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Polymer 45 (2004) 6779–6787
polymer
www.elsevier.com/locate/polymer

Hydrolytic degradation of oligo(lactic acid): a kinetic and mechanistic study

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Received 23 February 2004; received in revised form 28 July 2004; accepted 2 August 2004
Available online 20 August 2004

Abstract

This study shows that the degradation mechanism and kinetics of monodisperse oligo(lactic acid)s esterified with *N*-(2-hydroxypropyl)methacrylamide (HPMAm) are strongly influenced by the nature of the chain end. Oligomers with free hydroxyl chain ends degraded predominantly by chain end scission via a backbiting mechanism with a pseudo first-order rate constant $k_{bb}=2.7\ h^{-1}$ in aqueous buffer (37 °C, pH 7.2). Once the hydroxyl groups were protected by acetylation, random chain scission became the rate limiting step with $k_r=0.022\ h^{-1}$ under the same conditions. Using these rate constants, the theoretical time-resolved degradation profile was calculated for every (intermediate) degradation product and corresponded very well with the experimental results. The rate of formation of HPMAm was independent of the chain length for the acetylated oligomers, while the hydroxyl terminated oligomers with an even number of lactic acid units formed HPMAm more rapidly than oligomers with an odd number of units. The possibility to fine-tune the degradation rate is relevant when applied as e.g. hydrogels for controlled release or tissue engineering materials.

Keywords: Polylactic acid; Degradation; Kinetics

1. Introduction

Poly(lactic acid) (PLA) and related copolymers with glycolic acid (PLGA) are the most widely studied biodegradable polyesters used in various biomedical, pharmaceutical and technical applications including packaging materials, implants, scaffolds for tissue engineering, and drug delivery devices. Solid PL(G)A degrades through hydrolysis of the ester bonds, typically taking a few months to years for complete degradation, depending on a.o. the composition of the copolymers [1]. The initial degradation process can be described as bulk erosion, meaning that hydrolysis occurs homogeneously through the matrix of the solid material because the penetration of water is faster than the hydrolysis rate of the ester bonds.

In order to predict and control the degradation rate of PL(G)A based systems, it is important to have detailed information on the mechanism and kinetics of degradation. The degradation mechanism of PLA derivatives is believed to occur mainly through random chain scissions, causing a gradual decrease in the number average molecular weight. Dissolution of the material starts when the molecular weight has decreased to a certain level and, as a consequence, soluble degradation products are formed [2,3]. There have been a number of reports recently that provide strong indications that the degradation kinetics cannot only be described by random chain scissions, but that the end groups may play an important role in the process [4]. For example, the phenomenon of auto-catalysis by the carboxylic chain ends which are formed during degradation is well-documented [5]. Shih reported data that suggested chain end scission to be faster than random chain scission at acid conditions [6,7]. A more detailed computational analysis of the degradation profile of PLGA (containing 50:50 lactic acid and glycolic acid) microspheres by Batycky et al. revealed that chain end scission at either the COOH or OH end is approx. 4 orders of magnitude faster than random chain scission [8]. An unambiguous mechanistic explanation can, however, not be given for this phenomenon,

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doi:10.1016/j.polymer.2004.08.001

since the rate constants are influenced by many factors, including water accessibility, molecular mobility, local dielectric constant, local pH, etcetera. It can be anticipated that the local environment of the chain ends is much different from the bulk chains in the solid state.

Obviously, the contribution of chain end scission increases with decreasing molecular weight of the polymer since the fraction of chain ends increases, e.g. as a consequence of the degradation process. In recent applications low molecular weight PLAs are used instead of relatively high molecular weight polymers, for example, when they are used as degradable crosslinks in hydrogels [9–15]. Moreover, oligomers of lactic acid are water-soluble to some extent, which is expected to have a dramatic influence on the degradation kinetics with respect to solid polymers. The degradation rate of water-soluble oligo(lactic acid)s (degree of polymerization <10) has been studied by capillary electrophoresis [16], which indicated the preferential formation of lactoyllactate, subsequently slowly degrading to lactic acid. However, a detailed kinetic and mechanistic analysis of the hydrolysis process of lactic acid oligomers has not been reported so far.

Recently, we reported the preparation of degradable hydrogels based on stereocomplex formation of oligo(lactic acid) grafted to dextran [11]. The hydroxyl end group of each oligo(lactic acid) is coupled to dextran via a carbonate ester group, the carboxyl ends are esterified with a primary alcohol (methoxyethoxyethanol). It was shown that the degradation of the hydrogels under physiological pH and temperature was initiated by the cleavage of the carbonate ester bond [17], and that the resulting free oligo(lactic acid) chains further degraded to the monomers. We demonstrated that the latter process was dominated by chain end scission at the hydroxyl terminus [18]. It was concluded from the degradation profiles that at $pH \leq 2$ the ultimate ester bond was the most labile one, while the penultimate ester bond was preferentially hydrolyzed in neutral or alkaline pH resulting in the stepwise removal of lactoyllactate probably via its cyclic dimer (i.e. lactide, which is degraded further into lactic acid). This latter phenomenon was explained by the backbiting mechanism, which is shown in Scheme 1. Our conclusions were based on observations, but no detailed kinetic analysis of the degradation profiles was made yet. More recently, Schliecker et al. reported that random chain scission is the mode of degradation of lactic acid oligomers at alkaline pH, which contradicted our observations [19]. Their conclusions were, however, based on a questionable interpretation of their ^{1}H NMR data [20].

In a recent paper, we described stereocomplex hydrogels formed from oligo(lactic acid)-grafted poly-*N*-(2-hydroxypropyl) acrylamide (*p*HPMAm-*g*-oLA) [21]. Interestingly, the degradation times of these hydrogels where much increased as compared to corresponding dextran hydrogels. Moreover, we observed a strong effect of the nature of the side chain terminus on the degradation time. In the present paper, we report results that provide important information in order to be able to adjust and predict the degradation rates of low molecular weight PLAs and hydrogels based on them. We performed an in depth study of the degradation kinetics and mechanism of monomers used to prepare the aforementioned hydrogels, i.e. HPMAm-oligo(lactic acid). The results corroborate the previously suggested backbiting mechanism at physiological pH.

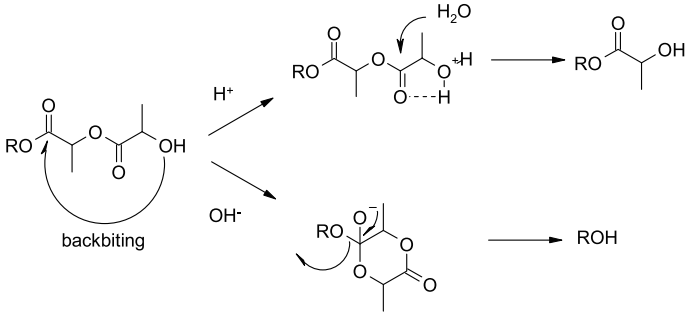
2. Experimental

2.1. Materials

All chemicals were used as received without further purification. L-Lactide ((3*S*-*cis*)-3,6-dimethyl-1,4-dioxane-2,5-dione, >99.5%) was obtained from Purac Biochem BV (Gorinchem, The Netherlands). *N*-(2-hydroxypropyl)methacrylamide (HPMAm) was purchased from Polysciences, Inc (Warrington, USA). Stannous octoate (tin(II) bis(2-ethylhexanoate), $SnOct_2$, 95%) was purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). Hydroquinone monomethyl ether (4-methoxyphenol) was obtained from Fluka (Zwijndrecht, the Netherlands). Acetonitrilee (HPLC gradient grade) was purchased from Biosolve LTD (Valkenswaard, The Netherlands). Acetic anhydride (>98.5%) was obtained from Merck (Darmstadt, Germany).

2.2. ^{1}H NMR spectroscopy

^{1}H NMR spectra were recorded on a Gemini



Scheme 1. Degradation of oligo(lactic acid) by chain end scission. Backbiting is catalyzed by hydroxyl ions.

spectrometer (Varian Associates Inc. NMR Instruments, Palo Alto, CA) operating at 300 MHz. Samples were measured in $CDCl_3$, (Cambridge Isotope Laboratories (Andover, MA)). Chloroform (at 7.25 ppm) was used as the reference line.

2.3. Mass spectrometry

Mass spectrometric (MS) analysis (positive ion mode) was carried out with a Shimadzu QP 8000 LC-MS. The probe voltage was +4.5 kV with a detector voltage of 1.5 kV. The CDL voltage was set at −55 V, and the CDL temperature was 230 °C. A deflector voltage of 50 V was used. The nebulizing gas (N_2) had a flow of 4.5 l/min. Instrumental control was performed with a CLASS 8000 LCMS Software Package.

2.4. HPLC

HPLC analysis was carried out on a Waters system (Waters Associates Inc., Milford, MA, USA) consisting of a pump Model 600, an autoinjector Model 717, and a variable wavelength absorbance detector Model 996. The injection volume was 100 μl, the flow was set at 1.0 ml/min, and the detection wavelength was 210 nm. An analytical column, LiChrospher 100 RP-18 (5 μm, 125×4 mm i.d.) with an RP-18 guard column (4×4 mm) (Merck) was used. A gradient was run from 100% A (water/acetonitrilee 95:5) to 100% B (acetonitrilee/water 95:5 (w/w)) in 30 min. The chromatograms were analysed with Millennium 32 Version 3.05 software (Waters Associates Inc.).

2.5. Synthesis of polydisperse HPMAm-oligo(L-lactic acid)

HPMAm-oligo(L-lactic acid) was prepared essentially as described by Neradovic et al. [22]. In brief: a mixture of 10.0 g L-lactide (69.4 mmol), 1.66 g HPMAm (11.6 mmol) and 1.5 mg hydroquinone monomethyl ether (0.012 mmol) was stirred at 120 °C until the lactide was molten, and stannous octoate (0.23 g; 0.58 mmol) was added. The mixture was stirred for 4 h at 130 °C and subsequently cooled to room temperature, to yield HPMAm-oligo(L-lactic acid). ^{1}H NMR ($CDCl_3$): δ (ppm) = 5.65 (d, 1H, $H^aH^bC{=}C$), 5.27 (d, 1H, $H^aH^bC{=}C$), 4.90–5.20 (m, C(=O)–C*H*(–CH_3)–O, CH_2–C*H*(–O)–CH_3), 4.35 (q, 1H, C(=O)–C*H*(–CH_3)–OH), 3.6 (m, 1H, CH^aH^b–CH(–O)–CH_3), 3.25 (m, 1H, CH^aH^b–CH(–O)–CH_3), 1.90 (s, 3H, C=C(–C*H*$_3$)), 1.35–1.60 (m, C(=O)–CH(–C*H*$_3$)–O), 1.20 (d, 3H, CH_2–CH(–O)–C*H*$_3$).

2.6. Acetylation of HPMAm-oligo(L-lactic acid)

Directly after the ring opening polymerization of L-lactide with HPMAm (described above), the mixture was cooled to 90 °C and a cooler was placed on the reaction flask. 15 ml of acetic anhydride was added and the mixture was stirred for 1 h. Subsequently, the unreacted acetic anhydride was removed under reduced pressure. The conversion was quantitative according to ^{1}H NMR. ^{1}H NMR ($CDCl_3$): δ (ppm) = 5.65 (d, 1H, $H^aH^bC{=}C$), 5.27 (d, 1H, $H^aH^bC{=}C$), 4.90–5.20 (m, C(=O)–C*H*(–CH_3)–O, CH_2–C*H*(–O)–CH_3), 3.6 (m, 1H, CH^aH^b–CH(–O)–CH_3), 3.25 (m, 1H, CH^aH^b–CH(–O)–CH_3), 2.07 (s, 3H, O–C(=O)–C*H*$_3$), 1.90 (s, 3H, C=C(–C*H*$_3$)), 1.35–1.60 (m, C(=O)–CH(–C*H*$_3$)–O), 1.20 (d, 3H, CH_2–CH(–O)–C*H*$_3$).

2.7. Isolation of monodisperse HPMAm-oligo(L-lactic acid)s (*n*, *nac*)

Monodisperse (acetylated) HPMAm-oligo(L-lactic acid) was obtained by fractionation of polydisperse products using an ÄKTA purifier (Pharmacia Biotech AB, Sweden) with a preparative HPLC column (Econosphere C8, 10 μm, 250×22 mm; Alltech, Illinois, USA). Polydisperse oligomer (1.0 g) was dissolved in 1.5 ml of water/acetonitrilee (5/95 %w/w) and filtered over a 45 μm filter. 1.5 ml of this solution was injected onto the column. A gradient was run from 50% A (water/acetonitrilee 95:5 (w/w)) to 100% B (acetonitrilee/water 95:5 (w/w)) in 120 min. The flow rate was 10.0 ml/min; detection by UV (λ = 215 nm). The chromatograms were analyzed with Unicorn Analysis module (version 2.30) software. The individual oligomers were collected and fractions with corresponding degrees of polymerization were pooled. The solvent was removed under reduced pressure. The oligomers were characterized by HPLC (analytical column), ^{1}H NMR and mass spectrometry.

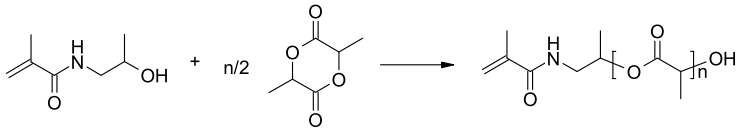
2.8. Degradation studies

The degradation experiments were carried out in 20 ml glass bottles, placed in a thermostated water bath at 37 °C. The pH was measured before and after degradation at the temperature of the experiment. For the standard degradation experiments 5 ml of stock solution of monodisperse (acetylated) HPMAm-oligo(L-lactic acid) in acetonitrilee (2 mg/ml) was diluted to a final concentration of 1 mg/ml with 5 ml phosphate buffer (pH 7.2, 100 mM, the ionic strength (μ) adjusted to 0.3 with sodium chloride). The buffer concentrations need to be at least 100 mM to keep the pH at a fixed value. Samples of 400 μl were drawn at regular time intervals and adjusted to pH 4 with 150 μl ammonium acetate buffer (pH 4, 1 M) to inhibit further degradation. The samples were stored at 4 °C prior to analysis with HPLC.

3. Results and discussion

3.1. Synthesis of monodisperse oligomers

Polydisperse oligolactide was obtained by ring opening polymerization of L-lactide initiated by HPMAm based on a



Scheme 2. Synthesis of HPMAm-oligo(lactid acid)s **n**.

procedure reported elsewhere (Scheme 2) [22]. Part of the product was reacted with acetic anhydride to end-cap the oligomers with acetyl groups. Monodisperse products were isolated by preparative HPLC essentially as described previously [11]. The identity and purity (>95%) of the final products were proven by HPLC, ^{1}H NMR and MS. The compounds mentioned in this paper are numbered according to the number of lactic acid units (n) in the oligomers. The acetylated oligomers contain the suffix 'ac'.

3.2. Hydrolysis rates

The degradation of monodisperse oligo(lactic acid)s with two different chain lengths and with or without an acetyl chain end (compounds **7**, **7ac**, **12**, and **12ac**) was carried out according to a method previously reported by us [18], i.e. they were dissolved in a 1:1 mixture of acetonitrile and buffer (100 mM PBS, pH 7.2) and incubated at 37 °C. A strong buffer was used to avoid a pH drop by the lactic acid produced during hydrolysis and thereby to prevent auto-catalytic effects. We choose to use acetonitrile as a cosolvent instead of pure water to avoid differences in hydrolysis rates due to differences in aqueous solubility of the oligomers. Later on (vide infra) we will correct our data for the presence of acetonitrile. Previously, detection of oligo(lactic acid) degradation products has been performed by capillary electrophoresis [16], which is a well-established technique to allow separation of charged (e.g. carboxylated) water-soluble compounds [23]. We choose to use HPLC analysis of the reaction mixtures because it allows easy and high-resolution separation of oligomers up to a degree of polymerization of 16 [18]. However, degradation products containing free carboxylic chain ends could not be separated by the HPLC method used here, and only the degraded segments still carrying the HPMAm chain end were detected. All peaks occurring in the HPLC chromatograms were identified by comparing with the retention times of monodisperse oligomers obtained by preparative HPLC.

The concentration of starting oligomers decreased according to first order kinetics, as indicated by the straight line obtained from plots of the natural logarithm of the amounts versus time (Fig. 1). The first order reaction rates (k_{obs}) calculated from the slopes of these lines are given in Table 1.

The most clear observation is that the degradation of the acetylated oligomers occurred much slower than the oligomers with the free hydroxyl chain end, which corroborated our previously published results with succinylated oligo(lactic acids) [18]. Moreover, the HPLC chromatograms of the degradation mixtures showed the prevalence of degradation products with odd and even number of lactic acid units in the case of **7** and **12**, respectively (vide infra). These phenomena can be ascribed to the stepwise degradation from the hydroxy terminus via the previously suggested backbiting mechanism in neutral and basic media, as described in Section 1.

The higher k_{obs} for **12ac** than for **7ac** can be ascribed to the higher number of ester bonds in the oligomer, which increases the chance of random chain scission. Assuming equal reactivity of all ester bonds one can derive the following equation:

$$k_{obs} = n \times k_r \quad (1)$$

where n is the number of ester bonds and k_r is the pseudo first order rate constant of random chain scission. However, we have previously established that the reactivity of the ester bond between HPMAm and lactic acid (with rate constant k_{HPMAm}) is considerably lower than the reactivity of the ester bond between two lactic acid units. To be precise, the reaction rate constant of the latter bond in HPMAm-di(lactic acid) (compound **2**) $k_{2,1} = 0.032\ h^{-1}$, while k_{HPMAm} of HPMA-mono(lactic acid) (**1**) is $0.0079\ h^{-1}$ (37 °C, 10% DMSO in buffer pH 7.5) [22]. Therefore, Eq. (1) is transformed in:

$$k_{obs} = (n-1) \times k_r + k_{HPMAm} \quad (2)$$

For the value of k_{HPMAm} in Eq. (2), the above mentioned number has to be recalculated in order to conform to the different conditions used here. Going from pH 7.5 [22] to pH 7.2 (this work) and knowing that the hydrolysis is first order in [OH^-] concentration above pH 5 [18], the reaction

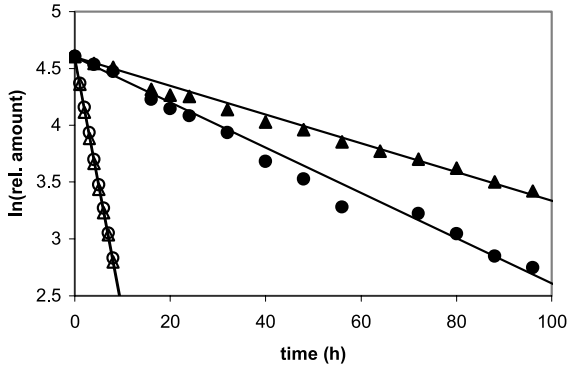


Fig. 1. Natural logarithm of the relative amounts (% with respect to the amounts at $t=0$) of monodisperse HPMAm-oligo(lactic acid)s during degradation (pH 7.2, 37 °C). Closed symbols: acetylated oligomers; open symbols: non-acetylated oligomers; triangles: **7(ac)**; circles: **12(ac)**.

Table 1
Reaction rate constants and half life times for the hydrolysis of HPMAm-oligo(lactic acid) at 37 °C, pH 7.2

	Acetonitrile/PBS (1:1) (measured)		PBS (calculated)	
	k (h^{-1})	$t_{1/2}$ (h)	k (h^{-1})	$t_{1/2}$ (h)
7	0.223[a]	3.1	2.90	0.24
12	0.222[a]	3.1	2.89	0.24
7ac	0.0126[a]	55	0.164	4.2
12ac	0.0199[a]	35	0.259	2.7
k_r	0.0017		0.022	
k_{HPMAm}	0.00042		0.0056	
k_{bb}	0.21		2.7	

[a] k_{obs}.

is expected to slow down by a factor 2. Furthermore, it was previously shown that linear relationships exist between the cosolvent content (and thereby the dielectric constant ε) of the medium and $\log(k_{obs})$ of ester hydrolysis. In DMSO the slope of the corresponding linear plot was 0.087 ε^{-1} [22], while for acetonitrile a slope of 0.055 ε^{-1} was found [18]. Therefore, going from 10% DMSO [22] to 50% acetonitrile (this work) one can calculate the value of k_{HPMAm} in Eq. (2) according to the following formula:

$$\log k' = \log k + \Delta\mathrm{pH} + \Delta\varepsilon_{DMSO} \times 0.087 + \Delta\varepsilon_{ACN} \times 0.055 \quad (3)$$

in which k' is the corrected value in the presence of acetonitrile (ACN), k is the measured value in the presence of DMSO, ΔpH is the increase in pH, $\Delta\varepsilon_{DMSO}$ and $\Delta\varepsilon_{ACN}$ are the increase in the dielectric constant going from the corresponding cosolvent mixture to pure aqueous buffer. From this equation k'_{HPMAm} was estimated to be 4.2×10^{-4} h^{-1}. Using this value and k_{obs} for compounds **7ac** or **12ac** from Table 1, it can be calculated with the help of Eq. (2) that $k_r = 1.7\times10^{-3}$ h^{-1}. Interestingly, when the previously reported value of $k_{2,1}$ ($=0.032$ h^{-1}, corresponding to the hydrolysis of the dilactate ester bond in HPMAm-dilactate) is recalculated in the same way to the conditions used in our experiments using Eq. (3), exactly the same value as for k_r is obtained, meaning that the bond between the lactic acid units in HPMAm-dilactate has the same susceptibility for hydrolysis as the corresponding ester bonds in the lactic acid oligomers.

In order to derive a similar equation as above for the compounds **7** and **12** with the free hydroxyl chain ends, one has to take the backbiting reaction into account:

$$k_{obs} = (n-2)\times k_r + k_{HPMAm} + k_{bb} \quad (4)$$

where k_{bb} is the rate constant for the backbiting reaction. Using this equation, it can be calculated that k_{bb} is equal to 0.21 h^{-1}, for both **7** and **12**.

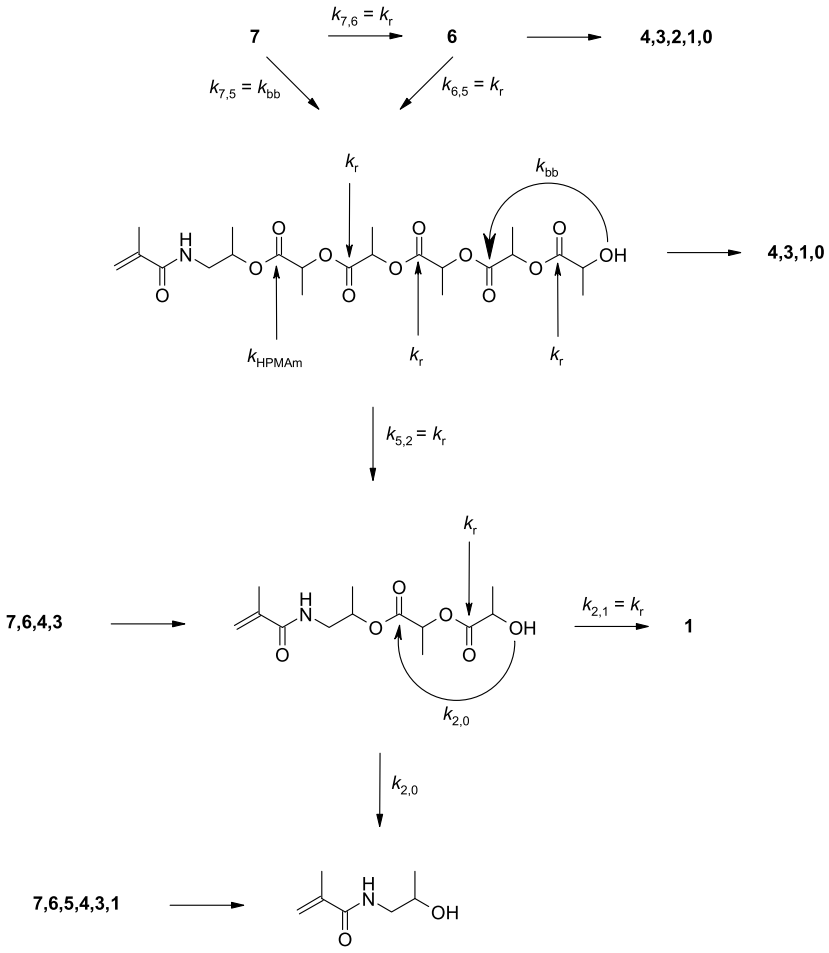
It must be stressed that the values reported here are the pseudo first order rate constants for the hydrolysis in acetonitrile/water (1:1) mixtures. In water, when corrected for the difference in the dielectric constant of the medium and assuming full water-solubility, the rate constants are anticipated to be 13-fold higher according to previous observations [18]. The reaction rate constants and half life times in both media are summarized in Table 1. It can be concluded from our data that the backbiting is more than 100 times faster than the random chain scission of a hydroxy-terminated lactic acid oligomer, which is a very important conclusion in order to explain the degradation behavior of oligo- and PLAs.

3.3. Calculation and interpretation of degradation profiles

In theory, one can predict the amounts of any of the degradation products at any time point during the hydrolysis using the reaction rate constants reported above (Table 1). The change in the amount of HPMAm-oligolactate with x number of lactic acid units (dc_x/dt) equals its rate of formation minus the rate of further degradation of that particular product to shorter chains. This can be expressed in the following formula, which is similar to the equations previously derived by Shih [6] and by Batycky et al. [8]:

$$\frac{dc_x}{dt} = \sum_{i=x+1}^{n} c_i k_{i,x} - c_x \sum_{i=0}^{x-1} k_{x,i} \quad (5)$$

where c_x (c_i) = concentration of x-mer (i-mer), $k_{i,x}$ = rate constant of hydrolysis of i-mer to x-mer (see Scheme 3), and n = degree of polymerization of the starting oligomer. For $k_{i,x}$ either k_r, k_{bb}, k_{HPMAm} can be used depending on the ester bond involved, with one exception (Scheme 3): Considering the ~4-fold lower reactivity of the HPMAm ester bond with respect to the lactic acid ester bond (Table 1), one can expect $k_{2,0}$ (referring to the backbiting reaction in HPMAm-dilactate, i.e. attack of the hydroxyl end group on the HPMAm ester bond) to be slower than the corresponding reaction in the higher oligomers (k_{bb}, attack on a lactic acid ester bond). It appeared from our degradation studies of oligomers **7** and **12** that after a period of about 50 h hardly any higher oligomers were left than HPMAm-dilactate (**2**). After that time the latter compound degraded with pseudo-first order kinetics ($k_{obs} = 6.1\times10^{-3}$ h^{-1}). Since in this case $k_{obs} = k_{2,1} + k_{2,0}$, and knowing that $k_{2,1}$ is equal to k_r (see above), one can calculate $k_{2,0}$ to be equal to 4.4×10^{-3} h^{-1}. This is indeed substantially lower than k_{bb} (Table 1)



Scheme 3. Part of the reaction scheme of the stepwise hydrolysis of HPMAm-oligo(lactic acid) **7**, highlighting the conversion of intermediate oligomers **5** into **2** and subsequently **0** (=HPMAm). The scheme shows the reaction rate constants corresponding to the hydrolysis of the indicated ester bonds.

and therefore has to be taken into account when calculating the degradation profile according to Eq. (5). For the acetylated starting compounds, we assume for the hydrolysis of the acetyl ester bond the same reaction rate constant as for the lactic acid ester bonds (i.e. $k_{7ac,7} = k_{12ac,12} = k_r$). Furthermore, the backbiting reaction cannot occur with **7ac** and **12ac** because the hydroxyl groups are acetylated, and we took for the hydrolysis of the penultimate ester bond of these starting compounds again the same k_r (i.e. $k_{7ac,6} = k_{12ac,11} = k_r$).

The degradation profiles were calculated by a stepwise procedure. Starting with the known composition at time $t = 0$, the change in concentration dc_x/dt for each compound present in the degradation mixture was calculated according to Eq. (5), whereupon the new composition at time $t + dt$ was calculated. This procedure was repeated with relatively small time increments with respect to the half life time of the oligomers; $dt < 6$ min for compound **12** ($t_{1/2} = 186$ min) did not show a noticeable change in the calculated degradation profile.

The results of these calculations are plotted in Fig. 2 for the oligomers used in this study, together with the measured compositions of the degradation mixtures. As can be seen, the calculated profiles match the measured amounts reasonably well. This implies that the kinetic model discussed above and illustrated in Scheme 3 can very well describe the mechanism and rate of degradation of oligo(lactic acid)s with different chain ends.

The following conclusions can be drawn from the degradation profiles. The oligomers **7** and **12** with the hydroxyl chain ends hydrolyze to form mainly odd and even degradation products, respectively. This can be clearly seen in Fig. 3(A), where the composition is plotted at three different time points during the degradation of **12**. This remarkable behavior can be ascribed to the stepwise backbiting reaction, being the main mechanism of degradation of these oligomers as discussed above. The presence of small amounts (i.e. not more than a few percent) of the odd oligomers in the reaction mixture of **12** is due to random chain scissions occurring less frequently than backbiting ($k_r \ll k_{bb}$). Interestingly, after extended reaction times compounds **1** and **2** appear to accumulate in the reaction mixture of **7** and **12**, respectively. This is due to the fact that once these products are formed, further degradation is slowed down because of the more stable HPMAm-lactic acid ester bond ($k_{HPMAm} < k_r$, and $k_{2,0} < k_{bb}$). Another

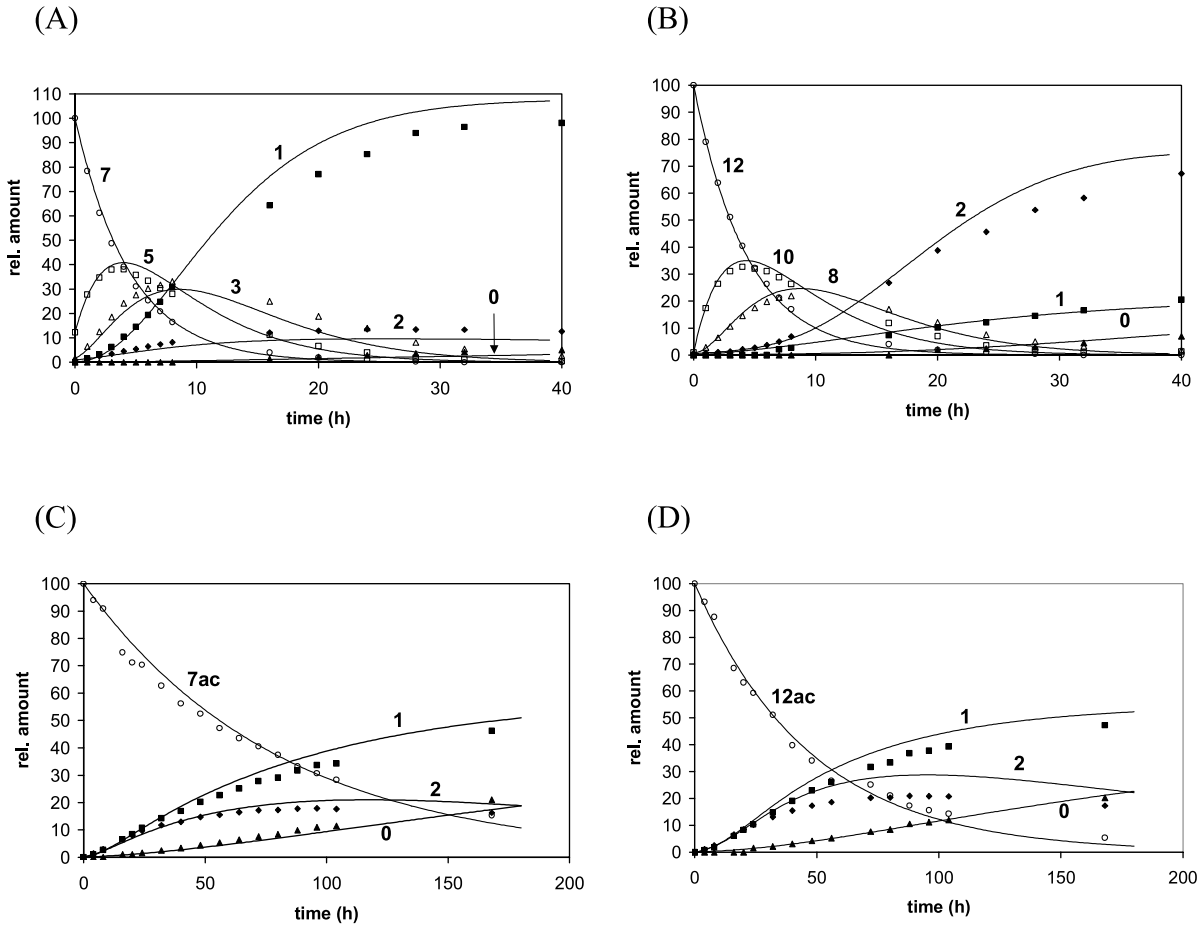


Fig. 2. Calculated (lines) and measured (symbols) degradation profiles of HPMAm-oligo(lactic acid)s **7** (A), **12** (B), **7ac** (C) and **12ac** (D). The given numbers are the amount of lactic acid units in the corresponding HPMAm-containing oligomeric degradation products. For clarity, only the most relevant degradation products are plotted in the graphs. The ones that are not shown are only minor constituents of the degradation mixtures.

interesting observation is that 35% of **12** was fully degraded to HPMAm after 168 min, while only 24% of **7** was completely hydrolyzed in the same time despite the shorter chain length of the latter oligomer (results not shown). This can be explained by the fact that **7** preferentially degrades via the backbiting mechanism to **1**, which hydrolyses more slowly than **2** formed from **12** ($k_{1,0} < k_{2,0}$).

The degradation profiles of the acetylated oligomers **7ac** and **12ac** are even more remarkable, but fully understandable in terms of the kinetics described above. The degradation of the starting oligomers resulted in the formation of almost exclusively the products **2**, **1** and **0** (see Figs. 2(C) and (D) and 3(B)); the intermediate oligomers could hardly be detected in the reaction mixtures. This can be explained by the fact that backbiting of the starting oligomers does not occur because the hydroxyl groups are protected. Therefore, these oligomers can only hydrolyze through the relatively slow random chain scission. Once one of the ester bonds is hydrolyzed, a shorter oligomer is formed with a free hydroxyl end group, and this product can rapidly hydrolyze through backbiting until either **1** or **2** is formed. Then the final degradation to **0** is slowed down again because of the stability of the HPMAm ester bond, as explained above. This mechanism is shown in Scheme 4. An interesting effect of protecting the chain end is that the rate of formation of the final degradation product HPMAm is independent of the chain length of the starting oligomer, i.e. 20% conversion after 168 h for both **7ac** and **12ac**, which is in clear contrast to the degradation of the non-acetylated oligomers. This difference is due to the fact that the initial chain scission occurs randomly in **7ac** and **12ac**, resulting in equal ratios of odd and even oligomers. Therefore, the intermediate compounds **1** and **2** are formed in similar ratios by the subsequent backbiting steps (compare Fig. 3(C) and (D)), and the formation of HPMAm, which is the final but rate determining step, occurs equally rapid for both initial chain lengths.

It must be stressed that the backbiting mechanism described here is a phenomenon which is typical for lactic acid oligomers in aqueous environment. The results cannot be directly correlated to the degradation profiles of high

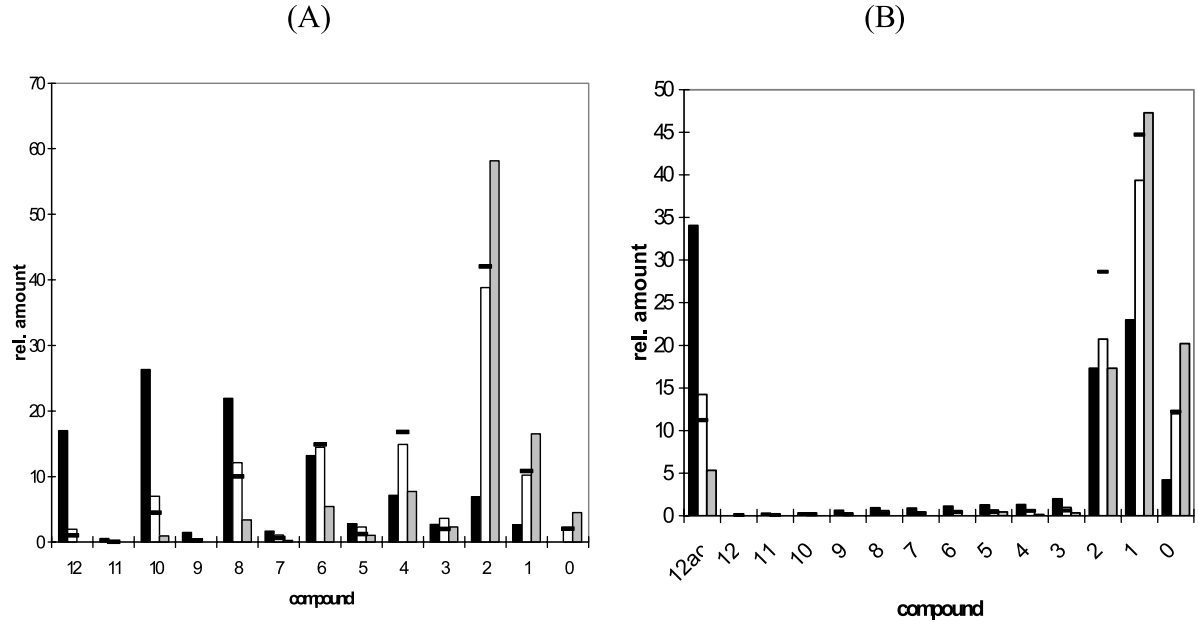
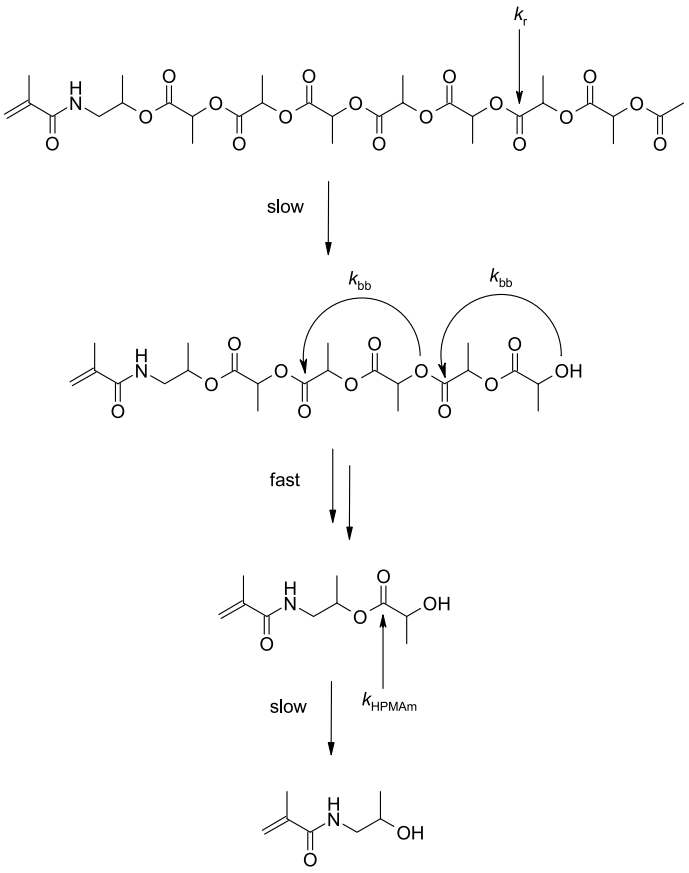


Fig. 3. Measured amounts of HPMAm-oligo(lactic acid)s during the degradation of (A): **12** after 8 h (black bars), 20 h (white bars) and 32 h (gray bars); (B): **12ac** after 48, 104 and 168 h, respectively. The horizontal lines in plot A and B are the calculated amounts after 20 and 104 h, respectively.

molecular weight PLAs (HMW PLA). For HMW PLA it can be anticipated that initially random chain scission will prevail due to the small fraction of chain ends present, as has indeed been reported by others [2]. Moreover, in pure aqueous environment, solubility limitations will have a huge influence on the degradation rates of HMW PLA.



Scheme 4. Reaction scheme showing one of the possible degradation routes of **7ac**.

Nevertheless, we gained more insight in the mechanism of oligo(lactic acid) degradation and our results can very well explain the degradation behavior of oligomers in the final stages of HMW PLA degradation, or explain and predict degradation rates of stereocomplex hydrogels based on these oligomers [18,21]. For example, Braud et al. observed accumulation of lactoyllactate during the degradation of oligo(lactic acid) [16]. They ascribed this phenomenon to the relatively high stability of lactoyllactate with respect to higher oligomers, without giving a reason for that, but from our results it becomes clear that it can be attributed to the fact that this compound is preferentially formed as a result of the backbiting reaction.

4. Conclusions

The kinetic study of the degradation products formed from monodisperse lactic acid oligomers presented in this work revealed unambiguously the mechanism of degradation at physiological pH. Chain end scission by backbiting is the main route of degradation with hydroxyl-terminated oligomers. Protecting these chain ends, e.g. by acetylation, has a significant influence on the rate and mechanism of degradation because the rate limiting step for degradation is then the slow random chain scission.

In conclusion, the studies presented here provide very important information on ways to tailor the degradation rate of oligo(lactic acid)s, which is relevant for applications where these compounds are used in hydrogels for controlled release systems.

Acknowledgements

This work was supported by the Netherlands Organisation for Scientific Research—Medical Sciences (MW-NWO) grant 0316/014-81-101.

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