Department of Biotechnology and Chemical Technology

Crosslinked poly(ester anhydrides) for controlled drug delivery

Risto Hakala

Aalto University

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Abstract

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Abstract

Bioresorbable polymers are extensively studied materials for medical applications. In this work, novel bioresorbable polymers, hydrophobically-modified crosslinked poly(ester anhydride) networks, were developed and characterized with the aim of obtaining suitable matrices for controlled drug release applications. In these free-radically crosslinked poly(ester anhydride) networks were combined the favorable characteristics of synthetic aliphatic polyesters and polyanhydrides.

The properties of the poly(ester anhydride) networks were altered by modifying the structure of the poly(epsilon-caprolactone) oligomers which were used in the preparation of the crosslinkable poly(ester anhydride) precursors. The molecular weight and architecture of the poly(epsilon-caprolactone) oligomers were controlled by using different types and amounts of co-initiator in the ring-opening polymerization. The hydrophobicity of the oligomers was modified by end-functionalizing the hydroxyl-telechelic oligomers with different alkenylsuccinic anhydrides. Crosslinkable precursors with reactive double and labile anhydride bonds were obtained by methacrylating the acid-terminated oligomers with methacrylic anhydride. Finally, the poly(ester anhydride) precursors were crosslinked to networks either thermally or by using visible light.

The change in the molecular architecture of the precursor from linear to star-shaped increased crosslinking density and raised the gel contents. In the hydrolysis studies the more hydrophobic samples, i.e., networks containing alkenyl chains, eroded slower than the networks without the alkenyl chains. In addition, some of the crosslinked samples exhibited clear signs of surface erosion: a linear mass loss but practically intact core. Increase in the molecular weight and hydrophobicity of the precursor changed the erosion mechanism of the networks from surface toward bulk erosion.

The mild photocrosslinking conditions of these poly(ester anhydride) networks enable the incorporation of sensitive active agents, such as peptide- and protein-based drugs, with reduced risk of inactivation. The photocrosslinked networks provided sustained and surface erosion controlled *in vivo* release of model peptide YY3-36. Furthermore, the *in vivo* release could be tailored by modifying the hydrophobicity of the network. These novel polymeric drug delivery materials exhibit high potential for applications requiring controlled release of sensitive macromolecular pharmaceutical agents.

Keywords biopolymer, poly(ester anhydride), crosslinking, hydrolysis, drug delivery

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Tiivistelmä

Biohajoavat polymeerit ovat laajasti tutkittuja materiaaleja lääketieteellisiin käyttökohteisiin. Tässä työssä kehitettiin ja tutkittiin uusia biohajoavia polymeerejä, hydrofobisesti modifioituja silloitettuja polyesterianhydridejä, matriiseiksi kontrolloituun lääkeaineiden annosteluun. Modifioitavan hydrofobisuuden lisäksi näissä silloitetuissa polyesterianhydrideissä yhdistettiin synteettisten alifaattisten polyestereiden ja polyanhydridien hyviä ominaisuuksia.

Silloitettujen polyesterianhydridien ominaisuuksia muokattiin muuttamalla poly(epsilon-kaprolaktoni)-pohjaisten esipolymeerien rakennetta. Esipolymeerien moolimassaa ja muotoa säädeltiin renkaanavaavassa polymeroinnissa käytettävillä koinitiaattoreilla ja niiden pitoisuuksilla. Hydrofobisuutta vastaavasti muokattiin funktionalisoimalla hydroksipäätteiset esipolymeerit happopäätteisiksi erilaisilla alkenyyliketjuja sisältävillä meripihkahappoanhydrideillä. Metakryloimalla happopäätteet metakryylihappoanhydridillä saatiin reaktiivisia kaksoissidoksia ja hydrolyyttisesti herkkiä anhydridisidoksia sisältäviä esipolymeerejä. Lopuksi nämä metakryloidut esipolymeerit silloitettiin verkkorakenteeksi joko lämmön tai valon avulla.

Esipolymeerin muodon vaihtaminen suoraketjuisesta tähtimäiseksi kasvatti silloitustiheyttä ja nosti geelipitoisuutta. Hydrolyysikokeissa hydrofobisemmat näytteet, eli ne jotka sisälsivät alkenyyliketjuja, erodoituivat hitaammin kuin alkenyyliketjuttomat näytteet. Lisäksi osa silloitetuista näytteistä osoitti merkkejä pintaeroosiosta: lineaarista massan menetystä näytteen sisäosan pysyessä muuttumattomana. Esipolymeerien moolimassan ja hydrofobisuuden kasvattaminen muutti hydrolyyttistä eroosiomekanismia pintaeroosiosta massaeroosion suuntaan.

Silloitettujen polyesterianhydridien miedot valosilloitusolosuhteet mahdollistavat herkästi inaktivoituvien aktiiviaineiden, kuten peptidi- ja proteiinilääkkeiden, käytön polymeerisissä lääkeannostelumateriaaleissa. Mallipeptidin YY3-36 *in vivo* vapautusta silloitetuista polyesterianhydrideistä pystyttiin modifioimaan muuttamalla polymeerin hydrofobisuutta. Nämä kehitetyt uudet polymeerit ovat potentiaalisia materiaaleja suurien lääkemolekyylien annosteluun.

Avainsanat biohajoava polymeeri, polyesterianhydridi, silloittaminen, hydrolyysi, lääkeannostelu

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Preface

This work was carried out in the Polymer Technology Research Group at Aalto University School of Chemical Technology (Helsinki University of Technology until January 2010). I wish to express my gratitude to my supervisor Professor Jukka Seppälä for his advice, interest and enthusiasm for my work, and for providing me the opportunity to work in his research group.

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Espoo, December 2012

Risto Hakala

Contents

List of publications

This thesis is based on the following five publications (Appendices I-V), which are, throughout the summary, referred to by their Roman numerals.

I Hakala, R. A., Korhonen, H., Holappa, S., Seppälä, J. V., Hydrophobicities of poly(ε-caprolactone) oligomers functionalized with different succinic anhydrides, *Eur. Polym. J.* 45 (**2009**) 557-564.

II Hakala, R. A., Korhonen, H., Seppälä, J. V., Hydrolysis Behaviour of Crosslinked Poly(ester anhydride) Networks Prepared from Functionalised Poly(ε-caprolactone) Precursors, *React. Funct. Polym.* 73 (**2013**) 11-17.

III Hakala, R. A., Korhonen, H., Meretoja, V. V., Seppälä, J. V., Photo-cross-linked biodegradable poly(ester anhydride) networks prepared from alkenylsuccinic anhydride functionalized poly(ε-caprolactone) precursors, *Biomacromolecules* 12 (**2011**) 2806-2814.

IV Mönkäre*, J., Hakala*, R. A., Vlasova, M. A., Huotari, A., Kilpeläinen, M., Kiviniemi, A., Meretoja, V., Herzig, K. H., Korhonen, H., Seppälä, J. V., Järvinen, K., Biocompatible photocrosslinked poly(ester anhydride) based on functionalized poly(ε-caprolactone) prepolymer shows surface erosion controlled drug release *in vitro* and *in vivo*, *J. Control. Rel.* 42 (**2010**) 349-355. **These authors share equal contribution*

V Mönkäre*, J., Hakala*, R. A., Kovalainen, M., Korhonen, H., Herzig, K. H., Seppälä, J. V. and Järvinen, K., Photocrosslinked poly(ester anhydride)s for peptide delivery: effect of oligomer hydrophobicity on PYY3-36 delivery, *Eur. J. Pharm. Biopharm.* 80 (**2012**) 33-38. **These authors share equal contribution*

Author's contribution in the appended publications

I Risto Hakala planned and carried out the experiments and wrote the manuscript.

II Risto Hakala planned and carried out the experiments and wrote the manuscript.

III Risto Hakala planned and carried out the experiments, excluding the cytotoxicity evaluation, and wrote the manuscript.

IV Risto Hakala planned and carried out the polymerizations and with Juha Mönkäre is responsible for the research plan, interpretation of the results, and the preparation of the manuscript.

V Risto Hakala planned and carried out the polymerizations and with Juha Mönkäre is responsible for the research plan, interpretation of the results, and the preparation of the manuscript.

Abbreviations and symbols

^{1}H NMR	proton nuclear magnetic resonance
^{13}C NMR	carbon nuclear magnetic resonance
8-ASA	(+/-)-2-octen-1-ylsuccinic anhydride
12-ASA	2-dodecen-1-ylsuccinic anhydride
18-ASA	n-octadecenylsuccinic anhydride
ASA	alkenylsuccinic anhydride
ATR	attenuated total reflectance
AUC	area under curve
BD	1,4-butanediol
CL	ε-caprolactone
CPP	1,3-bis(*p*-carboxyphenoxy)propane
CPH	1,3-bis(*p*-carboxyphenoxy)hexane
CQ	camphorquinone
DBPO	dibentzoyl peroxide
DS	degree of substitution
DSC	differential scanning calorimetry
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FTIR	Fourier transform infrared spectroscopy
HPLC	high-performance liquid chromatography
i.v.	intravenous
MAAH	methacrylic anhydride
NMR	nuclear magnetic resonance
MWD	molecular weight distribution
PCL	poly(ε-caprolactone)
PDLA	poly(D-lactide)
PDLLA	poly(D,L-lactide)
PERYT	pentaerythritol
PGA	polyglycolide or poly(glycolic acid)
PHB	poly(hydroxybutyrate)
PLA	polylactide or poly(lactic acid)
PLGA	poly(lactide-co-glycolide)
PLLA	poly(L-lactide)
PMAA	poly(methacrylic acid)
PYY3-36	peptide YY3-36
ROP	ring-opening polymerization

SA	sebacic acid
s.c.	subcutaneous
SAH	succinic anhydride
SEC	size exclusion chromatography
$SnOct_2$	stannous octoate
UV	ultraviolet
US	United States

Postfixes

-OH	hydroxyl-terminated
-a	acid-terminated
-m	methacrylated
-nw	network

Symbols

ΔH	melting enthalpy (J/g)
M_n	number average molecular weight (g/mol)
M_r	relative molecular mass
M_w	weight average molecular weight (g/mol)
T_g	glass transition temperature (°C)
T_m	melting temperature (°C)
X	monomer conversion (%)

Designation of polymer samples

The polymer samples are designated in terms of the shape of the oligomer (LIN for linear and STAR for star-shaped molecular architecture); the molecular weight of the hydroxyl-terminated oligomer (1000, 1500, 2000 or 4000 mol/g); the number of carbons in the alkenyl chains of the different succinic anhydrides (0, 8, 12, or 18); and the type of functionality (OH and a for hydroxyl- and acid-terminated oligomers, m for methacrylated precursor and nw for crosslinked network). For example, acid-terminated ε-caprolactone oligomer polymerized with 10 mol-% of 1,4-butanediol and functionalized with n-octadecenylsuccinic anhydride is designated LIN-1000-18a.

1. Introduction

1.1 General background

Drug development has classically targeted on new and more powerful drugs to improve the overall survival rate and quality of life of humans. In addition to drug development, there has been increased attention on the delivery systems by which the drugs are administered.[1-6] One attractive way to deliver drugs in a controlled manner is to use implantable polymeric drug delivery systems. In conventional drug delivery (e.g., oral administration or injections), the drug concentration in the blood rises after each administration and then decreases until the next administration (Figure 1, left).[4,7,8] Because each drug has an optimal plasma concentration range, above which the drug is toxic and below which the drug is ineffective, the plasma drug concentration in a patient at a particular time depends on the patient's compliance with the prescribed routine.[4,6,8] In a controlled drug delivery designed for long-term administration, the plasma drug level remains relatively constant and within the desired therapeutic range with a single administration for an extended period of time as shown in Figure 1 (right).[6,8] To achieve constant drug concentration in plasma it is necessary to achieve relatively constant and prolonged drug release rate. In addition to the maintenance of drug levels within a desired range, other potential advantages of controlled release systems include 1) localized delivery of drugs to the site where the therapeutic drug is needed (Figure 1, right), thereby lowering the systemic drug level; 2) reduced drug toxicity and side effects; 3) decrease in the number of dosages; 4) protection of biologically sensitive drugs from the surrounding environment; 5) increased patient comfort and/or improved compliance; and 6) reduced need for follow-up care.[2,4,6,7,9-12]

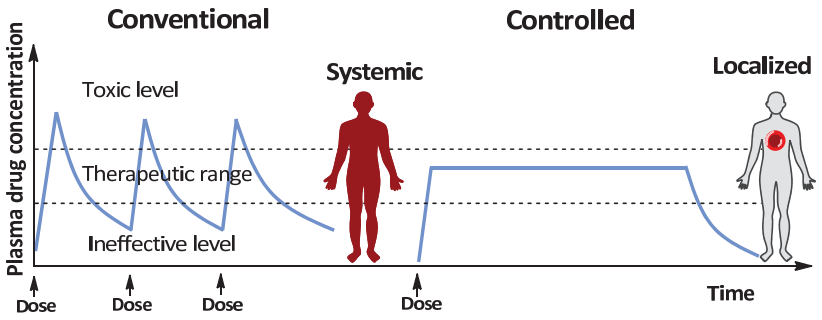


Figure 1. Drug release profiles for conventional systemic and controlled localized drug release (modified from refs. 4,6-8) .

Polymers have played an important role in the advancement of controlled drug delivery technology.[2,4,13] Polymeric drug delivery systems can be produced with natural or synthetic polymers, which can be biodegradable or non-biodegradable (Figure 2).[7,14-16] Generally, in many contexts the term biodegradable polymer is referred to a polymer in which the degradation requires the action of biological agents (enzymes, cells, or microorganisms) for causing the chemical degradation.[16] In this thesis, the term biodegradable polymer is used to polymers which degrade in the physiological environment (mainly through enzymatic or non-enzymatic hydrolysis), and if the degradation products are eliminated from the body by metabolism or excretion the polymers are defined as a bioresorbable polymers.[17] Bioresorbable polymers are particularly attractive for drug delivery systems because once they are introduced into the human body, they do not require removal or additional manipulation.[7,18-20] However, they must produce non-toxic degradation products that are easily eliminated from the body and possess a degradation time appropriate to ensure their timely function.[7,20,21] Among the desirable characteristics of a bioresorbable polymeric system used for drug delivery are a reliable drug release profile, high polymer purity and reproducibility, minimal tissue reactions after implantation, low likelihood of provoking a sustained inflammatory or toxic response, an acceptable shelf life, and ease in sterilization, as well as appropriate mechanical properties, processability, and permeability properties for their intended use.[20-23]

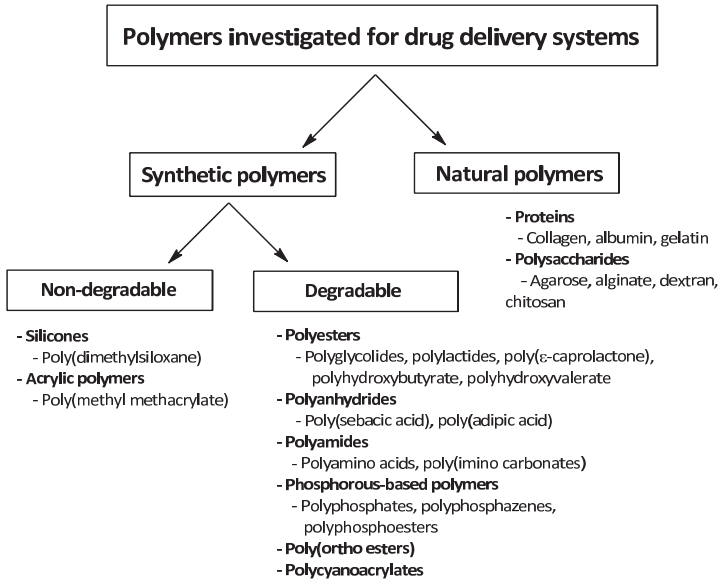


Figure 2. Examples of polymers investigated for polymeric drug delivery systems (modified from refs. 7,14-16).

The polymers used in medicine are usually thermoplastic and their properties are primarily modified by changing the molecular weight or by copolymerization with different monomers.[13,24,25] One way to further modify polymers is crosslinking. In a crosslinked structure (also referred often to as a *network*, *thermoset* or *cured polymer*), the polymer chains are connected to each other chemically (e.g., by free-radical polymerization induced by heat or light, polycondensation, ring-opening polymerization (ROP), or high energy irradiation) where covalent bonds are formed between the molecules and/or physically, e.g., by hydrophobic, ionic, or protein interactions, stereocomplex formation, hydrogen bonds, or crystallization.[26-33] Due to the covalent bonds, chemically crosslinked polymers are insoluble in nearly all common polymer solvents but have the capacity to swell in compatible solvents.[26-29] Likewise, the effects of physical crosslinking can be reversed by the action of solvent.[26] During the crosslinking process, the polymeric system can undergo a series of deformations, for example from a liquid to a loosely crosslinked rubbery gel and further to a highly crosslinked solid network, with ideal conditions resulting in the molecular weight of the crosslinked material approaching infinity.[34-36]

While newer active agents, such as peptide- and protein-based drugs, continue to be discovered, new requirements have been set for the delivery systems. The aim of this study was to develop a novel polymeric controlled drug delivery system. This thesis focuses on crosslinked poly(ester anhydrides) in which properties from both polyesters and polyanhydrides are applied. Because the control of polymer degradation rates can extend to the control of drug delivery kinetics, the degradation properties of the developed crosslinked poly(ester anhydride) networks were modified by altering the molecular size, architecture, and hydrophobicity of the crosslinkable poly(ester anhydride) precursors. In the literature review, a background on hydrolytic degradation and erosion of polymers is provided, in addition to the introduction of crosslinking and two classes of biodegradable polymers, aliphatic polyesters and polyanhydrides, which are widely studied for controlled drug delivery applications.

1.2 Hydrolytic degradation and erosion

Understanding the degradation and erosion behaviors of polymers is essential for drug release applications because the release of the drug is dependent upon the erosion process and the drug release rates become more predictable if the erosion process of the polymer is being understood.[11,37] The term degradation is commonly used to describe the chain scission process in which polymer chains are finally cleaved to oligomers and monomers, whereas erosion refers to the loss of material owing to the loss of monomers and oligomers from the polymer.[11,38-41] Thermal, radiation, enzymatic, and hydrolytic degradation are examples of processes resulting in polymer chain scission.[40,42,43] Thermal degradation can occur during different polymer processing phases, and γ-radiation is commonly known cause chain scission.[40,42] Enzymatic and hydrolytic degradations are the most important degradation mechanisms in the biomedical field because the majority of the bioresorbable polymers contain hydrolytically or enzymatically susceptible labile chemical bonds, such as esters, carbonates, anhydrides, orthoesters, amides, and urethanes, in their backbone (Figure 3).[2,21,26,40] Upon implantation into the body these chemical bonds degrade forming biologically compatible moieties that are eventually metabolized and removed from the body.[21,44] However, because the rate of *in vivo* degradation of enzymatically degradable polymers may vary with the site of implantation, depending on the availability and concentration of the enzymes, hydrolytically degradable polymers are generally preferred as bioresorbable materials, due to their minimal site-to-site and patient-to-patient variations compared to enzymatically degradable polymers.[21]

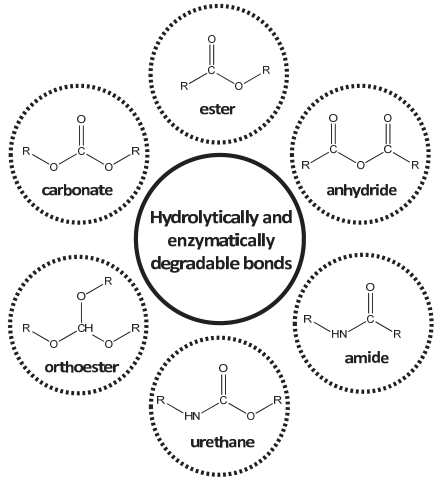


Figure 3. Chemical bonds sensitive to hydrolysis and enzymatic actions (modified from ref. 7).

Polymer erosion (i.e., mass loss) is a more complex phenomenon than degradation because erosion is a sum of several elementary processes, including 1) degradation; 2) dissolution; 3) diffusion of dissolved species; 4) swelling; and 5) morphological changes.[41,45] Polymer erosion can be categorized as surface (or heterogeneous) erosion (Figure 4a), bulk (or homogeneous) erosion (Figure 4b), or a combination of the two.[9,11,38,39,46] In surface erosion, the rate of water penetration into the matrix is lower than the rate of polymer degradation, and the material is consequently lost from the outside as in a bar of soap.[47,48] The erosion rate is dependent on the surface area of the polymer rather than on its volume and therefore thicker samples possess a longer lifetime.[47,48] In contrast, bulk erosion processes occur when the rate of water penetration into the matrix is higher than the rate of polymer degradation, such that polymer mass is lost uniformly throughout the matrix.[11,46] The erosion rate is dependent on the volume of the polymer rather than its thickness.[47,48] Furthermore, when degradation is for example acid catalyzed and the diffusion of the acidic degradation products is restricted, they can accelerate the internal degradation compared with the surface (autocatalysis), thereby depositing an exterior "skin" of higher molecular weight while producing a degraded interior with components of lower molecular weight (Figure 4c).[41,47,48]

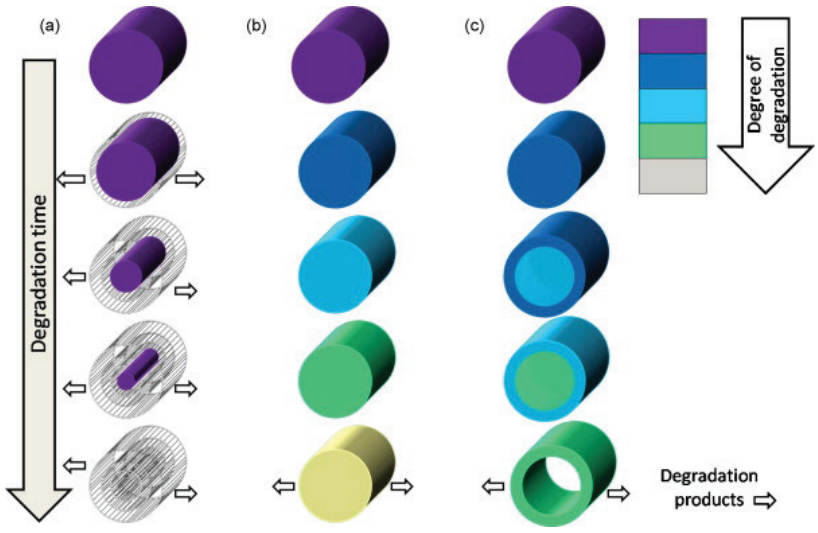


Figure 4. Schematic illustration of a) surface degradation, b) bulk degradation, and c) bulk degradation with autocatalysis (from ref. 48 , reprinted with permission from Elsevier Ltd.)

There are a number of polymer characteristics affecting the overall hydrolytic degradation and erosion rate of polymers. One of the most important characters is the nature of the bonds within the polymer backbone.[26,37,40,47,49] For example, anhydride and orthoester bonds are more susceptible to hydrolysis than ester or amide bonds, although the reactivity of the bonds can change markedly as a result of alterations in their chemical environment.[40,45,46] Another important character is the hydrophobicity of the polymers, which affects the extent of water penetration.[49,50] Hydrophilic polymers take up large quantities of water into the matrix and degrade uniformly throughout the matrix.[40,47] In contrast, hydrophobic polymers cannot take up large amounts of water and accordingly degrade very slowly.[47] If hydrophobic polymers contain hydrolytically labile bonds, as is often the case with polyanhydrides, they can degrade hydrolytically from the surface first. However, in this case the degradation products are usually so hydrophobic that they are deposited onto the polymer matrix itself; thus, the overall mass of the matrix may not decrease significantly.[47] In addition, hydrophobic degradation products often slow down hydrolytic degradation by preventing the diffusion of water into the matrix.[40,47,51]

Hydrolytic degradation and erosion are also influenced by other polymer characteristics, such as flexibility, molecular weight, the glass transition temperature (T_g), and crystallinity.[11,26,41,42,46,52-54] Increased polymer flexibility translates to increased degradation rate because the flexibility allows greater accessibility to water.[54] An increase in the molecular weight decreases the degradation rate because higher molecular weight polymers have a greater number of bonds to be broken and they may be glassier and less flexible.[11,42,47,54] A high T_g corresponds to a relatively limited molecular motion, and a high degree of crystallinity limits hydration through the ordered packing of polymer chains.[55] Water cannot diffuse through crystalline regions, while more flexible amorphous regions are accessible to water.[11,41,42,52,56] Among amorphous polymers of the same class, the increase in T_g indicates a higher molecular weight or, oftentimes, a more stable polymer, probably due to hydrogen bonding or hydrophobic interactions, thus decelerating the rate of degradation.[47,49]

In addition to polymer characteristics, hydrolytic degradation and erosion can be influenced by altering the chemical composition of the polymers.[11,40,42,57,58] Incorporating additional hydrophilic comonomers increases water penetration, resulting in an increase in the degradation rate.[49,52,59] For example, various linear polyester copolymers are reported in

the literature in which hydrophilic poly(ethylene glycol) blocks have been introduced into poly(ε-caprolactone) or polylactide blocks.[60-73] In addition, comonomers can increase the irregularity of the polymer chains, thereby reducing crystallinity and increasing degradation rate. Likewise, comonomers can also feature groups that increase the rigidity of the polymer, which will decrease the degradation rate.[11,40,42] For example, in an aliphatic-aromatic poly(anhydride) copolymer, the degradation rate is highly dependent on the rigid aromatic content.[40,42] Finally, additional factors affecting hydrolytic degradation and erosion include device dimensions (size, shape, surface to volume ratio, porosity), additives (drugs, acidic or basic components, solvents), processing (sterilization, heat, force), site(s) of application, and enzymes.[11,26,40-42,52] The effect of the crosslinking on the degradation is discussed in more detail in Chapter 1.5.

1.3 Synthetic biodegradable polymers

A wide range of drug delivery systems have been investigated using synthetic biodegradable polymers, such as aliphatic polyesters based on glycolic acid or glycolide, lactic acid or lactide, and ε-caprolactone, polyanhydrides based on sebacic and apidic acid, as well as polycarbonates, polyacetals, polyamides, polyorthoesters, polyurethanes, and phosphate-based polymers.[1,2,20,21,44] These polymers have bonds that are susceptible to hydrolytic or enzymatic degradation, are often non-toxic in nature, and bioresorb in the body over various time periods, from a few days up to several years.[1,2,7,20,21]

1.3.1 Aliphatic polyesters

Synthetic biodegradable aliphatic polyesters are one of the most extensively studied and characterized biodegradable polymers for biomedical applications (Figure 5).[9,24,74] This is mainly due to their bioresorbability, non-toxicity, good mechanical properties, easy processability, commercial availability, and relative ease of synthesis.[20,24,31] Polycondensation of difunctional monomers, such as glycolic acid or lactic acid, preferentially yields low molecular weight polymers, whereas ring-opening polymerization of corresponding cyclic dimers of glycolic acid or lactic acid (i.e., glycolides or lactides) and lactones is preferred when high molecular weight polymers are desired.[21,74] The degradation timeline of aliphatic polyesters varies from some weeks to years, and the erosion of the

polymers proceeds mostly homogenously throughout the polymer matrix (bulk erosion mechanism).[9,21,38-41]

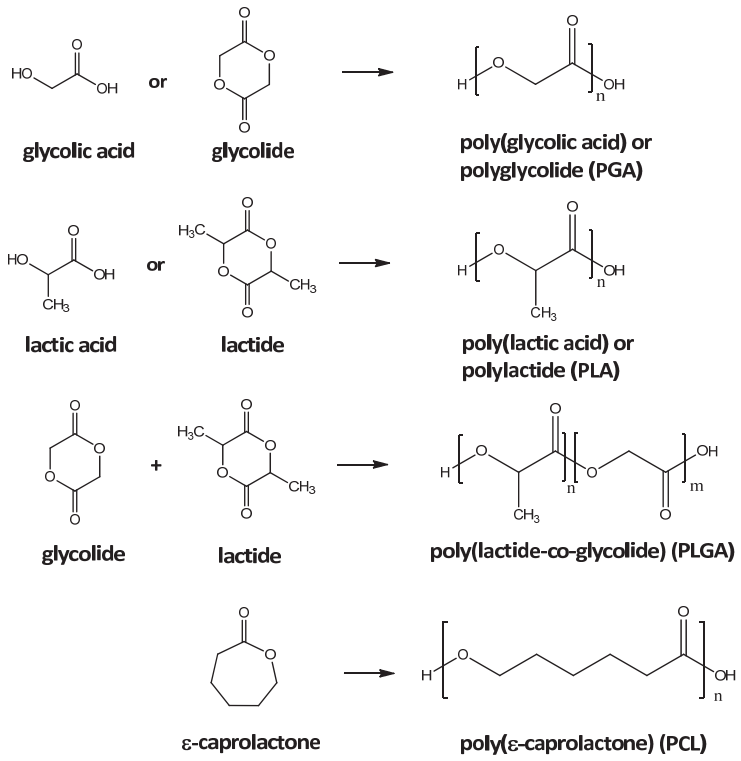


Figure 5. Chemical structures of common synthetic aliphatic polyesters.

Polyglycolides and polylactides

Polyglycolide (PGA) is one of the first biodegradable synthetic polymers investigated for medicine.[21] Polyglycolide is a hard, tough, and highly crystalline polymer (45-55% crystallinity) with a melting temperature (T_m) greater than 200 °C and a glass transition temperature (T_g) ranging from 35 to 40 °C.[21,22] In hydrolysis polyglycolide lose its strength within 1-2 months and losses its mass within 6-12 months.[21] Due to its excellent fiber forming properties, polyglycolide was developed for biodegradable sutures.[21,22] However, the high melting temperature and low solubility in most common polymer solvents of polyglycolide limits its use for drug delivery applications, because it is difficult to prepare films, rods, capsules, or microspheres using melt or solvent techniques.[75] In addition, its applications are limited by its relatively high degradation rate yielding

acidic degradation products, which have been linked to a strong and undesired inflammatory response.[20,21,74]

Unlike glycolide, lactide is a chiral molecule and exists in two optically active forms: L-lactide and D-lactide.[20,21] Polymerization of these molecules leads to four forms, which are poly(L-lactide) (PLLA), poly(D-lactide) (PDLA), poly(D,L-lactide) (PDLLA), and meso-poly(lactide).[20,21] PLLA is a crystalline polymer (of approximately 37% crystallinity, depending on molecular weight).[20,21] In hydrolysis high molecular weight PLLA has been reported to be bulk erodible and lose its strength in six months, although its completely resorption *in vivo* can take longer than two years.[20,21] Differing from PLLA, PDLLA is an amorphous polymer due to the random distribution of L- and D-lactide units. PDLLA lose its strength within 1-2 months when hydrolyzed and undergoes a loss in mass within 12-16 months through bulk erosion.[21] The steric shielding effects of the additional methyl group in polylactides render the polymer more hydrophobic and stable against hydrolysis than polyglycolides.[20,21,74] In the body, the polymeric chains of polylactides are randomly cleaved by hydrolysis, ultimately releasing lactic acid, which is natural body metabolite and is eliminated from the body via the citric acid cycle in the form of CO_2 and H_2O or excreted in the urine.[21,76] Degradation of the polylactides is often tailored by varying the stereochemistry of the used lactides or copolymerizing lactides with glycolides.[7]

Poly(ε-caprolactone)

Among aliphatic polyesters, poly(ε-caprolactone) (PCL) has been predominantly considered for drug delivery systems, because 1) it has an exceptionally low glass transition temperature of approximately -60°C and a low melting temperature ranging between 55 and 64°C depending on its degree of crystallinity; 2) it has a high permeability for small drug molecules; 3) it is compatible with a wide range of drugs, which enables constant levels of drug distribution in the formulation matrix; 4) its degradation environment is not as acidic compared with polylactides and polyglycolides; 5) it has an exceptional ability to form blends with other polymers, thereby affecting drug release profiles; 6) it is non-toxic; 7) it is cyto-compatible with several body tissues; and 8) it is already United States Food and Drug Administration (US FDA) approved in medical devices and formulations.[7,21,31,44,48,74-78] In addition, PCL can be obtained by ring-opening polymerization of relatively cheap ε-caprolactone (CL) monomers,

PCL has proven to be an easy material to process due to its low T_g and T_m and good organic solvent solubility, and it has flexible mechanical properties (e.g., low tensile strength (~23 MPa) but very high elongation at breakage (>700%)).[20,21,74-78]

Hydrolytic degradation of PCL can be divided into two phases. In the first step, random hydrolytic chain scission of the ester linkage leads to an initial decrease in the molecular weight, without any significant weight loss.[21,48,76,77] The non-enzymatic hydrolytic cleavage starts in the amorphous region, auto-catalyzed by the acid end groups of the fragmented polymeric chains.[78] In the second step, when the length of the PCL fragments produced becomes small enough (number average molecular weight (M_n) ~5000 g/mol), the weight loss begins due to diffusion of small polymeric fragments out of the polymer bulk to the medium.[48,76-78] In *in vivo* this bulk erosion process is generally accompanied by enzymatic surface erosion characterized by grooves and cracks on the surface.[78] Finally, hydrolysis yields 6-hydroxycaproic acid, which enters the citric acid cycle and is completely metabolized and eliminated from the body (Figure 6).[31,48,78] The hydrolytic degradation of the PCL homopolymer takes two to four years, depending on the molecular weight of the polymer. The degradation rate of PCL is slower than polyglycolides and polylactides due to its more hydrophobic character; thus, to increase overall PCL erosion rate, it is often blended or copolymerized with other polymers, such as polylactides and polyglycolides.[20,21] In the field of drug delivery, PCL homopolymer is mainly suited to diffusion-based long-term delivery devices.[20,21,31,44,48,55,74,78]

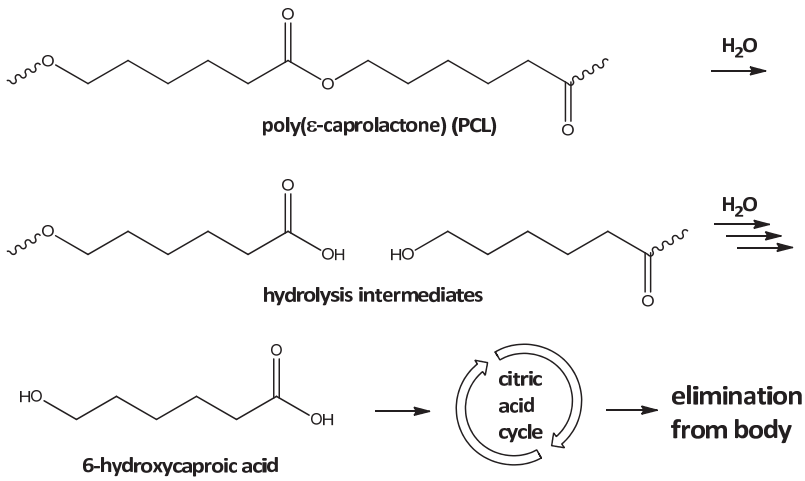
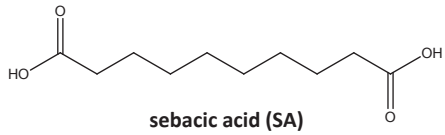


Figure 6. The hydrolysis of PCL and elimination from the body (modified from ref. 48).

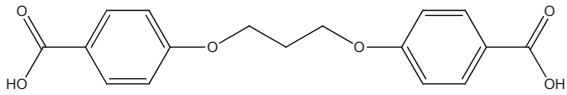
1.3.2 Polyanhydrides

Similar to aliphatic polyesters, polyanhydrides are interesting biodegradable materials as matrices for controlled drug release applications.[3,20,21,74,79] This is mainly because of their surface erosion behavior, which is due to the hydrophobicity of the polymer combined with the hydrolytic instability of the anhydride bond.[3,9,21,19,38,79-81] Surface-eroding devices can deliver entrapped drugs at sustained, steady rates, and such rates are achievable for a wide range of molecular sizes.[10,46,47] Especially, surface erosion enables the release of macromolecular active agents, such as peptides, proteins, and genes, which cannot generally diffuse from the polymer matrix.[3,20,79,80] In addition to the surface erosion behavior, the advantages of the polyanhydrides include preparation from low cost and generally considered safe dicarboxylic acid building blocks and processability by injection molding or extrusion for mass production.[79] The main limitations of polyanhydrides are low mechanical strength, hydrolytic instability, which requires storage under moisture-free conditions, and poor film- or fiber-forming properties.[79,81-84]

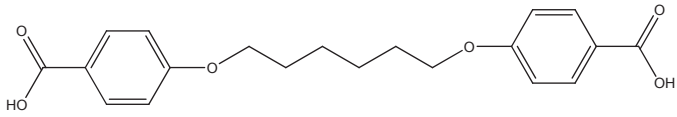
In general, thermoplastic polyanhydrides are composed of hydrophobic aliphatic and aromatic diacids, such as sebacic acid (SA), 1,3-bis(p-carboxyphenoxy) propane (CPP) and 1,3-bis(p-carboxyphenoxy) hexane (CPH) (Figure 7), which are linked via anhydride bonds. Aliphatic polyanhydrides degrade in a few days, while some aromatic polyanhydrides degrade over a few years.[3,19] Because aliphatic polyanhydrides have been found to have limited applications due to their crystalline structure and fast degradation, they have been copolymerized with aromatic diacids, to change their processability, degradation, and drug release properties.[20,21,19,74] The most extensively investigated polyanhydride is the CPP-SA copolymer.[3,21] The polymer has been found to exhibit a zero-order release of incorporated drug over periods of time ranging from days to years, depending on the ratio of the comonomers used and the molecular weight of the polymer.[21] The degradation products of the CPP-SA copolymers have been found to be non-toxic and biocompatible, and the US FDA has approved it for use in the localized controlled delivery of the chemotherapeutic agent carmustine for treatment of brain cancer (Gliadel®).[3,21]



sebacic acid (SA)



1,3-bis(p-carboxyphenoxy)propane (CPP)



1,6-bis(p-carboxyphenoxy)hexane (CPH)

Figure 7. Examples of diacid monomers involved in the synthesis of polyanhydrides.

To combine the advantageous properties of polyesters (e.g., good mechanical and processability properties) and polyanhydrides (e.g., surface erosion controlled drug release), several thermoplastic poly(ester anhydrides) have been developed which have two types of hydrolytically cleavable bonds, ester and anhydride, in the polymer backbone.[85-107] For example, Storey and Taylor[108] have prepared high molecular weight poly(ester anhydrides) from PCL oligomers, and Silvianic and Domb[109] have coupled PCL, PLA, and poly(hydroxybutyrate) (PHB) oligomers with sebacic acid via anhydride linkages. Krasko *et al.*[110-113] have prepared poly(ester anhydrides) for potential use as drug carriers from poly(sebacic acid) and ricinoleic acid. These waxy or pasty thermoplastic poly(ester anhydride) drug delivery systems have been studied for delivery of paclitaxel[114-116], antineoplastic proteins[117], and tamsulosin hydrochloride[118].

1.4 Free-radical crosslinking

Various synthetic methods can be used for the preparation of biodegradable chemically crosslinked networks.[26,27,30-33,49,119-125] One of the most common methods is the free-radical crosslinking of multifunctional biodegradable monomers and/or precursors (also referred to as *macromers, oligomers* or *prepolymers*) that form biodegradable polymer networks.[126-132] Free-radical crosslinking requires the use of initiators that dissociate into radicals upon the absorption of energy.[49,120,133] These free radicals, in turn, react with functional groups, such as acrylic[134-141],

methacrylic[142-145] fumaric[146-155], and maleic[156-159] double bonds, which polymerize via an addition-type reaction to form polyvalent crosslinks and, eventually, a crosslinked structure.[160] Free-radical crosslinking can be initiated by various methods, e.g., thermally, chemically, via exposure to electromagnetic radiation, or a combination of these initiation methods, with the ultimate result of generating free radicals to initiate the polymerization process.[161]

Thermally initiated crosslinking is typically initiated with organic peroxides, such as benzoyl peroxide, which form radicals upon heating.[161] Thermal crosslinking provides the advantage of being initiated by temperature change and is easily applied to polymers that sustain heat and are highly viscous at room temperature.[120] These characteristics make thermal crosslinking especially suitable in areas where the absorption and scattering of the polymerizing light is restricted, such as for very thick specimens or complex three-dimensional structures.[162-164] The drawbacks of thermally initiated systems include poor control of the crosslinking reaction and often excessively long crosslinking times and undesired high temperatures for biomedical applications.[50,120,165-167]

Chemically initiated crosslinking can be used to reduce polymerization time and temperature. In chemically initiated crosslinking, accelerators (also referred to as *activators*, *promoters*, or *co-initiators*) such as an aromatic tertiary amine (commonly, N,N-dimethyl-p-toluidine) is combined with peroxides to carry out a reduction-oxidation (redox) initiation.[161,168-170] Redox initiation is commonly used in poly(methyl methacrylate)-based bone cements.[169,170] Syringeability is one of the main advantages of the redox systems; however, disadvantages of the redox initiation in drug delivery include heat generation due to the exothermic polymerization reaction and often an excess of unreacted monomers remaining after crosslinking, which may result in toxicity.[161,170,171]

Initiation of crosslinking by electromagnetic radiation is commonly carried out with ultraviolet or visible light (i.e., photo initiated crosslinking, photocrosslinking, or photocuring), yielding several advantages.[37,49,133,172-179] Firstly, the crosslinking rate can be controlled, for example, by the structure of the monomers and/or precursors, number of double bonds, the light source (e.g., wavelength and intensity of the light), the choice and concentration of the initiator and accelerator, and the temperature.[37,120,172-174,180-182] Secondly, photocrosslinking possesses spatial control over the

crosslinking process, i.e., photocrosslinking only occurs in illuminated areas when the monomers and/or precursors are in the proximity of the light and initiator.[172-174,176] Because the initial materials are often liquid solutions or moldable putties, the systems are easily placed in complex-shaped voids and subsequently react to form a polymer of the exactly required dimensions.[171,175,176] Thirdly, crosslinking is temporally controlled, i.e., the reaction can be triggered by switching on the light source, and the reaction can be terminated by turning off the light.[172,174,176] By controlling the exposure time, the properties of the material can be adjusted.[34,173] Temporal control can also be used to minimize temperature increases during the exothermic radical polymerization.[133] Fourthly, the photocrosslinking reaction is rapid and crosslinking is effective even under the gentlest reaction conditions, such as at room temperature or under physiological temperature and pH, and is not inhibited by water or moisture.[49,133,162,171,172,175,176,179] Thus, photocrosslinking is a suitable method for *in situ* applications.[133,162,175,176,183,184] Finally, photocrosslinking can be performed without the need for heat or solvents; thus, a wide variety of drugs and heat-sensitive active agents can be entrapped as solid powders in the polymer network.[37,175,177,179,185] In addition, the invasiveness of surgical techniques can be minimized as the liquid precursor can be injected at the defect site and photocured with fiber optic cables, or even through tissues.[175,176] Although photocrosslinking does offer many advantages, some issues can be considered as weaknesses, such as light attenuation in forming thick materials, the insurance of non-toxic initiator residues, and (atmospheric) oxygen inhibition.[133,173,174,186,187] During the process of oxygen inhibition, both the initiating and the polymer radicals are rapidly scavenged by oxygen molecules to yield peroxyl radicals, which are not reactive toward double bonds and therefore cannot initiate or participate in the polymerization reaction.[186,187]

Free-radically crosslinked biodegradable networks for biomedical applications have been typically prepared from linear and star-shaped precursors based on lactides[119,121,129,144,145,160] and lactones[55,119,122,130,132,134,188-191]. For example, polyesters based on crosslinked poly(ε-caprolactone fumarates) have recently been developed as alternatives to acrylated and methacrylated poly(ε-caprolactones).[192-196] Furthermore, polyester networks based on poly(ε-caprolactone-co-D,L-lactides) have been extensively studied for tissue engineering and drug delivery applications.[49,197-203] In addition to crosslinked polyesters, incorporation of crosslinkable functional groups in polyanhydrides has led to the development of crosslinked

polyanhydride networks.[176,185,204-216] Muggli *et al.*[217,218] and Anseth *et al.*,[34,162] for example, have prepared hard and rigid polyanhydride networks mainly for orthopedic and bone tissue applications by photocrosslinking methacrylated SA, CPH, and CPP precursors. As for thermoplastic polyanhydrides, the degradation time of photocrosslinked polyanhydrides could be adjusted between two days and one year by varying the network composition.[34,162,176,185] The mass loss of these crosslinked polyanhydrides decreased linearly with time, supporting a surface erosion type mechanism.[185] Recently, Weiner *et al.*[219-221] prepared photocrosslinked polyanhydride networks from SA and CPH dimethacrylates and modified the degradation of and drug release from the networks by adding reactive diluent (poly(ethylene glycol) dimethacrylate) and an buffering agent (calcium carbonate).

1.5 Degradation and erosion of crosslinked biodegradable polymers

Crosslinking provides an effective tool for tailoring biodegradable polymers and it is often used to enhance mechanical properties.[127,160,162-163164165,166,217,222-227] For example in tissue engineering applications mechanical properties are important; hard and rigid networks are developed mainly for orthopedic and bone tissue applications,[160,162,164,166,176,217] whereas elastic networks are aimed toward soft-tissue applications.[49,141,168,224,225] However, in drug delivery, control of the degradation and erosion properties of the networks is particularly important because matrix degradation and erosion influence the drug release kinetics of the delivery system.

Similarly to biodegradable thermoplastic polymers, there are a number of ways to modify the degradation and erosion of crosslinked biodegradable networks. These include modifying the precursors as well as the overall chemical composition (e.g., varying concentration of reactive monomers, diluents, and additives). One feasible way to affect the degradation and erosion of the networks is to modify hydrophobicity of the networks by incorporating additional hydrophilic or hydrophobic comonomers and/or copolymers to the precursors or composition.[50,126,183,228-230] For example, Storey *et al.*[50] and Kim *et al.*[127] have decreased the water uptakes and degradation rates of lactide-based networks by incorporating more hydrophobic trimethylene carbonate comonomers into the precursor or by changing the hydrophilic poly(ethylene glycol) co-initiator to the more

hydrophobic poly(propylene glycol) or poly(tetramethylene glycol) co-initiators.

Although the degradation and erosion of the crosslinked networks are governed by the properties of the constituent precursors, the crosslinking density (i.e., the chain length between crosslinks) has an additional effect on the degradation properties of the networks.[126,127,160,231] At a high crosslinking density, the chain lengths between the crosslinks are short and the network is tightly crosslinked.[37] The crosslinking density can be modified by varying the molecular weight and architecture of the precursor, changing the chemical composition by altering the concentration of added comonomer/copolymer, and altering the crosslinking reaction (e.g., crosslinking method, time, temperature, initiation method, and initiator type and concentration).[34,49,120,160] An increase in the polymer crosslinking density typically leads to slower degradation. This response is due to a decrease in water diffusion because the degradable linkages are hindered within the densely crosslinked network, and to an increase in the number of bonds that must be cleaved to break the network into water-soluble components.[37,49,133] As the crosslinking density increases, the T_g increases because T_g, in addition to the limited molecular motion, corresponds to a low free volume within the polymer network.[55] In other words, a high T_g value indicates that there is less space available for water molecules to penetrate the matrix and the degradation time increases because the diffusion of water is hampered.

In addition to the degradation rate, crosslinking density has been also reported to affect the hydrolytic erosion mechanism.[49,225] Amsden *et al.*[225] have found that tightly photocrosslinked networks prepared from low molecular weight (1250 g/mol) acrylated star-shaped poly(ε-caprolactone-co-D,L-lactide) precursors eroded from the surface, whereas less-crosslinked networks prepared from corresponding high molecular weight (7800 g/mol) precursors eroded in a bulk erosion fashion. This finding was explained by a slower rate of water penetration into the bulk of the highly crosslinked networks compared with the rate of hydrolysis of the ester bonds.[225]

Finally, the effect of the kinetic chain length on degradation of the crosslinked networks has been studied recently by Jansen.[232] Kinetic chains are carbon-carbon chains that are formed through radical polymerization of the double bonds via an addition-type polymerization and remain present

after degradation.[232-234] Networks with a shorter kinetic chain length exhibited a lower T_g and crosslinking density and thus faster degradation. Kinetic chain length can be decreased by increasing the polymerization initiation rate (e.g., by varying the initiator concentration or light intensity) and/or adding chain transfer agents.[133,160,232-234] In conclusion, it is rather challenging to tailor the degradation behavior of crosslinked networks. As is the case with most polymer properties, influencing one characteristic usually directly affects another characteristic and these two characteristics may exhibit opposing forces on the degradation properties.[133] For example, if the T_g is decreased with the addition of more hydrophobic comonomers the degradation process is controlled by two opposing effects: a decrease of T_g, which increases water uptake and degradation, and an increase in hydrophobicity, which hinders water uptake and degradation.[49,50]

1.6 The scope of the thesis

The aim of this thesis work was to develop novel surface erodible polyesters with adjustable erosion rates to be used in controlled drug delivery. The surface erosion property of a polymeric drug delivery device is highly desired because the drug release rate in the surface erodible device is directly proportional to the polymer erosion rate and thus predictable, the release is not dependent on the diffusion or swelling properties of the drug and/or the polymer, unreleased drug remains intact and "protected" inside the polymer matrix, and by surface erosion can deliver molecules with wide range of molecular sizes.[10,40,46-48] One feasible way to achieve surface erosion is to produce hydrophobic polymers with hydrolytically labile bonds as in the case of polyanhydrides. Similarly, in this thesis the erosion of the polyesters was tailored by introducing hydrophobic moieties with easily hydrolysable anhydride bonds.

The research of biodegradable polymers based on aliphatic polyesters has expanded to polyesters containing anhydride bonds in the polymer research group at Aalto University.[98,99,235-247] We have previously reported that in hydrolysis studies thermally crosslinked poly(ester anhydrides) exhibited signs of surface erosion, but these networks eroded in few days, which is too short for most controlled drug delivery applications.[245] This was followed by a study in which the degradation rate of the thermoplastic poly(ester anhydrides) was decreased by functionalizing polyester oligomers with hydrophobic alkenylsuccinic anhydrides, but these polymers did not exhibit surface erosion.[247] In this work, the findings from these two previous studies were combined and the main objective was to slow down the erosion process of crosslinked poly(ester anhydrides) while maintaining their surface erosion property by increasing the hydrophobicity of the poly(ester anhydride) precursors with alkenylsuccinic anhydrides. This thesis also focused on crosslinking and characterization of the crosslinked networks. These novel hydrophobically-modified crosslinked poly(ester anhydride) networks were especially aimed for the delivery of sensitive macromolecular active agents, such as protein- and peptide-based drugs.

This thesis summarizes the research reported in the five appended publications. The synthesis of the crosslinked poly(ester anhydride) networks containing labile anhydride bonds and hydrophobic moieties is reported in publications I-III. In publication I was investigated the effect of the poly(ε-caprolactone) based oligomer structure (i.e., molecular weight,

molecular architecture, and hydrophobic side chain length of the functionalizing agent) on the hydrophobicity and thermal properties of the oligomers. In publications II and III, these hydrophobically-modified oligomers were methacrylated to precursors and the precursors were crosslinked to networks. Thermal crosslinking of the precursors was reported in publication II and in publication III the thermal crosslinking was replaced by photocrosslinking to achieve milder crosslinking conditions.

The properties, especially hydrolytic erosion, of the crosslinked networks were reported in publications II-IV. The effect of the hydrophobic alkenyl chain as well as the molecular weight and architecture of the precursor on the thermal properties and hydrolytic erosion of the crosslinked networks was studied in publications II and III, whereas the effect of the *in vivo* environment on erosion was studied in the publication IV. The preliminary safety of the crosslinked networks was evaluated *in vitro* and *in vivo* in publications III and IV.

The drug release properties of the crosslinked poly(ester anhydrides) were studied in publications III-V. The *in vitro* release of a high molecular weight model compound (dextran, relative molecular mass (M_r) ~ 2 000 000) through surface erosion was demonstrated in publication III and the release of a water-soluble small molecular weight model drug (propranolol HCl, 296 g/mol) with different drug loading degrees *in vitro* was studied in publication IV. Finally, the *in vivo* investigation of a photocrosslinked poly(ester anhydride) implant for controlled delivery of peptide YY3-36 (PYY3-36, 4050 g/mol) and examination of the effect of the increased hydrophobicity of the implant on the drug release was reported in publication V.

2. Synthesis of crosslinked poly(ester anhydrides)

Crosslinked poly(ester anhydride) networks were prepared from hydrophobically-modified ε-caprolactone based poly(ester anhydride) precursors (Figure 8). Nomenclature in the field of crosslinked polymers can be confusing because different names for the same meanings are frequently used. In this thesis the term “hydroxyl-terminated oligomer” is used for products resulting from ring-opening polymerization of ε-caprolactone monomers (Figure 8a). Functionalization of the hydroxyl-terminated oligomers with different succinic anhydrides produces “acid-terminated oligomers” (Figure 8b). When the acid-terminated oligomers are methacrylated and ready for crosslinking they are called “precursors” (Figure 8c). The term “prepolymers” refers to oligomers (hydroxyl- and acid-terminated) and precursors all together.

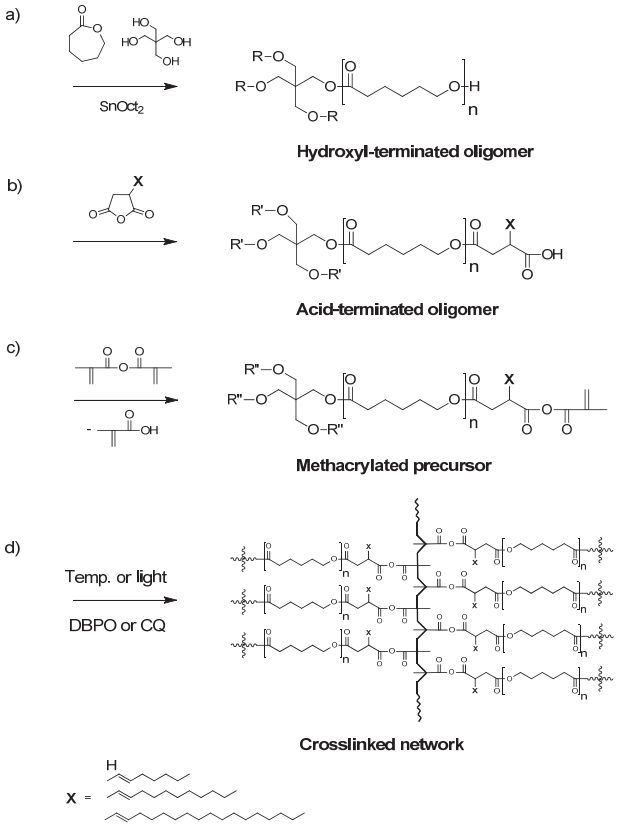


Figure 8. Reaction scheme for the synthesis of crosslinked poly(ester anhydride) networks.[I-III]

2.1 Synthesis of prepolymers

The reaction scheme for the preparation of hydrophobically-modified methacrylated precursors for crosslinking is shown in Figure 8a-c. The synthesis of precursors consisted of three steps. First, ε-caprolactone monomers were polymerized to hydroxyl telechelic oligomers by ring-opening polymerization. In the next step, hydroxyl termination was changed to acid termination through reaction with succinic anhydride (SAH) or alkenylsuccinic anhydrides (ASAs). In the final step, acid-terminated oligomers were allowed to react with methacrylic anhydride (MAAH) to obtain crosslinkable poly(ester anhydride) precursors with labile anhydride bonds. The prepolymers were analyzed with nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and size exclusion chromatography (SEC). The main feature of these characterizations was to confirm the proposed structures and to calculate the degree of substitution (DS) in the acid-functionalized oligomers and methacrylated precursors. In this summary, the denotation of the prepolymers indicates the molecular structure of the prepolymer, i.e., “LIN” and “STAR” indicate linear and star-shaped molecular architecture, being followed by the molecular weight of the hydroxyl-terminated oligomer (1000-4000 g/mol) and the number of carbons in the alkenyl chain of the acid functionalizing agent (0-18 carbons). Finally, “OH” indicates hydroxyl termination, “a” indicates acid end group, “m” indicates methacryl end group, and “nw” indicates crosslinked network. As an example, in the denotation STAR-1000-8nw, STAR indicates star-shaped molecular architecture of the oligomer, 1000 indicates molecular weight of the oligomer before acid functionalization, 8 indicates acid functionalization with alkenylsuccinic anhydride containing alkenyl chain of 8 carbons and nw indicates crosslinked network.

2.1.1 Synthesis of hydroxyl-terminated oligomers

The reaction scheme and overview of the polymerizations of the hydroxyl-terminated oligomers are presented in Figure 8a and Table 1. Hydroxyl-terminated oligomers were prepared by ring-opening polymerization of ε-caprolactone monomers in the presence of stannous octoate ($SnOct_2$) initiator and a co-initiator containing hydroxyl groups. The polymerization was carried out at 160 °C for four hours. The molecular weight of the hydroxyl-terminated oligomers was controlled by varying the amount of the co-initiator (2.5 to 10 mol-%), whereby molecular weights calculated from

the NMR spectra coincided well with theoretical molecular weights calculated from the feed ratios (Table 1). The molecular architecture of the oligomers was controlled by the type of co-initiator; 1,4-butanediol (BD) contains two hydroxyl groups and was used in the preparation of linear oligomers while pentaerythritol (PERYT) contains four hydroxyl groups and was used for the preparation of the star-shaped oligomers with four arms. In general, changing the molecular architecture of the polymers from linear to star-shaped can be used to tailor flow properties of the polymers and reduced viscosity may yield even liquid polymers.[248-250]

Table 1. Molecular weights of the hydroxyl-terminated oligomers.[247,I]

	Hydroxyl-terminated oligomer				NMR			SEC			State[b]
	Co-init. type	Co-init. amount (mol-%)	Segm. (theor.) (CL units)	M_n (theor.) (g/mol)	Segm. (CL units)	M_n (g/mol)	X[a] (%)	M_n (g/mol)	M_w (g/mol)	MWD	
LIN-1000-OH	BD	10	4.5	1120	4.26	1060	>99	2400	2800	1.2	Waxy
LIN-2000-OH	BD	5	9	2150	8.93	2130	>99	4600	5500	1.2	Solid
STAR-1000-OH	PERYT	10	2.25	1160	2.18	1130	>99	1800	2600	1.4	Visc. Liq.
STAR-1500-OH	PERYT	7.5	3	1510	3.02	1510	>99	2600	3600	1.4	Waxy
STAR-2000-OH	PERYT	5	4.5	2190	4.46	2170	>99	3900	5500	1.4	Solid
STAR-4000-OH	PERYT	2.5	9	4250	9.05	4270	>99	7100	11800	1.6	Solid

[a] Monomer conversion.

[b] Physical state at room temperature

2.1.2 Synthesis of acid-terminated oligomers

For varying the hydrophobicity of the hydroxyl-terminated oligomers, and thus the hydrolytic erosion properties of the final crosslinked networks, the oligomers were acid-functionalized with succinic anhydride (SAH) or alkenylsuccinic anhydrides containing 8, 12 or 18 carbons in the alkenyl chain, i.e., with (+/-)-2-octen-1-ylsuccinic anhydride (8-ASA), 2-dodecen-1-ylsuccinic anhydride (12-ASA), or n-octadecenylsuccinic anhydride (18-ASA) (Figure 8b). These alkenylsuccinic anhydrides have been reported to increase the hydrophobicity of soy protein and gelatin surfactants and to improve the hydrophobicity performance of chitosan and starch surfaces.[251-254] In addition, in drug delivery alkenylsuccinic anhydrides has been used as starch modifiers to enhance drug dissolution properties in the hydrophilic matrix tablets.[255]

An overview of the acid functionalizations is presented in Table 2. The functionalization of hydroxyl end groups to carboxylic acids was carried out

in bulk without a catalyst within three hours at 160 °C. The chemical structures of the resulting oligomers were confirmed from FTIR and NMR spectra. In the FTIR spectra, the original broad band for the hydroxyl absorption disappeared upon incorporation of the succinate, and a new broad peak at 3000-3500 cm^{-1} for carboxylic acid appeared. The degree of substitution was calculated from proton nuclear magnetic resonance (^1H NMR) spectra. Since the terminal hydroxyl group in the PCL chain is a primary hydroxyl, its reactivity with cyclic anhydrides is good.[157] Thus, the degree of substitution (DS) for all the acid-functionalized oligomers was over 96% (Table 2).

Table 2. Molecular weights of the acid-terminated oligomers and methacrylated precursors.[247,I-III]

	Acid-terminated oligomer									Methacrylated precursor					
	Co-init. type	Co-init. amount (mol-%)	Segm. (theor.) (CL units)	Alkenyl chain (carbons)	NMR DS (%)	SEC M_n (g/mol)	SEC M_w (g/mol)	SEC MWD	State[b]	NMR DS (%)	Theor. M_n (g/mol)	SEC M_n (g/mol)	SEC M_w (g/mol)	SEC MWD	State[b]
LIN-1000-0	BD	10	4.5	0	>99	2900	3600	1.3	Waxy	93	1450	3300	3900	1.2	Waxy
LIN-1000-8	BD	10	4.5	8	>99	2700	3400	1.3	Visc.Liq.	97	1670	3400	4000	1.2	Visc.Liq
LIN-1000-12	BD	10	4.5	12	>99	2900	3700	1.3	Visc.Liq.	94	1790	3900	4300	1.1	Visc.Liq
LIN-1000-18	BD	10	4.5	18	96	3200	4200	1.3	Visc.Liq.	95	1950	4800	5500	1.2	Visc.Liq
LIN-2000-0	BD	5	9	0	>99	5000	6700	1.3	Solid	97	2480	5600	7200	1.3	Solid
LIN-2000-8	BD	5	9	8	>99	5100	6400	1.3	Waxy	98	2700	6300	7800	1.2	Waxy
LIN-2000-18	BD	5	9	18	97	5600	7000	1.2	Waxy	98	2980	7600	10800	1.4	Waxy
STAR-1000-0	PERYT	10	2.25	0	>99	3100	4500	1.4	Visc.Liq.	94	1830	3800	4800	1.3	Visc.Liq
STAR-1000-8	PERYT	10	2.25	8	>99	3400	4700	1.4	Visc.Liq.	98	2280	4300	5300	1.2	Visc.Liq
STAR-1000-12	PERYT	10	2.25	12	>99[a]	3900[a]	5300[a]	1.4[a]	Visc.Liq.[a]	95	2500	4800	5600	1.2	Visc.Liq
STAR-1000-18	PERYT	10	2.25	18	>99	4800	7000	1.4	Visc.Liq.	96	2840	6400	7600	1.2	Visc.Liq
STAR-1500-0	PERYT	7.5	3	0	>99	3700	5600	1.5	Visc.Liq.	94	2180	6300	9900	1.6	Visc.Liq
STAR-1500-8	PERYT	7.5	3	8	>99	4100	5500	1.3	Visc.Liq.	99	2620	5500	7200	1.3	Visc.Liq
STAR-1500-18	PERYT	7.5	3	18	99	5200	8100	1.5	Visc.Liq.	98	3180	8400	13800	1.6	Visc.Liq
STAR-2000-0	PERYT	5	4.5	0	>99	5300	7800	1.4	Waxy	95	2860	7100	11100	1.6	Waxy
STAR-2000-8	PERYT	5	4.5	8	99	5700	7600	1.3	Waxy	98	3300	7000	9500	1.4	Visc.Liq
STAR-2000-18	PERYT	5	4.5	18	98	6900	10200	1.5	Visc.Liq.	98	3860	9300	15100	1.6	Visc.Liq
STAR-4000-0	PERYT	2.5	9	0	>99	9000	16100	1.7	Solid	97	4920	12900	20100	1.6	Solid
STAR-4000-8	PERYT	2.5	9	8	98	10000	14500	1.5	Solid	99	5360	11500	16600	1.4	Waxy
STAR-4000-18	PERYT	2.5	9	18	96	11300	18300	1.6	Solid	98	5920	14300	22500	1.6	Waxy

[a] Unpublished data
[b] Physical state at room temperature

2.1.3 Synthesis of methacrylated precursors

Crosslinkable poly(ester anhydride) precursors with hydrophobic moieties, labile anhydride bonds, and double bonds were obtained by allowing acid-terminated oligomers to react with methacrylic anhydride (Figure 8c). Double bonds were introduced into the oligomers to make them crosslinkable through radical polymerization. Methacrylation was

carried out at 60 °C for 24 hours with an excess of methacrylic anhydride. The chemical structures of the methacrylated precursors were confirmed from the FTIR, ^{1}H NMR, and carbon nuclear magnetic resonance (^{13}C NMR) spectra. The FTIR spectra were dominated by the ester carbonyl absorbance of lactone units at around 1728 cm^{-1}. The methacrylation created absorbance due to the anhydride bond and the methacryl double bond at 1788 cm^{-1}, 1803 cm^{-1} and 1636 cm^{-1} and, as expected, the broad acid absorbance from 3000 to 3500 cm^{-1} disappeared. The characteristic peaks of a methacrylate double bond were seen in the ^{1}H NMR spectra at 6.20 and 5.79 ppm and in the ^{13}C NMR spectra at 129.41 ppm.[II]

Table 2 summarizes the degrees of substitution and molecular weights of the methacrylated precursors. The degree of substitution for the methacrylations was found to be over 93% for all precursors. The changes in molecular weight during methacrylation were followed by using SEC. In some cases the molecular weight of the precursors increased after methacrylation and drying in vacuum. Helminen *et al.*[245] and Kim *et al.*[212] found the molecular weight of dimethacrylated precursors containing anhydride bonds to increase during drying under high vacuum and suggested that this may be due to polycondensation of the anhydride linkages. When the vacuum drying was done as rapidly as possible and the disappearance of the solvent was followed by ^{1}H NMR, the vacuum drying did not significantly change the molecular weights, and the molecular weight distributions remained narrow. An increase in either the molecular weight of the precursor or the length of the alkenyl chain did not have an effect on the methacrylations.

2.2 Synthesis of crosslinked networks

The synthesis of the photocrosslinked poly(ester anhydride) networks is illustrated in Figure 8d. Crosslinking of the higher molecular weight precursors was performed by thermal initiation while the viscous liquid lower molecular weight precursors were crosslinked by photoinitiation (Table 3). Characterizations available for polymers are often carried out with polymer solutions. Due to the insolubility of the crosslinked samples, the structures were analyzed by FTIR and/or FTIR equipped with attenuated total reflectance apparatus (ATR-FTIR) as well as by applying extraction and swelling tests. FTIR and ATR-FTIR revealed the changes in reactive groups and double bond conversion. Gel content measured by the extraction tests gave information on the degree of crosslinking, whereas

swelling behavior revealed differences in crosslinking density. The gel contents and degrees of swelling of the networks are presented in Table 3.

Table 3. The initiation methods, gel contents, and degrees of swelling of the networks.[II-III]

	Crosslinked network				
	Initiation method	Temp. (°C)	Time (min)	Gel cont. (%)	Swell. (%)
LIN-1000-0nw	Photo	23	28	48	800
LIN-1000-8nw	Photo	23	30	83	380
LIN-1000-12nw	Photo	23	32	84	380
LIN-1000-18nw	Photo	23	32	78	490
LIN-2000-0nw	Thermal	120	60	9	800
LIN-2000-8nw	Thermal	120	60	14	2000
LIN-2000-18nw	Thermal	120	60	49	2200
STAR-1000-0nw	Photo	23	18	95	250
STAR-1000-8nw	Photo	23	20	96	190
STAR-1000-12nw	Photo	23	22	97	190
STAR-1000-18nw	Photo	23	22	96	220
STAR-1500-0nw	Thermal	120	60	94	450
STAR-1500-8nw	Thermal	120	60	96	360
STAR-1500-18nw	Thermal	120	60	95	360
STAR-2000-0nw	Thermal	120	60	96	460
STAR-2000-8nw	Thermal	120	60	98	360
STAR-2000-18nw	Thermal	120	60	95	360
STAR-4000-0nw	Thermal	120	60	96	640
STAR-4000-8nw	Thermal	120	60	98	470
STAR-4000-18nw	Thermal	120	60	97	470

2.2.1 Thermal crosslinking

Thermal crosslinking was used for the higher molecular weight precursors due to their waxy or solid physical state at room temperature. Before the thermal crosslinking the precursors were first heated to 60 °C, whereby all precursors were in the liquid state and the dibentzoyl peroxide (DBPO, 2 wt-%) initiator could be added as solid powder. The mixture was stirred and applied to a steel mould. The precursors were cured in the mould for one hour at 120 °C to produce crosslinked poly(ester anhydride) networks.

Based on the FTIR spectra of the thermally crosslinked networks, all of the double bonds reacted during the crosslinking, as indicated by the disappearance of the absorbance of the double bond peak at 1637 cm^{-1}. These results indicated a high yield of the double bond conversion. However, in the extraction tests, the gel contents for the thermally crosslinked networks prepared from linear precursors (LIN-2000) were low (9-49%, Table 3). Since the FTIR results indicated that all of the double bonds had reacted, the anhydride bond must have a depressant effect on the gel contents. In addition, Lendlein *et al.*[256] have reported high gel contents of 94% for networks that were crosslinked from similar methacrylated ε-caprolactone based linear precursors with corresponding molecular weights but lacking anhydride bonds.

Changing the molecular architecture of the methacrylated precursor had a clear-cut increasing effect on the gel content. The gel contents of the networks that were prepared from star-shaped precursors were all greater than 94% (Table 3). This was clearly due to the amount of methacrylate end groups which was higher for the star-shaped precursors. In the thermal crosslinking of the star-shaped precursors all the double bonds did not have to react, whereas in the linear precursors the reaction of both double bonds was required in order to contribute to crosslinking. Increasing the molecular weight of the star-shaped precursor or adding the alkenyl chain did not have an effect on the gel contents.

The thermally crosslinked networks that were prepared from the star-shaped precursors also exhibited a low degree of swelling (360-640%) as shown in Table 3. Similarly, Lendlein *et al.*[256] report for networks that were crosslinked from poly(ε-caprolactone) based dimethacrylates that at high gel contents the degree of swelling values are low (between 410 and 490%). This result indicates high crosslinking densities, i.e., tightly crosslinked networks. As expected, decreasing the molecular weight of the precursor also decreases the swelling degree of the networks. This decrease was clearly due to the shorter poly(ε-caprolactone) arms, which makes the network denser.

2.2.2 Photocrosslinking

Photoinitiation of low molecular weight liquid precursors was utilized to prepare crosslinked poly(ester anhydride) matrices at room temperature and without solvents, because one of the aims of the thesis was to prepare matrices for controlled delivery of sensitive protein- and peptide-based drugs. Before the photocrosslinking the photoinitiator (1 wt-% of camphorquinone) was stirred with the methacrylated precursors at room temperature and the mixture was applied to a Teflon® mold. Photocrosslinking was done with visible light with exposure times of 10 to 30 minutes depending on the precursor and the light source. Visible light instead of ultraviolet (UV) curing systems was chosen, because it is considered safer to use and provides greater depth of cure.[161,162] Transparent Teflon® film was applied to the top and bottom of the discoids to assist the molding and to prevent oxygen inhibition. The photocrosslinking reaction was followed nearly on-line by ATR-FTIR. During 20-minute photocrosslinking the anhydride bond shifted from 1788 cm^{-1} and 1803 cm^{-1} to 1814 cm^{-1}, as seen in Figure 9a, and the methacryl double bond at 1636 cm^{-1} decreased in intensity, as shown more closely in Figure 9b. In general, by changing the molecular architecture of the precursor from linear to star-shaped, or by decreasing the alkenyl chain length of ASA from 18 to 8 carbons, the crosslinking time decreased and the amounts of reacted double bonds increased.[III]

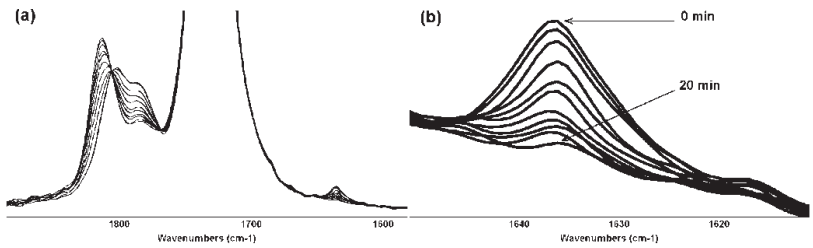


Figure 9. ATR-FTIR spectra of a) the anhydride bond at 1814 cm^{-1} and b) the double bond at 1636 cm^{-1} of STAR-1000-18m precursor recorded during photocrosslinking.[III]

As in the thermal crosslinking, changing the molecular architecture of the methacrylated precursor from linear to star-shaped had an increasing effect on the gel content and a decreasing effect on the swelling degree of the photocrosslinked networks. As seen in Table 3, the gel contents of the

networks prepared from star-shaped precursors were all above 94% whereas those of the corresponding networks prepared from linear precursors were from 48 to 84%. The difference was evidently due to the larger amount of double bonds in the star-shaped precursors. The swelling results proved the networks formed from star-shaped precursors to be denser (swelling degrees 190-250%) than those formed from the corresponding linear precursors (swelling degrees 380-800%). The denser network was due to the chain length of the precursor arm, which was half as long in the star-shaped precursor as in the linear counterpart. Adding an alkenyl chain or increasing the alkenyl chain length in the photocrosslinked networks prepared from the star-shaped precursors did not markedly affect the gel content or the swelling degree.

3. Properties of the prepolymers and networks

The goal of this thesis was to prepare hydrophobically-modified crosslinked poly(ester anhydrides) with tunable degradation and erosion properties to be used as drug delivery implants. This part of the thesis addresses the effect of oligomer molecular structure and acid functionalization with alkenylsuccinic anhydrides on hydrophobicity and thermal properties of the crosslinked polymers. These properties have a strong effect on the hydrolytic degradation and erosion, and thus on the drug release behavior of the networks. The hydrophobicities of the oligomers and networks are studied in the publication I and II and the thermal properties are reported in publications I-III. The last part of this chapter deals with the safety of the oligomers and networks, as reported in publications III and IV.

3.1 Hydrophobicity

One of the most important factors affecting degradation and erosion of the polymers is the hydrophilic or hydrophobic nature of the polymer, since it influences to the water penetration into the polymer.[49,50] Especially hydrophobic polymers containing hydrolytically labile bonds, as often is the case with polyanhydrides, can degrade from the surface, which is a very desirable property for drug release. In this thesis the hydrophobicity of the oligomers and crosslinked networks was evaluated by using the sessile drop contact angle method. Low contact angles indicate that a surface is hydrophilic and high contact angles that the surface is hydrophobic. The water contact angles of the oligomers were determined from thin films spin-coated from chloroform onto silica surface, whereas contact angles of the networks were determined from the surface of the crosslinked discoids.

3.1.1 Hydrophobicity of the oligomers

Molecular weight and architecture of the hydroxyl-terminated oligomer have a clear effect on the contact angles. As expected, the contact angle increased when the molecular weight of the linear oligomer increased. This was evidently due to the increased amount of the more hydrophobic PCL segments and the lower amount of hydrophilic hydroxyl end groups. If the

molecular weight was the same but the molecular architecture was changed from linear to star-shaped, the contact angle dropped due to the doubly higher number of polar hydroxyl end groups. However, comparison of hydroxyl-terminated oligomers with the same PCL segment length showed the contact angles to be at the same level despite the half-lower molecular weight of the star-shaped oligomer. Numata *et al.*[257] report similar results for poly(lactides): the contact angle values of linear and branched PLLA oligomers increase with the molecular weight, and the contact angles of linear and branched PLLAs with the same segment length are almost identical.

The hydrophobicity of the hydroxyl-terminated oligomers was modified by using ASAs. An overview of the contact angles of the star-shaped oligomers is shown in Figure 10. In general, the length of the alkenyl chain affected contact angles: oligomers containing a 18-carbon alkenyl chain resulted in higher contact angles than corresponding oligomers having 8-carbon alkenyl chains. Similarly to the hydroxyl-terminated oligomers, the molecular weight and the molecular architecture of acid-terminated oligomers also had a clear effect on the contact angles. Due to the higher concentration of hydrophobic alkenyl chains, the star-shaped oligomers had considerably higher contact angles than their linear counterparts with the same molecular weight. However, comparison of the contact angles of the oligomers requires that the physical state of the sample is the same, i.e., the viscous liquid STAR-1000 oligomers exhibited clearly lower equilibrium contact angle values than the corresponding solid STAR-4000 oligomers (Figure 10), although the instantaneous contact angles were clearly larger. The lower equilibrium contact angles for liquids than solids are due to the easier rearrangement of the molecules into more energetically favorable conformation in the liquid.[258]

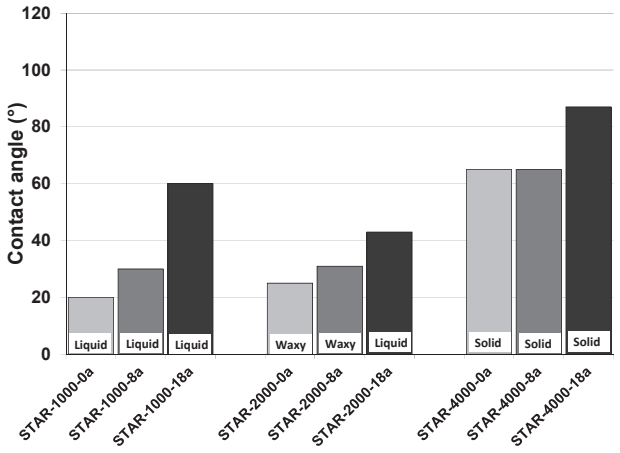


Figure 10. Equilibrium contact angles and physical appearance at room temperature of acid-functionalized star-shaped oligomers. The standard deviations for all the contact angle measurements were under 2°.[1]

3.1.2 Hydrophobicity of the crosslinked networks

All the crosslinked networks possessed a similar solid physical state and the contact angles were more reliably comparable with each other. The contact angles of the thermally crosslinked poly(ester anhydride) networks prepared from star-shaped precursors are presented in Figure 11. Crosslinking increased the equilibrium contact angles remarkably, especially when compared with the lower molecular weight oligomers and networks prepared from them as seen from Figures 10 and 11. In addition to crosslinking, the alkenyl chain and the molecular weight of the precursor influenced the contact angles of the crosslinked networks. In the networks that were crosslinked from the highest molecular weight precursors (STAR-4000), the 8-carbon alkenyl chain had no effect on the contact angle value, whereas the more hydrophobic 18-carbon alkenyl chain increased the contact angle from 82 to 96° (Figure 11). In the networks crosslinked from the lowest molecular weight precursors (STAR-1500), the short 8-carbon alkenyl chain was hydrophobic enough to increase the contact angle from 68 to 86°. This effect was due to the high proportion of 8-carbon alkenyl chains relative to the short ε-caprolactone chains. An increase in the alkenyl chain length to 18 carbons increased the contact angles to 110° (Figure 11). The contact angle of 110° is clearly larger than the contact angle of crosslinked poly(ε-caprolactone) di- and triacrylates, which has been reported to be between 52 and 77°,[134] or thermoplastic high molecular weight poly(ε-caprolactone) thin polymer films, which has been reported to be 78°.[259] In conclusion, the hydrophobicity of the crosslinked poly(ester

anhydride) networks can be easily modified by the molecular weight of the precursor and with different alkenylsuccinic anhydrides.

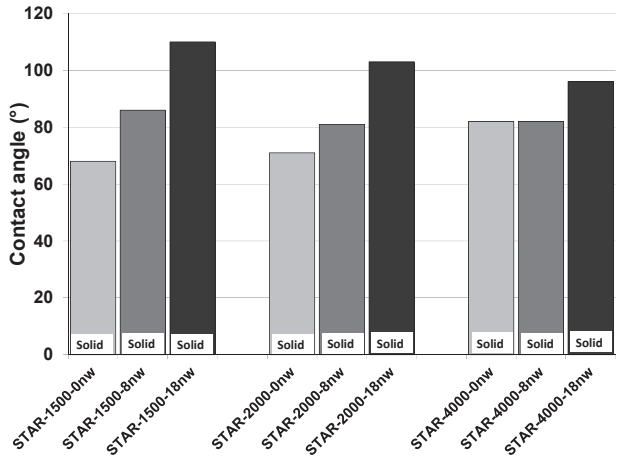


Figure 11. Equilibrium contact angles of the solid networks prepared from star-shaped poly(ester anhydride) precursors. The standard deviations for all the contact angle measurements were under 2°.[II]

3.2 Thermal properties

In addition to the hydrophobicity of the networks, thermal properties have great influence on the degradation and thus the release properties of crosslinked networks. The thermal properties of the prepolymers and the crosslinked networks were evaluated by differential scanning calorimetry (DSC). The acid-terminated oligomers were evaluated because the oligomers are degradation products of the networks, and their solubility, which in turn affects the degradation, depends on their T_m. The T_m of the methacrylated precursors was of importance, since it was used in determining initiation method and temperature, i.e., precursors with low T_m were suitable for photocrosslinking at room temperature. For the crosslinked networks, DSC was used to evaluate the possible melting enthalphy (ΔH) changes due to the crystallinity and changes in T_g, yielding information of the crosslinking density; a higher T_g indicates a more tightly crosslinked network which will degrade more slowly.

3.2.1 Thermal properties of the prepolymers

Thermal properties of the crosslinked polyesters can be controlled widely by the structure of the crosslinkable precursor.[157,166,260] The results of the

thermal properties of the hydroxyl-terminated oligomers are summarized in Table 4. Depending on the molecular weight, the physical state of the hydroxyl-terminated oligomers varied from solid to viscous liquid. Star-shaped hydroxyl-terminated oligomers had lower crystallinity and melting temperatures compared with the corresponding linear oligomers with the same molecular weights. This is due to the fact that the star-shaped polymers have a different entanglement structure and possess a higher density of chain-ends than linear polymers of comparable molecular weights.[250] This affects the crystallinity, which decreases due to the increase in the number of the free chain ends disrupting the crystalline structure.

Table 4. Thermal properties of the oligomers, precursors, and crosslinked networks determined by DSC.[I-III]

	Oligomer			Precursor			Network		
	T_g (°C)	T_m (°C)	ΔH (J/g)	T_g (°C)	T_m (°C)	ΔH (J/g)	T_g (°C)	T_m (°C)	ΔH (J/g)
LIN-1000-OH	-66	36	84						
LIN-1000-0	-56	38	58	-60	25	66	[c]	[c]	[c]
LIN-1000-8	-58	15	47	-63	12	42	-61	14	34
LIN-1000-12	-58	16	49	-64	12	46	-61	14	38
LIN-1000-18	-57	10	43	-60	8	36	-57	22	27
LIN-2000-OH	-62	48	85						
LIN-2000-0	-56	50	83	-59	41	76	-55	43	72
LIN-2000-8	-57	33 & 42	65	-60	36	45	-52	42	55
LIN-2000-18	-57	30 & 40	65	-63	35	56	-53	40	51
STAR-1000-OH	-64	-1 & 16	38						
STAR-1000-0	-51	-	-	-54	-	-	-44	-	-
STAR-1000-8	-50	-	-	-58	-	-	-48	-	-
STAR-1000-12	-50[b]	-	-	-58	-	-	-48	-	-
STAR-1000-18	[a]	19	42	[a]	9	39	[a]	16	35
STAR-1500-OH	-61	18 & 30	51						
STAR-1500-0	-50	-	-	-61	-	-	-46	-	-
STAR-1500-8	-51	-	-	-62	-	-	-46	-	-
STAR-1500-18	[a]	17	39	[a]	-4	35	[a]	6	20
STAR-2000-OH	-61	32 & 41	60						
STAR-2000-0	-53	34	50	-62	22	32	-51	-	-
STAR-2000-8	-54	26	14	-63	19	5	-52	-	-
STAR-2000-18	-53	16	34	-63	-14	22	-53	5	17
STAR-4000-OH	-61	46 & 51	70						
STAR-4000-0	-53	43 & 49	60	-64	41	46	-52	41	40
STAR-4000-8	-54	46	54	-62	41	41	-52	38	35
STAR-4000-18	-56	45	49	-63	38	40	-53	36	33

[a] Not detected.

[b] Unpublished data.

[c] Not measured because of low gel content.

Acid functionalization of the hydroxyl-terminated oligomers increased the T_g of all oligomers and the increase was greater for the lower molecular weight oligomers (Table 4). In addition, acid functionalization in general lowered the melting temperatures and enthalpies and the decrease was again greater for the lower molecular weight oligomers and if the functionalization agent contained an alkenyl chain, i.e., if ASAs were used (Table 4). Although the alkenyl chains lowered the melting enthalpies of the oligomers in general, the 18-carbon alkenyl chain increased melting enthalpies in the case of lower molecular weight star-shaped oligomers. This was apparently due to the long alkenyl chain that was able to crystallize. Similar crystallization of the alkenyl chain has been reported for stearyl side chains.[261,262] Methacrylation of acid-terminated oligomers resulted in lower T_m and T_g values and changed the physical state at room temperature to less viscous. This is especially favorable for photocrosslinking. Finally, thermal properties of the poly(ester anhydride) precursors can be modified, in addition to the molecular weight and architecture of prepolymers, by the choice of the acid-functionalizing agent.

3.2.2 Thermal properties of the crosslinked networks

The glass transition temperatures (T_g's) of the crosslinked poly(ester anhydride) networks were influence by the molecular weight and architecture of the precursors (Table 4). For the networks prepared from the linear precursors, the crosslinking increased the T_g by 2-10 °C. However, T_g's of these networks were not comparable due to wide variation in gel contents of the networks. When the structure of the precursor was changed from linear to star-shaped, the crosslinking increased the T_g by 10-15 °C (Table 4). Furthermore, the networks that were formed from the lower molecular weight precursors had higher T_g's than networks formed from larger precursors which was clearly related to the higher crosslinking density. The presence of the alkenyl chains, regardless of the molecular weight of the precursor, did not markedly affect the T_g.

Melting temperatures and enthalpies were only observed for networks that were prepared from the linear precursors, highest molecular weight star-shaped precursors (STAR-4000) and for networks containing a 18-carbon alkenyl chain (Table 4). Evidently in these networks the polymer main chain or the 18-carbon alkenyl chain was long enough to crystallize. In the networks crosslinked from the STAR-4000 precursors the addition of the alkenyl chain decreased the melting temperatures and enthalpies

(Figure 12). Similarly, decrease of T_m and ΔH values have been observed earlier for thermoplastic poly(ε-caprolactone)-based poly(ester anhydride) polymers containing alkenyl chains.[246]

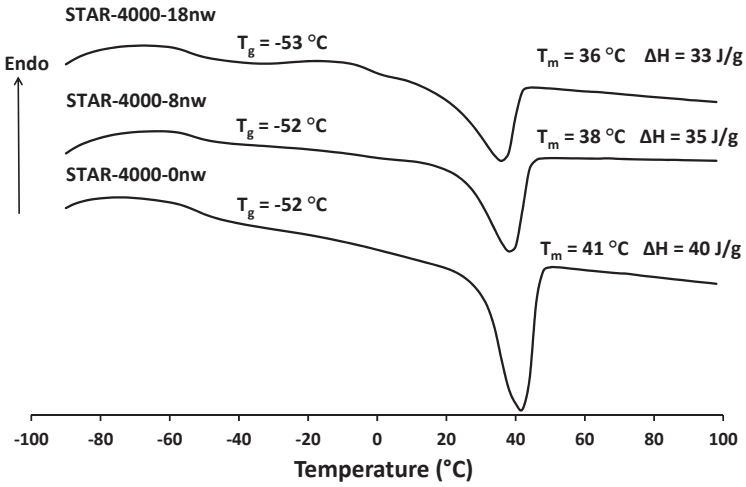


Figure 12. The DSC curves of the STAR-4000 networks prepared from star-shaped poly(ester anhydride) precursors.[II]

3.3 *In vitro* and *in vivo* cytotoxicity

The aim of *in vitro* and *in vivo* cytotoxicity studies was to evaluate the preliminary safety of crosslinked poly(ester anhydrides) based on methacrylated poly(ε-caprolactone) based precursors. Because poly(ε-caprolactone) is comprehensively studied and considered as safe material (already US FDA approved in medical devices), we were mainly interested of the effect of the alkenyl chain to the toxicity of the oligomers. *In vitro* cytotoxicity of the acid-terminated oligomers was evaluated by the AlamarBlue test. The acid-terminated oligomers were non-cytotoxic and cell activities were in the range of 85 to 108% (Figure 13). In addition, the methacrylated poly(ester anhydride) precursor without the alkenyl chain was evaluated and it was also non-cytoxic.[IV] These results are in agreement with those obtained from crosslinked polyanhydride networks which are generally well tolerated.[176,263]

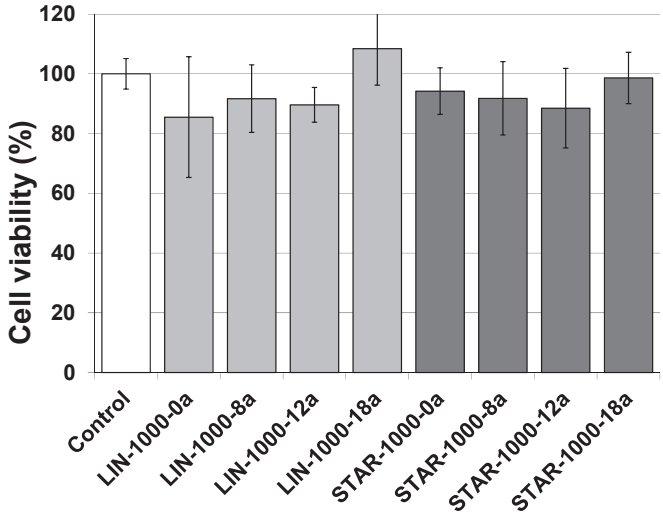


Figure 13. Cell viabilities of acid-terminated oligomers (50 μg/ml) after incubating with human gingival fibroblasts for 24 h.[III]

It must be noted that the degradation products of the studied crosslinked poly(ester anhydride) networks have not yet been characterized. However, it is presumed that similarly to crosslinked polyanhydrides, during hydrolytic degradation of the poly(ester anhydride) networks, the anhydride bonds are cleaved, leading to the formation of constituent acid-terminated oligomers and water-soluble poly(methacrylic acid)(PMAA) kinetic chains.[176] As mentioned above, acid-terminated oligomers were non-cytotoxic in *in vitro* cytotoxicity tests. In addition, water-soluble polymers, such as PMAA, with molecular weights up to 50 000 g/mol are cleared by the kidneys within a few days and through liver when the molecular weight is above 50 000 g/mol.[160] Burkoth and Anseth[176] and Burdick *et al.*[233] have analysed the degradation products of photocrosslinked polyanhydrides and reported very different outcomes: the average molecular weights of PMAA chains were 500-3500 g/mol or 58 000-410 000 g/mol and the lengths of the PMAA chains were highly depended on the crosslinking conditions. However, Melchels *et al.*[160] have recently studied kinetic chains of photocrosslinked lactide-based networks with solid-state NMR and reported that molecular weights of polymethacrylate kinetic chains were maximally 8800 g/mol, which is far below the threshold for renal clearance.

The preliminary *in vivo* safety of poly(ester anhydride) networks was evaluated in rats by measuring cytokine concentrations in plasma after

subcutaneous implantation of poly(ester anhydride) discoids. Discoids dipped in ethanol (100%) were implanted subcutaneously dorsally into 10-week-old Wistar male rats weighing 200-300 g. Cytokines are cell signaling proteins released in many pathological conditions, e.g., infection and inflammation and produced in response to microbes and other antigens. After the implantation of foreign materials, cytokines are secreted by many cells, such as macrophages or neutrophils, promoting inflammation and wound healing processes.[264] Thus, increased plasma concentrations of cytokines may indicate immunogenic responses.[265,266] The studied photocrosslinked poly(ester anhydride) discoids eroded *in vivo* within 120 hours and did not provoke any major immunogenic responses. These results are similar to previously reported thermoplastic poly(ester anhydride) implants and injectable pastes which have indicated no or only minor inflammatory reactions in mice and rats.[112,114,267] In conclusion, although the preliminary safety evaluations suggested that these crosslinked poly(ester anhydrides) should be non-toxic materials, before proceeding to clinical studies, the characterization of the degradation products and more extensive safety studies will be required.

4. Erosion and drug release studies

The main focus of this thesis was to prepare new biodegradable polymers with tunable degradation and erosion properties for controlled drug release. PCL has many attractive features and is a highly suitable material for drug release applications but degrades slowly by a bulk erosion mechanism and the drug release is mainly based on the diffusion of the drug. Hydrolytically susceptible anhydride bonds have been incorporated into the PCL in order to modify its degradation rate and mechanism.[245,246] This part of the thesis addresses the effect of molecular structure (i.e., molecular weight and architecture) and hydrophobic modification (i.e., acid functionalization with alkenylsuccinic anhydrides) of the oligomers on hydrolytic erosion of the crosslinked networks as well on their drug release behavior.

4.1 Erosion of the crosslinked networks

Evaluation of hydrolytic degradation and erosion is one of the most important factors when the biodegradable polymers are considered as materials for controlled drug release. In this thesis the hydrolytic erosion rate of PCL-based polyester networks has been modified by introducing labile anhydride bonds and hydrophobic alkenyl chains into the polymers. In addition to tailoring the erosion rate, the goal was to change the bulk erosion mechanism of PCL towards surface erosion. The effect of precursor structure on the hydrolytic erosion of crosslinked poly(ester anhydrides) was studied in publications II and III. In publication II the hydrolysis studies of thermally crosslinked networks is reported. These networks were mainly prepared from higher molecular weight precursors and the melting temperatures of the prepolymers and networks varied on both sides of the dissolution temperature of 37 °C. Publication III focused on the hydrolysis of photocrosslinked networks prepared from low molecular weight, low viscosity precursors in which the melting temperatures of the prepolymers and networks were clearly below the dissolution temperature. Finally, in publication IV *in vivo* degradation was compared with *in vitro* degradation.

The hydrolytic erosion of the networks was studied by monitoring the change in the mass loss of the crosslinked discoids. All hydrolysis studies were carried out in a pH 7.4 buffer solution with orbital shaking at a frequency of 120 strokes/min at 37 °C. During the studies no change in pH

was observed. The hydrolytic solubility of the acid-functionalized oligomers, i.e., potential degradation products, was evaluated before the hydrolysis studies of the networks. The molecular weight and the alkenyl chain clearly influenced the solubility of the acid-functionalized oligomers. The higher molecular weight oligomers (i.e., LIN-2000 and STAR-4000 oligomers) did not lose any mass in 28 days. These oligomers had melting temperatures above the hydrolysis temperature of 37 °C. In addition, none of the oligomers containing alkenyl chains exhibited any mass loss in seven days even though their melting temperatures were below 37 °C. In other words, the only oligomers which dissolved in the buffer solution were low molecular weight acid-functionalized oligomers without the hydrophobic alkenyl chain.

4.1.1 Mass loss of thermally crosslinked networks

Based on the obviously lower gel contents (under 50%), the thermally crosslinked networks that were prepared from the linear precursors (LIN-2000) were not studied in the hydrolysis test. The mass losses of the networks that were prepared from the higher molecular weight star-shaped precursors (STAR-4000) are presented as a function of time in Figure 14a. All mass losses remained below 20% in eight weeks, these losses occurring mainly during the first weeks. The low mass losses of the crosslinked networks were likely due to the melting temperatures (above 43°C) of the acid-terminated oligomers, i.e., degradation products that are formed after the hydrolysis of the anhydride bonds. Because the T_m of the oligomers was above the experimental dissolution temperature of 37 °C, mass loss of the networks requires also hydrolysis of the ester bonds. After cleavage of the ester bonds the polyester blocks are short enough to dissolve into the buffer solution. The mass loss of networks depended also on the hydrophobicity, because the more hydrophobic network containing 18-carbon alkenyl chains did not exhibit any mass loss during the hydrolysis tests. This was evidently due to the hydrophobic degradation products which were insoluble in the buffer solution. Göpferich *et al.*[268] have also reported for polyanhydride copolymers consisting of a fatty acid dimer and sebacic acid that poorly soluble degradation products can accumulate on the surface of the crosslinked discoids.

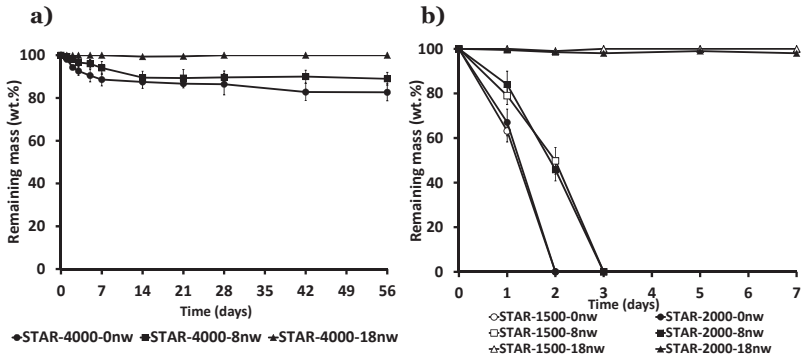


Figure 14. Mass loss of thermally crosslinked poly(ester anhydride) networks prepared from a) higher molecular weight star-shaped precursors (STAR-4000) and b) lower molecular weight star-shaped precursors (STAR-2000 and STAR-1500).[II]

A decrease of the molecular weight of the oligomers to 2000 g/mol or lower had a clear effect on the thermal properties and thus on the mass loss and erosion behaviour of the crosslinked networks. The lower molecular weight oligomers and the networks prepared from them were either amorphous or had melting temperatures below the dissolution temperature. The mass losses of the thermally crosslinked networks prepared from the lower molecular weight (STAR-2000 and STAR-1500) precursors are shown in Figure 14b. The networks without an alkenyl chain lost all of their mass rapidly in two days. The addition of the 8-carbon alkenyl chain increased the hydrophobicity of the networks and prolonged the mass loss by one day. Interestingly, the samples exhibited an almost linear mass loss with intact core, which are clear signs of surface erosion. The mass loss of the most hydrophobic networks, i.e., networks prepared from precursors containing a 18-carbon alkenyl chain, was insignificant, without any mass loss in eight weeks. Despite the increased hydrophobicity water could still penetrate to the matrix and cleave the anhydride bonds because, according to the FTIR spectra, the peak of the anhydride bond at 1815 cm^{-1} disappeared in three days.[III] Mass loss of the networks did not occur, because the hydrophobic degradation products did not dissolve in the buffer solution. We obtained similar results for thermoplastic poly(ester anhydrides) containing 18-carbon alkenyl chains.[246]

4.1.2 Mass loss of photocrosslinked networks

Mass losses of the photocrosslinked samples prepared from linear precursors are shown in Figure 15a. In view of the clearly lower gel content (under 50%), the network without an alkenyl chain was not studied in the hydrolysis test. As shown in Figure 15a, lengthening of the alkenyl chain from 8 to 12 or 18 carbons, i.e., increasing the hydrophobicity, did not significantly affect the erosion time and the networks eroded in approximately two days. Evidently, as for the thermal properties, the increase in hydrophobicity was not sufficient to affect the erosion rate of the discoids prepared from linear precursors. However, similarly to some of thermally cured samples they exhibited clear signs of surface erosion.

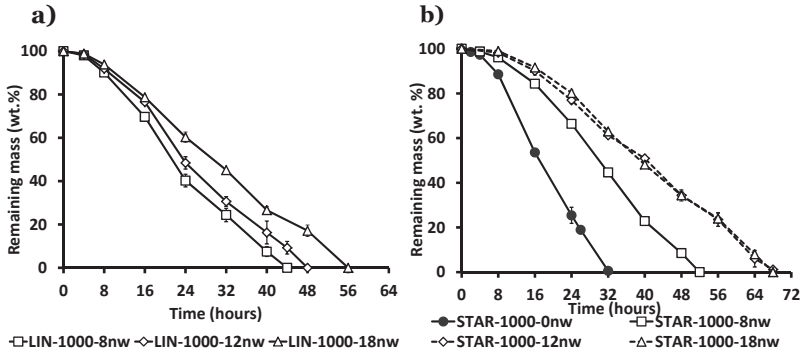


Figure 15. Mass loss of photocrosslinked poly(ester anhydride) networks prepared from low molecular weight a) linear precursors and b) star-shaped precursors.[III]

Changing the molecular architecture of the oligomer from linear to star-shaped tightened the network due to the shorter PCL segments and doubled the amount of alkenyl chains and thus increased the hydrophobicity. Mass losses of the photocrosslinked poly(ester anhydride) networks prepared from star-shaped low molecular weight precursors are shown in Figure 15b. The discoid without alkenyl chains eroded linearly from the surface, after four hours lag time, in 32 hours. Adding the 8-carbon alkenyl chain doubled the lag time to eight hours and the erosion time to 52 hours. As shown in the photograph of the eroded discoids in Figure 16, crosslinked discoid containing 8-carbon alkenyl chain eroded from the surface, similarly to the discoid without the alkenyl chain. However, due to the differences in the solubility of the acid-functionalized oligomers, the degradation products of the networks with 8-carbon alkenyl chains caused

the buffer solution to become slightly opaque, whereas the buffer solution was transparent even after complete degradation of networks without the alkenyl chains. Increase of the alkenyl chain length in the star-shaped precursors from 8 to 12 or 18 carbons prolonged the erosion to 68 hours. The degradation products of the more hydrophobic networks, i.e. networks containing 12- and 18-carbon chains, precipitated on the surface of the discoids and beneath the degradation products the discoids remained intact, the erosion having occurred only on the surface (Figure 16). As in the thermally crosslinked networks, the precipitation resulted from the poor solubility of the hydrophobic degradation products, which accumulated on the surface of the crosslinked discoids.

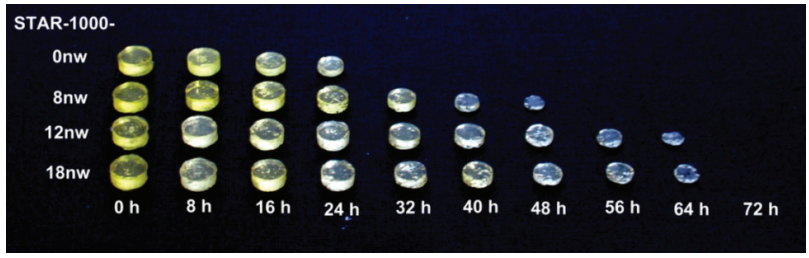


Figure 16. Photograph of eroded poly(ester anhydride) discoids prepared from star-shaped precursors.[III]

4.1.3 *In vivo* mass loss of the crosslinked networks

Because the studied crosslinked networks were aimed to be used as implantable drug release devices it was important to evaluate their *in vivo* erosion. In this study were used photocrosslinked discoids which were prepared from STAR-1000-0m precursors. The discoids were rinsed with ethanol/water solution and implanted subcutaneously in three different dorsal locations in 12-week-old male Wistar rats. The *in vivo* erosion compared with the *in vitro* erosion as a function of time is shown in Figure 17. The *in vivo* erosion followed closely the *in vitro* erosion profile of the corresponding discoid. The similarity between the *in vitro* and *in vivo* erosion suggests that the *in vivo* erosion is based on hydrolytic degradation of the anhydride bonds. If enzymes or other biological factors were playing a significant role in the erosion process, the erosion would then be quicker *in vivo* than *in vitro*.[269] In addition, the discoids exhibited surface erosion,

because the dimensions of discoids shrunk steadily and the discoid core kept its shape during both *in vitro* and *in vivo* studies.

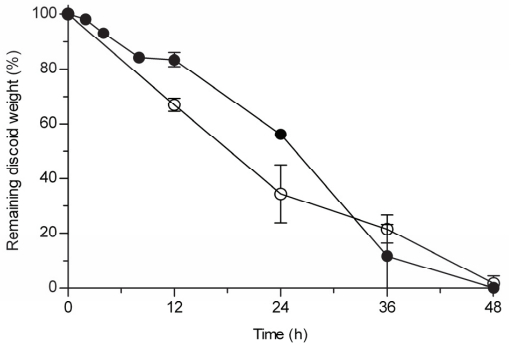


Figure 17. Erosion of poly(ester anhydride) discoids *in vitro* (●) and *in vivo* (○).[IV]

4.2 Drug release from the crosslinked networks

Drug release of the networks was studied in publications III-V. In publication IV the *in vitro* and *in vivo* release of small molecular weight propranolol HCl model drug (296 g/mol) is studied. The *in vitro* release of the macromolecular model compound dextran (M_r ~ 2 000 000) is reported in publication III. The size of the dextran ruled out the diffusion-based release mechanism which might occur with small molecular weight drugs, such as propranolol HCl. Publication V focused on the *in vivo* release of the peptide-based drug PYY3-36 which is a promising drug candidate for the treatment of obesity.[270]

All samples for the release studies were prepared by photocrosslinking low molecular weight STAR-1000 precursors. These precursors were chosen due to their low viscosity which enabled photocrosslinking and mixing of the drug powder at room temperature without any solvents. In typical preparation methods of biodegradable polymeric drug delivery systems, such as hot melt extrusion and solvent casting methods, the polymer must be heated or solvents used in order to mix the polymer and the drug.[271] The heat and the solvent may result in loss of drug activity, particularly, in the cases of sensitive peptide- and protein-based drugs.[271] Organic solvents can also be problematic due to toxic solvent residues.[271]

After the photocrosslinking with visible light either in the light curing oven (220 mW/cm^2) or with a 11 W lamp (16 mW/cm^2), the samples

appeared rubbery and extraction of the samples revealed gel contents above 95% indicative of nearly complete crosslinking of precursors. In addition, final double bond conversions of the photocrosslinked discoids were above 92%. In analogy with the hydrolysis studies, the *in vitro* release studies for the propranolol HCL and dextran were carried out in buffer solution at pH 7.4 and 37 °C with orbital shaking at a frequency of 120 strokes/min. The *in vitro* PYY3-36 release was studied by using USP Apparatus I (basket) under sink conditions in buffer solution at pH 7.4 and 37 °C and with a basket rotation speed of 50 rpm. In the *in vivo* delivery of PYY3-36 one implant was implanted subcutaneously in the back of each male Wistar rat.

4.2.1 Small molecular propranolol HCl release

Well-known small water-soluble drug, propranolol HCl (296 g/mol, solubility 50 mg/ml), was used as the model drug to evaluate whether release from photocrosslinked poly(ester anhydrides) was erosion-controlled. In the propranolol HCl release studies the crosslinked networks were prepared from low molecular weight precursors without the alkenyl chains. The goal was first to find out the effect of the anhydride bond to the release properties. The low viscosity of the precursors enabled mixing of the drug as a plain powder at room temperature and with high drug loading degrees of up to 60 wt-%. The samples were crosslinked in a light curing oven with visible light in 10 minutes. The released propranolol HCl in the buffer was analyzed with a high performance liquid chromatograph equipped with of an ultraviolet detector (HPLC-UV).

The propranolol HCl release from the photocrosslinked poly(ester anhydride) networks with 10-60 wt-% loads as a function of time is shown in Figure 18a. The corresponding network without anhydride bonds with a 10 wt-% drug load is shown for comparison (Figure 18a, dashed line). After a eight hours' lag time the *in vitro* release was linear with 10, 20, and 40 wt-% drug loads. Recently Mönkäre *et al.*[272] have showed that this linear *in vitro* propranolol HCL (10 wt-%) release was primarily controlled by surface erosion. Increasing the drug loading degree to 60 wt-%, abolished the lag time in the release profile, instead an initial burst release within the first hour was observed (Figure 18a). The steady-state propranolol HCl (10 wt-%) release rate from the poly(ester anhydride) network was clearly faster compared with the corresponding polyester network without anhydride bonds; adding the anhydride bonds increased the release rate from 0.04 to 3.3 %/h within the first 48 hours.

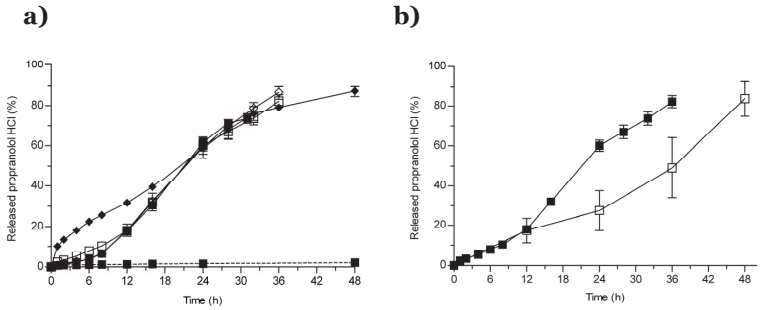


Figure 18. a)*In vitro* drug release from poly(ester anhydride) discoids with drug loading degrees of 10 wt-% (■, solid line), 20 wt-%(◇, solid line), 40 wt-%(□, solid line) and 60 wt-% (♦, solid line) and corresponding polyester discoids without anhydride bonds with drug loading degree of 10 wt-% (■, dashed line), and b) *in vitro* (■)and *in vivo* (□)drug release with drug loading degree of 40 wt-%.[IV]

The *in vivo* drug release with drug loading degree of 40 wt-% was conducted similarly to the earlier mentioned *in vivo* erosion test (without the drug) in 12-week-old male Wistar rats. The *in vivo* drug release, in addition to the *in vitro* drug release, from crosslinked discoids loaded with 40 wt-% propranolol HCl as a function of time is shown in Figure 18b. *In vivo* experiments revealed a linear drug release between 12 and 48 hours. However, the drug release rate of the linear release phase was lower *in vivo* than *in vitro*. The *in vivo* drug release is consistent with the *in vivo* erosion (Figure 17), both lasting approximately 48 hours, indicating drug release controlled by surface erosion.

4.2.2 Macromolecular dextran release

High molecular weight dextran (M_r ~ 2 000 000, 10 wt-%) was used as a model compound to simulate the release of macromolecular drugs that cannot diffuse from the crosslinked matrix. Dextrans are highly water-soluble polysaccharides made from glucose.[273] The *in vitro* release study was carried out on networks prepared from the small molecular weight precursors without or with 8-carbon alkenyl chains. These precursors were selected because the crosslinked networks prepared from them had high gel contents and exhibited clear surface erosion *in vitro* without precipitation of degradation products. The samples were again crosslinked in a light curing oven with visible light in 10 minutes. Due to the large molecular size of the dextran the release was characterized by using SEC equipped with Ultrahydrogel water columns.

The mass loss and the quantity of dextran released during the erosion as a function of time are shown in Figure 19. Dextran release was nearly linear, and proportional to the decrease in the mass. In addition, the network containing 8-carbon alkenyl chains released dextran slightly slower. Modification of the degradation and release rate of crosslinked samples was thus possible by varying the hydrophobicity of the crosslinkable precursor.

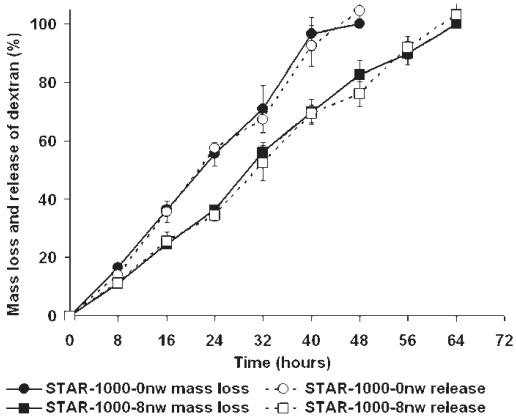


Figure 19. *In vitro* dextran release from (dashed line) and mass loss of (solid line) the dextran-containing photocrosslinked poly(ester anhydride) discoids prepared from STAR-1000-0m (circles) and STAR-1000-8m precursors (squares).[III]

4.2.3 Peptide YY3-36 release

Human peptide YY3-36 (PYY3-36, 4050 g/mol) is a promising candidate for treatment of obesity.[270] In the *in vivo* PYY3-36 release studies, crosslinked networks were prepared from the small molecular weight precursors without or with 12-carbon alkenyl chains. The goal was to decrease the peptide release rate with a more hydrophobic network. The low viscosity of the precursors again enabled mixing of the drug as plain powder at room temperature. To ensure the mildest possible reaction conditions for the sensitive peptide drug, the light curing oven was changed to the 11W visible light lamp and the crosslinking time was 20 minutes. The *in vitro* PYY3-36 release and *in vivo* PYY3-36 plasma concentrations were analyzed using total human PYY ELISA kit according to the manufacturer's instructions.

The *in vivo* PYY3-36 release from the two poly(ester anhydride) networks with 10 wt-% loads as a function of time is shown in Figure 20. The administration of PYY3-36 via a subcutaneously (s.c.) implanted discoid significantly sustained the PYY3-36 release. After subcutaneous injection of solution, PYY3-36 was detected in plasma only for four hours, whereas administration via poly(ester anhydride) implants prolonged the detection period up to even nine days (Figure 20a). The duration of the *in vivo* release was also analyzed by the percent area under the curve (AUC) method which describes drug input to the systemic circulation by combining release and absorption phases.[274] A comparison of implants without the alkenyl chains and with 12-carbon alkenyl chains indicated that hydrophobic modification sustained the PYY3-36 release from three to seven days (Figure 20b). In addition, the linear *in vivo* release rate halved from two to one %/h by the addition of 12-carbon alkenyl chains. However, the *in vitro* release of PYY3-36 from the network containing 12-carbon alkenyl chains was less than 20% in 60 hours, despite the fact that the samples were completely eroded within that time. This indicated that the degradation products might interact with the peptide under the *in vitro* conditions, which does not happen *in vivo*. At the end of the *in vivo* experiments, rats were sacrificed and the implantation site was visually inspected and no traces of polymer could be detected from the s.c. tissue, indicating complete erosion of the implants.

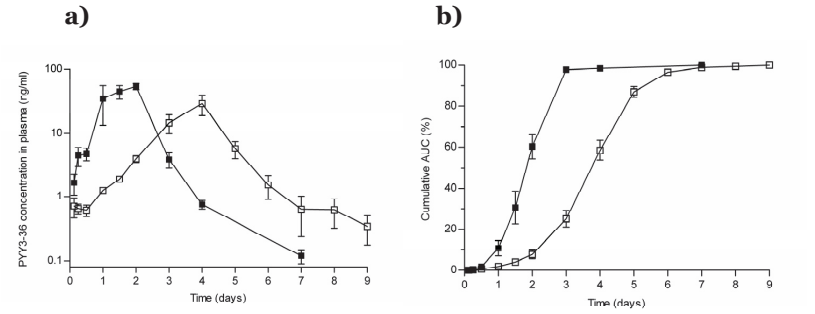


Figure 20. a)PYY3-36 concentration in plasma after s.c. administration of PYY3-36-loaded (10 wt-%) photocrosslinked poly(ester anhydride) discoids without the alkenyl chains (■) and with 12-carbon alkenyl chains (□)and b) *in vivo* cumulative release, by using the percent AUC method, of PYY3-36 from photocrosslinked poly(ester anhydride) implants without the alkenyl chains (■) and with 12-carbon alkenyl chains (□). The profile indicates the percent of PYY3-36 released and absorbed into plasma.[V]

The increased bioavailability was clear evidence of improved delivery when using crosslinked poly(ester anhydride) implants. After administration as a solution the subcutaneous bioavailability of PYY3-36 was dose-dependent and low (4-29%), whereas it was practically 100% when administered in photocrosslinked poly(ester anhydride) implants. The fundamental difference between solution and implant administration is the availability of peptide for absorption. After administration of solution, PYY3-36 was immediately available for absorption, but with the implant the release rate is controlled such that the release rate is constantly 5-10 μg/h during the linear release phase. Based on differences in bioavailability between implant and solution administration it is postulated that this low and steady release rate would be beneficial for the absorption of PYY3-36. In addition, the unreleased peptide was located in the polymer matrix where it would be protected from degradation in the subcutaneous space.[275,276] Finally, it seems that subcutaneous administration via a hydrophobically-modified crosslinked poly(ester anhydrides) represents an potential route for sustained and controlled delivery of PYY3-36.

5. Conclusions

The main focus of the thesis was to prepare novel hydrophobically-modified crosslinked poly(ester anhydrides) for controlled drug delivery. In the crosslinked poly(ester anhydrides) the favorable properties of synthetic aliphatic polyesters and polyanhydrides are combined. The network precursors were synthesized by ring-opening polymerization of ε-caprolactone to hydroxyl telechelic oligomers which were acid-functionalized and methacrylated. In addition to the different amount and type of co-initiator used, the structure and properties of the precursors and thus the properties of the final crosslinked networks were modified by using different succinic anhydrides. The major findings of the thesis are summarized as follows:

- The hydrophobicity, viscosity, and thermal properties of the poly(ε-caprolactone) based prepolymers could be easily modified by the oligomer structure, i.e., molecular weight, molecular architecture, and hydrophobic side chain length of the functionalizing agent.

- Methacrylated poly(ester anhydride) precursors were crosslinked by means of temperature or radiation. Change in the molecular structure of the precursor from linear to star-shaped increased the crosslinking density and raised the gel contents. Photoinitiated crosslinking enabled solvent-free, light-induced liquid-to-solid transition at room or body temperature for sensitive drugs. The prepolymers and the networks did not exhibit toxicity in *in vitro* cytotoxicity and *in vivo* cytokine secretion studies.

- Crosslinked networks had different erosion properties depending on hydrophobicity and structure of the precursors. The hydrolytic erosion rate and mechanism, important factors for drug release properties, of the hydrophobically-modified crosslinked networks could be tailored by the length of the hydrophobic alkenyl chain, as well as by the molecular weight and molecular architecture of the precursor. Networks prepared from lower molecular weight precursors containing shorter alkenyl chains exhibited faster erosion that occurred by the surface erosion mechanism.

- The *in vitro* and *in vivo* release studies with model drugs revealed potential for high-load administration and surface erosion controlled drug release for macromolecular active agent delivery. In addition, the release could be tailored by modifying the hydrophobicity of the network.

- Photocrosslinked poly(ester anhydride) networks provided sustained and surface erosion controlled *in vivo* release of peptide PYY3-36 up to 9 days. The release was tailored by modifying the hydrophobicity of the network.

This thesis has been a part of ongoing research in the field of crosslinked biodegradable polymers and the developed novel hydrophobically-modified poly(ester anhydride) precursors have been patented[277]. In summary, the studied crosslinked poly(ester anhydrides) are promising materials for controlled drug delivery, especially for protein- and peptide-based drugs. Although the poly(ester anhydride) networks were in this thesis mainly developed for controlled release of macromolecular active agents, they could also be used in the field of tissue engineering as fast eroding scaffold materials or drug releasing coatings for scaffolds. In this case it would be important to concentrate on the mechanical properties of the networks. Decreasing the drug release rate further to weeks and months while maintaining the surface erosion mechanism, the development of the *in situ* crosslinking, potential interactions of the degradation products with active agents, and more detailed safety studies can be mentioned as future challenges for the studied crosslinked biodegradable networks.

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Bioresorbable polymers are extensively studied materials for medical applications. In this thesis, novel bioresorbable polymers, hydrophobically-modified crosslinked poly(ester anhydride) networks, were developed and characterized with the aim of obtaining suitable matrices for drug release applications. In these poly(ester anhydride) networks were combined the favorable characteristics of synthetic aliphatic polyesters and polyanhydrides. The properties of the networks were altered by modifying the structure of the poly(epsilon-caprolactone) oligomers which were used in the preparation of the crosslinked poly(ester anhydride) networks. The developed crosslinked poly(ester anhydride) networks were demonstrated to have high potential for applications requiring controlled release of sensitive macromolecular pharmaceutical agents.

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