be less toxic than the cured homopolymer. The latter was cured via the thermal method while the porous material via UV-curing. Keeping in mind that the porous polymer is a copolymer and the solid material a homopolymer, UV-curing seems to result in lower toxicity than the thermal method. Due to the low toxicity of the uncured polymer, the curing step must be responsible for the toxicity. One possibility is that despite the leaching in THF, there are still rests of curing agents in the scaffolds. Another explanation might be the toxicity of the scaffold itself. By introducing thiol ethers in the polymer, toxicity may be introduced. To obtain further insight into the biocompatibility, cytotoxicity of the UV initiator and the di-thiol should be investigated.

6.3.3 Degradation

![Cumulative weight loss vs. Time graph](Figure 6.5)

Figure 6.5 Degradation of P(Am-co-DXO) (50:50). Experiments were performed in duplo, the mean and the error bars are shown. ■ porous material, • solid polymer.

Enzymatic degradation of aliphatic polyesters is determined by the rate of surface erosion. Therefore the surface per unit volume has a large influence on the degradation rate. By creating porous materials this surface area per unit volume is increased significantly. Degradation experiments were conducted by putting 100 mg of P(Am-co-DXO) (50/50) scaffold in Phosphate Buffered Saline (PBS) with 0.1 mg/mL Lipase PS and a pH of 7.4. Swelling of the material was observed and degradation was faster compared to the solid polymer (Figure 6.5).
Within 100 days the solid material showed only 30 % degradation, whereas 30 % of the porous material degraded in 55 days. The degradation curves both seem linear. Although only 40 % degradation is obtained within 70 days, it is expected that full degradation can be reached.

6.4 Conclusions

Different porogens were tested in the construction of permeable polyester scaffolds. Sugar was found to give the best results. Varying the ratio sugar/polymer, different porosities were obtained. However, for creating an interconnected porous network at least a ratio of 10:1 has to be used. One drawback of using sugar as porogen is that sugar crystals are opaque, what blocks the UV-light. Curing of thick samples can thus be troublesome. Using PEG resulted in molecular mixing of the two polymers and Pluronic® block-copolymers show fast phase separation upon mixing with the polyester.

The biocompatibility of these scaffolds is found to be low. Further investigation is needed to check whether the curing agents are completely removed from the material. A second option can be that the cross-linked material is toxic itself. Introducing thiol groups into the network can have a negative influence on the cytotoxicity.

Degradation of a porous scaffold made from P(Am-co-DXO) (50/50) was found to be faster than the solid material. However, degradation is still slow for the application as nerve guide tubes.

6.5 Outlook

In this chapter, it is described how a degradable porous polyester scaffold can be made. However, there are many aspects which still need improvement. First of all a leaching method needs to be standardized in order to make reproducible batches of the porous material. Furthermore the relation between porosity, cross-link density and degradation behavior needs to be investigated. Different amounts of dithiol can be mixed with the polymer and the porogen, to tune the cross-link density. Afterwards degradation can be studied over time. Leaching of these curing agents should be investigated further. Materials remain toxic after leaching. Incorporating less cross-link agent may create a more open network and the toxicity may decrease.
The degradability of the materials needs to be enhanced further. In order to make these materials suitable for nerve guides, degradation should be complete within 2 – 3 months. This can be done by increasing the DXO content in the copolymer. Moreover, the material properties of the formed networks will need to be investigated.

To make these porous materials bioactive, different peptide sequences can be grafted from the polymer backbone. These can promote cell growth in the pores. Small sequences as IGAV (Isoleucine – Glycine – Valine – Alanine – Valine) and RGD (Arginine – Glycine – Aspartic Acid) are known to promote cell growth.

### 6.6 Experimentals

#### 6.6.1 Materials

P(Am-co-DXO) was synthesized via the same route as described for all other copolymers in Chapter 4. PEG of different masses were purchased from Aldrich. Sugar was purchased from Van Gilze and grinded to < 125 μm particles using a microgrinder purchased from IKA and a sieve with a threshold of 125 μm. THF was purchased from Biosolve and used without further purification. 4-hydroxybenzophenone was purchased from Fluka and ethylene glycol bis(3-mercaptopropionate) was purchased from Wako.

#### 6.6.2 Methods

Scanning electron microscopy was performed on a FEI Quanta 3D FEG in the high vacuum mode. Samples were coated with gold before measurements.

#### 6.6.3 General procedure for obtaining porous materials

Porous structures were obtained by dissolving 0.5 g of polymer in a minimal amount of THF. After dissolving the polymer, the predetermined amount of porogen, dithiol and 4-hydroxybenzophenone were added to the solution and radiated with UV light using a Philips HPR-125 mercury discharge lamp with an output of 125 W/ 1.15 A. After curing the samples
were put in THF for 4 hours and dried under vacuum. A second leaching step was performed in distilled water for 3 days.

### 6.6.4 Biocompatibility tests

Extracts of the polymers were made by incubating them (0.1 g/mL) for 48 h in cell culture medium (DMEM/NUT mix F12 with glutamax-1 supplemented with 10 % inactivated fetal calf serum and 100 U/mL penicillin and 100 μg/mL streptomycin) at 37 °C/5 % CO₂. These extracts were added to cultures of a mouse fibroblast 3T3 cell line, which had been plated 18 h before. Cell cultures were subsequently maintained for 4 days after which an MTT assay was performed to assess the metabolic activity of the cell cultures. Next to the biomaterial extracts, which were used undiluted, 1:1 diluted, and 1:3 diluted, normal cell culture medium (as a positive control for ‘normal’ metabolic activity of cells) and latex extract (48h incubation of 0.1 g latex per mL culture medium), which served as a negative control for strongly decreased metabolic activity in a toxic environment) were used in these cytotoxicity experiments. In addition, all tests were performed on two different plating densities of cells (10K and 5K 3T3 cells per well in a 96-wells plate).

**MTT assay**

The cell cultures were incubated with 0.5 mg/mL MTT for 90 minutes. Endogenous enzymes in the cells metabolize MTT thereby producing blue formazan crystals, which can be measured by spectrometry using a wavelength of 570 nm. The values regarding this test are calculated as the optical density of the tested cell culture minus the optical density of a well with cell culture medium but no cells (background). Every condition (3T3 cell culture with respective conditions; cell culture medium only or extracts of undiluted polymer, 1:1 diluted polymer, or 1:3 diluted polymer) was tested two-fold (in duplo). The metabolic activity of the cell cultures under the various conditions was statistically compared using a one-way analysis of variance (ANOVA) with Bonferroni post-hoc correction.

### 6.6.5 Enzymatic degradation in PBS

100 mg of porous P(Am-co-DXO) was added to 4 mL of 0.1 M PBS (pH = 7.4) containing 0.1 mg/mL Lipase PS. Ten samples were prepared and put in a Grant GLS400 shaking bath at 37
°C. Every week one sample was analyzed and the rest of the samples were provided with new buffer/enzyme solution. The weight loss of every sample was measured.

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TECHNOLOGY ASSESSMENT
7.1 Biomedical materials: From lab bench materials to final products

Biomaterials must meet many requirements, and before implantation in humans, years of research and development are needed. It can take up to a decade from a lab bench design to the actual use in the hospitals.\textsuperscript{1} During this time several stages can be distinguished as shown in Figure 7.1. This chapter tries to put the materials and processes used in this thesis into the perspective of this flow chart. The aim is to provide a critical evaluation and positioning of the results obtained in this thesis in the bigger scheme of commercial biomedical materials. Obviously this was done with limited knowledge of the biomedical market and from the perspective of a polymer scientist.

![Diagram of development process](image)

**Figure 7.1** Development process for a new biomedical device or material.\textsuperscript{1}

### 7.1.1 Designing product concept and investigation of market need

The first step should contain a laborious study of the market that the product is aimed for. Basically all better and cheaper materials are very welcome on the medical market, due to the fact that many materials used nowadays have some drawbacks. Polymers as implants and other medical devices were only introduced in the last century, which leaves much room for improvement by implementing new developments in the field. After the market has been
investigated, first a product should be designed. With designing this concept one should take all
material demands into account (e.g. biocompatibility, morphology, stability, degradability etc.).

**Position of this thesis**

There is a clear need for better nerve guide tubes because nowadays the standard is to use
autologous nerve grafts from somewhere else in the human body. Often the sensory nerves
from the leg are used. This means that at the donor place loss of functionality occurs and
instead of only one incision, two sites of surgery are created. Therefore, longer recovery periods
are needed.

The design criteria for the materials choice were driven by the fact that polymers from
(unsaturated) macrolactones are easily accessible by metal-free enzymatic polymerization, have
good mechanical properties and the degradation products resemble natural fatty acids. The
materials are also processible into porous scaffolds and ideally provide opportunities to
introduce growth factors and other nutrients by using the possible unsaturation in the main
chain of the polymer.

### 7.1.2 Lab bench prototype development

After designing the concept, the first materials can be made. This step includes selecting
the right monomers, polymerization method and possible post-polymerization modifications.
Here it has to be taken into account which supporting chemicals are used in the process of
synthesizing the products as these should be completely removable or biocompatible. The use
of organic solvents should be considered wisely. In this step the prototype should be tested on
performance and an evaluation of the material properties should be done.

**Position of this thesis**

Polymers from macrolactones could be obtained utilizing a biocompatible synthesis route
avoiding any metal catalyst contamination. Using Novozym 435 as a catalyst in ring opening
polymerization of lactones, a variety of polymers based on macrolactones could be obtained. By
selecting the proper monomers, copolymers can be obtained for which the properties, e.g.
hydrophilicity, crystallinity, degradation and mechanical properties could be tuned. Moreover,
by choosing unsaturated macrolactones as comonomers it was possible to cross-link and process
the obtained polyesters into porous structures. The results obtained in this thesis confirm that
these materials almost fulfill these design criteria set out at the beginning of the work. One point of attention is the biocompatibility after curing of the polymers. All base polymers were tested to be biocompatible, however, curing these materials led to introducing toxicity, most probably related to the curing agents.

### 7.1.3 Production on factory scale

Synthesis on lab-scale can vary significantly from production on factory scale. Heat development and mixing are two examples of factors that should be taken into account. For many materials the best way to up-scale the synthesis is to design a continuous process. On the other hand, medical materials are not bulk polymers but specialty polymers, which means that the volumes are lower and the prices of these materials can be significantly higher. Under these presumptions a batch process is economically more feasible. However, the different batches must be of constant quality.

**Position of this thesis**

To be able to design a proper production process for these nerve guide tubes (NGTs), the production volume must be determined. However, estimation of the production volume per year is not straightforward. It is difficult to determine the actual number of nerve repair operations done per year only in the Netherlands. In an annual report of the department of neurosurgery of the VU medisch centrum in 2002, an overview is given on the operations done in the years 1998 – 2002. On average there are 100 operations on peripheral nerve repair per year. These operations are performed by neurosurgeons in all hospitals over the country. There are 93 general hospitals in the Netherlands. Assuming that these numbers are the same for all hospitals, it is estimated that ca. 93 000 operations are done in the Netherlands every year. For one tube 0.1 g of polymer is needed. For the Netherlands this means a production of 9.3 kg of polymer per year. Since this quantity is not high, a batch process would be most suitable.

However, nerve repair in humans is not the only application of these NGTs. These tubes can be used as a standard in clinical trials of other biomedical materials. Upon clinical testing multiple test groups are considered, namely a control group, a standard group and the group consisting of the tested material itself. The standard used nowadays is an autologous nerve graft. However, if a good alternative is found it is very likely that this alternative will be used as the new standard, which could even increase the total volume of polymer needed.
In the following, we sketch the production process of nerve guide tubes as a batch process, from monomers to the actual product. According to this research, the best material at the moment is a copolymer based on 1,5-Dioxepan-2-one (DXO) and ambrettolide (Am). The industrial production of such NGTs consists of four parts: the synthesis of the comonomer DXO, the eROP of DXO and Am, the post polymerization process (curing) and the formation of the actual tube (Figure 7.2).

**Production of DXO**

1,5-Dioxepan-2-one is obtained by a Baeyer Villiger oxidation of tetrahydro-4H-pyran-4-one (THP) on lab-bench scale. DXO is commercially available, but there is only one supplier and the quantities are low and the prices are high\(^4\) (1341 euros, 25 gram). Therefore it is economically more feasible to produce the monomer on site. THP is available at a much lower price\(^5\) (286 USD, 25 gram), in larger quantities\(^6\) (1 kg) and there are several suppliers in the world.\(^7\)
The synthesis and purification of DXO consists of three steps on bench scale; the oxidation step, the filtration and washing steps and the distillation of the pure monomer. The oxidation step involves suspending 3-chloro-benzenecarboperoxoic acid (mCPBA) in dichloromethane at 0 °C. When a stable suspension is obtained, THP is added slowly at 0 °C. When everything is added, the solution is refluxed at 40 °C overnight.

An upscaled process can be performed batch wise in a stirred tank reactor (Figure 7.2, I-V). After filtration or decantation, the product mixture is extracted twice, once with sodium sulfite and once with sodium carbonate. After extraction the product is distilled and is ready for use in the polymerization process.

Enzymatic ring opening of DXO and ambrettolide

Up-scaling of the enzymatic ring opening polymerization of $\omega$-pentadecalactone was investigated by De Geus. The synthesis on bench-scale was converted to a continuous process on factory scale using continuous tubular reactors in series. However, the anticipated volume for NGTs favors a batch process using a stirred tank reactor (Figure 7.2, VI). The synthesis of P(Am-co-DXO) (50/50) on 10 kg scale would require 6.85 kg of macrolactone, 3.15 kg of DXO and 0.1 kg of immobilized enzyme. 10 liters of solvent would be needed to dissolve the monomers. Polymerization will be performed at 70 °C, what is above the melting point of the produced polymer. Agitation can be done by a mechanical stirrer. After polymerization the product mixture should be filtered and after cooling the polymer solution can be precipitated in methanol (70 L) Filtration can be difficult by the pressure drop caused by the enzyme. When the enzyme can be immobilized e.g. in a fixed bed in the reactor, no filtration step is needed. A second solution is to use a free enzyme. One drawback is that the enzymes then stay in the final product. This can interfere with the biocompatibility of the product. After polymerization, toluene and methanol can be separated from the product and the solvents can be recovered by distillation (Figure 7.2, VII). The product stream will be heated to 60 °C and the polymer will be melt processed.

Curing and the formation of tubes

The polymer melt is mixed with sugar particles < 125 μm, a curing agent and a UV-initiator in an extruder (Figure 7.2, VIII). Several routes towards the final product exist: [1] the mixture is pressed into a mold containing holes of the exact dimensions of the tubes (Figure 7.2, IX). The polymer is cooled to 0 °C and excreted from the mold. The tubes are put under UV light for...
curing (Figure 7.2, X). [2] The mixture is pressed into long tubes of the diameter of the final product. Upon cooling the long tubes are excreted from the mold and cured. After curing the rods are cut into small tubes. The next steps are leaching the remaining initiator and curing agent in THF and the sugar in water. The product will be finished after drying. The whole production process needs to be sterile and even packaging of the tubes should take place under sterile conditions. The tubes should be packed separately and before use, the tubes should be swelled in buffered solution.

However, this cross-linkable polyester can be used for other applications, such as tendon replacement and suture material. To meet all different requirements for all different applications, a variety of polymers can be made. The designed process is widely applicable for the synthesis of all kinds of polyesters made by eROP. By varying the feed ratio of the reactor, different polymers can be made. Different lactones can be fed to the reactor. In this way various product lines can be operated. Instead of making cured porous tubes, the product from the reactor can be spun into fibers or extruded into curable films/tapes.

### 7.1.4 Preclinical tests

In this phase the material is tested in vitro as well in vivo on the following points (according to ISO 10993):¹⁰

- Carcinogenity
- Cytotoxicity
- Degradation
- Genotoxicity
- Hemocompatibility
- Implantation
- Irritation
- Sensitization
- Systemic toxicity

When using completely new materials, which have never been used in medical applications before, extensive studies need to be performed to prove efficacy and safety. These tests can take several years and the costs are considerable and therefore often already tested raw materials are used.
Position of this thesis

The monomers used here are ambrettolide and DXO, the first being approved by the FDA for the use in flavors and fragrances. However, this only counts for the monomer and not for the polymer. DXO has not yet been approved by the FDA, however is reported non-toxic. Copolymers based on these monomers have still to be tested for approval. In our research we investigated the degradation and cytotoxicity in vitro, of which the degradation can be tuned by altering the hydrophilicity and/or crystallinity of the material by altering the ratio of the comonomers added to the feed of the polymerization. Cytotoxicity of the polymers was tested to be negative, however, upon adding curing agents, the material shows toxicity. By improving the washing procedure and minimizing the amount of curing agents needed or by searching for non-toxic alternatives, toxicity should be lowered significantly.

7.1.5 Clinical trials

This part is divided into four phases:

Phase 1: The product is tested on a small number of healthy volunteers. These people should be well informed about the risks of the usage. In this phase the most effective treatment and application methodology are determined.

Phase 2: Trials on a small group of patients are performed. More knowledge on the side effects of the product is obtained because the product is tested on non-healthy subjects.

Phase 3: Larger groups of patients are involved in this study. Normally there are several groups of patients: the control group, the placebo group and the group which is actually treated with the product.

Phase 4: This phase includes tests that are done after the product is approved and introduced into the market. These involve tests for long term effects of the product.

Position of this thesis

Before all these clinical tests can be performed, first in vivo testing should be done. However, these materials are well suited for their application and no severe side effects are expected. Only 0.1 g of polymer is placed in the body. The metabolized products resemble naturally occurring fatty acids, which are excreted from the body in the normal ways. Inflammation can be an issue using poly(lactic acid)s due to the local drop in pH caused by the highly acidic degradation products. However, the materials used here have less acidic groups
per unit of chain length and therefore a smaller decrease in pH is expected. Complete degradation should be possible since the degradation curves shown in chapter 4 and 6 are linear and reach high degradation levels. Therefore, it is expected that the material will be excreted by the body completely and no residual material will be left. Only two additives are used: the UV-initiator, 4-hydroxybenzophenone and the dithiol, ethylene glycol bis(3-mercaptopropionate). The first is washed out after curing, only negligible traces of this initiator will be present in the final product. The dithiol is incorporated in the network and only consists of ester functionalities and thio ethers. These are both naturally occurring moieties and therefore no major issues are expected. The toxicity observed in the materials is expected to come from remaining UV-initiator.

7.1.6 Market sale

After the clinical trials, the product must be approved by the authority of the locations where the product will be sold. Afterwards a marketing offensive must be planned to introduce the product to the end-user (surgeons). In every step unforeseen problems may be encountered and proper evaluation of the product is therefore an important step.

Position of this thesis

Since ambrettolide has already been approved and DXO\textsuperscript{12} is reported to be non-toxic, for the further discussion approval of the NGT material by the FDA is assumed.

The market offensive should be directed towards health insurance companies and hospital surgeons. The insurance companies have a financial benefit when these materials are used in surgery for three reasons. Firstly because using these tubes as implants instead of autologous nerve grafts is saving operation time and hospital time. Only one operation is needed instead of two and therefore there is also only one area in the patient’s body that needs healing. This takes less time and patients recover better. The second reason is that the donor nerve is still intact. No complications can occur here. By using an autologous nerve, the donor place can cause acute pain. Upon cutting the nerve, there is a chance that the nerve bundles accumulate electrical charge and start to discharge spontaneously, what causes acute pain. This can cause severe discomfort for the patient, who is not able to function properly and this costs money. The third reason is that upon major injury, the autologous nerve does not provide enough material to heal all nerves. Therefore body functions can only be recovered partially. Using
these nerve guide tubes, all nerves can be recovered and body functions can recover completely.

The surgeons using this material save time by only performing surgery on the site of injury. This means a lower chance of complications and it saves time.

For the patient being treated with these nerve grafts a better chance of complete recovery is expected and less hospital time and less chance of complications are foreseen.

7.2 Conclusions and outlook

Here the different steps starting from a bench material to the final product have been described. The results described in this thesis are put into perspective of the very complicated process of introducing a new biomedical product. At this moment this research is in the stage of lab bench prototype development. However, the prospective of the future steps could be positive. There is a clear need for new NGT material as it would save time, costs and surgical risks for all stakeholders.

An estimation of the material volumes needed suggests that a batch process is most feasible. Upscaling of the complete manufacturing process of porous polyester scaffolds appears doable but the development of a good process needs more research. However, the feasibility of using Novozym 435 should be investigated. The active lipase is leached out of the carrier to a certain extend. This could make re-use of this catalyst troublesome, although the catalyst can be easily replaced in a batch process. Another option is to use a free lipase as a catalyst. Also here, the catalyst can not be reused and will stay in the product. Immobilization of the catalyst in a different, preferably covalent way could be a solution.

7.3 References

6. www.specbiochem.com
These nerve guide tubes, all nerves can be recovered and body functions can recover completely. The surgeons using this material save time by only performing surgery on the site of injury. This means a lower chance of complications and it saves time. For the patient being treated with these nerve grafts a better chance of complete recovery is expected and less hospital time and less chance of complications are foreseen.

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References


Inge van der Meulen was born in Veldhoven on December 11th, 1982. In 2001 she finished gymnasium at the Sondervick College in Veldhoven. Subsequently, she started her master studies in Chemical Engineering and Chemistry at the Eindhoven University of Technology. In the summer of 2005 she started her graduation project in organic chemistry under supervision of Prof. dr. E. W. Meijer, where after she performed her internship within N.V. Organon in Oss. She graduated in February 2007. In December 2006 she was employed as a PhD-student at the Eindhoven University of Technology in the polymer chemistry group under guidance of Prof. dr. C. E. Koning and Dr. A. Heise.
CURRICULUM VITAE


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List of publications


• ‘Synaptic Plasticity in the Substantia Gelatinosa in a Model of Chronic Pain’ Robby J.P. Jaken, Elbert A.J. Joosten, Martin Knüwer, Ritanne Miller, Inge van der Meulen, Marco A.E. Marcus, Ronald Deumens, Neuroscience Letters, 2010, 469, 30 – 33.

• ‘High Molecular Weight Poly(pentadecalactone) for Fiber Applications’ Matthijs de Geus, Inge van der Meulen, Bart Goderis, Kristof Vanhecke, Marko Dorschu, Harm van der Werff, Cor E. Koning, Andreas Heise, Polymer Chemistry, 2010, 1, 525 – 533.


• ‘Copolymers from Unsaturated Macrolactones – Towards the Design of Cross-linked Biodegradable Polyesters’ Inge van der Meulen, Yingyuan Li, Ronald Deumens, Elbert A.J. Joosten, Cor E. Koning, Andreas Heise, submitted to Biomacromolecules.

• ‘Enzymatic Routes to Polymers’ Andreas Heise, Inge van der Meulen, chapter in Green Polymerization Techniques, Michael Meier (editor) 2010, Wiley VCH. In preparation.
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