Kaleidoscope

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Oswald Physical and Engineering Sciences Second Place: Multiple Macromer Hydrogels for Multiphase Drug Release

David Spencer

Abstract

Biodegradable hydrogels are of interest for drug delivery applications due to their resemblance to biological tissue and their ability to absorb large amounts of biological fluids. Here, hydrogels were synthesized from multiple macromers to demonstrate step-wise degradation and multiphase drug release profiles. Control over the degradation and release profiles of multiple macromer hydrogels has potential applications in implantable, extended release drug delivery devices in which removal would not be needed after administration. Herein, macromers were synthesized from diethylene glycol diacrylate (A), poly(ethylene glycol) diacrylate (n=400) (H), and isobutylamine (6) in 1.2:1 molar ratios of total diacrylate to amine with diacrylate ratios of A:H (0:1), (1:1), and (2:1). Multiple macromer hydrogels were synthesized via UV photo polymerization with a 365nm UV flood source and an intensity of 8-10mW/cm^2. Degradation and swelling studies were conducted gravimetrically, and fluorescence correlation spectroscopy (FCS) was used to track diffusion coefficients at different stages of degradation. Degradation for the fully degradable systems used was inconclusive, while degradation for the non-degradable systems demonstrated a two-stage release. Swelling was found to increase with hydrophilic character of the hydrogels. Fluorescently tagged lysozyme, trypsin, and bovine serum albumin were loaded into the multiple macromer hydrogels and release was tracked using fluorescence spectroscopy. A triphasic drug release was not achieved for the systems used; however, release was tuned by varying the mass ratios of the hydrogel components.

Introduction

The use of multiple macromers in hydrogel synthesis allows for a stage-wise degradation profile and a controlled multiphase release. Over the past few decades, biodegradable hydrogels have been extensively investigated for biomedical applications.[1] Hydrogels are defined as three dimensional cross-linked hydrophilic polymer systems that have the ability to imbibe large amounts of water or biological fluids. These systems have become of great interest for medical applications due to their tunable mechanical properties and degradation profiles, as well as their resemblance to biological tissue. Biodegradable hydrogels are the subset of hydrogels that will degrade under physiological conditions, and they are therefore attractive for *in vivo* applications such as tissue scaffolding and drug release. In addition, they are desirable as there is no need to remove the biodegradable system after application.[2,3] One such system of biodegradable hydrogels can be synthesized using poly(β-amino ester) macromers. Recently, a combinatorial library of 120 poly(β-amino esters) (PBAE) encompassing combinations of twelve acrylates and ten amines was developed by Anderson et al. via condensation reactions that combined amines with diacrylates.[4] Degradation of the PBAE hydrogels occurs via hydrolytic of the backbone ester bonds and varies depending on the relative hydrophobicity of the systems.

PBAEs have been studied for applications such as polymeric gene delivery[5], pulsatile drug release via PBAE iron oxide nanocomposites[6], and synergistic cancer therapy via co-loading of paclitaxel and iron oxide nanoparticles.[7] Additionally, protein release has been studied in poly(ethylene glycol) and poly(acrylic acid) cross-linked hydrogels. [8, 9] Recently, degradation profiles of hydrogels synthesized from single macromers and multiple macromers were investigated. Single macromer systems exhibited relatively linear degradation profiles, while multiple macromer systems displayed a stepwise degradation profile.[10] Here, multiple

macromer hydrogels were synthesized, and a multiphase drug release was demonstrated utilizing changes in swelling states and diffusivity during degradation. Figure 1 illustrates the concept that smaller molecular weight drugs would initially be released at a higher rate, and then as the hydrogel degrades, the higher molecular weight drugs would be released.

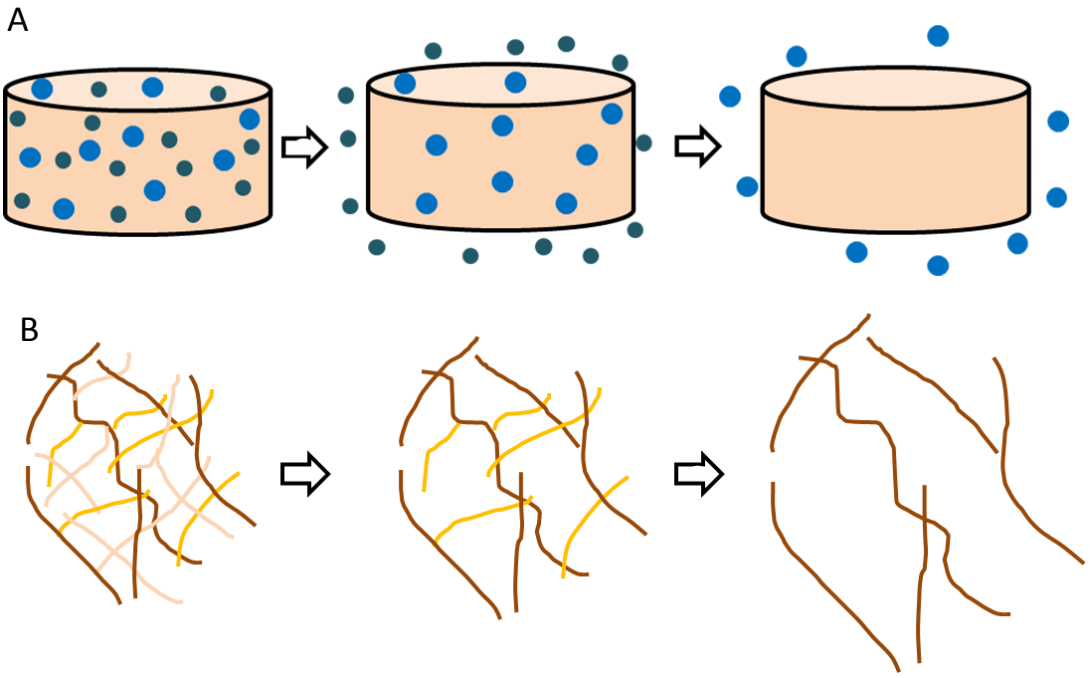

Figure 1. Illustration of multiphase release via changes in swelling and diffusion with degradation. (A) illustrates the concept that smaller molecular weight drugs will release first, smaller circles, followed by drug of larger sizes, larger circles. (B) illustrates the concept of enhanced swelling and diffusion as components degrade.

Materials

Isobutyl amine (IBA), dimethyl sulfoxide (DMSO), 2-dimethoxy-2-phenylacetophenone DMPA, lysozyme from chicken egg white, trypsin from porcine pancreas, and bovine serum albumin (BSA) were purchased from Sigma. Poly(ethylene glycol)400 diacrylate (PEG400DA) and diethylene glycol diacrylate (DEGDA) were purchased from Polysciences, Inc. Alexa 488 carboxylic acid succinimidyl ester, Alexa 594carboxylic acid succinimidyl ester, and Alexa 680 carboxylic acid succinimidyl ester were purchased from Invitrogen.

Methods

Macromer Synthesis

From the library of PBAE macromers developed by Anderson et al., two acrylates and one amine were selected for hydrogel synthesis.[4] Based on the desired properties and previous work, poly(ethylene glycol)400 diacrylate (PEG400DA; H), diethylene glycol diacrylate (DEGDA; A), and isobutylamine (IBA; 6) were utilized. Four AH6 macromers were synthesized with 1.2:1 molar ratios of acrylate to amine: A:H (0:1), (1:1), (2:1), and (0:1). Figure 2 shows a schematic of the species involved in macromer synthesis as well as the macromer synthesis reaction set-up. The amine was added to the combination of acrylates and the reaction was carried out at 85°C in a round bottom flask. The reaction was allowed to occur for 48hrs under stirring at 300rpm, after which the macromer was removed and stored at 4°C. [11]

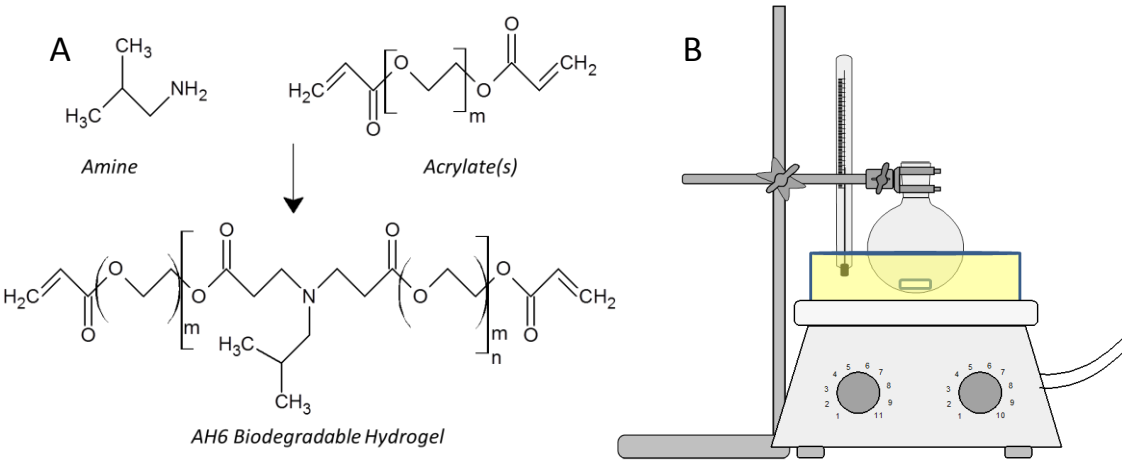

Figure 2. Macromer synthesis reagents (A) and reaction set-up (B).

Hydrogel Synthesis

Biodegradable hydrogels were synthesized using AH6 macromers and PEG400DA through UV photo polymerization between glass plates. All hydrogels consisted of three components with varying mass ratios of PEG400DA, AH6(2-1), AH6(1:1), and/or AH6(0:1).

Macromer was weighed in a 20mL glass vial. Proteins were dissolved in 95wt% DMSO with respect to total macromer. The relatively high amount of solvent was demanded by the solubility of BSA in DMSO. [12,13] The protein solution was added to the macromer and vortexed for 30 seconds. DMPA at 1 wt% of total macromer was dissolved in 5wt% DMSO. The photo-initiator was added to the reaction solution and vortexed for 30 seconds and immediately transferred into glass plates separated by a Teflon spacer. The hydrogels were polymerized using a 365nm UV flood source for 5 minutes at an intensity of 8-10mW/cm^2.[14] After polymerization, gels were allowed to cool for 15 minutes before transferring to ethanol to wash overnight. Hydrogels were cut into discs in the swollen state, and placed in a desiccator until dry.

Degradation

The rate of hydrolytic degradation for the multiple macromer hydrogel systems was measured gravimetrically. Phosphate buffered saline (PBS) was added at 37°C to pre-weighed hydrogel discs. Temperature was held constant using a water bath. At desired time points, excess PBS was removed before the samples were frozen and freeze dried to determine a final weight. All samples were run in triplicate.

Swelling

Percent swelling of the multiple macromer hydrogel was determined gravimetrically. The hydrogels were cut into discs and 0.5mL of 37°C PBS was added to each sample. Temperature was held constant using a water bath. At desired time points, excess PBS was removed, gels were weighed, and 0.5mL of fresh PBS was added to each sample.

Protein Labeling

Lysozyme, trypsin, and BSA were tagged with Alexa Fluor dyes (Invitrogen). Lysozyme was tagged with Alexa 594 carboxylic acid succinimidyl ester, trypsin was tagged with Alexa

680 carboxylic acid succinimidyl ester, and BSA was tagged with Alexa 488 carboxylic acid succinimidyl ester. The desired protein was dissolved in 0.1M sodium bicarbonate buffer at 10mg/mL. The Alexa Fluors were dissolved in DMSO at 10mg/mL. The dye was slowly added to the protein while mixing, and was then continually mixed for 1 hour. The solution was filtered using Millipore centrifuge tubes and washed thrice with PBS to remove unreacted dye. The concentrated protein solution was lyophilized and stored at 4°C.

Protein release

The rate of release of protein was studied with fluorescence spectroscopy. Protein loaded hydrogel discs were weighed and immersed in 0.5mL PBS at 37°C. At desired time points, the PBS solution was removed and replaced. The removed aliquot was analyzed at the respective excitation/emission wavelengths for the fluorescently tagged proteins using a BioTek SynergyMx plate reader.

Fluorescence Correlation Spectroscopy

The multiple macromer hydrogel discs were immersed in 3mL (10nM) rhodamine in PBS. At desired time points, the hydrogel discs were removed from the solution, excess solution was removed and samples were observed with FCS.

Results and Discussion

Hydrogel Synthesis

Two sets of hydrogels were successfully synthesized. The first set of hydrogels consisted of two biodegradable components and one non-biodegradable component, and they were composed of PEG400DA:AH6(1-1):H6 with mass ratios of 20:40:40, 50:25:25, and 80:10:10. The multiple macromer gels were synthesized with and without fluorescently tagged lysozyme and BSA. The second set of multiple macromer hydrogels consisted of three biodegradable

components. The systems synthesized were AH6(2-1):AH6(1-1):H6 with mass ratios of 40:10:50, 40:30:30, and 40:50:10. The gels were synthesized with and without fluorescently tagged lysozyme, trypsin, and BSA.

Degradation of multiple macromer hydrogels

Degradation was conducted for PEG400DA:AH6(1-1):H6 multiple macromer hydrogels with mass ratios of 20:40:40, 50:25:25, and 80:10:10. Figure 3 shows the degradation profile for 7 days of degradation. The values shown are adjusted for incomplete drying of the hydrogels before starting degradation. The average weight of each set of hydrogel discs was adjusted to the average weight of a set of dry hydrogel discs. After 12 hours of degradation, the H6 fraction of the multiple macromer hydrogel appeared to be degraded, which is consistent with the degradation rate of control H6 hydrogels. Control AH6(1-1) hydrogels fully degrade in 36 hours, however, after 7 days of degradation, the fraction of the multiple macromer hydrogel remaining was greater than expected. Two distinct phases of degradation were present in these systems. The similar nature of the H6 and AH6(1-1) components may have masked an additional step of degradation. The higher than expected remaining fraction could be a result of incomplete conversion or incomplete removal of degradation products from the hydrogel. Conversion was measured using Fourier transform infrared spectroscopy (FTIR). The ratio of the area under the curve of the C=C bond at 1637cm^{-1} to the area under the curve for the C=O bond at 1724cm^{-1} was compared for the reaction solution and unwashed hydrogels. Conversion of PEG400DA:A6:H6 systems with mass ratios of 20:40:40, 50:25:25, 80:10:10 indicated that conversion decreased with increasing PBAE character. Conversion of the 80:10:10 system was approximately 90%, while conversion for the 50:25:25 and 20:40:40 systems were 80% and 70% respectively. The lower conversion associated with the PBAE components would cause the mass

ratio of the non-degradable component to be higher than reported. However, it is unlikely that this phenomenon is the only aspect affecting the degradation. A second possibility for the higher than expected degradation rate is entrapment of degradation products in the non-degradable portion of the gel. If the degraded fractions were unable to escape, the fraction remaining would be higher than the theoretical based on mass ratios.

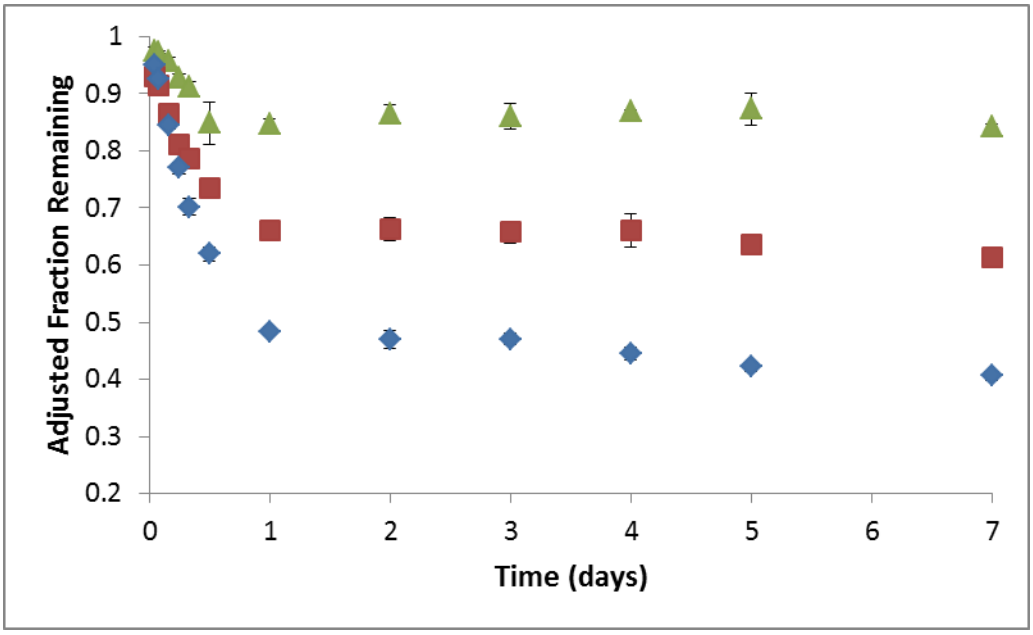

Figure 3. Degradation plot for PEG400DA:AH6(1-1):H6 with mass ratios of 80:10:10 (triangles), 50:25:25(squares), and 20:40:40(diamonds).

Degradation was carried out for a set of fully degradable AH6(2-1):AH6(1-1):H6 multiple macromer hydrogel with mass ratios of 40:10:50, 40:30:30, and 40:50:10. The degradation profiles are shown in Figure 4. The values shown on the plot are adjusted for incomplete drying of the hydrogels before the start of degradation. Similar to the PEG400DA:AH6(1-1):H6 systems, the theoretical H6 fraction seemed to degrade in 12 hours. However, all three systems degraded in less than 36 hours. The similar and rapid degradation times for the H6 and AH6(1-1) components made it difficult to determine if a step-wise degradation was occurring with these systems.

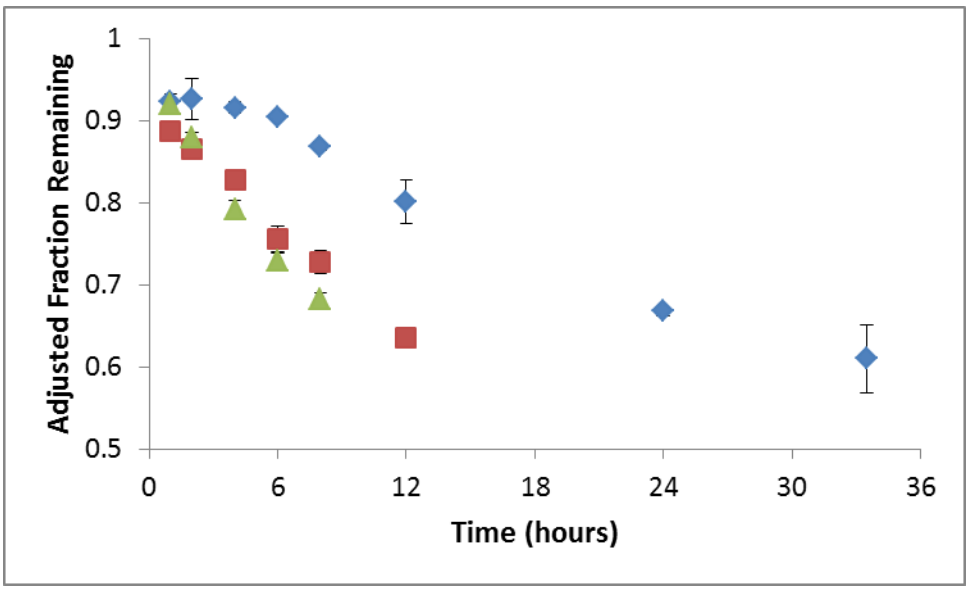

Figure 4. Degradation plot for AH6(2-1):AH6(1-1):H6 with mass ratios of 40:10:50 (triangles), 40:30:30 (squares), and 40:50:10 (diamonds).

Swelling

Swelling studies were conducted for the fully degradable AH6(2-1):AH6(1-1):H6 systems, but have not yet been conducted for other systems.. Swelling was found to increase with increasing hydrophilic character of the PBAEs. The results are shown in Figure 8.

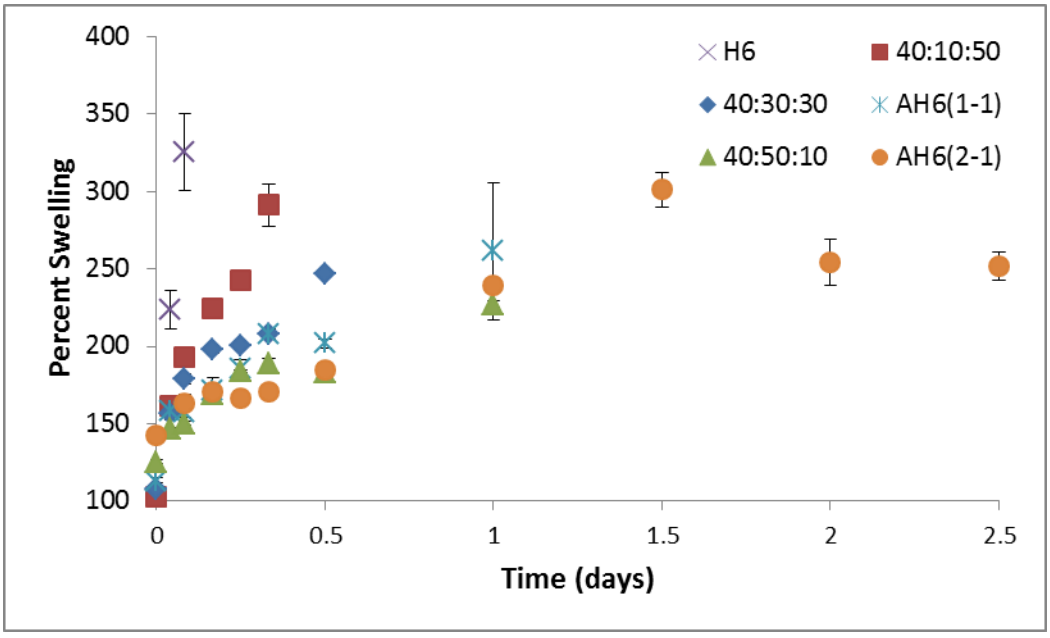

Figure 5. Plot of percent swelling for the AH6(2-1):AH6(1-1):H6 multiple macromer hydrogel systems.

Release

Lysozyme and BSA were released from PEG400DA:AH6(1-1):H6 hydrogels with mass ratios of 20:40:40, 50:25:25, and 80:10:10. The lysozyme and BSA were loaded at 0.2wt% and 100wt% DMSO was used as the solvent. The mass percent of the non-degradable component was sufficient in all three systems to keep the gel intact over 3 weeks of degradation. Additionally, some protein remained entrapped in the network at that time point. The cumulative release per gel weight is shown in Figure 5. The loading of the proteins was theoretically the same, however, different amounts of photo-bleaching may have occurred.

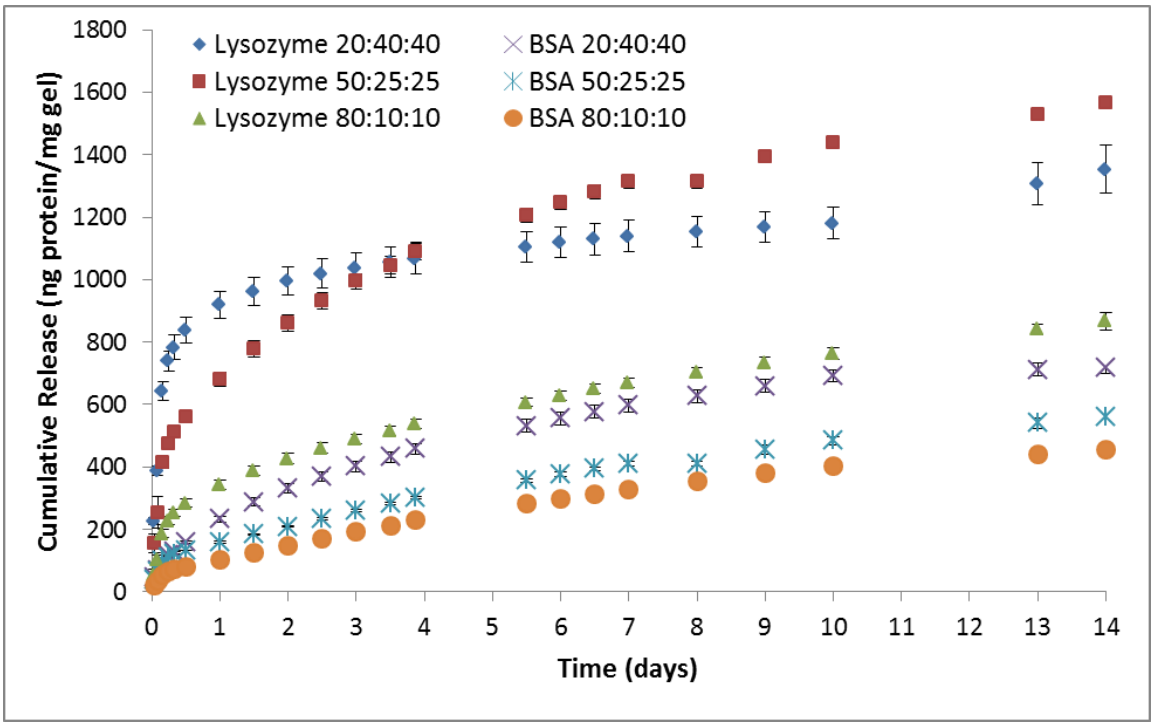

Figure 6. Release profile of lysozyme and BSA from PEG400DA:AH6(1-1):H6 multiple macromer hydrogel.

Lysozyme, trypsin, and BSA were released from fully degradable AH6(2-1):AH6(1-1):H6 multiple macromer hydrogels with mass ratios of 40:10:50, 40:30:30, and 40:50:10 as well as AH6(2-1), AH6(1-1), and H6 single macromer hydrogels. The three multiple macromer systems fully degraded and therefore released all the proteins in less than 36 hours. Figure 6

plots A-C show the rate of release for lysozyme, trypsin, and BSA. The rate of release of the proteins was dependent upon the composition of the hydrogels. As the hydrophobic character of the hydrogels increased the time for degradation and release increased. As shown in plot D of Figure 6, very little difference is seen between the release profiles of the three systems. This result could be due to the minimal difference in degradation times of the three systems.

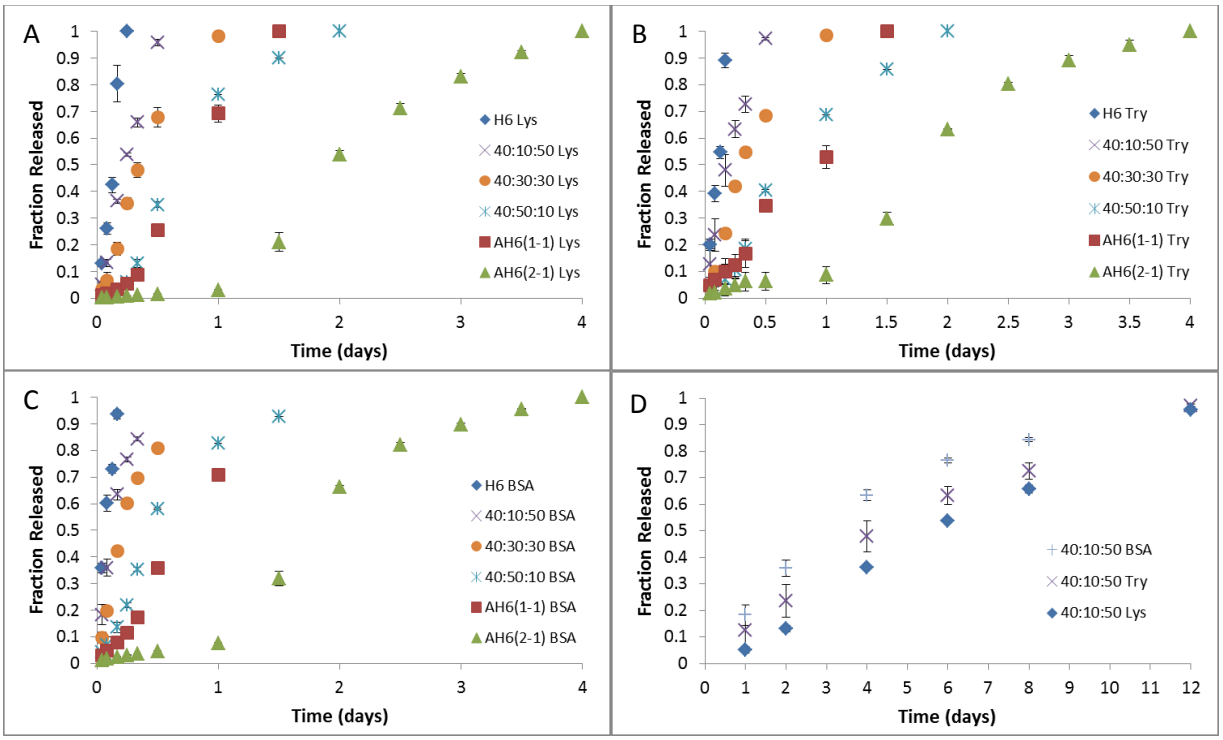

Figure 7. Release of proteins from AH6(2-1):AH6(1-1):H6 multiple macromer hydrogels and AH6(2-1), AH6(1-1), and H6 single macromer hydrogels. Lysozyme, trypsin, and BSA release are shown in plots A, B, and C respectively for the six systems. A comparison of the release of lysozyme, trypsin, and BSA for the AH6(2-1):AH6(1-1):H6 40:10:50 system is shown in plot D.

Fluorescent Correlation Spectroscopy

Fluorescence correlation spectroscopy was conducted on the PEG400DA:AH6(1-1):H6 system. The study was done at room temperature, and the diffusion coefficients were somewhat surprising. The diffusion coefficients were expected to increase with time, however, diffusion coefficients at some of the intermediate time points were greater than those at 7 days. Only one set of samples were run for each system, therefore more trials are needed before the results have statistical significance.

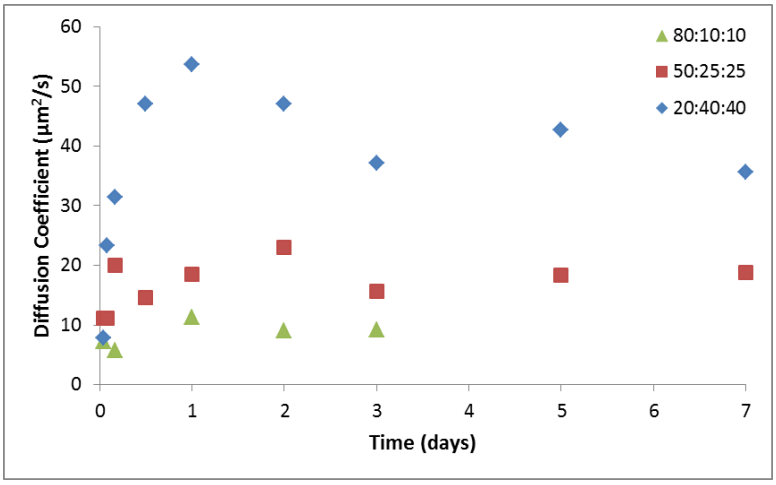

Figure 8. Preliminary FCS results for PEG400DA:AH6(1-1):H6 multiple macromer hydrogels with varying mass ratios.

Conclusion

A step wise degradation profile was observed for the PEG400DA:AH6(1-1):H6 multiple macromer hydrogels, but not for the AH6(2-1):AH6(1-1):H6. A multiphase degradation profile was not observed for the fully degradable hydrogel systems. However, two stages of release were seen in the PEG400DA:AH6(1-1):H6 systems, characterized by an initial rapid phase of release and then a slower sustained release. Swelling was found to increase with increasing 'H' character in the fully degradable system, and the diffusion coefficients yielded interesting results in the PEG400DA:AH6(1-1):H6 systems. The use of PBAE components with more diverse characteristics, and a smaller amount of overlapping degradation, may allow for a multi-phase release profile to be observed. Overall, the multiple macromer systems allow for a tunable degradation profile, and show promise for use in drug delivery type applications. Slight adjustments to the components and mass ratios used for synthesis should allow for a multi-phase release profile, which can then be tuned further for the release of multiple drugs for a specific disease state.

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