Effect of Macromer Synthesis Time on the Properties of the Resulting Poly(β-amino ester) Degradable Hydrogel

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ABSTRACT: Poly(β-amino ester) biodegradable hydrogels are common in biomedical applications because of their tunable properties and similarities to natural soft tissue. Previous work has shown property adjustments through the choice of monomers, the ratio between monomers and the addition of a crosslinking component. Here, we show that the reaction time for the creation of the macromer can affect the resulting hydrogel properties, and thus provides another method of tuning properties. Macromer was created through the reaction of isobutylamine with poly(ethylene glycol) diacrylate (n = 400). The reaction progress was analyzed using IR and GPC analysis. Hydrogels were created through UV photopolymerization from macromers synthesized for 24, 36, and 48 h. The degradation, compressive moduli, and swelling were measured in an aqueous solution. All showed significant differences between hydrogels of different macromer synthesis times. These differences likely stem from the incomplete macromer synthesis reaction and resulting PEG-rich regions in hydrogels from shorter synthesis times. These regions will not readily degrade, but do increase the mechanical properties and extent of swelling. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 1420–1426, 2011

Key words: biodegradable; hydrogels; poly(β-amino esters); biological applications of polymers; kinetics (of polymerization)

INTRODUCTION

In recent years, biodegradable hydrogels have been a rapidly expanding area of research for a wide variety of applications including tissue engineering and drug delivery. Hydrogels are widely applied for biological applications as they exhibit tunable properties that can mimic that of natural tissue, and the high water content allows waste and nutrient materials to pass through readily.[1–3] Biodegradable hydrogels possess similar properties to traditional hydrogels yet avoid the necessity of removing the implant after its useful lifetime and concerns regarding the long-term effects of the implant on the body.[2] The main disadvantage in using hydrogels is the inherent heterogeneity in their structure, which can cause inconsistencies in the macromolecular properties (i.e., mass transfer, degradation, and mechanical properties).[1]

Poly(β-amino ester) (PBAE) polymer systems have been extensively studied because their properties (i.e., degradation rate, mechanical strength, cellular response, etc.) are easily tunable, the synthesis reaction of a diacrylate and an amine is relatively simple, and they can degrade in physiological conditions. When created with equal amounts of diacrylate and amine or excess amine, these systems have been proven effective for gene therapy applications.[4–7] In other work, excess diacrylate was used in the initial synthesis reaction to create an acrylate-terminated linear macromer, which can then be crosslinked using free radical polymerization.[8] Using these systems, electrospun fibrous scaffolds have been created which allow for better control over cell growth on the surface and tunable properties, specifically by using hydrogels of different properties in the same scaffold.[9,10]

There are a variety of ways PBAE systems can be tuned to give the target hydrogel properties for a given application. In the initial work, the choice of chemistry was highlighted as one method of tuning; a range of diacrylates and amines were shown to produce hydrogels with varying degradation profiles and mechanical strengths.[8] In subsequent work, an increase in the ratio of diacrylate to amine in the initial condensation reaction was shown to have a substantial effect on resulting properties, specifically yielding macromers with smaller molecular weights. This results in hydrogels with higher crosslinking density, exhibiting slower degradation and increased strength.[11,12] In other work, the addition of a separate crosslinking agent was shown to enhance the mechanical properties while maintaining a similar degradation profile.[13] However, due to the nature

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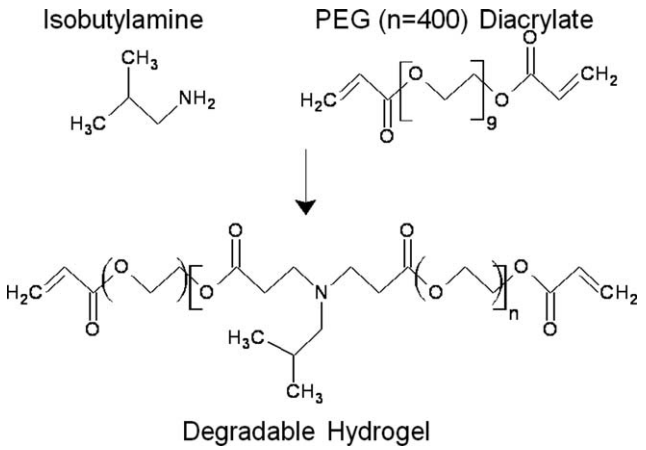

Figure 1 Reaction of isobutylamine and PEG400DA to produce the biodegradable hydrogel.

of the step-wise polymerization reaction the time period of macromer synthesis is yet another avenue to achieve tunable properties. Control of the molecular weight and heterogeneity of the resulting hydrogel structure can be attained by adjusting this variable.

In this article, one PBAE degradable hydrogel system was studied to analyze the effect the synthesis time has on the resulting gel properties. The progression of the reaction during synthesis was analyzed using FTIR and GPC analysis. Then, the resulting hydrogel properties were examined. Specifically, the degradation profile of the gels in physiological saline was quantified, which may be the most defining property of these systems for their use in both drug delivery and tissue engineering applications. The compressive properties and the swelling behavior were also measured to better understand the synthesis time effects and in turn be able to create optimal systems for tissue-specific applications. For the particular system studied, synthesis time played a major role in the macromolecular properties of the hydrogel. Thus, not only is it important that the reaction kinetics be analyzed when working with the PBAE systems, but a new variable is introduced that can potentially be used for further control of hydrogel properties.

EXPERIMENTAL

Macromer synthesis procedure

Macromers were synthesized similar to a method previously described.[8] This article focused on a degradable hydrogel system made from poly(ethylene glycol) $n = 400$ diacrylate (PEG400DA, Polysciences, Inc.) and isobutylamine (Sigma) in a molar ratio of 1.2:1 (Fig. 1). This system in particular had been previously used in pilot studies and was known to exhibit different properties as a result of the synthesis time period and further investigation was necessary. Each chemical was pipetted into a 100mL round bottom flask with magnetic stirrer arranged in a heating mantle attached to a temperature controller (J-Kem Scientific). Once the chemicals were added, the controller was set to ramp to a temperature of 85°C. The synthesis time period is defined as the point where the chemicals are first mixed at room temperature and includes the time it takes the controller to ramp to the set temperature. To maintain consistency in the mixing and heat transfer, the total mass of macromer ranged between 20 and 22 g. Throughout the synthesis, samples (~ 0.5mL) were taken every 6 h and stored at 4°C.

Analysis of synthesis

To examine the changes occurring in the reaction vessel and map the reaction kinetics, IR and GPC analyses were carried out on all samples. The samples were analyzed using the attenuated total reflectance setup of a Fourier transform infrared spectrometer (Varian 700e FTIR) to determine the functional groups present. Briefly, macromer samples were pipetted onto the crystal, and the spectrum was analyzed. For quantitative measurements, the areas under the carbon double bond peak (~ 1635 cm^{-1}) and the amine peak (~ 3300–3400 cm^{-1}) were taken as a ratio of the area under the carbon–oxygen double bond peak (~ 1710 cm^{-1}), which is constant throughout the synthesis. In addition, macromer samples were dissolved in tetrahydrofuran and then analyzed for molecular weight and distribution using gel permeation chromatography (Shimadzu GPC).

Gel polymerization

To create biodegradable hydrogels for this study, macromer was polymerized into the gel form using UV-initiated free radical photopolymerization. Hydrogels were polymerized from macromer batches removed at 24, 36, and 48 h of synthesis, and the resulting hydrogel systems are denoted H-24, H-36, and H-48, respectively. Macromer was combined with a solution of 1 weight% 2,2-dimethoxy-2-phenyl acetophenone (DMPA, Sigma) in 50 weight% ethanol (amounts based on initial macromer weight). The initiator-macromer mixture was then pipetted between prepared glass slides with 1.5 mm Teflon spacers and exposed to a UV flood source (14.0 mW/cm^2) for 5 min. Gels were washed in ethanol overnight to remove any unreacted macromer, and then placed at 4°C for ethanol evaporation. The lower temperature was used to slow the

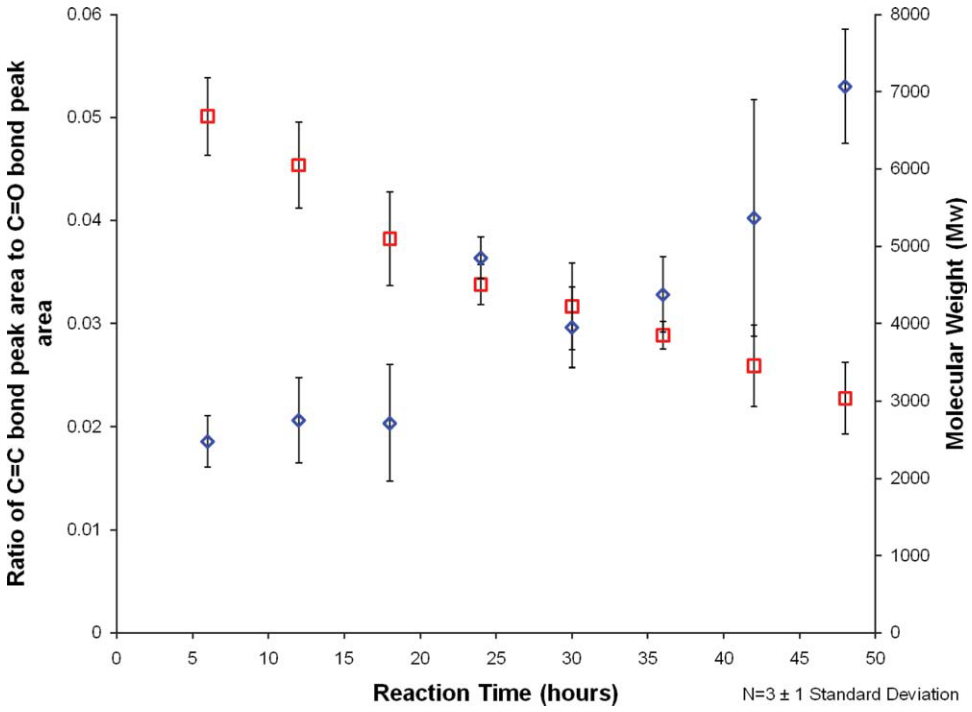

Figure 2 GPC and IR analysis of the macromer system throughout the synthesis. The peak molecular weight is plotted on the right vertical axis ◇, and the carbon double bond peak ratio is plotted against the left vertical axis □. $N = 3 \pm 1$ standard deviation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

evaporation of ethanol, thereby avoiding cracking of the gels.

Degradation studies

Samples of the hydrogel systems were cut (9.6 mm diameter), weighed, and placed into individual 50 mL centrifuge tubes with 20 mL of 37°C phosphate-buffered saline (PBS). The tubes were placed into a 37°C water bath, and samples were removed every hour. The samples were placed into petri dishes and stored at −20°C prior to freeze drying (Labconco FreeZone 2.5 Plus). The final dry sample mass was weighed and reported as a fraction of the original mass (M_t/M_o).

Mechanical testing

Unconfined compression testing of dry hydrogel samples was completed using a Bose ELF 3300 test system. Samples were positioned between the platens and loaded at a rate of 3 mm/min with an initial preloading of approximately 8*N*. The compressive modulus was calculated from the initial slope of the resulting stress–strain curve. Compression studies were completed on gels that had degraded for up to 4 h for the 24 and 48 h synthesis times. Sample preparation was similar to that of the degradation work; instead of freeze drying, however, the samples were immediately tested on the Bose system.

Swelling studies

The macromer molecular weight will control the crosslinking density of the resultant hydrogel, this can be examined through the analysis of the swelling of the hydrogel in PBS. For this study, samples of the hydrogel from all three macromer synthesis times were cut into 9.6 mm diameter disks and weighed prior to immersion in PBS. At predetermined time points, the samples were quickly removed from the PBS, patted dry with a Kim-wipe, and weighed to determine the mass gained from the swelling in water. The samples were then returned to the PBS until the next time point.

Statistical analysis

Analysis was completed using GraphPad InStat software. Analysis of variance was used to determine the differences between groups; this was followed by the Tukey-Kramer Multiple Comparisons Test. Results were considered significant if $P < 0.05$.

RESULTS

Analysis of synthesis

The change in molecular weight and C=C double bond peak of the macromer as a function of synthesis time are shown in Figure 2. The changes in the C=C and amine peak are shown in Figure 3. There

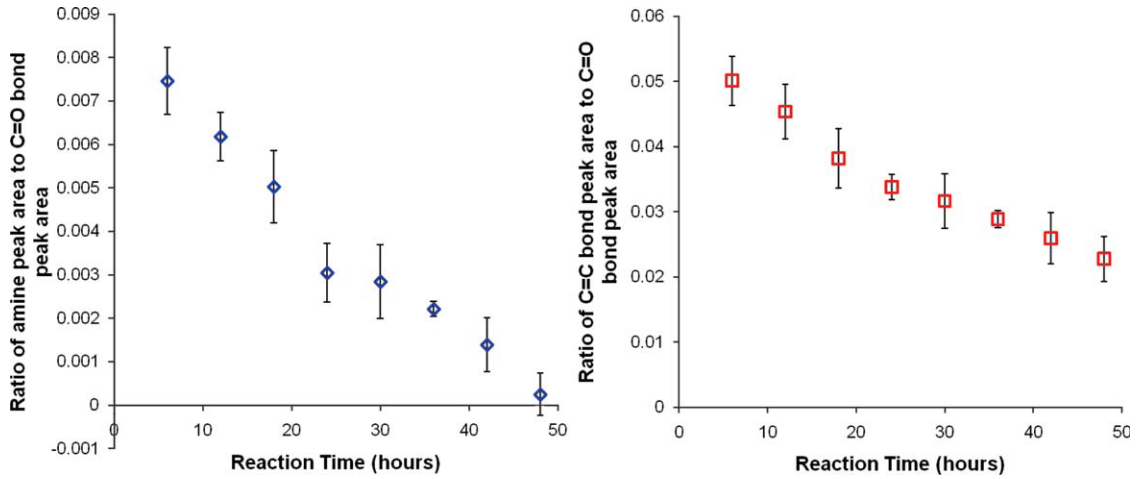

Figure 3 FTIR data plots for the secondary amine (A) and carbon double bond (B) peaks in comparison to the carbon–oxygen double bond peak. $N = 3 \pm 1$ standard deviation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

was an almost linear decrease in both the carbon double bond peak as well as the amine peak throughout the reaction time period. At the 48-h mark, the amine peak had decreased to nearly zero, indicating that the majority of the amine was incorporated into the macromer structure. In the GPC chromatograms obtained, a distinct second peak was visible in the early time point samples due to the unreacted PEG400DA. The intensity of this peak decreased as the reaction progressed, indicating that the compound reacted through conjugate addition, resulting in increased molecular weight.

Degradation studies

The degradation plot of mass remaining with time is shown in Figure 4. In the first 4 to 5 h of degradation, the three systems followed a similar trend. The H-48 gel then degraded rapidly, reaching complete degradation in approximately 7 h. The H-36 and H-24 gels degraded until a plateau was reached where a portion of the gel remained intact throughout the duration of the experiment. In these later time points, the gels were in a very swollen state and difficult to handle, increasing the uncertainty of analysis. However,

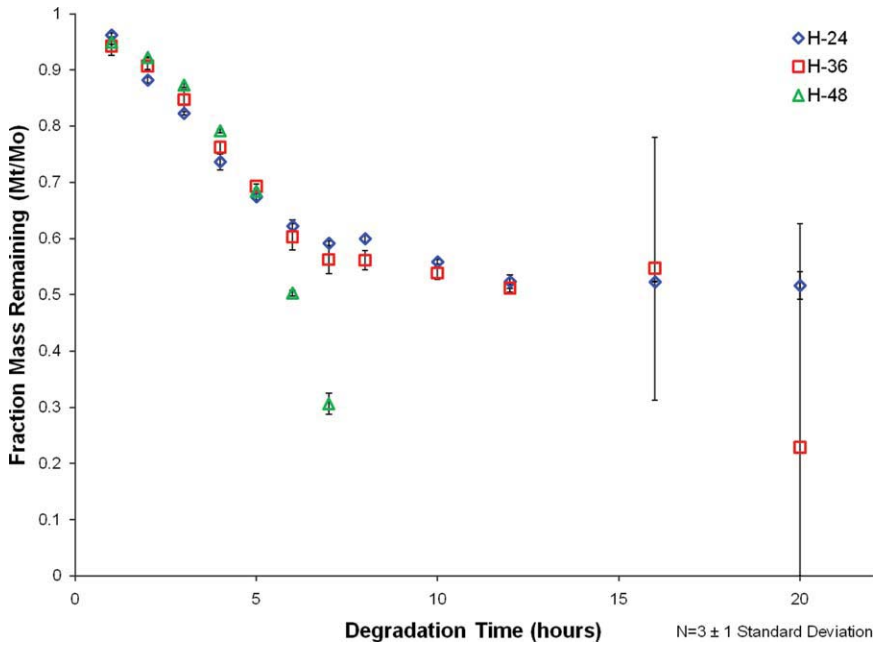

Figure 4 Degradation plot for the hydrogel system in 37°C PBS. Plot shows the H-24 system □, the H-36 system◇, and the H-48 system △. $N = 3 \pm 1$ standard deviation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

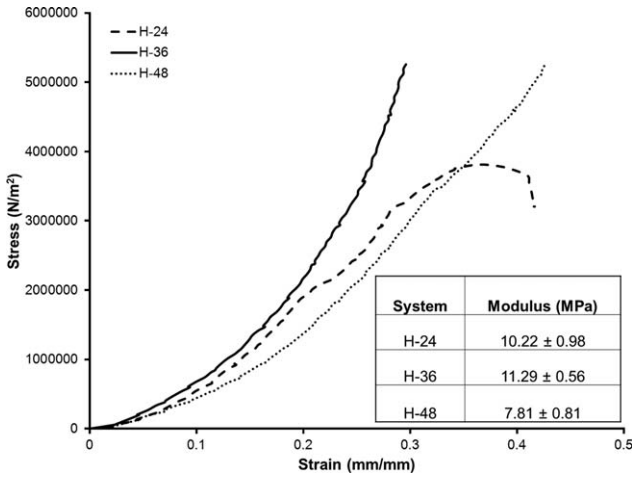

Figure 5 Dry state mechanical testing for the hydrogel system. Plot shows a representative curve for each system H-24 system (dashed line), the H-36 system (solid line), and the H-48 system (dotted line). Inset shows the average modulus calculated for each system. $N = 3 \pm 1$ standard deviation.

visual observations indicated that the samples of the H-24 and H-36 systems were still present after 24 h.

Mechanical testing

The mechanical testing was completed on all systems in the dry state and then analyzed throughout the degradation of the 24 and 48 h systems. The initial compressive modulus and representative plots of each system are shown in Figure 5. The slope of the stress–strain curve was reported for strain values between 0.07 and 0.17. As shown, the H-24 and H-36 samples had similar moduli, whereas the H-48 samples had a significantly lower modulus ($P < 0.05$) when compared with either the H-24 or H-36. As expected, the gel strength decreased with degradation; however, the H-24 and H-48 gels had very similar moduli through the first 4 h of degradation (data not shown). This is explainable as the two gels have very similar degradation in this time period.

Swelling studies

The swelling studies were carried out until the samples were too degraded and swollen to handle and perform the testing adequately. The results for the first six hours are shown in Figure 6. The H-24 system had the greatest percentage of swelling initially and throughout the degradation. The H-48 had the least amount of water uptake throughout the entire degradation and reached a maximum value before the mass loss outweighed water uptake. As expected, the H-36 system curve fell between the H-24 and H-48 systems.

DISCUSSION

Analysis of synthesis

For these particular macromer synthesis reactions, the amine adds to the diacrylate through a stepwise conjugate addition.[8] As the reaction proceeds, we expected the disappearance of the reactant peaks and an increase in the molecular weight of the macromer product. IR analysis was used to monitor the carbon double bond peak as well as the secondary amine peak, which represents the remaining isobutylamine reactant. The carbon double bond peak was the largest in the initial time points as pure diacrylate was present, and as the reaction progressed, this peak diminished as the amine was added to the macromer. A gradual decrease was observed later in the reaction as the macromer chains continued to react and increase the molecular weight of the macromer. The carbon–oxygen double bond peak was used as the reference because this functional group does not participate in the addition reaction. Our observations are consistent with a step-growth polymerization reaction, a relatively slow process in which the polymer chains slowly build up to form a large molecular weight linear polymer.[14]

Degradation studies

In the degradation study, the H-48 system was the only one to reach complete degradation in the time scale of the experiment. This is likely a result of the lack of complete reaction in the macromer synthesis in the other systems. This is evidenced by the secondary peak in the GPC chromatograms at the 24-h time point, indicating a portion of unreacted PEG400DA. Thus, these unreacted PEG chains can

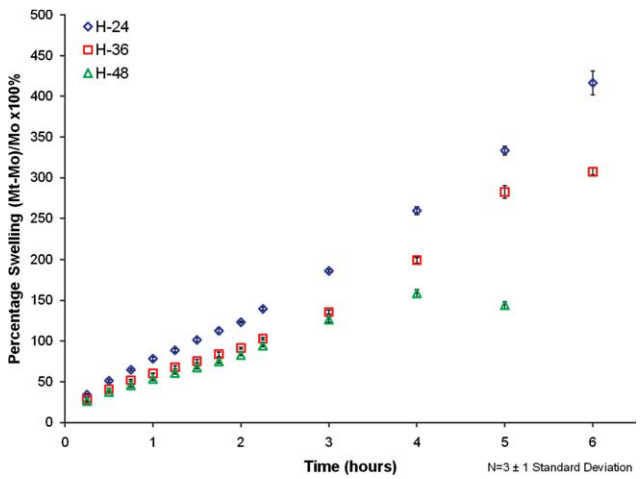

Figure 6 Swelling plot for the biodegradable hydrogel system in 37°C PBS. Plot shows the H-24 system ◇, the H-36 system □, and the H-48 system △. $N = 3 \pm 1$ standard deviation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

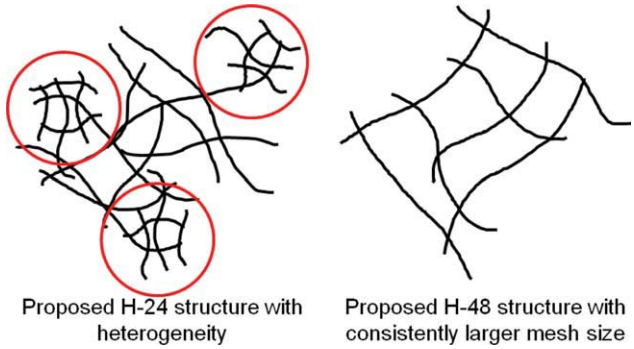

Figure 7 Schematic showing the proposed structure of the H-24 and H-48 gels. The H-24 gel has more heterogeneity with densely crosslinked regions composed primarily of PEG, whereas the H-48 system has a relatively uniform structure because the macromer synthesis reaction was allowed to go to completion. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

polymerize into the hydrogel creating PEG-rich regions, as shown in Figure 7. In the figure the circled regions are composed of macromer that is pure PEG with no amine units in the chain, thus they are more densely crosslinked than the surrounding material. These PEG-rich regions are embedded in a surrounding PBAE matrix that has longer chains and a lower crosslinking density. These areas will then likely behave as pure PEG diacrylate gels as opposed to biodegradable PBAE systems. PEG diacrylate gels are known to not readily degrade in physiological conditions, and therefore the PEG-rich areas allow portions of the gel to remain intact through the duration of the experiment.[15]

Mechanical testing

In the mechanical testing, it was found that the H-48 hydrogel had a significantly lower modulus than both the H-24 and H-36 systems. This is understandable, as the H-48 gel was made from higher molecular weight macromer chains, and thus a lower crosslinking density, whereas the presence of PEG-rich, highly crosslinked regions in the other systems provide mechanical integrity. Similar results have been observed in previous work. In work with similar PBAE systems the macromer molecular weight was varied by an adjustment in the diacrylate to amine ratio in the synthesis, an increase in the diacrylate to amine ratio created lower molecular weight systems with higher compressive moduli.[11] In other work with PEG-based hydrogels a decrease in the molecular weight of the PEG component caused an increase in the compressive moduli.[16]

Another interesting observation was that the H-24 gels failed and crumbled, whereas the H-36 and H-48 gels were able to return to near their original shape after the compression. Once again, this is a result of the highly crosslinked PEG-rich regions that are unable to absorb the applied force as well as the H-36 and H-48 gels, which are primarily composed of the larger molecular weight chains and thus a lower crosslinking density, giving them the ability to deform during compression and return to their original shape.

Swelling studies

Hydrogel swelling showed a trend that is counterintuitive in that the gels with larger macromer molecular weights and lower crosslinking densities swelled the least of the three samples. The PEG-rich regions in the H-24 gel are likely the cause of the increased swelling. When the isobutylamine attaches to the macromer, it increases the hydrophobicity of the system. At 24 h, there was still a significant fraction of unreacted amine and PEG diacrylate, and thus this gel will in part behave as a pure hydrophilic PEG gel. Literature values for a 20% pure PEG 400 gel report an equilibrium swelling percentage in water of approximately 300%,[17] and thus a maximum value of slightly over 400% is reasonable considering the extent of degradation and the concentration of remaining PEG chains. On the other hand, the H-48 system had the larger molecular weight and decreased crosslinking, yet the increased hydrophobicity due to the amine side group resulted in less swelling. These swelling study results are quite significant in designing these systems for potential applications in drug delivery. When loaded with a drug that releases via diffusion, the extent of swelling has a strong influence on the release rate.[18]

CONCLUSIONS

In this report, the properties of PBAE hydrogels resulting from different macromer synthesis time periods were studied. In the system studied, the macromer synthesis time period was found to have a significant effect on degradation, mechanical, and swelling properties. As the macromer synthesis time increased, the gels exhibited less swelling in PBS and increased degradation rates, both properties of utmost importance in common biodegradable hydrogel applications. Thus, it is important to understand the macromer synthesis reaction kinetics and the resulting properties attained when working with these degradable hydrogel systems.

The effect of macromer synthesis time on the resulting properties can also provide another avenue of tunability for these types of systems in addition to the choice and ratio of monomers used. In some cases, a hydrogel with an initial burst of degradation followed

by a prolonged period of a loose swollen state could be ideal, such as for multiphase drug release. With further understanding of the kinetics and application requirements, optimal devices can be created using these PBAE biodegradable hydrogel systems.

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