ESI-MS Reveals the Influence of Hydrophilicity and Architecture on the Water-Soluble Degradation Product Patterns of Biodegradable Homo- and Copolyesters of 1,5-dioxepan-2-one and ε-Caprolactone

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ABSTRACT: The hydrolytic degradation process and degradation product patterns of biodegradable homo- and copolyesters of 1,5-dioxepan-2-one (DXO) and ε-caprolactone (CL) were monitored by electrospray ionization mass spectrometry (ESI-MS). The degradation product patterns were compared to mass loss, molecular weight changes, copolymer composition, and pH changes after various hydrolysis times. Water-soluble oligomers up to heptadecamer were identified after hydrolysis of hydrophilic PDXO, while only oligomers up to hexamer were detected after hydrolysis of the more hydrophobic PCL. The product pattern of DXO−CL−DXO triblock copolymer mainly consisted of DXO-based oligomers, whereas the CL/DXO multiblock copolymer degradation product pattern contained DXO and CL oligomers as well as oligomers containing both DXO and CL units. The DXO-rich oligomers, however, dominated the product patterns. ESI-MS gave valuable insights into the hydrolysis process of hydrophobic and hydrophilic polyesters and showed that hydrophilicity of the polymer as well as copolymer architecture both greatly influenced the water-soluble degradation product patterns.

Introduction

Continuous progress in the synthesis of aliphatic polyesters such as polylactide (PLA), poly(ε-caprolactone) (PCL), polyglycolide (PGA), and various copolymers has led to a broad array of materials with sophisticated architectures and controlled properties.[1] Water-soluble hydroxyacids and their oligomers are commonly formed as degradation products when aliphatic polyesters are hydrolyzed.[2] The nature and amount of these products influence the biocompatibility during the use in biomedical applications. Gas chromatography−mass spectrometry (GC-MS),[3–5] high performance liquid chromatography (HPLC),[5,6] and capillary zone electrophoresis (CZE)[7,8] have been used to monitor the water-soluble products liberated during the hydrolytic and enzymatic degradation of polyesters. Recently, electrospray ionization mass spectrometry (ESI-MS) has proven itself as a useful analytical tool for analysis of individual (co)polyester oligomers released during the hydrolysis. The structure of the end groups and repeating units of oligomers may also be inferred from the mass spectra. Oligomers formed during degradation of PLA,[9,10] poly(ε-caprolactone−δ-valerolactone),[11] poly(ε-caprolactone-L-lactide),[11] poly(butylene succinate-*co*-butylene sebacate/adipate),[12] and poly(hydroxyalkanoates) and their synthetic analogues as well as their copolymers and blends[13–15] have been analyzed by ESI-MS. The application of mass spectrometry to evaluate the changes in chemical composition for different PLA, PCL, and PGA copolymers during hydrolytic degradation has also been reported.[16]

In previous work we used GC-MS to analyze monomeric hydroxyacids formed during hydrolysis of various poly(ester−ether)s.[17,18] It was shown that migration of monomeric hydroxyacids from the material, a potential cause of inflammatory responses in the body, was controllable through macromolecular design.[17] In this work, focus was shifted toward the water-soluble oligomers, another potential factor which may influence the cytotoxicity.[19] Our hypothesis was that the water-soluble degradation product patterns are highly dependent on the hydrophilicity of the polyester and its oligomeric degradation products as well as the arrangement of the hydrophilic units in the copolymer. Homo- and copolymers of hydrophobic PCL and hydrophilic poly(1,5-dioxepan-2-one) (PDXO) were, thus, exposed to hydrolytic degradation for up to 147 days. Water-soluble oligomers released into the degradation medium were identified on the basis of ESI-mass spectra to evaluate the effect of hydrophilicity and macromolecular structure on the water-soluble degradation product patterns.

Experimental Section

Materials. ε-Caprolactone (CL) (Aldrich, Germany) was dried and distilled over calcium hydride (CaH_2) at reduced pressure before use.1,5-Dioxepan-2-one (DXO) was synthesized via a Bayer−Villiger oxidation according to the literature.[20] Purification of DXO was performed by recrystallization from dry ether and two subsequent distillations under vacuum. Prior to the final distillation, the monomer was dried over CaH_2 for 24 h. All monomers were stored in an inert atmosphere in an Mbraun MB150B-G-I (Germany) glovebox before use. Chloroform (BergmanLabora, Sweden) stabilized with 2-methyl-2-butene was dried over CaH_2 for at least 24 h and distilled under vacuum in an inert atmosphere just before use. The cyclic tin alkoxide initiator 1,1,6,6-tetra-*n*-butyl-1,6-distanna-2,5,7,10-tetraoxacyclodecane was synthesized from dibutyltin oxide and 1,2-ethanediol as described in the literature.[21]

Polymerization Technique. The reaction vessels were silanized, and all glassware and syringes were flame-dried prior to use. The synthesis of the CL and DXO homopolymers and the CL/DXO copolymers (triblock and multiblock) were performed as described in literature.[21,22] The first monomer, initiator, and chloroform were added to the reaction vessel in an inert atmosphere and were allowed to react until complete conversion via ring-expansion polymerization before the second monomer was added. Only CL and DXO were added for the synthesis of the homopolymers and allowed to react completely. DXO was added as the first monomer for the multiblock

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Table 1. Polymers and Their Characteristics Prior to Hydrolysis

		polymer composition (%)		average block length[b]						
polymer name	polymer architecture	CL	DXO	CL	DXO	M_n[c] (g/mol)	PDI[c]	T_m[d] (°C)	w_c[d] (%)	T_g[d] (°C)
H-D100	homopolymer	0	100			5 500	1.6			
H-C100	homopolymer	100	0			17 500	2.0	66.0	72.6	
M-C60D40	multiblock	62[a]	38[a]	7.3	4.1	20 600	1.9	53.6	49.9	−49.7
M-C75D25	multiblock	74[a]	26[a]	11.6	3.1	17 200	2.1	56.7	55.4	−54.6
T-C60D40	triblock	62[a]	38[a]			19 500	2.3	60.0	45.0	−45.7

[a] Determined by ^{1}H NMR using $\delta_{CL} = 2.30$ ppm and $\delta_{DXO} = 3.75$ ppm. [b] Determined by ^{13}C NMR. [c] Determined by DMF SEC calibrated with narrow MWD polystyrene standards. [d] Determined by DSC from the first heating scan.

copolymers and CL as the second monomer, whereas the reaction order was reversed for the triblock copolymer. The obtained polymers were precipitated in a 95:5 mixture of cold hexane and methanol. Films from the triblock and multiblock copolymers and the homopolymers were prepared by dissolving the polymers in chloroform and thereafter solution-casted into thin films (0.1 mm) on glass plates. The solvent was evaporated, and the films were allowed to dry under vacuum for at least 1 week before analysis.

Hydrolysis. The different polymers were subjected to hydrolytic degradation in deionized water at 37 °C. Approximately 10 mg of polymer and 5 mL of water were added to a 20 mL glass vial. The sample vials were sealed with septa and placed in a thermostatically controlled incubator with temperature set to 37 °C and rotation to 60 rpm. Vials were withdrawn from the test environment after 1, 7, 21, 49, and 147 days of degradation. The remaining solid polymer was dried to constant weight under reduced pressure for 2 weeks, and the water fractions were analyzed for water-soluble oligomers by ESI-MS. Triplicate samples were withdrawn and analyzed after each aging time.

ESI-MS and ESI-MS/MS Analysis. Electrospray mass spectrometry analysis was performed using a Finnigan LCQ ion trap mass spectrometer (Finnigan, San Jose, CA). The polyester samples were dissolved in a water/methanol system (2:1 v/v), and the solutions were introduced to the ESI source by continuous infusion using the instrument syringe pump at a rate of 3 μL/min. The ESI source of the LCQ was operating at 4.5 kV, and the capillary heater was set to 200 °C. Nitrogen was used as nebulizing gas. For ESI-MS/MS experiments the ions of interest were isolated monoisotopically in the ion trap and collisionally activated. The helium damping gas present in the mass analyzer was acting as collision gas. The significant voltage range RF amplitude was set to a value that caused the peak height of the parent ion to decrease by at least 50%. The analyses were performed in negative-ion mode.

Mass Loss. The mass loss of the polymer samples was determined by comparing the dry weight (m_d) after hydrolysis with the initial weight (m_0) according to eq 1. The samples were dried for 2 weeks at reduced pressure (0.5×10^{-3} mbar) before determination of the dry weight.

$$\Delta m_d = \frac{m_0 - m_d}{m_0} \times 100 \quad (1)$$

Nuclear Magnetic Resonance (NMR). The composition of the materials was determined using ^{1}H NMR by comparison of the peak intensities of the comonomers ($\delta_{CL} = 2.30$ ppm and $\delta_{DXO} = 3.75$ ppm). The average sequence lengths of the multiblock copolymers were determined by ^{13}C NMR spectroscopy and calculated according to the equations[23]

$$\bar{L}_C = \frac{I_{CC}}{I_{CD}} + 1 = \frac{I_{CC}}{I_{DC}} + 1 \quad (2)$$

$$\bar{L}_D = \frac{I_{DD}}{I_{DC}} + 1 = \frac{I_{DD}}{I_{CD}} + 1 \quad (3)$$

^{1}H NMR and ^{13}C NMR were obtained using a Bruker Advance DPX-400 nuclear magnetic resonance spectrometer operating at 400 and 100 MHz, respectively. 5 mg samples for ^{1}H NMR and 100 mg samples for ^{13}C NMR were dissolved in 1 mL of deuteriochloroform ($CDCl_3$) in a 5 mm diameter sample tube. Nondeuterated chloroform was used as an internal standard ($\delta = 7.26$ ppm for ^{1}H NMR and $\delta = 77.0$ ppm for ^{13}C NMR).

Size Exclusion Chromatography (SEC). The molecular weights of the polymers after polymerization and after different hydrolysis times were determined by SEC. *N,N*-Dimethylformamide (Fischer, Sweden) was used as the eluent with a flow rate of 1.0 mL/min. The injection volume was 50 μL. The instrument comprised a Waters 717 Plus autosampler and a Waters model M-6000A solvent pump equipped with a PL-EMD 960 light scattering evaporative detector, two PLgel 10-mm mixed B columns (300 × 7.5 mm) from Polymer Laboratories, and one Ultrahydrogel linear column (300 × 7.8 mm) from Waters, connected to an IBM-compatible computer, were used. Polystyrene standards with narrow molecular weight were used for calibration. Millennium software version 3.20 was used for data processing.

Differential Scanning Calorimetry (DSC). The thermal properties of the materials were examined using a DSC (Mettler Toledo DSC 820 module) under nitrogen atmosphere. Approximately 5 mg of the polymer was encapsulated in a 40 μL aluminum cap without pin. Samples were heated under a nitrogen gas flow of 50 mL/min from −65 to 80 °C at a rate of 10 °C/min. The samples were then cooled from 80 to −65 °C at a rate of 10 °C/min before being heated again from −65 to 80 °C at a rate of 10 °C/min. From the first heating scan, the melting temperatures, T_m, were noted as the maximum values of the melting peaks and the midpoint temperature of the glass transition was determined as the glass transition temperature, T_g. When determining the crystallinity of the copolymers, it was assumed that the only contribution to the heat of fusion was from CL units. PDXO has earlier been shown to be a fully amorphous polymer having a T_g between −35 and −40 °C.[20] The approximate crystallinity of the copolymers was calculated according to the equation

$$w_c = \frac{\Delta H_f}{\Delta H_f^0} \quad (4)$$

where w_c is the degree of crystallinity, ΔH_f is the heat of fusion of the sample, and ΔH_f^0 is the heat of fusion of 100% crystalline polymer. 139.5 J/g was used as ΔH_f^0 value for PCL.[24]

Results and Discussion

Homo- and copolymers of caprolactone (CL) and 1,5-dioxepan-2-one (DXO) were subjected to hydrolytic degradation in deionized water for up to 147 days. The hydrolytic degradation and especially the effect of copolymer composition and architecture on the degradation process were followed by monitoring the water-soluble degradation products by ESI-MS. In addition, mass loss, molecular weight, copolymer composition, and pH were determined after various degradation times. The polymer type, copolymer compositions, average block lengths, and molecular weights are presented in Table 1. The polymer names are denotations of their structure and copolymer composition; i.e., M-C60D40 is a multiblock copolymer comprising 60% CL and 40% DXO.

Structural Characterization of Water-Soluble Degradation Products by ESI-MS. After different hydrolysis times the water fractions were analyzed by electrospray ionization mass

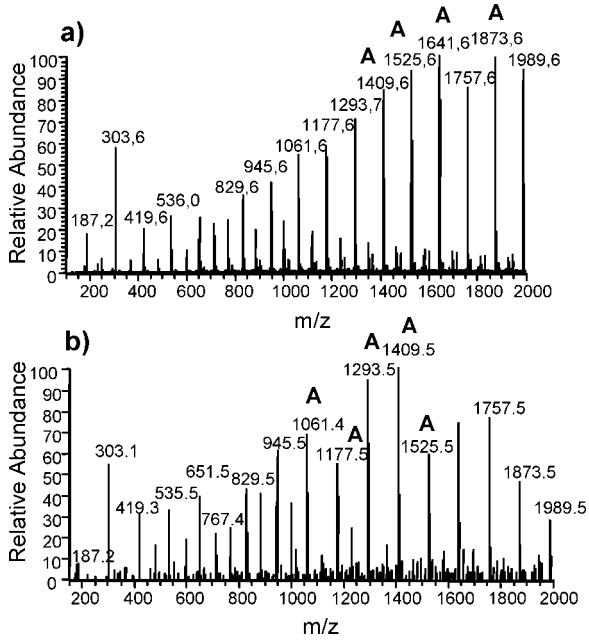


Figure 1. Negative ESI-MS spectra of water-soluble compounds migrating from poly(1,5-dioxepan-2-one) to water after (a) 1 day and (b) 21 days degradation at 37 °C.

spectrometry (ESI-MS) to identify the water-soluble degradation products formed during the hydrolysis of homo- and copolyesters of 1,5-dioxepan-2-one and ε-caprolactone. The expected hydrolysis products are the oligomers terminated by hydroxyl and carboxyl end groups and their respective monomeric hydroxyacids.

ESI-MS Analysis of Water-Soluble Oligomers and Hydrolysis Products of PDXO. Parts a and b of Figure 1 show negative ESI-MS spectra of the low molecular weight compounds that had migrated from poly(1,5-dioxepan-2-one) (PDXO) homopolymer (H-D100) to water after 1 day and after 21 days of hydrolysis, respectively.

The figure clearly shows how the water-soluble product pattern changes as the hydrolysis proceeds. The mass spectrum acquired after 1 day shows increasing signal intensities for the oligomers as a function of increasing molecular weight (Figure 1a), whereas the concentration of oligomers with lower molecular weight increases as a function of hydrolysis time (Figure 1b). The low original molecular weight and short hydrolysis time, together with the significant increase in the number-average molecular weight and decrease in the polydispersity index observed for H-D100 after only 1 day in water (see the Molecular Weight Changes section), support the conclusion that the compounds detected after 1 day are low molecular weight oligomers originally present in the sample and not degradation products formed due to hydrolysis. The shift of the signals toward lower mass range shows that the hydrolytic degradation process progressed rapidly over the 21 day period leading to formation of shorter oligomers (Figure 1b).

Figure 2 shows ESI-MS spectrum of H-D100 hydrolysis products after 21 days in two spectral expansions of *m/z* 50−500 (Figure 2a) and *m/z* 820−1450 (Figure 2b). One main set of anions was observed with a peak-to-peak mass increment of 116 Da (labeled A). This series correspond to 3-(2-hydroxyethoxy)propanoic acid (at *m/z* 133) and its water-soluble oligomers terminated by carboxyl and primary hydroxyl end groups. The general chemical structure of the oligomers is also presented in Figure 2.

Two additional series of anions, labeled B and C, were present predominantly in the low mass range. With the exception of some ions from B series at the low mass range, these series generally had significantly lower abundance (especially for series C). These series of anions are probably formed by fragmentation of 3-(2-hydroxyethoxy)propanoic acid oligomers (series A), which occurs during the ionization process in the ESI-MS spectrometer and do not correspond directly to real hydrolytic degradation products of PDXO. These products can be formed by the cleavage of an $-CH_2-O-CH_2-$ ether bond with transfer of a hydrogen atom and formation of the product ions: one bearing a carboxyl and an unsaturated end group and the other terminated with carboxyl and hydroxyl end groups, series B and C, respectively (Scheme 1). The formation of unsaturated (Scheme 1, pathway a) and hydroxyl (Scheme 1, pathway b) end groups due to the ether bond cleavage was previously reported in the case of fragmentation of PEG oligomers.[25]

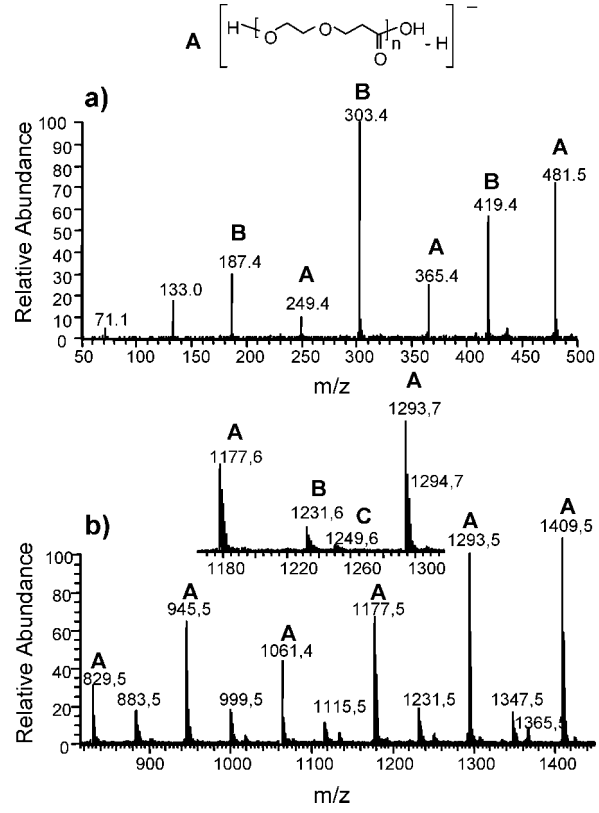
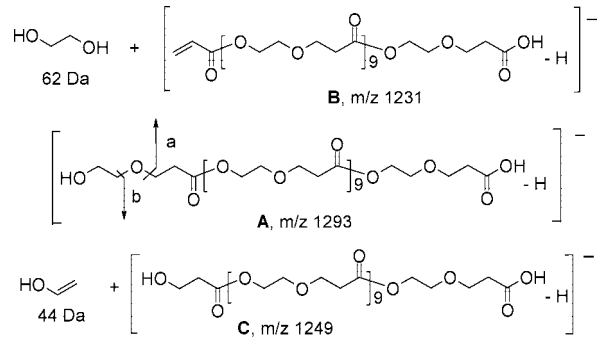


Figure 2. Expanded ESI-mass spectra of water-soluble degradation products of poly(1,5-dioxepan-2-one) after 21 days of hydrolytic degradation at 37 °C in the mass range (a) *m/z* 50−500 and (b) *m/z* 820−1450.

Scheme 1. Possible Fragmentation Pathway of DXO Oligomers to (a) Unsaturated and (b) Hydroxyl-Terminated Products during the ESI-MS Analysis



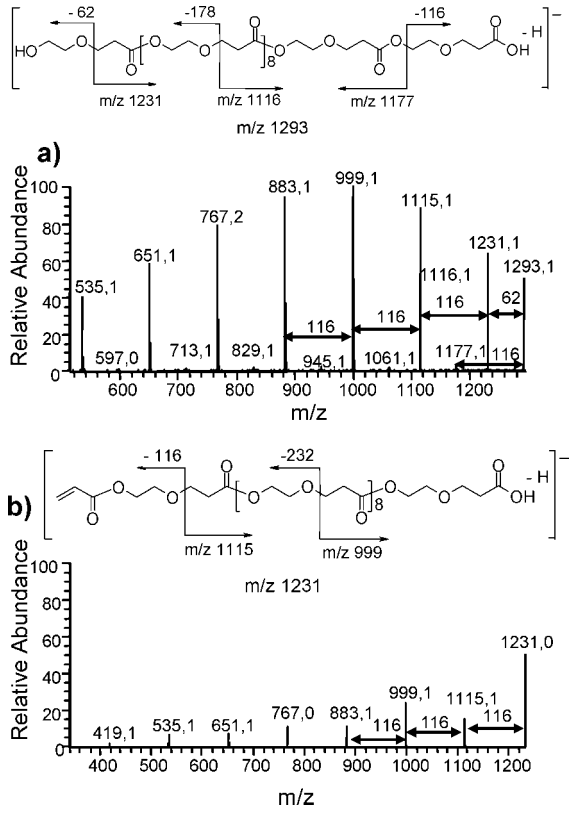


Figure 3. ESI-MS/MS spectra of DXO oligomers: (a) an oligomer at *m/z* 1293 terminated by hydroxyl and carboxyl end groups selected from the series A (Figure 2) and (b) an oligomer at *m/z* 1231 terminated by unsaturated and carboxyl end groups selected from the series B (Figure 2).

Confirmation of the Structure of the Hydrolysis Products by ESI-MS/MS. Additional insight into the structure of individual oligomer chains is achieved by multistage mass spectrometry (MS^n), where the molecular ion of interest is separated from all other ions formed during ionization and further fragmented. The structural assignment of the obtained water-soluble PDXO degradation products was confirmed by the ESI-MS/MS experiments which were performed for the two anions at *m/z* 1293 and *m/z* 1231 selected from the series A and B, respectively (Figure 3a,b).

The fragmentation of the ion at *m/z* 1293, which may occur from both sides of the oligomer, produces two sets of fragment ions (see fragmentation pathway in Figure 3a). According to the assigned structures, the main series of product ions at *m/z* 1231, 1115, 999, 831, 767, 651, and 535 correspond to the DXO oligomers containing unsaturated and carboxyl end groups. The product ions at *m/z* 1177, 1061, 945, 829, 713, and 597 (with low intensities) correspond to the DXO oligomers containing hydroxyl and carboxyl end groups. Thus, the fragment ion at *m/z* 1231 corresponds to the oligomer formed by the expulsion of a neutral molecule (62 Da), and the further fragmentation of this ion proceeds, mainly via ether bond cleavage, and creates a set of product ions which also contain unsaturated and carboxyl end groups (product ions at *m/z* 1115, 999, 831, 767, 651, and 535; see fragmentation pathway Figure 3a and Scheme 1a).[25] The series of product ions at *m/z* 1177, 1061, 945, 829, 713, and 597 (with low intensities) are formed by hydrogen rearrangement mechanisms via a six-membered intermediate, preferentially from carboxyl side, and leads to the selective cleavage of the −O−CH− bond between the two monomer units in the macromolecule (see fragmentation pathway Figure 3a). A similar fragmentation pathway was previously reported for poly[(*R*,*S*)-3-hydroxybutyrate-*co*-hydroxyhexanoate] copolyester.[26]

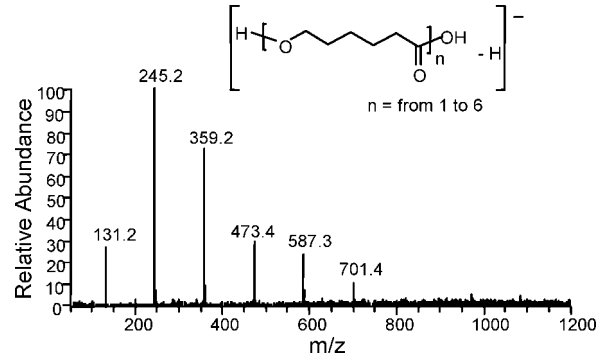


Figure 4. Negative ESI-MS spectra of water-soluble degradation products of poly(ε-caprolactone) after 147 days of hydrolytic degradation at 37 °C.

The formation of unsaturated end groups by the ether bond cleavage was demonstrated by ESI-MS/MS experiment provided for the parent ion at *m/z* 1231 selected from the series B (Figure 3b). The exclusive fragmentation via −O−CH_2− ether bond cleavage was observed, shoving the higher susceptibility of ether bond with respect to ester bond cleavage under the fragmentation conditions (Figure 3b, Scheme 1a).

ESI-MS Analysis of Water-Soluble Hydrolysis Products of PCL. Figure 4 shows the ESI-MS spectrum of water-soluble degradation products of poly(ε-caprolactone) (PCL) homopolyester H-C100 after 147 days of hydrolytic degradation. One set of singly charged anions with a peak-to-peak mass increment of 114 Da, which is equal to the molecular weight of the CL repeating unit, is observed in the mass range *m/z* 50−900 in this spectrum. The signals observed correspond to the 6-hydroxyhexanoic acid (signal at *m/z* 131) and its water-soluble oligomers with 2−6 repeating units terminated by carboxyl and hydroxyl end groups. Longer oligomers might also be formed during the hydrolysis, but due to the hydrophobic nature of PCL, they are not water-soluble and thus not detected by ESI-MS. A large difference in the product pattern is, thus, observed compared to the ESI-MS spectra of PDXO hydrolysis products, where water-soluble products with molecular weights up to 1990 g/mol, i.e., oligomers with 17 DXO units, were observed. As the upper mass range of the ESI-MS was 2000 g/mol, it is likely that even longer DXO oligomers are water-soluble and present in the water fractions.

The ESI-MS/MS experiment performed for the molecular anions at *m/z* 701 selected from ESI-MS of water-soluble degradation products of poly(ε-caprolactone) (Figure 4) as well as the fragmentation experiment ($ESI\text{-}MS^3$) performed subsequently for the ion at *m/z* 359 presented in Figure 5 confirmed the structural assignments above. The fragmentation of the selected anions of CL oligomers occurs by the hydrogen rearrangement mechanism, preferentially from the carboxyl side, and leads to the selective cleavage of the −O−CH− bond between the two monomer units in the oligomer. Thus, the fragment ion at *m/z* 587 corresponds to the CL oligomer product ions terminated by hydroxyl and carboxyl end groups which are formed by the expulsion of 5-hexenoic acid (114 Da) from the carboxyl side (see fragmentation pathway in Figure 5). The subsequent fragmentation experiment of the anion at *m/z* 359 and corresponding MS^3 mass spectrum is also presented in Figure 5. The further fragmentation of this anion also occurs from the carboxyl side and leads to the formation of two ions: CL dimer (hydroxyl- and carboxyl-terminated, signal at *m/z* 245) and 6-hydroxyhexanoic acid (signal at *m/z* 131).

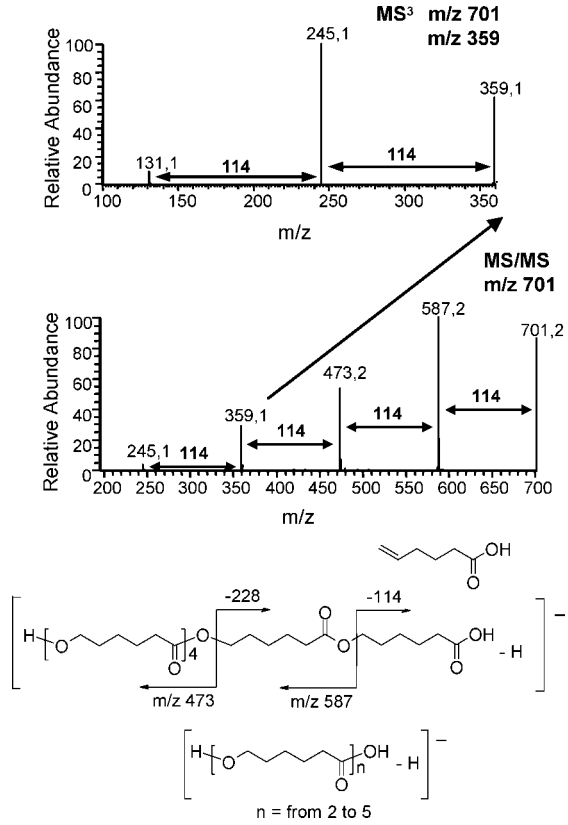


Figure 5. ESI-MS³ experiments of the hydroxyl- and carboxyl-terminated CL oligomer anion at *m/z* 701 selected from ESI-mass spectrum of water-soluble degradation products of poly(ε-caprolactone) (Figure 4).

ESI-MS Analysis of Water-Soluble Hydrolysis Products of the CL/DXO Copolyesters. Figure 6a−c shows three spectral expansions of the ESI-mass spectra of the water-soluble products obtained after 147 days of hydrolytic degradation of CL/DXO copolyesters with different architectures: (a) triblock DXO−CL−DXO with 60% CL (T-C60D40), (b) multiblock CL−DXO with 60% CL (M-C60D40), and (c) multiblock CL−DXO with 75% CL (M-C60D40). The ESI-mass spectrum of the water-soluble degradation products of T-C60D40 (Figure 6a) is similar to the spectrum obtained for the water-soluble degradation products of H-D100 (compare Figure 1 and Figure 6a). The set of anions observed in this spectrum correspond to the water-soluble DXO oligomers terminated by carboxyl and primary hydroxyl end groups. These results together with the decreasing DXO content determined by NMR clearly show that the DXO side blocks were preferentially hydrolyzed. Figures 6b,c demonstrate the ESI-mass spectra of water-soluble products of multiblock copolyesters with different composition, i.e., M-C60D40 and M-C75D25. Besides the signals corresponding to the DXO oligomers, signals corresponding to oligomers containing both CL and DXO units terminated by hydroxyl and carboxyl end groups were observed in these spectra. Moreover, in each mass spectra signals corresponding to oligomers terminated by unsaturated and carboxyl end groups formed probably under the ionization condition were detected (Scheme 1). The assignments of the peaks observed in the expanded region of the negative spectra (Figures 6a−c) are reported in Table 2.

Additionally, in the low mass range of ESI-mass spectra of water-soluble products of each copolyester, signals corresponding to 3-(2-hydroxyethoxy)propanoic acid (at *m/z* 133) and its fragmentation product (at *m/z* 71), 6-hydroxyhexanoic acids (*m/z* 131) as well as small amounts of 6-hydroxyhexanoic acid oligomers with hydroxyl and carboxyl end groups were detected (Figure 7). Independently of architecture or composition of copolyesters studied the detected water-soluble products were enriched with DXO and DXO/CL oligomers with low number

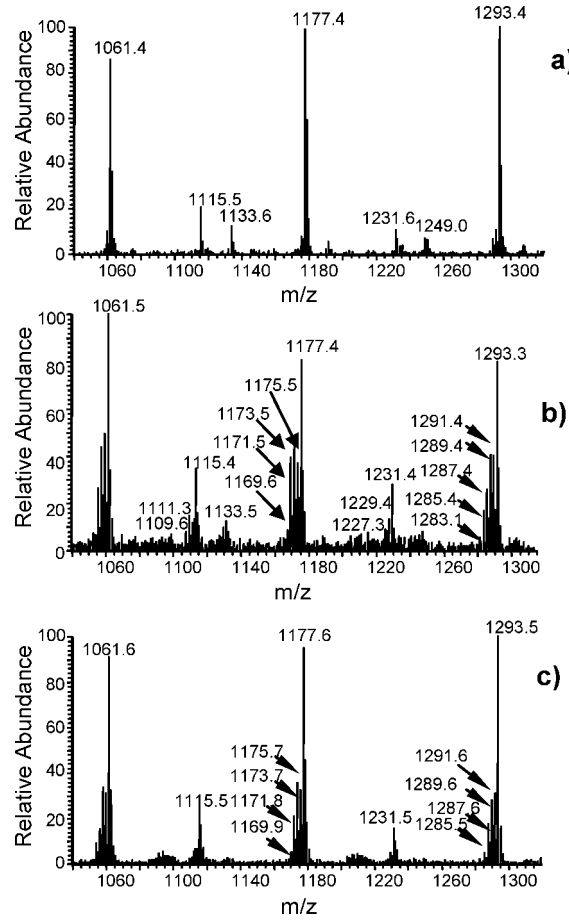


Figure 6. Expanded region *m/z* 1040−1320 of the ESI-mass spectra of the water-soluble products of (a) T-C60D40, (b) M-C60D40, and (c) M-C75D25 obtained after 147 days of hydrolysis at 37 °C.

Table 2. Calculated *m/z* Values for Assumed Chemical Compositions for the Peaks Observed in the Expanded Regions of the Negative ESI-Mass Spectra of the Water-Soluble Degradation Products of CL/DXO Copolyesters with Different Architectures

chemical compositions of hydrolytic degradation products of CL/DXO copolyesters	negative ions *m/z* observed in ESI-mass spectra of CL/DXO copolyesters	
	products of hydrolytic degradation	products of fragmentation
DXO_9	1061	
CL/DXO_8	1059	
CL_2/DXO_7	1057	
CL_3/DXO_6	1055	
CL_4/DXO_5	1053	
DXO_{10}	1177	1115
CL/DXO_9	1175	1113
CL_2/DXO_8	1173	1111
CL_3/DXO_7	1171	1109
CL_4/DXO_6	1169	1107
DXO_{11}	1293	1231
CL/DXO_{10}	1291	1229
CL_2/DXO_9	1289	1227
CL_3/DXO_8	1287	1225
CL_4/DXO_7	1285	1223
CL_5/DXO_6	1283	

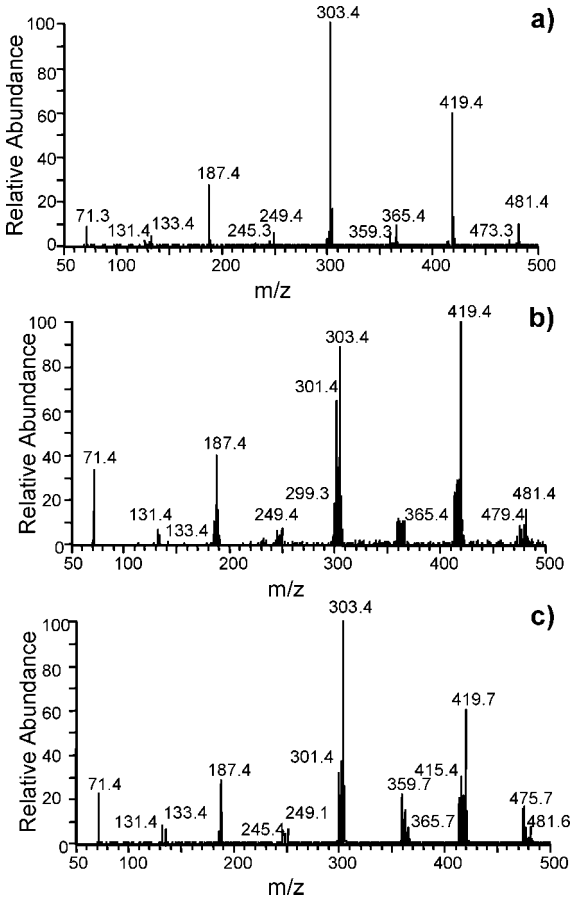


Figure 7. Expanded region (low mass range) of the ESI-mass spectra of the water-soluble products of (a) T-C60D40, (b) M-C60D40, and (c) M-C75D25 obtained after 147 days of hydrolysis at 37 °C.

of CL units. The ESI-MS results are, thus, in agreement with the significant decrease in DXO content detected by NMR.

It is known that in the case of applying the ESI-MS technique for the analysis of mixtures containing hydroxyacids and their oligomers the intensities of the signals corresponding to the acids are lower than the intensities of the signals of their respective oligomers. In order to estimate oligomer and hydroxyacid content quantitatively, separated calibration for acid and respective oligomers would be necessary. However, the mass spectrometry analysis confirmed that both hydroxyacid oligomers as well as their respective monomeric hydroxyacids are formed during hydrolytic degradation of CL/DXO copolyesters. The ESI-mass spectrometric determination of the structures of water-soluble oligomers released into the degradation medium was supported by NMR and SEC analysis of the degraded samples.

Mass Loss. The mass loss for the different polymers during hydrolysis is shown in Figure 8. Copolymer composition and polymer architecture both influenced the mass loss. The homopolymer of DXO (H-D100) exhibited the largest mass loss with 40% mass loss already after 7 days of degradation. This is explained by the hydrophilic and amorphous character of PDXO, which facilitates water uptake and subsequent hydrolysis, and the low initial molecular weight of H-D100. Because of the low molecular weight and hydrophilicity, many water-soluble oligomers are present in the material already before the hydrolysis. New water-soluble oligomers are also more rapidly formed by hydrolysis of PDXO. The PCL homopolymer showed a small mass loss of only 2% after 147 days of degradation. In contrast to PDXO, the hydrophobic and semicrystalline material properties of PCL suppress water absorption, and therefore the degradation rate is substantially reduced.

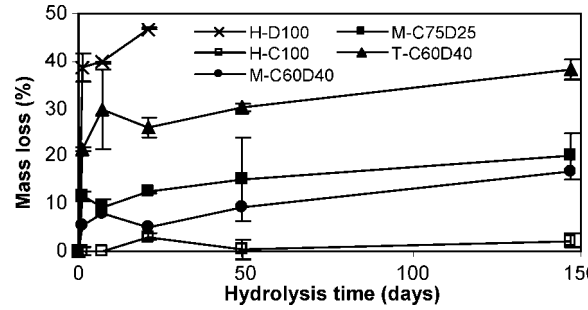


Figure 8. Mass loss profiles for the different CL/DXO polymers during hydrolysis.

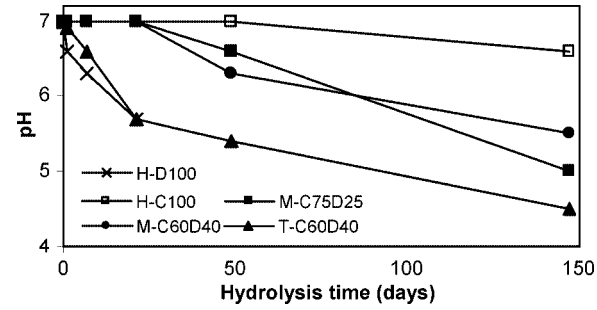


Figure 9. pH of the sample solutions during hydrolysis.

For the copolymers with the same composition, i.e., 60% CL and 40% DXO, the polymer architecture clearly affected the degradation rate. The triblock copolymer, T-C60D40, exhibited twice as high mass loss compared to the multiblock copolymer after 147 days. This is due to the long DXO blocks in the triblock copolymer which like PDXO are amorphous and hydrophilic and therefore more susceptible toward hydrolysis compared to the short DXO blocks in the multiblock copolymer. These mass loss results are in accordance with previous studies on these materials in phosphate buffer at pH 7.4 and 37 °C.[17] Although the difference decreased with hydrolysis time, M-C75D25 unexpectedly showed a somewhat larger mass loss compared to M-C60D40. The initially slightly lower molecular weight and higher polydispersity of M-C75D25 could be one reason for the somewhat faster mass loss compared to M-C60D40.

pH. The pH of the sample solutions after different hydrolysis times was measured, and the results are shown in Figure 9. This gives an indication of the amount of hydroxyacids released to water. In accordance with ESI-MS and mass loss results the largest pH decrease was observed for H-D100 and T-C60D40. The pH of H-D100 decreased from 7.0 to 6.6 already after 1 day in water, while pH of the other samples remained unchanged during the first day. This is explained by the high water solubility of DXO oligomers also shown by ESI-MS analysis. The sample solutions of these polymers showed a continuous decrease in pH and reached 4.5 for the triblock copolymer after 147 days. For the multiblock copolymers, pH started to decrease after 21 days of degradation and reached 5−5.5 at the end of the study. The sample solution of the homopolymer of PCL showed a small pH decrease from 7 to 6.6 between 49 and 147 days. The overall faster degradation rate of H-D100 and T-C60D40 combined with larger solubility of DXO oligomers induced larger amounts of water-soluble acidic compounds and hence a larger decrease in pH.

Copolymer Composition. ^{1}H NMR was used to monitor the changes in copolymer composition during hydrolysis. The results are presented in Figure 10. The two flanking DXO blocks in the triblock copolymer were preferentially hydrolyzed with preservation of the intermediate CL block. Already after 7 days

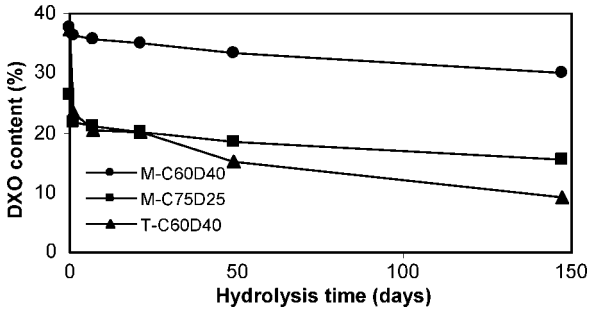


Figure 10. DXO content of the different CL/DXO copolymers after different hydrolysis times.

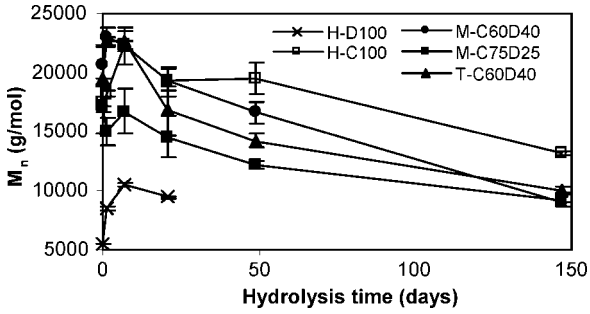


Figure 11. Molecular weight changes of the different linear CL/DXO polymers during hydrolysis.

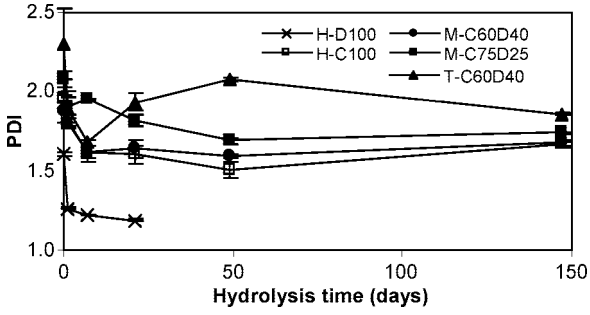


Figure 12. Changes in polydispersity index (PDI) of the different linear CL/DXO polymers during hydrolysis.

the DXO content had decreased from 38% to 20%. At the end of the study, after 147 days, the DXO content of the triblock copolymer had decreased to only 9%, whereas the multiblock copolymer with the same composition, i.e., M-C60D40, still had a DXO content of 30%. The DXO content of M-C75D25 had decreased from 26% to 15% at the end of the study. In general, although the multiblock copolymers showed a continuous decrease in DXO content, their composition was considerably less affected by the hydrolysis compared to the triblock copolymer. These results are in correlation with previously reported results after hydrolysis of CL/DXO copolymers in phosphate buffer.[17] In addition, similar results have also been reported for caprolactone/valerolactone copolymers.[11]

Molecular Weight Changes. Figure 11 shows the molecular weight of the studied homo- and copolyesters as a function of hydrolysis time. During the first 7 days of hydrolysis the molecular weight increased for all the polymers, probably due to the release of water-soluble oligomers. This is endorsed by the large initial decrease in polydispersity index (PDI) for all materials after 1 day of degradation as presented in Figure 12. Largest initial increase in molecular weight was observed for T-C60D40 and H-D100 which indicates that mainly DXO oligomers were released. In accordance these two polymers also showed the largest decrease in PDI. However, after 7 days the molecular weight of all polymers continued to decrease at approximately the same rate, although slightly slower for H-C100. The PDI values of the various polymers showed a continuous decrease during the first 47 days where after the values were less affected with the exception of the triblock copolymer which exhibited a fluctuating PDI with time.

Conclusions

Hydrolytic degradation products of DXO and CL homo- and copolymers were established at the molecular level by electrospray ionization multistage mass spectrometry. One main series of oligomers with a peak-to-peak mass increase of the corresponding repeating unit was observed in the spectra of the respective homopolymers. The oligomer patterns shifted toward the lower mass range with increasing degradation time. A large difference in the water-soluble fraction was observed between the PCL and PDXO homopolymers. Oligomers up to heptadecamer were detected after hydrolysis of more hydrophilic PDXO, while only oligomers up to hexamer were detected after hydrolysis of hydrophobic PCL. This demonstrates the considerably higher water solubility of the DXO oligomers, which facilitates the rapid mass loss during hydrolysis. Even longer DXO oligomers are expected to be found in the water fraction, but the molecular weight of these oligomers is above the upper mass range of the ESI-MS. The ESI mass spectrum obtained from the triblock copolymer was similar to the spectrum of the PDXO homopolymer degradation products, showing preferential hydrolysis of DXO blocks, which was confirmed by NMR results. The hydrolysis of multiblock copolymers resulted in formation of DXO, CL, and "mixed" DXO/CL oligomers, where DXO-rich oligomers dominated in the product patterns. ESI-MS gave valuable insights into the hydrolysis process of CL and DXO homo- and copolymers with different hydrophilicities and architectures. The ESI-MS^n fragmentation experiments enabled determination of molecular masses and structures of fragment ions of mass-selected oligomers containing ether and ester linkages. In addition to the primary hydrolytic degradation products, i.e., oligomers terminated by hydroxyl and carboxyl end groups, partial oligomer fragmentation in the ESI mass spectrometer was observed which resulted in the formation of unsaturated and carboxyl-terminated products. The exclusive fragmentation via $-O-CH_2$ ether bond cleavage observed for DXO oligomers showed higher susceptibility for ether bond with respect to ester bond cleavage under the fragmentation experiments.

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