In-vitro Degradation of Polyesteramides Containing Poly- ϵ -caprolactone Blocks

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Abstract: Most biodegradable polymers have been designed to have hydrolytically instable bonds in the polymer backbone. By cleavage of these labile bonds, the generally water-insoluble macromolecules are converted into water soluble oligomers. This process may occur in the bulk of the material as well as at the surface depending on the hydrophobicity of the polymeric backbone and stability towards hydrolysis of the functional groups incorporated. Polyester-amides, copolymers of α -amino acids and hydroxy acids have been synthesized by the ring-opening copolymerization of cyclo(α -amino acid- α -hydroxy acids) and lactones as lactide and ϵ -caprolactone. The in vitro degradation of polyesters and poly(ester-amides) can be described by bulk hydrolysis of ester bonds which results in a decrease of the molecular weight. Weight loss starts when the molecular weight of the polymer chains has sufficiently decreased. The period required for complete degradation depends on the type of polymer, the crystallinity, the initial molecular weight of the samples, and the glass transition temperature. The kinetics of the hydrolysis of ester bonds in polyesters and polyesteramides have been analyzed by determining the molecular weight as a function of time before the start of weight loss. During this period the molecular weight distribution and overall composition does not change. The degradation kinetics of the polyesters and polyesteramides can be described either as a process autocatalyzed by the generated carboxylic acid end groups, or as a non-catalyzed hydrolysis.

INTRODUCTION

The design and synthesis of biodegradable materials for applications like drug delivery devices, surgical sutures, pins, rods and screws for fixation devices have become important in biomaterials research. The developments and prerequisites that should be fulfilled by biodegradable polymers have been recently discussed.^{1,2} Most biodegradable polymers have been designed to have hydrolytically instable bonds in the polymer backbone. By cleavage of these labile bonds, the generally water-soluble macromolecules are converted into water-soluble oligomers. This process may occur in the bulk of the material as well as at the surface, depending upon the hydrophobicity of the polymeric backbone and hydrolytic stability of bonds incorporated. The rate of hydrolytic cleavage will not only be dependent upon the chemical structure of the main chain (e.g. the presence of substituents) but also on the physical

properties of the material, such as crystallinity and glass transition temperature. These properties can be further influenced by plasticization, blending and copolymerization.

Part of our research on sustained drug delivery systems^{3–5} has been focused on the development of biodegradable polymers for the release of hydrophobic drugs. Poly- ϵ -caprolactone (PCL) does have an excellent permeability for hydrophobic drugs. However, its long degradation time (over 3 years) usually is a disadvantage for medical applications like drug delivery systems. Our objective of developing biodegradable polymers with a permeability for hydrophobic drugs comparable to PCL, but with much shorter degradation times, prompted us to investigate novel biodegradable PCL-based polymers.

The properties of poly- ϵ -caprolactone and copolymers of ϵ -caprolactone and other lactones such as lactide and glycolide have been extensively studied.⁶ Copolymers of poly- ϵ -caprolactone with other

Fig. 1. Synthesis of a polyester-amide from D,L-lactide and 6-methylmorpholine-2,5-dione.

lactones turned out to be much more sensitive to hydrolysis or enzymatic degradation.^{7,8}

During the past years we have shown that the incorporation of glycine units in the polymer backbone of poly-D,L-lactide (PDLLA) yielded copolymers which after exposure to a phosphate buffer solution at 37°C were degraded to oligomers which dissolved faster than the degradation products of PDLLA.⁹ The incorporation of glycine units in PDLLA was accomplished by copolymerizing D,L-lactide with 6-methylmorpholine-2,5-dione, a cyclic depsipeptide built from lactic acid and glycine residues (Fig. 1). Dissolution of oligomers formed from these copolymers upon hydrolysis of ester bonds was governed by the increased hydrophilicity due to the incorporation of glycine units. We hypothesized that the incorporation of depsipeptide units in PCL might yield PCL-based polymers, which will either more rapidly degrade or give oligomers with a higher solubility compared to degradation products of PCL. Because the properties of PCL should not be greatly altered, copolymers containing only a small amount of depsipeptide units were investigated.

Recently we have investigated the synthesis and ring-opening polymerization of several cyclo-(α-

Fig. 2. Alternating polydepsipeptides prepared by ring-opening polymerization of morpholine-2,5-diones in the melt using stannous octate as an initiator.

Fig. 3. Synthesis of polyester-amides from ε-caprolactone and 3-substituted 6-methylmorpholine-2,5-diones.

amino acid-α-hydroxy acids) (Fig. 2). These new monomers were copolymerized with ε-caprolactone to give various polyester-amides. An example is given in Fig. 3.

DEGRADATION OF POLYESTER-AMIDES BASED ON D,L-LACTIDE

In our early work on the development of novel biodegradable polymers, we showed that the degradation of amorphous polymers like PDLLA in aqueous solution is a bulk degradation process. As described above, the incorporation of glycol units in the polymer backbone of PDLLA yields polymers which degrade to more hydrophilic oligomers than formed from pure PDLLA. Oligomers formed from the copolymers will already dissolve at higher molecular weight. However, the changes in the composition of the remaining copolymer upon degradation showed that these copolymers did not have a random distribution of the glycol units in the polymer chains. As a result, oligomers with a high glycol content diffused from the polymer matrix, leaving the bulk of the material with an increased lactic acid content. The time profiles of the remaining weight of these copolymers show that the *rate of dissolution* of oligomers is more gradual than for poly-D,L-lactide (Fig. 4).

SYNTHESIS OF POLYESTER-AMIDES BASED ON PCL

The ring-opening copolymerization of ε-caprolactone and cyclic ester-amides may be used as a tool to prepare copolymers of ε-caprolactone which will degrade more rapidly and/or lose their weight more rapidly than PCL. Especially, random copolymerization using small amounts of cyclic depsipeptides might be used to prepare copolymers with properties

Fig. 4. Degradation of PDLLA and poly(glycine D,L-lactic acid) (8/2) expressed as remaining weight as a function of time. Polymer discs of ~ 40 mg and ~ 400 μm thickness were incubated in a 0.1 M phosphate buffer (pH 7.4) at 37°C.

comparable to PCL but with optimized behaviour with respect to degradation.

Copolymers were prepared by the ring-opening copolymerization in the bulk of ϵ -caprolactone and cyclic depsipeptides (molar ratios were varied from 0.98–0.02 to 0.85–0.15) using stannous octoate as an initiator (Fig. 3). Cyclic ester-amides composed of a D,L-lactic acid residue and either the α -amino acid residue glycine, alanine, valine or aspartic acid were used in the copolymerization reactions.

All copolymers prepared had apparent molecular weights of $\sim 6 \times 10^4$ (as determined with GPC against polystyrene standards) and were further characterized by NMR and DSC. The sequence analysis using ^{13}C NMR revealed a random distribution of depsipeptide units in the polymer chains. DSC analysis showed that the copolymers were semicrystalline, having melting points between 56 and 66°C. It was concluded that the

Fig. 5. Heat of fusion of retained PCL samples after different times of exposure to 0.1 M phosphate buffer (pH 7.4) at 37°C.

copolymer chains consist of large caprolactone blocks subdivided by single depsipeptide units.^{10,11}

DEGRADATION OF POLYESTER-AMIDES BASED ON PCL

Small polymer discs of PCL and the copolymers weighing about 20–30 mg and having a thickness of about 200–300 μm were cut from dense polymer films that were prepared by casting copolymer solutions in dichloromethane onto glass and subsequently evaporating the solvent. The polymer discs were incubated in a 0.1 M phosphate buffer at pH 7.4 at 37°C.

In crystalline polymers like poly(glycolic acid) (PGA), poly(L-lactic acid) (PLLA) and poly(ϵ -caprolactone) (PCL), degradation first occurs in the amorphous regions and later in the crystalline domains. The crystallinity increases in time, due to an increased mobility of polymer chains formed upon degradation and (re)crystallization of PCL. This is illustrated in Fig. 5 by the increment in the heat of fusion concurrent with the degradation.

The degradation of PCL and polyester-amides can be described by the penetration of water into the polymer matrix followed by bulk hydrolysis of ester bonds which results in a decrease in the molecular weight. Characteristic for a bulk degradation is that the weight of the sample will remain constant for a relatively long period and that weight loss only starts when the molecular weight of the polymer chains has sufficiently decreased. The degradation profiles of the polyester-amides showed similar trends. Two examples are presented

Fig. 6. Plot of apparent molecular weight (M_n) in time for PCL and copolymers composed of caprolactoyl and glycyll-lactyl units (molar ratio 9/1, G10) and a copolymer composed of caprolactoyl and aspartyl-lactyl units (molar ratio 0.98/0.02, H2).

Fig. 7. Plot of percentage remaining weight in time for PCL and copolymers composed of caprolactoyl and glyceryl-lactyl acid units (molar ratio 9/1, G10) and a copolymer composed of caprolactoyl and aspartyl-lactyl units (molar ratio 0.98/0.02, H2).

in Figs 6 and 7, together with data obtained for PCL. In the initial stages of the degradation, the rate of hydrolysis of the polyester-amides appeared to be higher than that found for PCL.

The kinetics of the hydrolysis of ester bonds in these polyester-amides has been analyzed by determining the apparent molecular weight (M_n) as a function of time (t) before the start of weight loss. The rate of degradation k can be determined from either one of the following equations describing, respectively, the uncatalyzed process and the process catalyzed by the generated carboxylic acid end groups.¹²

For uncatalyzed

$$\text{hydrolysis: } 1/M_n = 1/M_n^0 + k_1 t$$

For catalyzed

$$\text{hydrolysis: } \ln(M_n) = \ln(M_n^0) - k_2 t$$

On the basis of the equations given above the rates of hydrolysis were determined. A plot of $\ln(M_n)$ versus immersion time for PCL according to autocatalytic hydrolysis gave higher correlation coefficients than a plot of $1/M_n$ versus time based on uncatalyzed hydrolysis. However, it appeared that the changes in molecular weight (M_n) of the polyester-amides like G10 and H2 (see Fig. 6) in time were consistent with either an autocatalytic or uncatalyzed hydrolysis. The rate constant for copolymer G10 was higher ($k = 5 \times 10^{-2} \text{ week}^{-1}$) than determined for PCL ($k = 1 \times 10^{-2} \text{ week}^{-1}$). Incorporation of only 2 mol % aspartyl units (copolymer H2) induces an even larger increase in the degradation rate ($k = 10 \times 10^{-2} \text{ week}^{-1}$).

Analysis of the copolymer composition of G10

Fig. 8. Heat of fusion of a copolymer composed of caprolactoyl and glyceryl-lactyl acid units (molar ratio 9/1, G10) and a copolymer composed of caprolactoyl and aspartyl-lactyl units (molar ratio 0.98/0.02, H2).

during the period of degradation revealed that upon weight loss, the composition is shifted towards higher PCL contents. This can only be caused by dissolution of formed oligomers having a relatively high amount of depsipeptide units. These fragments will generally only be present in the amorphous regions, which will degrade first. Obviously depsipeptide units are rejected from the crystals present in the polyester-amides. As shown in Fig. 8 for the two copolymers, the crystallinity of the polymer samples increased in time as a result of the change in composition.

Introduction of a pendant carboxylic acid functional group as present in the incorporated aspartyl units in PCL thus appears to be a good method of increasing the degradation rate of PCL. The mechanism of degradation cannot be clearly distinguished and can be described by either the uncatalyzed or catalyzed kinetic equation. Incorporation of 10 mol % glyceryl-lactyl units in PCL appears to cause a higher degradation rate than observed for PCL, but on the other hand causes a change in composition of the remaining polymer.

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