Influence of Network Structure on the Degradation of Photo-Cross-Linked PLA-*b*-PEG-*b*-PLA Hydrogels

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Triblock copolymers of functionalized poly(lactic acid)-*b*-poly(ethylene glycol)-*b*-poly(lactic acid) (PLA-*b*-PEG-*b*-PLA) have been widely investigated as precursors for fabricating resorbable polymeric drug delivery vehicles and tissue engineering scaffolds. Previous studies show degradation and erosion behavior of PLA-*b*-PEG-*b*-PLA hydrogels to rely on macromer chemistry as well as structural characteristics of the cross-linked networks. In this research, the degradation kinetics of diacrylated PLA-*b*-PEG-*b*-PLA copolymers as soluble macromers and cross-linked gels are directly compared as a function of macromer concentration, buffer pH, and ionic strength. The pseudo first-order rate constants for degradation of soluble macromers increase with water concentration and show a minimum at intermediate pH values, but are insensitive to ionic strength. The degradation rate constants for covalently cross-linked gels display a greater sensitivity to local water concentration and a minimum at lower pH values than corresponding soluble macromers. In addition, ionic strength significantly affects the rate of gel degradation due to the direct correlation between the degree of network ionization and gel water content.

Introduction

Hydrogels based on the free-radical polymerization and cross-linking of acrylated or methacrylated poly(lactic acid)-*b*-poly-(ethylene glycol)-*b*-poly(lactic acid) (PLA-*b*-PEG-*b*-PLA) macromers were designed to maintain all of the advantages of a PEG-based material while also permitting tunable degradability.[1] As the utilization of PLA-*b*-PEG-*b*-PLA gels has increased, this characteristic of tunable degradability has become extremely useful in the biomedical field to eliminate the need for physical removal of PLA-*b*-PEG-*b*-PLA implants as well as to modulate the delivery rates of drugs or growth rates of cells encapsulated within cross-linked PLA-*b*-PEG-*b*-PLA matrices. Therefore, it is important to understand the factors controlling the degradation behavior of chemically cross-linked PLA-*b*-PEG-*b*-PLA hydrogels.

According to previously developed degradation models, there are two main parameters that affect the form and rate of network degradation for chemically cross-linked PLA-*b*-PEG-*b*-PLA macromers.[2−4] The first is the hydrolysis kinetics of PLA ester bonds within the cross-linked hydrogels. The second is the physical structure of the gel. These two parameters are interdependent, making the entire gel degradation process very complex. Sawhney et al.[1] showed that increasing the molecular weight of the PEG segment decreases the cross-linking density (structural effect), which in turn increases the water content of PLA-*b*-PEG-*b*-PLA gels. Increases in water content increase the rate of hydrolysis of PLA ester bonds (kinetic effect) and the overall gel degradation rate.[1]

The ability to tailor hydrogel cross-linking density can also be used to control network mesh size and diffusivities of encapsulated solutes. Lu et al.[5] have shown that the initial mesh size of the hydrogel network as well as the degradation rate affect the drug release profile. Faster degradation rates result in faster increases in mesh size and more rapid drug release. Metters et al.[4] demonstrated that changing the macromer weight percent during polymerization also affects the cross-linking density and the resulting water content of the degradable gel. Lowering the macromer content during gel fabrication lowers the cross-linking density of the hydrogel while increasing both its water content and degradation rate.[5−7]

In addition to its cross-linking density, water content, and elastic modulus, the chemical nature of a degradable PLA-*b*-PEG-*b*-PLA hydrogel also changes with degradation. For example, as PLA-*b*-PEG-*b*-PLA gels degrade, acidic species are generated along the backbone chains of the network. These immobilized anionic groups should make the swelling and degradation behavior of the resultant gels sensitive to changes in solution pH and ionic strength. Since previous studies of PLA-*b*-PEG-*b*-PLA hydrogel degradation behavior have been conducted at constant pH and ionic strength,[1−5,7−10] the results obtained from these investigations cannot provide insight as to how alterations in pH or ionic strength affect the kinetics of ester bond degradation or the nanoscopic architecture of the gel. To better understand the behavior of photocrosslinked PLA-*b*-PEG-*b*-PLA hydrogels and to evaluate their potential as environmentally responsive biomaterials, the sensitivity of their degradation behavior to local changes in pH and ionic strength needs to be understood.

To help unravel the dependence of PLA-*b*-PEG-*b*-PLA hydrogel degradation behavior on macromer chemistry versus network structure, the goal of this work is to compare the pseudo first-order degradation kinetics of *soluble* PLA-*b*-PEG-*b*-PLA macromers to those of identical yet cross-linked macromers contained within insoluble, highly swollen gels. For this study, gels were synthesized from PLA-*b*-PEG-*b*-PLA macromers by photopolymerization, and their hydrolytic degradation was compared to the degradation of the same *unpolymerized* macromers in buffered solutions as a function of time. Mass-transfer effects are minimized in both cases due to the low

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Table 1. ^{1}H NMR Characterization of PLA-*b*-PEG-*b*-PLA Macromers

macromer	number of LA units per end group (*j*)	percent acrylation (%)
PEGPLA1	3.1	83
PEGPLA2	2.8	96
PEGPLA3	2.7	86

macromer concentrations of the chosen solutions and highly swollen nature of the gels investigated.[4,9] Degradation of uncross-linked macromer in solution eliminates association of neighboring chains or gel structure. Therefore, any significant differences in degradation behavior between the soluble and insoluble systems can be attributed to the supramolecular character of the cross-linked gel. Such a direct comparison should provide a better understanding of the complex set of parameters controlling the process of hydrogel degradation and enable one to independently design hydrogel chemistry and structure for specific applications.

Materials and Methods

Macromer Synthesis and Characterization. The detailed procedure for the synthesis of the PLA-*b*-PEG-*b*-PLA diacrylate macromer has been described previously.[1] Briefly, it was synthesized by first reacting 20 g of dry PEG 4600 Da (Aldrich; $M_n = 4600$) with 3.13 g of d,L-lactide (Polysciences Inc., PA; $M_w = 144.1$ Da) at 130 °C to give PLA-*b*-PEG-*b*-PLA through a ring-opening polymerization. In the second step, this triblock macromer was dissolved in a minimum amount of dichloromethane (Fluka; purity ≥ 98%) and end capped with acrylate functionalities by reaction with 2.83 mL of acryloyl chloride (Aldrich; Purity > 98%) and 5.33 mL of triethylamine (Aldrich; Purity = 99.5%) under vigorous agitation at room temperature for 4 h. The synthesized macromer was then purified by precipitation in ice-cold ether followed by filtration. The remaining solvent was removed by drying the purified product under vacuum at room temperature for 48 h. All materials were used as received.

^{1}H NMR (Bruker 300) analysis of the synthesized macromers dissolved in deuterated chloroform (Aldrich; purity = 99.9%) was used to determine the number of lactic acid groups (*j*) added to each end of the 4600 Da PEG chain and the percent acrylation of the final triblock macromer. The number of lactic acid groups *j* is also the number of ester bonds at each end of the 4600 Da PEG chain. Characteristics of the three batches of PLA-*b*-PEG-*b*-PLA macromer synthesized for the current study are given in Table 1.

Hydrogel Polymerization. Hydrogels were synthesized by solution photopolymerization of PLA-*b*-PEG-*b*-PLA diacrylate macromer in deionized water. In brief, the macromer was dissolved in deionized water to form 7.0, 10, 20, or 30 wt % solutions by weight, which correspond to water contents of 93, 90, 80, and 70 wt %. Photoinitiator, Irgacure 2959 (Ciba, NY), was added to the solution at a final concentration of 0.5 wt %. This solution was then injected between two glass slides separated by 0.8 mm Teflon spacers. Photopolymerization and gel formation was initiated by exposure of this solution to 365 nm UV light of intensity ~8.5 mW/cm^2 for 10 min (Black-Ray: B 100 AP, Upland, CA). Identical disk-shaped gel samples having a volume of ~160 μL were then cut for subsequent degradation studies using a 5/16 in. hollow punch.

Hydrogel Degradation. To measure the effect of buffer pH on the rate of gel degradation, identical undegraded gel samples synthesized from a 30 wt % PLA-*b*-PEG-*b*-PLA macromer (PEGPLA3) solution were placed in a large excess of 50 mM buffer (approximately 3 mL) at pH 2, 3, 4, 5, or 7.4 and allowed to degrade at a constant temperature of 37 °C. The pH 2 and 3 buffer solutions were made using phosphoric acid (Sigma), the pH 7.4 buffer solution was made using sodium phosphate dibasic (Riedel-de Haën, Germany), and the pH 4 and 5 buffer solutions were made using acetic acid (Aldrich). Sodium chloride (Sigma-Aldrich) was added to all buffers to maintain a constant ionic strength of 0.135 M. To measure the effect of ionic strength on the rate of gel degradation, additional gel samples synthesized from 30 wt % macromer (PEGPLA1) solution were placed in 7.4 pH buffer solutions with ionic strengths of 0.135, 0.4, 0.7, 1, and 1.5 M. Finally, to study the effect of water content on the rate of gel degradation, gel samples were synthesized from solutions of 7.0, 10, 20, and 30 wt % macromer (PEGPLA2) and allowed to degrade in 7.4 pH buffer with an ionic strength of 0.135 M.

For each degradation experiment, gels were first allowed to equilibrate in buffer solution for 48 h. After equilibration, the swollen weights of all gel samples were measured. Some gels were dried in vacuum for 2 days to provide corresponding dry weights. The ratio of the swollen gel weight before drying (S_w) to the polymer weight after drying (D_w) provided the mass swelling ratio (Q_m) and the percent initial water content by mass (IWC) for each gel as shown by eqs 1 and 2, respectively

$$Q_m = \frac{S_w}{D_w} \tag{1}$$

$$\mathrm{IWC} = \frac{Q_{m,0} - 1}{Q_{m,0}} \times 100\% \tag{2}$$

where $Q_{m,0}$ is the initial, equilibrium mass swelling ratio measured after 48 h in buffer. It should be noted that the hydrogel IWC may differ from that of the macromer solution used to make it due to swelling (or deswelling) during equilibration. The swollen weights of the remaining gels were measured at regular intervals during the degradation experiment. Additional gel samples were dried at regular intervals to obtain the characteristic mass loss profile for the experiment.

It has been previously established that the change in gel swelling ratio with time is related to the degradation rate of the PLA-*b*-PEG-*b*-PLA hydrogels and can be used to calculate the pseudo first-order degradation rate constant of the ester bonds present in the cross-links of the highly swollen networks.[2−4,6−13] This relation is based on the assumption that the water concentration and pH inside the gel remain approximately constant during degradation. This assumption is reasonably valid for highly swollen gels degraded in an excess of buffer. The Flory-Rehner equation relates the dynamic mass-swelling ratio of this degrading system to the time-dependent cross-linking density of the gel, as given by eqs 3 and 4 below:[8,11,14]

$$v_c = \frac{-[\ln(1 - \nu_2) + \nu_2 + \chi_{12}\nu_2]}{(\nu_2^{1/3} - 2\nu_2/f_A)} \tag{3}$$

$$\nu_2 = \frac{1}{Q} = \frac{\overline{v}_2}{[(Q_m - 1)\overline{v}_1 + \overline{v}_2]} \tag{4}$$

where v_c is the gel cross-linking density, Q is the volumetric swelling ratio, ν_2 is the polymer volume fraction in the hydrogel, v_1 is the specific volume of water (1.006 cm^3/g at 37 °C), v_2 is the specific volume of dried PEG (0.92 cm^3/g at 37 °C), χ_{12} is the Flory−Huggins interaction parameter for a PEG-H_2O system (0.45[11,15]), and f_A is the functionality of the PLA-*b*-PEG-*b*-PLA macromers ($f_A = 4$ for all divinyl macromers used in the current studies).

Assuming pseudo first-order degradation kinetics, the time-dependent decrease in PLA-*b*-PEG-*b*-PLA cross-linking density can be expressed mathematically as

$$v_c = e^{-2jk'_{gel}t}[A]_0 \tag{5}$$

where k'_{gel} is the pseudo first-order degradation rate constant in h^{-1}, 2*j* is the number of ester bonds per macromer, *t* is the degradation time

in hours, and $[A]_0$ is the initial concentration of PLA-*b*-PEG-*b*-PLA diacrylate molecules in the system.[11]

Values of k'_{gel} were obtained by quantitatively matching theoretical and experimental values of v_c. Equations 3 and 4 were first used to calculate experimental values of the cross-linking density v_c from the gel mass swelling ratios (Q_m) obtained with eq 1. Using the solver function in Microsoft Excel, the values of k'_{gel} and $[A_0]$ were then independently adjusted until the residual error between the experimental v_c values and those predicted by eq 5 were minimized. The solver used a quasi-Newtonian iteration algorithm with linear interpolation and forward differentials.

Solution Degradation. To measure the effect of buffer pH and water content on the degradation rates of soluble PLA-*b*-PEG-*b*-PLA, unpolymerized macromer was dissolved in buffer solutions at the same pH and ionic strengths as the previously described gel samples. A total of 10 mL of macromer (PEGPLA1 and PEGPLA2) solutions at concentrations of 5.0, 10, 15, and 20 wt % were then allowed to degrade at 37 °C. These solutions correspond to IWC of 95, 90, 85, and 80 wt %, respectively. Unlike the hydrogel measurements, the bulk water contents of these macromer solutions remain constant throughout the degradation period. To measure the effect of ionic strength on the degradation rate of unpolymerized PLA-*b*-PEG-*b*-PLA, macromer (PEGPLA2) solutions at a concentration of 6.7 wt % (IWC = 93.3 wt %) were prepared and allowed to degrade in 7.4 pH buffer solutions at ionic strengths of 0.135, 0.7, and 1.5 M at 37 °C.

Soluble macromer degradation was observed by the formation of lactic acid, a well-known degradation product of PLA-*b*-PEG-*b*-PLA hydrolysis. The time-dependent increase in lactic acid concentration of each solution was measured with a sensitivity of 0.01 g/L using a YSI 2700 SELECT Biochemistry Analyzer (YSI Inc., OH). This analytical sensitivity is sufficient for this study, since 0.01 g/L corresponds to only 0.2% degradation for the 5 wt % macromer solution or just 0.03% degradation for the 20 wt % solution. The change in lactic acid concentration with time was used to calculate the degradation rate constant for the linear macromer in solution by assuming pseudo first-order reaction kinetics. This is a valid assumption since both buffer pH and water content are constant during the experiment. A single molecule of lactic acid is produced from the diacrylated PLA-*b*-PEG-*b*-PLA macromer only upon cleavage of two adjacent ester bonds. According to previously published pseudo first-order kinetic models for PLA-*b*-PEG-*b*-PLA degradation,[2−4,10,11] the statistical fraction of hydrolyzed or degraded ester bonds (P_{Ester}) is described by eq 6

$$P_{Ester} = 1 - \frac{[E]_t}{[E]_0} = 1 - e^{-k't} \quad (6)$$

where $[E]_t$ is the ester bond concentration at time t, $[E]_0$ is the maximum ester bond concentration at time $t = 0$, t is the degradation time, and k' is the pseudo first-order degradation rate constant. Release of one lactic acid molecule requires cleavage of two ester bonds. Therefore, the pseudo first-order rate constant for degradation can be related to the growing concentration of lactic acid in solution by eq 7

$$(P_{Ester})^2 = \left(1 - \frac{[E]_t}{[E]_0}\right)^2 = (1 - e^{-k'_{soln}t})^2 = \frac{[LA]_t}{[LA]_{max}} \quad (7)$$

where $[LA]_t$ is the lactic acid concentration in the solution at time t, $[LA]_{max}$ is the final lactic acid concentration after the macromer is completely degraded, and k'_{soln} is the pseudo first-order degradation rate constant.

Results and Discussion

Macromer Degradation. It is well established that hydrolysis of ester bonds is acid- as well as base-catalyzed.[16] Equation 8 represents the generalized kinetic equation for acid- and base-catalyzed hydrolysis of ester bonds such as those present within solubilized or cross-linked PLA-*b*-PEG-*b*-PLA macromers

$$\frac{d[E]}{dt} = -k_{H^+}[H^+][H_2O][E] - k_{^-OH}[^-OH][H_2O][E] \quad (8)$$

where, k_{H^+} is the kinetic rate constant for acid-catalyzed hydrolysis, $k_{^-OH}$ is the kinetic rate constant for base-catalyzed hydrolysis, [E] is the concentration of ester bonds in the PLA segments at time t, $[H_2O]$ is water concentration, $[H^+]$ is the hydronium ion concentration, and $[^-OH]$ is the hydroxyl ion concentration.

In the current study, $[H^+]$ and $[^-OH]$ remain essentially constant during the degradation of both the macromer solutions as well as hydrogels due to the use of excess buffer solutions to maintain a constant pH. Assuming no localized variations, $[H_2O]$ also remains constant during degradation of solubilized macromers. $[H_2O]$ does change as Q increases during hydrogel degradation. However, for the highly swollen hydrogels used in the current study with large initial water contents, this change is relatively minor. For example, the water content within a gel with an initial $Q = 5$ (equivalent to an initial water content of 80 wt %) will increase, at most, by 20%. Likewise, gels with an initial $Q > 10$ will experience changes in water concentration of less than 10% over the entire course of degradation. Assuming these variations are acceptable for the current analysis eq 8 can be simplified for the degradation of both soluble and cross-linked macromers to the following pseudo first-order kinetic equation:[2−4,9,17]

$$\frac{d[E]}{dt} = -k'[E] \quad (9)$$

where k' is the pseudo first-order rate constant equal to $(k_{H^+}[H^+][H_2O] + k_{^-OH}[^-OH][H_2O])$. Previously published studies on the degradation behavior of PLA oligomers and networks have clearly established the acceptance of using a pseudo first-order kinetic mechanism to accurately describe PLA hydrolysis kinetics under experimental conditions similar to those used in the current work.[18−22]

Equation 9 illustrates that, although k' is constant during pseudo first-order macromer degradation, the degradation rate ($d[E]/dt$) varies with time. As the macromer degrades, the ester bond concentration and the degradation rate decrease. These changes make it difficult to quantitatively compare the degradation behavior of gels synthesized with various ester bond concentrations or degraded under different conditions. However, under the proper conditions discussed above (constant pH and high initial gel swelling), the pseudo first-order degradation rate constant (k') remains constant throughout the degradation process, making it a useful tool for assessing and comparing the characteristic degradation behavior of various systems. The impact of important system parameters (e.g., pH, water concentration, etc.) on the gel structure and ester bond hydrolysis kinetics can be readily observed by monitoring apparent changes in k'. Furthermore, this analysis is applicable for comparing macromer degradation in solution and in highly swollen gels since k' is equivalent to both k'_{soln} and k'_{gel} in the appropriate environment.

Effect of pH. During degradation of soluble macromer, cleavage of the PLA ester bonds takes place through hydrolysis leading to the formation of PEG, lactic acid, and poly(acrylic acid) as degradation products. For a single lactic acid molecule to be released, two adjacent ester bonds along the macromer chain must be broken. Therefore, as macromer degradation

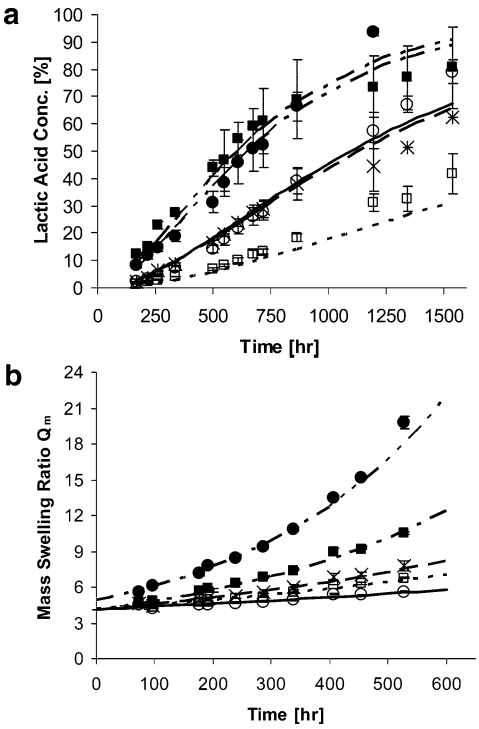


Figure 1. Experimental measurements of hydrolytic degradation of PLA-*b*-PEG-*b*-PLA macromer at constant pH. Points show experimental data, while solid and dashed lines indicate model predictions. (a) Change in lactic acid concentration with time as soluble PLA-*b*-PEG-*b*-PLA macromer is hydrolytically degraded. (b) Mass swelling ratios of PLA-*b*-PEG-*b*-PLA gels as functions of degradation time. (■, −-−) pH 2; (○, —) pH 3; (□, ---) pH 4; (×, −−−) pH 5; (●, —--—) pH 7.4, For all data: solution, 10 wt % PEGPLA1; gel, 30 wt % PEGPLA3; ionic strength = 0.135 M; $n = 3$, error bars = ± std. dev.

proceeds, the lactic acid concentration in solution increases in a sigmoidal fashion as shown in Figure 1a. The value of k'_{soln} for a given set of degradation conditions is obtained by fitting eq 7 to the data, which provides a very good fit to the measured data, as shown in Figure 1a. Additionally, the shift in the degradation curves shown in Figure 1a demonstrate that the degradation rate of soluble macromer and the calculated value of the pseudo first-order rate constant (k'_{soln}) depend strongly on the pH of the surrounding buffer solution.

Alternatively, degradation of PLA-*b*-PEG-*b*-PLA hydrogels takes place by cleavage of ester bonds located within the network cross-links. In these gels, only one PLA ester bond needs to be cleaved to break a cross-link. However, the release of lactic acid from a degrading network can be a complex function of hydrolysis kinetics and mass transfer limitations. Therefore, for gel degradation, it can be difficult to determine the degradation rate constant k'_{gel} by direct measurement of degradation products such as lactic acid (LA). Instead, the change in mass swelling ratio with time is used to calculate k'_{gel} using eqs 3−5.[3,4,9,11] The time-dependent exponential growth in the mass swelling ratio of the gels produced by these equations provides a good fit to the experimental data as shown in Figure 1b. Similar to the soluble degradation experiment, comparison of the multiple data sets in Figure 1b demonstrate that the rate of gel degradation and the measured value of k'_{gel} also depend strongly on buffer pH.

The effects of pH on k'_{soln} and k'_{gel} are summarized in Figure 2. As buffer pH is increased from a value of 2.0 to 4.0, the calculated value of k'_{soln} decreases. k'_{soln} then continually increases with buffer pH above 4.0. A minimum in k'_{soln} is therefore observed to occur at pH 4, close to the pK_a of lactic acid (pK_a = 3.85). This pH-dependent minimum for the degradation rate constant is consistent with observations made by Schliecker et al.[18] and de Jong et al.[23] during the degradation of pure PLA networks. In these previous studies, a degradation rate minimum was observed for pure PLA at a buffer pH of 4.0. This minimum was hypothesized to occur due to the hydrolysis of PLA being both acid as well as base-catalyzed. According to degradation mechanisms provided by de Jong et al.[23] and Shih,[24] at pH values below the pK_a of lactic acid, proton catalysis dominates due to protonation of the acid hydroxyl group leading to subsequent attack by H_2O and hence degradation. Similarly, at pH values above the pK_a, hydroxyl catalysis dominates due to nucleophilic attack by the hydroxyl end group on the second carbonyl group, also known as back-biting.[23,25] Though the current gel networks are not pure PLA systems, obtaining similar trends in degradation behavior can be expected because of the PLA-mediated degradation of the PLA-*b*-PEG-*b*-PLA macromers.

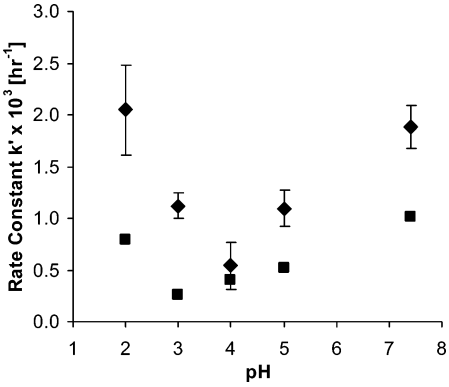
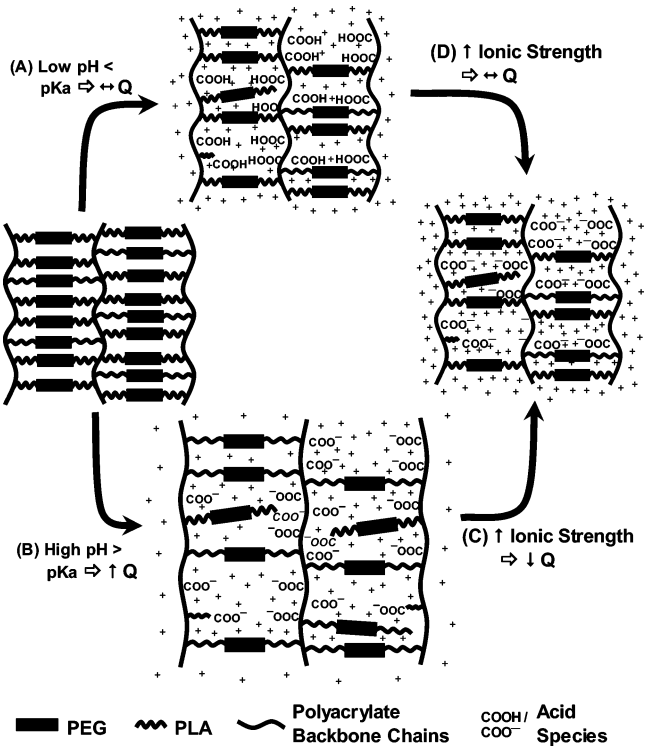


Figure 2. Experimentally determined degradation rate constants for PLA-*b*-PEG-*b*-PLA macromers in solution (♦; k'_{soln}) and in gels (■; k'_{gel}) as functions of pH. For all data: solution, 10 wt % PEGPLA1; gel, 30 wt % PEGPLA3; ionic strength = 0.135 M; $n = 3$, error bars = ± std. dev.

Degradation of both cross-linked and soluble macromers occurs through hydrolysis of PLA ester bonds. As a result, the measured pseudo first-order rate constants for hydrogel degradation agree reasonably well with those obtained for the soluble system, even though the two kinetic parameters were calculated using different methods (Figure 2). In addition, the range of values obtained in the current study for both degradation rate constants agrees with previously published results.[4,6] The values for k'_{gel} show a similar dependency on pH when compared to k'_{soln} with a minimum occurring between pH 2 and 7.4 (Figure 2). However, some differences do occur between the two data sets. The values of k'_{gel} are consistently lower than those of k'_{soln} at corresponding pH. One reason for this is that the initial water contents of the gels (79 wt % ± 1.5%) are lower than those of the macromer solutions (90 wt %) used in this experiment. In addition, the minimum value for k'_{gel} is shifted to a lower pH value compared to that of k'_{soln}. This shift of the observed minimum to a lower pH value is not consistent with pK_a differences between linear and cross-linked polymers. Previous studies indicate the acidity of carboxy groups in hydrogels to be much lower than linear polymers of identical composition.[26] In other words, the apparent pK_a's of carboxy groups in hydrogels are higher than those of linear polymers of the same composition. For example, the pK_a's of poly(acrylic acid) hydrogels have been measured at pH values much higher than 5.0 compared to a value of 4.7 for soluble poly(acrylic acid).[27]

Scheme 1. Sensitivity of PLA-*b*-PEG-*b*-PLA Hydrogel Swelling to Solution pH and Ionic Strength[a]



[a] Partial hydrolysis of PLA ester bonds creates an anionic network (A) network-immobilized acrylic acid and lactic acid species do not deprotonate at low pH ($<pK_a$). (B) At high pH values ($>pK_a$), these acid species deprotonate, leading to increased water contents, gel swelling ratios (Q), and kinetic rate constants (k_{gel}). (C) Increased buffer ionic strength at high pH shields any charged groups present and leads to gel deswelling and lower values of k'_{gel}. (D) Increased buffer ionic strength at low pH has no effect on gel swelling or k'_{gel} due to absence of ionized acid species.

If the location of the k'_{gel} minimum is simply a function of acid-group pK_a, then it would be expected to shift to higher pH values in Figure 2.

To understand why the pH dependence of k'_{soln} and k'_{gel} differs requires an analysis of the hydrogel structure and degradation kinetics. According to eq 8, any change in the water content of cross-linked PLA-*b*-PEG-*b*-PLA networks will alter the local environment of the hydrolytically labile ester bonds and affect k'_{gel}. Although a nondegraded PLA-*b*-PEG-*b*-PLA gel is considered to be relatively non-ionic, the water content of partially degraded PLA-*b*-PEG-*b*-PLA networks varies strongly with pH due to ionization of weakly acidic pendant groups created during ester bond cleavage.[28] Scheme 1 illustrates the impact of buffer pH and ionic strength on the swelling ratio (Q) and degradation rate constant (k'_{gel}) of a partially degraded PLA-*b*-PEG-*b*-PLA gel network.

After hydrolysis of some fraction of PLA ester bonds in the cross-links, network-immobilized acid groups are created: lactic acid groups at the ends of cleaved cross-links and acrylic acid groups along the backbone chains. Poly(acrylic acid) has a pK_a of 4.7[27] compared to a value of 3.85 for lactic acid. Therefore, in buffer solutions of pH greater than or equal to approximately 3.85, a significant portion of network-immobilized acid functionalities will be deprotonated (i.e., ionized). This ionization can alter the water content of the gel through two distinct mechanisms. First, the immobilized negative charges repel one another, causing the cross-linked polymer chains to stretch to a higher degree and increased gel swelling to occur[29−32] (part B in Scheme 1). In addition, the excess negative charge inside the gel attracts positive counterions from the buffer into the swollen gel environment. The ionic concentration inside the gel becomes greater than the surrounding buffer. Transport of water from the surrounding solution into the hydrogel then occurs to balance the osmotic pressure difference.[28,33] The end result is that the swelling of the partially ionized gel increases until the elastic forces of the stretched polymer network are in equilibrium with the increased osmotic forces.[34]

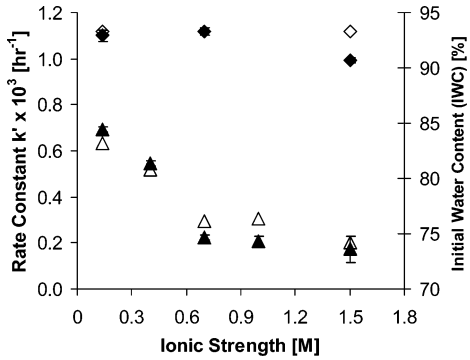


Figure 3. Dependence of kinetic rate constants for PLA-*b*-PEG-*b*-PLA macromer degradation on buffer ionic strength: k'_{gel} (▲) and water content (△) for cross-linked gels. k'_{soln} (♦) and water content (◊) for macromer in solution. For all data: solution, 6.7 wt % PEGPLA2; gel, 30 wt % PEGPLA1; pH = 7.4; $n = 3$, error bars = ± std. dev.

Therefore, although the water content of a single-phase macromer solution is fixed during degradation, the water concentration within a partially degraded PLA-*b*-PEG-*b*-PLA gel depends on local pH. Below the pK_a of lactic acid (pH = 3.85) negligible ionization of network-immobilized lactic acid as well as poly(acrylic acid) groups occurs (part A in Scheme 1). However, above pH 3.85, the degree of ionization and the amount of water transported inside the gel continuously increases. As a result, the values of k'_{gel} at pH values greater than 3.85 are increased compared to what they would be if ionization and additional water transport did not occur. This preferential increase in k'_{gel} values at higher pH adds to the inherent complexity of PLA degradation kinetics already observed in solution and results in shifting the pH at which the minimum kinetic constant value is observed from pH 4 to 3 (Figure 2).

Effect of Ionic Strength. As depicted in Scheme 1C, if ionization does occur inside the gel network, then it should be possible to nullify the increased swelling of partially degraded PLA-*b*-PEG-*b*-PLA gels observed at higher pH by increasing the ionic strength of the surrounding buffer solution. As the ionic strength of the buffer solution is raised, the osmotic pressure and degree of gel swelling should decrease.[28,33] In addition, the excess negative charges produced in the hydrogel will be screened or masked by the increased concentration of positive ions in the buffer, thus reducing the repulsive forces and further decreasing gel swelling. As shown by eqs 8 and 9, this decrease in gel water content will ultimately lower the gel degradation rate and the observed k'_{gel}.

To verify that the ionization of acid species within PLA-*b*-PEG-*b*-PLA hydrogels affects gel swelling and degradation behavior as described above, gels fabricated from 30 wt % macromer solutions were degraded in pH 7.4 buffer solutions of varying ionic strength. Within the range of pH values investigated, pH 7.4 represents the value where maximum ionization of the acrylic acid and lactic acid groups inside the partially degraded gel network occurs. Figure 3 shows that under these conditions the initial water content of the hydrogels decreases with an increase in buffer ionic strength up to 1.5M. The relatively small decrease in gel water content of ap-

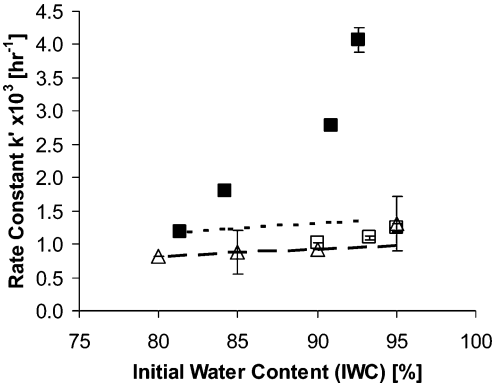


Figure 4. Dependence of kinetic rate constants for PLA-*b*-PEG-*b*-PLA macromer degradation on water content: k'_{soln} for two soluble PLA-*b*-PEG-*b*-PLA macromers (△, PEGPLA1; □, PEGPLA2); k'_{gel} of cross-linked PLA-*b*-PEG-*b*-PLA macromer (■, PEGPLA2); Predicted behavior of k'_{soln} (- - -) and k'_{gel} (———) according to pseudo first-order kinetics of ester bond degradation. All data at pH 7.4, ionic strength = 0.135 M, $n = 3$, error bars = ± std. dev.

proximately 11% leads to a rather large 75% decrease in the measured degradation rate constant k'_{gel}. This observed decrease in k'_{gel} is much greater than the effect predicted by the pseudo first-order relationship that assumes k'_{gel} to be directly proportional to gel water content (eq 9).

Contrary to what is observed with cross-linked macromer, no decrease in the degradation rate constant for the macromer solutions (k'_{soln}) is observed when the solution ionic strength is increased except for a slight decrease at the highest ionic strength of 1.5 M (Figure 3). This result is expected since no difference in osmotic pressure can be created in the single-phase soluble system and any repulsion between adjacent macromer molecules is minimized due to their high degree of mobility. Furthermore, when identical experiments were conducted in pH 2 buffer solutions where negligible ionization of the lactic or acrylic acid species occurs, increasing ionic strength had no significant effect on gel swelling or the degradation rate constants for the gel or solution-based systems (data not shown). These results indicate that ionic repulsion and osmotic pressure effects are significant only within cross-linked hydrogels at buffer pH values greater than the pK_a of the network-immobilized acid species.

Effect of Macromer Concentration. The bulk water contents of the two-component macromer solutions are determined solely by their macromer concentrations. Therefore, to directly observe the effect of water content on the degradation rate constant of soluble macromers (k'_{soln}), PLA-*b*-PEG-*b*-PLA solutions with macromer weight percents of 5, 10, 15, and 20 wt %, corresponding to water contents of 95, 90, 85, and 80 wt %, were prepared and allowed to degrade in a well-defined buffer solution (pH 7.4 and ionic strength 0.135M) at 37 °C. As described above, the degradation rate constant k'_{soln} was calculated by measuring the change in lactic acid concentration over time. The open symbols in Figure 4 show the increase in k'_{soln} with increase in water content. Extrapolating from the experimental data point at 80% water content ($k'_{soln} = 8.2 \times 10^{-4}$ h^{-1}), measured values of k'_{soln} increase proportionately with water content as predicted by the pseudo first-order kinetic assumption (dashed line). Similar values of k'_{soln} are obtained using a second macromer, indicating that within the range of macromers tested (see Table 1), variations in percent acrylation and PLA block size have no significant effect on the k'_{soln} of macromer solutions with relatively high water contents (open triangles and open circles in Figure 4).

Buffer ionic strength affects gel water content as shown previously in Figure 3. Gel water content can also be varied by altering macromer concentration in the prepolymer solution. A lower macromer concentration during network formation leads to lower cross-linking densities due to a greater degree of macromer cyclization and relaxation of the backbone polymer chains.[4,8−11,35] As described by the Flory−Rehner equation[14] (eqs 3 and 4), lower cross-linking densities lead to higher gel swelling ratios and water contents. Therefore, to systematically vary the water content experienced by the PLA ester bonds during degradation while maintaining a constant pH and ionic strength, gels with initial water contents of 93, 91, 84, and 81 wt % were prepared using 7, 10, 20, and 30 wt % macromer solutions. As shown by the solid squares in Figure 4, the values of k'_{gel} increase more rapidly with initial water content than the measured k'_{soln} values. This dramatic increase results from a corresponding decrease in macromer concentration during network formation and is similar to the trend seen in Figure 3 as ionic strength is decreased. The data in Figure 4 indicate a decrease in water content of ~12% results in a significant 71% decrease in k'_{gel} (from 4.1×10^{-3} to 1.2×10^{-3} h^{-1}). Therefore, in gel form the pseudo first-order kinetic constant for hydrolysis is much more sensitive to water content than predicted by the kinetic theory or seen in soluble systems. Furthermore, the increased sensitivity of the degradation kinetics to changes in the local water concentration is independent of whether those changes are due to variations in ionic strength (environmental parameter) or macromer concentration (structural parameter).

Conclusions

Degradation behavior of PLA-*b*-PEG-*b*-PLA macromers in soluble form and insoluble, photo-cross-linked form was compared by direct examination of the respective degradation kinetic constants, k'_{soln} and k'_{gel}. The time-dependent production of lactic acid from aqueous solutions of PLA-*b*-PEG-*b*-PLA was used to quantify k'_{soln}, whereas k'_{gel} was estimated from the change in the mass swelling ratio of photo-cross-linked PLA-*b*-PEG-*b*-PLA gels with degradation time. Kinetic constants obtained from both systems under a variety of degradation conditions ranged from 1.7×10^{-4} to 4.1×10^{-3} h^{-1} and were similar to one another and to previously reported values. Close examination of these kinetic constants demonstrates that network structure definitively influences macromer degradation behavior. Specifically, the apparent degradation kinetic constants of macromers assembled into cross-linked hydrogels are more sensitive to fluctuations in macromer chemistry as well as pH, ionic strength, and water content of the bulk environment compared to those of soluble macromers. Degradation of soluble macromer was observed to be a complex function of pH, displaying a minimum value of k'_{soln} at pH 4, similar to previous observations of pure PLA systems. However, this minimum was shifted to pH 3 during degradation of cross-linked macromers. Increasing buffer ionic strength counteracted the charge repulsion and osmotic pressure effects occurring inside cross-linked PLA-*b*-PEG-*b*-PLA gels at high pH, decreasing gel swelling and k'_{gel}. However, increasing the buffer ionic strength had no effect on values of k'_{soln} and the rate of soluble macromer degradation. These experiments indicate that the difference between the pH-dependent degradation behavior of soluble versus cross-linked PLA-*b*-PEG-*b*-PLA macromers results from ionization of network-immobilized lactic acid and acrylic acid species that increases the water content of cross-linked PLA-*b*-PEG-*b*-PLA networks in all but strongly acidic solutions.

The results of the current studies also indicate the rate of PLA-*b*-PEG-*b*-PLA macromer degradation in a buffered solution

at constant pH and temperature to be primarily a function of water content. For soluble macromer solutions, water content was shown to be a function of macromer concentration but not to vary to any significant extent with changes in ionic strength or pH. When the soluble macromer concentration was decreased, k'_{soln} increased linearly and in proportion to the corresponding increase in water content as predicted by the pseudo first-order kinetic equation. This behavior was independent of macromer chemistry within the range of macromers studied. However, increasing gel water content by decreasing macromer concentration during photo-cross-linking of PLA-*b*-PEG-*b*-PLA hydrogels lead to an approximately 6-fold greater increase in k'_{gel} than predicted by the pseudo first-order kinetic equation. The similar increases in k'_{gel} observed with a decrease in either ionic strength (environmental parameter) or macromer concentration (structural parameter) indicate that k'_{gel} for a given macromer is dependent on the absolute gel water content and independent of whether this water concentration is manipulated by changes in solution or network properties. Unlike the soluble system, measured values of k'_{gel} vary significantly with slight changes in macromer chemistry. Although water transport is identical in the highly swollen, cross-linked networks, slight differences in cross-linking density and network structure most likely affect the rates at which ionic degradation products are released from the gels. Differences in the concentration of ionic species within the degrading networks affect the response of the local gel environment to bulk solution conditions. Therefore, the results of this study indicate that the cross-linking of PLA-*b*-PEG-*b*-PLA macromers affects the rate and sensitivity of their hydrolytic degradation to bulk solution conditions such as water concentration, pH, and ionic strength. These differences must be taken into account when designing cross-linked degradable gels for clinical applications and indicates their sensitivity to external stimuli as well as their potential as stimuli-responsive materials.

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