Tuning the Degradation Rate of Poly(2-hydroxypropyl methacrylamide)-*graft*-oligo(lactic acid) Stereocomplex Hydrogels

Cornelus F. van Nostrum,* Theo F. J. Veldhuis, Gert W. Bos, and Wim E. Hennink

Department of Pharmaceutics, Utrecht Institute of Pharmaceutical Sciences, Utrecht University, P.O. Box 80082, 3508 TB Utrecht, The Netherlands

Received October 10, 2003; Revised Manuscript Received December 18, 2003

ABSTRACT: The degradation time of stereocomplex hydrogels based on poly(2-hydroxypropyl methacrylamide) (pHPMAm) with oligo(lactic acid) side chains of opposite chirality can be tailored from approximately 1 week to almost 3 months under physiological conditions by adjusting the grafting density and the side chain terminus. The polymers were prepared by free radical copolymerization of HPMAm−oligo(lactic acid) macromonomers and HPMAm. The viscoelasticity and degradation time of hydrogels formed by mixing aqueous solutions of two polymers containing side chains of opposite chirality increased with increasing grafting density. Gels based on polymers with side chains having acetyl chain ends degraded slower than those containing hydroxyl-terminating side chains, because of a change in the hydrolysis mechanism from slow random chain scission to more rapid chain end scission (backbiting), respectively. The ease of preparation and the possibility of tuning the degradation rate over a wide range make these pHPMAm-based hydrogels ideal materials for the controlled release of, e.g., therapeutic proteins.

Introduction

Biodegradable hydrogels are an important class of materials for tissue engineering and for the controlled release of pharmaceutically active compounds such as therapeutic proteins.[1,2−3]

Hydrogels are three-dimensional polymeric networks made by chemical or physical cross-linking of hydrophilic polymers. In chemically cross-linked gels, the polymers are connected by covalent bonds. In physically cross-linked gels, the network is formed by physical interactions, which exist between different polymer chains. In recent years, there is an increasing interest in physically cross-linked gels, especially in which the gel formation occurs under mild conditions in the absence of organic solvents. The main reason is that the use of cross-linking agents and organic solvents to prepare such hydrogels is avoided. These agents and solvents can not only affect the integrity of the substances to be entrapped (e.g., proteins, cells), but they are often toxic compounds which have to be removed/extracted from the gels before they can be applied. A great variety of methods have been applied to create physically cross-linked gels, including ionic, hydrophobic, and hydrogen bond interactions.[4] Also the formation of crystalline domains is a tool to create physical cross-links. The latter includes stereocomplex formation,[5] which is the subject of this contribution.

In a mixture of polymers of opposite chirality, the formation of racemic crystallites has been observed. In the literature, the formation of such racemic crystallites has been referred to as stereocomplex formation.[6] For example, blends of high molecular weight poly(L-lactic acid) (PLLA) and poly(D-lactic acid) (PDLA) have a higher melting temperature (T_m = 230 °C) than the individual enantiomers (T_m = 170 °C), due to a different crystal structure.[7,8] Stereocomplex formation between PLLA and PDLA has been applied by us and others for the preparation of biodegradable hydrogels.[9−13] The general feature of these hydrogels is that polymers or oligomers of either L-lactic acid or D-lactic acid are attached to a water-soluble polymer in the form of block or graft copolymers. Association takes place in crystalline domains (stereocomplexes) upon mixing the two polymers (one containing L-lactic acid, the other containing D-lactic acid), providing the physical cross-links. The resulting hydrogels are degradable due to the hydrolysis of the lactate chains. Polymers containing long lactic acid blocks and/or high grafting densities are insoluble in water and have the disadvantage of the need for using organic (co)solvents.[10,11] Therefore, we investigated the minimum length required for stereocomplex formation to be effective[14] and coupled these enantiomeric oligo(lactic acid)s to dextran via a carbonate linkage at various grafting densities to create water-soluble polymers which form stereocomplex hydrogels upon mixing, as illustrated in Figure 1.[9]

The degradation of the dextran stereocomplex hydrogels occurs through hydrolysis of the oligo(lactic acid) side chains. The carbonate ester bonds between the side chains and the backbone appeared to be quite sensitive to hydrolysis, resulting in a relatively rapid degradation of the hydrogels within a few days at physiological pH and temperature.[15] The most stable gels were the ones which contained side chains of low polydispersity and survived for approximately 6 days. To allow control of the degradation rate over a wider period of time, we searched for improved stereocomplex hydrogels and found one in which we replaced the backbone for another biocompatible polymer, i.e., poly(2-hydroxypropyl methacrylamide) (pHPMAm), to which the side chains are grafted via an ester instead of a carbonate bond. Degradation of these polymers results in the formation of lactic acid and pHPMAm. The latter polymer is nontoxic, is highly biocompatible, and can be excreted by the kidneys if the molecular weight is below the renal threshold. Moreover, drug delivery systems based on pHPMAm are currently in phase II clinical studies.[16]

* To whom correspondence should be addressed. Fax: +31 (0)-30 2517839. E-mail: C.F.vanNostrum@pharm.uu.nl.

10.1021/ma035534+ CCC: $27.50
Published on Web 02/14/2004

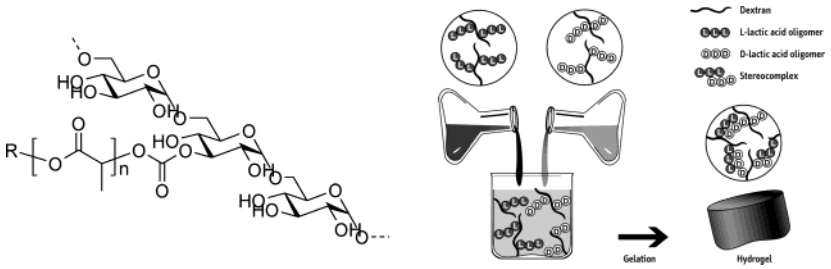

Figure 1. Chemical structure of dextran-*graft*-oligo(lactic acid), and schematic picture illustrating the formation of stereocomplex hydrogels from polymers containing either L- or D-lactic acid.

The graft copolymers are prepared by free radical copolymerization of HPMAm and HPMAm−esters of oligo(lactic acid)s. The grafting density can easily be controlled by the monomer ratio in the feed. This method is similar to the way Lim et al. prepared poly(2-hydroxyethyl methacrylate)-*graft*-oligo(lactic acid)s aiming at stereocomplex hydrogels.[11] However, they only investigated the degradation of solvent-cast films and no details on hydrogel characteristics were reported. In this work, we report the copolymerization of HPMAm and the HPMAm−oligo(lactic acid) macromonomer and investigated the rheological properties of hydrogels formed by mixing aqueous solutions of the two enantiomeric forms of the polymers. Swelling and degradation of the hydrogels were studied and the mechanism of degradation was elucidated by comparison with the degradation of the macromonomers reported elsewhere.[17]

Experimental Section

Materials. Preparation, and fractionation by preparative HPLC, of HPMAm−oligo(lactic acid)s with and without acetylated end groups has been carried out as described elsewhere.[17] α,α′-Azoisobutyronitrile (AIBN, >99%) was obtained from Fluka (Zwijndrecht, The Netherlands). Dioxane (distilled before use) and diethyl ether were purchased from Biosolve LTD (Valkenswaard, The Netherlands). D_2O and DMSO-d_6 were obtained from Cambridge Isotope Laboratories (Andover, MA).

Synthesis of pHPMAm-*graft*-oligo(lactic acid). A typical procedure to synthesize pHPMAm-*graft*-oligo(lactic acid) is the following. Polydisperse acetylated HPMAm−oligo(lactic acid) (10.0 g, 9.5 mmol), having an average number of lactic acid units $DP_{av} = 12$, and HPMAm (12.27 g, 85.7 mmol) were dissolved in 220 mL of freshly distilled dioxane at a temperature of 80 °C. Next, AIBN (156 mg, 0.95 mmol) was added. The solution was stirred for 2 h at 80 °C under a nitrogen atmosphere. The formed polymer was precipitated in 1 L of ice-cold diethyl ether. Next, the product was isolated by filtration and dried under vacuum at 40 °C, to yield 8.3 g pHPMA-*graft*-oligo(lactic acid) (37%).

^{1}H NMR Spectroscopy. ^{1}H NMR spectra were recorded on a Gemini spectrometer (Varian Associates Inc. NMR Instruments, Palo Alto, CA) operating at 300 MHz, using D_2O/DMSO-d_6, (1:7) as the solvent. The central peak of DMSO (at 2.50 ppm) was used as the reference line.

Chemical shifts of the polymers with acetylated side chains (δ, ppm): 4.9−5.25 (m, lactate methyne + HPMAm−ester methyne), 4.7 (br, HPMAm hydroxyl), 3.65 (br, HPMAm methyne), 2.9 (br, HPMAm(−ester) methylene), 2.05 (s, acetyl), 1.3−1.6 (m, lactate methyl), 0.7−1.3 (br m, backbone methyl and methylene, HPMAm(−ester) methyl).

Chemical shifts of the polymers, with hydroxylated side chains: δ (ppm) 4.9−5.25 (m, intrachain lactate methyne + HPMAm−ester methyne), 4.7 (br, HPMAm hydroxyl), 4.2 (quintet, chain end lactate methyne) 3.65 (br, HPMAm methyne), 2.90 (br, HPMAm(−ester) methylene), 1.3−1.6 (m, lactate methyl), 0.7−1.2 (br m, backbone methyl and methylene, HPMAm(−ester) methyl).

Grafting densities (or degree of substitution, DS, the average number of side chains per 100 HPMAm residues) were calculated from the ^{1}H NMR spectra from the sum of the integrals of the lactate and HPMAm−ester methyn groups divided by the number of protons involved, and the HPMAm hydroxyl integral at 4.7 ppm:

$$I_{\text{graft}} = (I_{4.9-5.25} + I_{4.2})/(\text{side chain } DP_{av} + 1)$$

$$DS = I_{\text{graft}}/(I_{\text{graft}} + I_{4.7}) \times 100$$

When residual monomers were still present (as seen by the single proton peaks at 5.6 and 5.65 ppm for HPMAm−oligo(lactic acid) and HPMAm, respectively), we corrected the integrals from the polymers with the amounts of monomers present: the value of I_{graft} was then decreased by $I_{5.6}$, and DS became equal to $I_{\text{graft}}/(I_{\text{graft}} + I_{4.7} - I_{5.65}) \times 100$.

Rheology. Polymer samples were hydrated during at least 1 day at room temperature in acetate buffer (pH 4, 100 mM). Solutions (equal volumes) containing equal amounts of pHPMAm-*graft*-oligo(L-lactic acid) and pHPMAm-*graft*-oligo(D-lactic acid) were mixed, homogenized and quickly applied on an AR 1000N rheometer (TA Instruments, Etten-Leur, The Netherlands) using a cone−plate measuring geometry (steel, 2 cm diameter with an angle of 1°; gap 31 μm). A solvent trap and a thin layer of silicon oil (110 mPa·s) were applied to prevent evaporation of the solvent. Gelation of the mixtures was monitored by measuring the storage modulus (G'), as well as the loss modulus (G'') at 20 °C over a period of 20−72 h. A frequency of 1 Hz and a controlled strain of 0.1% were applied.

Swelling and Degradation. Polymer solutions were made in acetate buffer (pH 4, 100 mM). Solutions containing equal amounts of L-lactic acid grafted polymer and D-lactic acid grafted polymer (of similar DS and DP) were mixed and transferred into 2 mL Eppendorf tubes, centrifuged (2 min, 13 000 rpm) for compression of the material and stored overnight at 4 °C to allow gel-formation. After gelation, the hydrogels were removed from the tubes, cut into a cylindrical shape (length 2 cm, radius 0.46 cm) and weighed accurately (W_0, approximately 1 g). The weighed gels were placed in vials containing 10 mL of phosphate buffer (pH 7.2, 100 mM, ionic strength adjusted to 0.3 with sodium chloride), which were placed in a water bath at 37 °C. At regular time intervals, the buffer solutions were completely removed and the weights of the gels (W_t) were determined to calculate the swelling ratio. After weighing, new aliquots of buffer were added to the gels. The swelling ratio (Z) is defined as W_t/W_0. The hydrogel dissolution time is defined as the time needed for complete degradation ($Z = 0$).

Results and Discussion

Synthesis and Copolymer Composition. First, HPMAm−oligo(lactic acid) macromonomers containing either hydroxyl (R = H) or acetyl (R = Ac) chain ends (Figure 2) with an average chain length $n \approx 12$ (DP_{av}, degree of polymerization) were synthesized as described elsewhere.[17] This DP was chosen because previous work on dextran-*graft*-oligo(lactic acid) hydrogels has shown that a minimum chain length of 11 lactic acid units is required for stereocomplex formation.[9] We used either

Table 1. Yields and Chemical Compositions of the Polymers Used in This Work

polymer	enantiomer	end group	DP of macromonomer[a]	macromonomer feed ratio (% mol/mol)	DS (%)[a]	yield (%)
p**1**-L-Ac	L	Ac	12.5[b]	9	2.6	40
p**1**-D-Ac	D	Ac	13.0[b]	9	2.6	46
p**2**-L-Ac	L	Ac	12.5[b]	10	3.2	n.d.[c]
p**2**-D-Ac	D	Ac	12.0[b]	10	2.5	n.d.
p**3**-L-Ac	L	Ac	12.5[b]	13	4.0	31
p**3**-D-Ac	D	Ac	13.0[b]	13	4.0	34
p**4**-L-Ac	L	Ac	12.0[b]	16	5.2	36
p**4**-D-Ac	D	Ac	12.0[b]	16	5.2	36
p**5**-L-Ac	L	Ac	11.5[b]	20	8.3	43
p**5**-D-Ac	D	Ac	12.0[b]	20	8.7	46
p**6**-L-Ac	L	Ac	11−14	16	5.8	29
p**6**-D-Ac	D	Ac	11−14	16	5.1	16
p**7**-L−H	L	H	12.0[b]	16	5.3	40
p**7**-D−H	D	H	12.0[b]	16	5.2	34
p**9**-L-Ac	L	Ac	12.5[b]	20	7.2	n.d.
p**9**-D-Ac	D	Ac	12.0[b]	20	8.1	n.d.
p**10**-L-Ac	L	Ac	8−10	20	7.2	33
p**10**-D-Ac	D	Ac	8−10	20	8.5	34

[a] Determined by ^{1}H NMR. [b] Polydisperse side chains, DP_{av} of the macromonomers as determined by ^{1}H NMR. [c] n.d. = not determined.

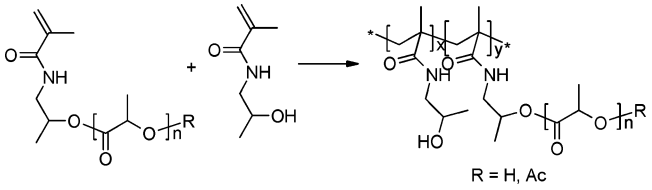

Figure 2. Synthesis of pHPMAm-*graft*-oligo(lactic acid)s.

polydisperse macromonomers, or macromonomers that were fractionated by preparative HPLC and contained oligomers with DP ranging from 11 to 14 (macromonomers with DP 8−10 where also isolated and used as a control).

Several graft copolymers containing poly-HPMAm as the backbone and acetylated or hydroxyl-terminating oligo(lactic acid) side chains were prepared by free radical copolymerization of HPMAm with HPMAm−oligo(lactic acid) in dioxane using AIBN as the initiator (Figure 2). The grafting density (DS, degree of substitution) was simply controlled by the ratio of the two monomers. For each variety of the graft copolymers we prepared two batches, one containing L-lactic acid grafts and one containing D-lactic acid grafts. Table 1 shows the polymers used in this work.

Table 1 shows that the DS of the synthesized polymers are lower than the feed ratios of the monomers. ^{1}H NMR analysis of the reaction mixture in time showed that the free radical copolymerization of the two monomers, HPMAm and HPMAm−oligo(lactic acid), was characterized by an unequal reactivity and consequently a composition drift during the polymerization occurred. HPMAm was the most reactive monomer since the peaks in the NMR spectrum belonging to this monomer changed most rapidly. This finding is different from the observations reported by Eguiburu et al.[18] and Shinoda et al.[19] with the copolymerization of methyl methacrylate (MMA) and methacrylate-terminated polylactic acid macromonomer, where the macromonomer was consumed approximately equally fast as MMA in conventional free radical polymerization. On the other hand, macromonomer reactivity was decreased in the copolymerization of MMA with methacrylate-terminated poly(dimethylsiloxane) (PDMS-MA), as reported by Shinoda et al. in another paper,[20] which is similar to our observations. This phenomenon was attributed to the effect of both limited diffusion associated with the large size of the macromonomer, and incompatibility of macromonomer with the propagating comonomer chain.[20] Poly(MMA) and poly(lactic acid) are indeed miscible,[18] whereas the incompatibility is probably an important factor determining the MMA/PDMS−MA reactivity ratios, as well as in our system in view of the large difference in polarity between (poly-)HPMAm and HPMAm−oligo(lactic acid).

Therefore, we can conclude that a reactive chain end containing a HPMAm unit reacts preferentially with another HPMAm monomer, probably due to incompatibility of the polar chain end with the apolar macromonomer or due to slow macromonomer diffusion. It can, however, not be concluded whether the radical chain end containing a macromonomer reacts preferentially with another macromonomer (because of compatibility reasons) or with HPMAm (because of slower diffusion of the macromonomer). This has some consequences for the expected copolymer structure. In the former case, we would expect the formation of copolymers with a blocky structure, whereas in the second case the synthesized polymer can be considered as a more or less random copolymer.

Rheology. Hydrogels were formed upon mixing an aqueous solution of a poly-HPMAm graft copolymer having oligo(L-lactic acid) side chains with a solution of a polymer containing oligo(D-lactic acid) of similar DS and DP, in a 1:1 ratio. Acetate buffer of pH 4 was used to minimize hydrolysis of the side chains.[21] Solid contents used were 22.5−30 wt %. Gelation due to the formation of stereocomplex crystallites (as explained in the Introduction) was accompanied by an increase of the storage modulus (G') and a decrease of tan δ of each mixture, due to increasing elasticity, which tend to level off after several hours. This behavior was also seen for the dextran-*graft*-oligo(lactic acid) stereocomplex hydrogels.[9] A typical rheology curve is shown in Figure 3. The end values of G' and tan δ are presented in Table 2 for several hydrogel formulations.

In the first series of experiments, polymers were used containing acetylated side chains, and the grafting density (DS) was increased while the average side chain length ($DP_{av} \approx 12$) and the water content (70 wt %) were

Table 2. Rheology, Swelling, and Degradation of pHPMAm-*g*-Oligo(Lactic Acid) Hydrogels

hydrogel	composition	DP[a]	DS (%)[b]	initial water content (%)	G′ (Pa)	tan δ	maximum swelling ratio	degradation time, pH 7.2 (days)
h**1**	p**1**-L-Ac + p**1**-D-Ac	poly	2.6	70	3700	0.40	3.1 ± 0.2[c]	8 ± 0[c]
h**2**	p**2**-L-Ac + p**2**-D-Ac	poly	2.9	70	4300	0.40	3.3	8
h**3**	p**3**-L-Ac + p**3**-D-Ac	poly	4.0	70	5100	0.40	2.8 ± 0.3[c]	8.5 ± 0.5[c]
h**4**	p**4**-L-Ac + p**4**-D-Ac	poly	5.2	70	33 000	0.20	2.9 ± 0.3[c]	43.5 ± 0.5 [c]
h**5**	p**5**-L-Ac + p**5**-D-Ac	poly	8.5	70	62 000	0.25	3.1 ± 0.2[d]	84 ± 0[d]
h**6**	p**6**-L-Ac + p**6**-D-Ac	11−14	5.5	70	20750 ± 250[c]	0.30	2.6 ± 0.0[c]	60 ± 7[c]
h**7**	p**7**-L-H + p**7**-D-H	poly	5.3	70	17500 ± 0[c]	0.30	2.8 ± 0.2[c]	14.5 ± 0.5 [c]
h**8**	p**4**-L-Ac + p**4**-D-Ac	poly	5.2	77.5	9150 ± 350[c]	0.25	1.9 ± 0.2[c]	15 ± 0[c]
h**9**	p**9**-L-Ac + p**9**-D-Ac	poly	7.8	77.5	11 200	0.25	1.3	15
h**10**	p**10**-L-Ac + p**10**-D-Ac	8−10	7.9	70	14 000	0.55	n.d.[f]	n.d.
	p**4**-L-Ac, p**4**-D-Ac, p**5**-L-Ac, p**5**-D-Ac	poly	5.2−8.7	70	n.d.	n.d.	1.3 ± 0.3[e]	7.3 ± 0.5[e]

[a] poly = polydisperse side chains, $DP_{av} \approx 12$. [b] Average DS of L and D enantiomers. [c] Result of two independent measurements ($n = 2$). [d] $n = 3$. [e] $n = 4$. [f] n.d. = not determined.

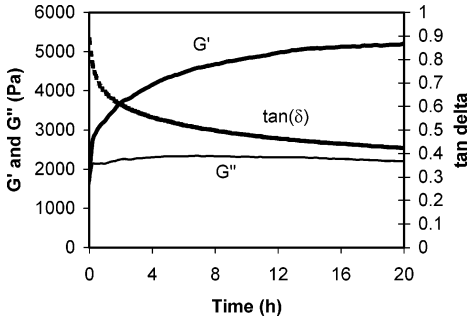

Figure 3. Typical rheogram of a pHPMAm-*g*-oligo(lactic acid) hydrogel (mixture of p**3**-L-Ac and p**3**-D-Ac, water content 70%).

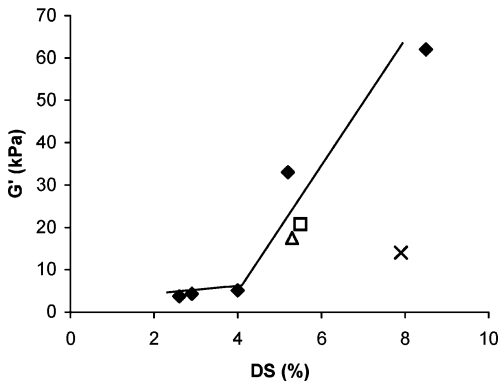

Figure 4. Storage modulus of pHPMAm-*g*-oligo(lactic acid) hydrogels (water content 70 wt %) vs the grafting density (DS): (◆) acetylated side chains, $DP_{av} \approx 12$; (□) acetylated side chains, DP = 11−14; (×) acetylated side chains, DP = 8−10; (Δ) hydroxyl-terminating side chains, $DP_{av} \approx 12$. The drawn line is a guide to the eye.

kept constant (h**1** − h**5**, Table 2). The storage modulus is plotted vs the DS in Figure 4. It is clear that strong gels with a high G' (and a relatively low tan δ (<0.40, Table 2)) are formed when DS > 4. The values of G' are the same order of magnitude as previously found for dextran-*graft*-oligo(lactic acid) hydrogels with similar DS, DP, and water content.[9] However, the tan δ values of the corresponding dextran hydrogels were 0.11−0.17, which is lower than the values we observed for the pHPMAm hydrogels. This indicates that the pHPMAm gels are less perfect than the dextran gels, probably due to the higher fraction of dangling chain ends in the hydrogels. This, in turn, can be explained by the copolymerization characteristics discussed above. The difference in monomer reactivity and, as a consequence, the composition drift during the synthesis of the polymers may be responsible for this relatively high tan δ. The polymer chains which are formed initially during the polymerization reaction may contain higher amounts of HPMAm than in a later stage and thereby contribute less to the hydrogel formation.

For a pHPMAm-*g*-oligo(lactic acid) gel with side chains of DP 11−14 (hydrogel h**6**) it was expected to give a stronger and more elastic gel (higher G', lower tan δ) due to the absence of short side chains which do not contribute to stereocomplex formation.[9,15] However, from Table 2 and Figure 4, it can be seen that the hydrogel containing the low-polydispersity grafts has a slightly lower G' than a hydrogel with a similar DS and high-polydispersity grafts (compare h**6** and h**4**). Probably the absence of side chains with DP > 14 is responsible for the lower G'. Since a minimum DP of 11 was required for stereocomplex formation in the previously reported dextran hydrogels,[9] we expected that a pHPMAm-*g*-oligo(lactic acid) gel with side chains of DP 8−10 (hydrogel h**10**) would not give gel formation. Indeed, Figure 4 and Table 2 clearly showed the low G' and high tan δ, respectively, for this hydrogel. Gelation in this case is probably only due to hydrophobic interactions between the side chains, which are expected to be weaker than stereocomplex interactions.

As expected, increasing water content (hydrogels h**8** and h**9**) gave hydrogels with lower G', but the same tan δ. Similar gels (DS of 7.0%) with 70% and 77.5% water displayed G' values of 33 and 9 kPa, respectively (compare h**4** and h**8**). Further increasing the water content reduced gel formation substantially (e.g., when increasing the water content for hydrogel h**2** (DS of 3.5%) from 70% to 85%, G' decreased from 4.3 to 0.3 kPa), but this could be counteracted by increasing the DS ($G' = 5.5$ kPa for a 85% gel with DS of 7.5). Finally, a gel formed from polymers with nonacetylated oligo(lactic acid) side chains showed a lower G' than the ones with acetylated side chains (see Figure 4). This may be due to the increased hydration of the more hydrophilic chain ends in the case of the nonacetylated compounds.

Swelling and Degradation. Degradation of the hydrogels is expected at physiological conditions due to the hydrolysis of the cross-links, i.e., the oligo(lactic acid) side chains.[15] Weight changes of the hydrogels were measured during incubation of the gels at 37 °C in a phosphate buffer of pH 7.2. Swelling (and degradation) was expressed as the weight ratio at time t with respect to the weight at time zero (W_t/W_0). A typical swelling curve is shown in Figure 5 for stereocomplex hydrogel h**5**, obtained by mixing enantiomers p**5**-L-Ac and p**5**-D-Ac, together with the curve for the weaker gels formed by the individual enantiomers. It can be seen that the stereocomplex hydrogels degrades much slower (lifetime of 84 days) than the individual enantiomers (lifetime

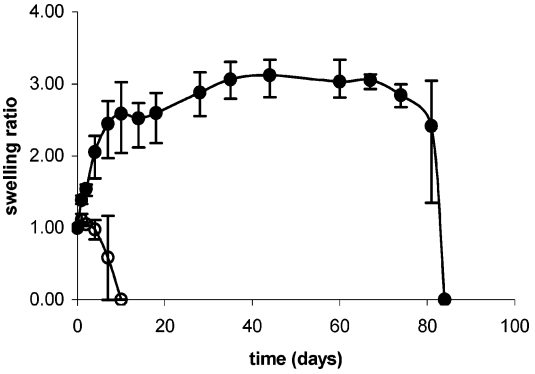

Figure 5. Typical swelling and degradation experiment of pHPMAm-*g*-oligo(lactic acid) hydrogels (water content 70%). Open circles: average of the individual enantiomers p**5**-L-Ac and p**5**-D-Ac. Closed circles: hydrogel h**5** (mixture of p**5**-L-Ac + p**5**-D-Ac, average of three measurements). Variations are indicated by the error bars.

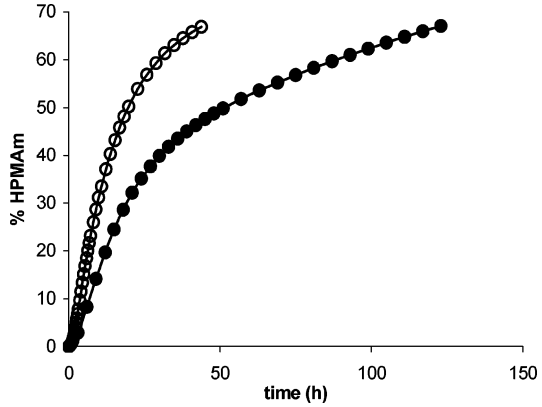

Figure 6. Relative amounts of HPMAm formed during the hydrolysis of acetylated (●) and nonacetylated (○) HPMAm−oligo(lactic acid) (DP = 12, monodisperse). The calculations are carried out as described in ref 17, using the reported rate constants in aqueous PBS buffer.

of 7 days), clearly indicating the contribution of stereocomplex formation on hydrogel stability. We previously showed for the dextran-*g*-oligo(lactic acid) hydrogels that the stereocomplex phase is crystalline, while the individual enantiomers form amorphous associates.[22] Therefore, it is likely that the ester bonds are less sensitive to hydrolysis in the stereocomplex phase.

During the first few days the stereocomplex hydrogels swelled due to the uptake of water. Gels with an initial water content of 70% absorbed an amount of water up to three times their initial weights, thereby increasing their water content to 90% independently of the grafting density of the polymers used (h**1**−h**5**, Table 2). The maximum swelling ratios of hydrogels with an initial water content of 77.5% (h**8** and h**9**) was lower than the gels initially containing 70% water, which is to be expected since they contained more water already from the start of the experiments. The maximum water content after swelling for these gels was 83−89%, which is slightly lower than the water content of the gels which started with 70% water.

After the initial swelling period, most hydrogels formed from the polymers with acetylated side chains remained stable for quite a long time. The final stage of the degradation was quite abrupt in most cases. The lifetimes of gels h**1**−h**5** increased with increasing DS, in agreement with the observations for the dextran-based hydrogels.[15] The gels formed from the polymers with a DS ≤ 4 had lifetimes that were close to the lifetimes of individual enantiomers (approximately 8 and 7 days, respectively), indicating that stereocomplex formation was not effective with these low DS values. This is in agreement with the rheology data (vide supra). Surprisingly, when the initial water content of the gels with high DS was increased from 70% to 77.5%, the lifetime decreased substantially from more than 40 days (gels h**4** and h**5**) to 15 days (gels h**8** and h**9**). This may be explained by less efficient stereocomplex formation when starting with more dilute polymer solutions, i.e., part of the side chains may not or not wholly overlap during gelation and therefore degrade relatively fast.

From a gel based on polymers with oligo(lactic acid) side chains of DP 8−10 (h**10**), it was not possible to monitor the swelling/degradation because the gels were too weak from the beginning (see rheology data). This is in strong contrast to hydrogels based on polymers with side chains of DP 11−14 (h**6**) and the same initial water content. The latter gels showed lifetimes of approximately 60 days, being even longer than corresponding gels having polydisperse side chains with similar DS (compare h**6** and h**4**). The difference between high and low polydisperse samples may be attributed to the absence of side chains with DP < 11 in the latter case, which are not contributing to stereocomplex formation.

One of the most interesting observations in our experiments is the fact that the lifetime of a hydrogel prepared from polymers containing hydroxyl-terminating side chains is substantially lower than corresponding gels from acetylated polymers (compare hydrogels h**7** and h**4**: degradation time 14.5 and 43.5 days, respectively). This is fully in line with the observed difference in degradation rate between acetylated and nonacetylated HPMAm−oligo(lactic acid) macromonomers which will be reported in another paper.[17] In the latter work, we demonstrated that degradation of nonacetylated chains occurs through rapid chain end scission (backbiting), while degradation of acetylated oligomers is initiated by slow random chain scissions. The lifetimes observed for the hydrogels are, however, much longer than the half lifetimes of the freely dissolved monodisperse macromonomers of DP 12. The latter $t_{1/2}$ are 0.24 and 2.7 h for the nonacetylated and acetylated oligomers in pure aqueous environment, respectively,[17] while for the corresponding hydrogels it takes 350 and 1050 h to fully dissolve.

Since partly degraded side chains may still contribute to hydrogel formation either by stereocomplexation or by hydrophobic interactions, it may be more relevant to correlate the hydrogel dissolution times to the rate of formation of fully hydrolyzed HPMAm units present in the polymer backbones. Figure 6 shows the calculated relative amount of HPMAm formed during the degradation in PBS buffer of nonacetylated and acetylated monodisperse HPMAm−oligo(lactic acid) macromonomers (DP = 12), using the kinetic model and rate constants as described elsewhere.[17] From these results, it can be seen that HPMAm formation is indeed faster in the case of the nonacetylated macromonomers. The times required to fully degrade 50% of the macromonomers are 20 and 51 h for the nonacetylated and acetylated side chains, respectively. At those levels of conversion, the remaining, not yet fully hydrolyzed macromonomers are only HPMAm−mono- and HPMAm−dilactate (not shown), which in theory can still form physical cross-links through hydrophobic interac-

tions.[23] The rates of HPMAm formation thus derived are still about 20 times faster than the corresponding hydrogel dissolution times. This can be perfectly explained by the fact that the calculated macromonomer degradation rates were based on fully dissolved and hydrated molecules, while the side chains in the hydrogels are associated into crystalline domains, thus slowing down the degradation. Nevertheless, we may conclude that hydrogel dissolution is probably the result of the increase of the amount of HPMAm units in the polymer backbone beyond a certain critical level, which can be controlled by the number of the side chains (DS) and their terminal groups (acetyl or hydroxyl).

Comparison with Dextran Hydrogels. The lifetimes of the pHPMAm-based hydrogels described above are much longer than the lifetimes of corresponding hydrogels based on the dextran backbone as described previously by us. For example, dextran-*graft*-oligo(lactic acid) hydrogels with similar characteristics (DP, DS, water content) as hydrogels h**4** or h**6** degraded within approximately 3 or 6 days, respectively,[15] while h**4** and h**6** had lifetimes of more than 40 days. Even hydrogel h**7** with the hydroxyl-terminating side chains degraded less rapidly than similar dextran hydrogels (i.e., in approximately 2 weeks). In view of the chemical structure of the dextran hydrogels (see Figure 1), degradation of their side chains must occur through random chain scissions, or through hydrolysis of the carbonate ester that links each side chain to the backbone, as suggested in ref 15. Backbiting is not possible because the side chains do not terminate in a hydroxyl group. Random chain scission is expected to be equally slow as in the pHPMAm-based hydrogels and can therefore not account for the difference in degradation rates. Therefore, the carbonate ester present in the dextran polymers must make the difference, and it has indeed been suggested to be the most hydrolytically sensitive group.[15] Besides, removal of the side chains occurred probably by neighboring assistance of hydroxyl groups being in a favorable position at the dextran backbone. Three reasons for the slower degradation of the pHPMAm-based hydrogels can thus be given: (i) the higher intrinsic stability of the ester linkage between the side chains and the backbone with respect to a carbonate ester; (ii) perhaps a less efficient assistance of the hydroxyl groups of neighboring HPMAm units; (iii) as a result of that, degradation of the side chains occurring through gradual shortening of the side chains by sequential chain end scission and/or random chain scission events, in contrast to the side chains of the dextran hydrogels which are removed as a whole after the rapid breaking of the carbonate ester.

Conclusion

The degradation times of stereocomplex hydrogels, previously based on dextran, has been largely extended by replacing the polymeric backbone by pHPMAm and connecting the oligo(lactic acid) side chains through an ester linkage instead of a carbonate bond. The degradation time can now easily be tailored from 1 week to almost 3 months by changing the grafting density of the polymers. Besides, the nature of the end groups of the side chains plays an important role in the degradation rate. Protecting the hydroxyl end group causes a significant retardation of the degradation rate by preventing rapid chain end scission (backbiting). The ease of preparation, the biocompatibility of the degradation products (pHPMAm and lactic acid), and the possibility of tuning the degradation rate over a wide range make these pHPMAm-based hydrogels ideal materials for the controlled release of bioactive compounds, e.g., therapeutic proteins, and for the encapsulation of living cells (tissue engineering).

Acknowledgment. This work was supported by The Netherlands Organization for Scientific Research−Medical Sciences (MW−NWO) Grant 0316/014-81-101.

References and Notes

(1) Hoffman, A. S. *Adv. Drug Delivery Rev.* **2002**, *54*, 3−12.
(2) Lee, K. Y.; Mooney, D. J. *Chem. Rev.* **2001**, *101*, 1869−1879.
(3) Peppas, N. A.; Bures, P.; Leobandung, W.; Ichikawa, H. *Eur. J. Pharmacol. Biopharm.* **2000**, *50*, 27−46.
(4) Hennink, W. E.; van Nostrum, C. F. *Adv. Drug Delivery Rev.* **2002**, *54*, 13−36.
(5) van Nostrum, C. F.; de Jong, S. J.; Kettenes-van den Bosch, J. J.; Hennink, W. E. In *Advances in Controlled Drug Delivery*; ACS Symposium Series 846; Dinh, S. M.; Liu P.; Eds.; American Chemical Society: Washington, DC, 2003.
(6) Slager, J.; Domb, A. J. *Adv. Drug Delivery Rev.* **2003**, *55*, 549−583.
(7) Ikada, Y.; Jamshidi, K.; Tsuji, H.; Hyon, S.-H. *Macromolecules* **1987**, *20*, 904−906.
(8) Okihara, T.; Tsuji, M.; Kawaguchi, A.; Katayama, K. *J. Macromol. Sci.—Phys.* **1991**, *B30*, 119−140.
(9) de Jong, S. J.; De Smedt, S. C.; Wahls, M. W. C.; Demeester, J.; Kettenes-van den Bosch, J. J.; Hennink, W. E. *Macromolecules* **2000**, *33*, 3680−3686.
(10) Lim, D. W.; Park, T. G. *J. Appl. Polym. Sci.* **2000**, *75*, 1615−1623.
(11) Lim, D. W.; Choi, S. H.; Park, T. G. *Macromol. Rapid Commun.* **2000**, *21*, 464−471.
(12) Fujiwara, T.; Mukose, T.; Yamaoka, T.; Yamane, H.; Sakurai, S.; Kimura, Y.; *Macromol. Biosci.* **2001**, *1*, 204−208.
(13) Grijpma, D. W.; Feijen, J. *J. Controlled Release* **2001**, *72*, 247−249.
(14) de Jong, S. J.; van Dijk-Wolthuis, W. N. E.; Kettenes-van den Bosch, J. J.; Schuyl, P. J. W.; Hennink, W. E. *Macromolecules* **1998**, *31*, 6397−6402.
(15) de Jong, S. J.; van Eerdenbrugh, B.; van Nostrum, C. F.; Kettenes-van den Bosch, J. J.; Hennink, W. E. *J. Controlled Release* **2001**, *71*, 261−275.
(16) Kopecek, J.; Kopeckova, P.; Minko, T.; Lu, Z. *Eur. J. Pharmacol. Biopharm.* **2000**, *50*, 61−81.
(17) van Nostrum, C. F.; Veldhuis, T. F. J.; Bos, G. W.; Hennink, W. E. Submitted for publication.
(18) Eguiburu, J. L.; Fernandez-Berridi, M. J.; Roman, J. S. *Polymer* **1996**, *37*, 3615−3622.
(19) Shinoda, H.; Matyjaszewski, K. *Macromolecules* **2001**, *34*, 6243−6248.
(20) Shinoda, H.; Miller, P. J.; Matyjaszewski, K. *Macromolecules* **2001**, *34*, 3186−3194.
(21) de Jong, S. J.; Arias, E. R.; Rijkers, D. T. S.; van Nostrum, C. F.; Kettenes-van den Bosch, J. J.; Hennink, W. E. *Polymer* **2001**, *42*, 2795−2802.
(22) de Jong, S. J.; van Nostrum, C. F.; Kroon-Batenburg, L. M.J.; Kettenes-van den Bosch, J. J.; Hennink, W. E. *J. Appl. Polym. Sci.* **2002**, *86*, 289−293.
(23) Neradovic, D.; van Nostrum, C. F.; Hennink, W. E. *Macromolecules* **2001**, *34*, 7589−7591.

MA035534+