Poly(ethylene glycol) Methacrylate/Dimethacrylate Hydrogels for Controlled Release of Hydrophobic Drugs

Jackie A. DiRamio,[†] William S. Kisaalita,*[,†] George F. Majetich,[‡] and Joel M. Shimkus[‡]

Department of Biological and Agricultural Engineering and Department of Chemistry, University of Georgia, Athens, Georgia 30606

Hydrogels have been successfully used to entrap hydrophilic drugs and release them in a controlled fashion; however, the entrapment and release of hydrophobic drugs has not been well studied. We report on the release characteristics of a model hydrophobic drug, the steroid hormone estradiol, entrapped in low (MW 360/MW 550) and high (MW 526/MW 1000) molecular weight poly(ethylene glycol) methacrylate (PEG-MA)/dimethacrylate (PEG-DMA) hydrogels. The cross-linking ratio, temperature, and pH ranged from 10:1 to 10:3, from 33 to 41 °C, and from 2 to 12, respectively. The gelation of the PEG-MA/PEG-DMA hydrogel was initiated with UV irradiation. The absence of poly(glutamic acid) in the hydrogel formulation resulted in a loss of pH sensitivity in the acidic range, which was displayed by the hydrogels' similarities in swelling ratios in the pH buffers of pH 2, 4, and 7. Use of high molecular weight polymers resulted in a higher hydrogel swelling (300%) in comparison to the low molecular weight polymers. Drug size was found to be a significant factor. In comparison to 100% estradiol (MW 272) release, the fractional release of insulin (MW 5733) was 12 and 24% in low and high molecular weight gels at pH 2, respectively, and 17% in low molecular weight gels at pH 7. On the release kinetics of the estradiol drug, the hydrogels displayed a non-Fickian diffusion mechanism, which indicated that the media penetration rate is in the same range as the drug diffusion. The synthesis, entrapment, and release of estradiol by the PEG-MA/PEG-DMA hydrogels proved to be successful, but the use of ethanol in the buffers to promote the hydrophobic release of the estradiol in the in vitro environment caused complications, attributed to the process of transesterification.

Introduction

Drug delivery technology is evolving through the creation of new techniques that deliver a variety of drugs effectively. These developments benefit numerous patients by achieving a higher compliance and quality of life. Hydrogels consist of three-dimensional polymeric networks with excellent water-absorbing capacity and biocompatibility (*1*). Depending on their formulation, hydrogels can exhibit a variety of drug release profiles determined by the release environment. Thermosensitive and pH-sensitive hydrogels are the most extensively studied gels because of their controlled-release characteristics (*2*). The release of large protein drugs such as insulin from hydrogels is of great interest because these high molecular weight drugs are normally delivered to the body through injections, with low patient compliance (*3*). Hydrogels, which have great swelling capacity, can entrap these high molecular weight drugs and thus release them in a controlled fashion (*4*). Although hydrogels appear to work well with large hydrophilic proteins such as insulin (*5*), their use for the release of hydrophobic drugs has not been well studied.

This study focuses on hydrophobic drug release from characteristically pH-sensitive hydrogels. A previous study by Yang et al. (*5*) presented a pH-sensitive hydrogel based on poly(ethylene glycol) methacrylate-graft-poly(glutamic acid) and poly(ethylene glycol) dimethacrylate. In a preliminary study, replication of the Yang et al. (*5*) hydrogel synthesis was unsuccessful. Subsequently, the protocol was altered (*6*) by removing the poly(glutamic acid) and creating a hydrogel composed only of poly(ethylene glycol) methacrylate [PEG-MA] and poly(ethylene glycol) dimethacrylate [PEG-DMA], hereafter referred to as PEG-MA/PEG-DMA. PEG-MA/PEG-DMA should retain the original pH-dependent release characteristic, and by varying the ratio between these polymers, the resultant cross-linking should alter the drug release characteristics. We chose estradiol as a model hydrophobic drug for this study, because it is an estrogen derivative. Although the use of estrogen replacement therapy for postmenopausal women has now come under question (*7*), estrogen is still needed by patients suffering from estrogen deficiency, e.g., young girls for maturation of their reproductive systems (*8*).

Estradiol, a hydrophobic steroid, represents an important hormone to the female reproductive system. Like protein drugs, when taken orally, estradiol is greatly degraded by the digestive enzymes, thus losing any therapeutic effects. A pH-sensitive hydrogel that char-

* Corresponding author. Phone: 706-542-0835. Fax: 706-542-8806. Email: williamk@engr.uga.edu.
[†] Department of Biological and Agricultural Engineering.
[‡] Department of Chemistry.

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acteristically releases at a high pH could protect estrogen in the low pH acidity of the stomach and thus release the drug in the more stable and basic environment of the intestine.

The purpose of this study was, therefore, twofold: first, to synthesize, characterize, and entrap estradiol in the PEG-MA/PEG-DMA hydrogels, and second, to determine the optimum release of estradiol from the PEG-MA/PEG-DMA hydrogels with respect to temperature, pH, and cross-linking ratio.

Materials and Methods

Materials. Estradiol, insulin, *n*-vinyl pyrrolidinone and 2,2-dimethoxy-2-phenylacetophenone (hydrogel initiator compounds), poly(ethylene glycol) methacrylate (MW 360 and MW 526), and poly(ethylene glycol) dimethacrylate (MW 550) were all obtained from Sigma-Aldrich (St. Louis). Poly(ethylene glycol) dimethacrylate with a molecular weight of 1000 was obtained from Monomer-Polymer & Dajac Labs, Inc. (Feasterville, PA).

Hydrogel Synthesis Procedure. Estradiol (1.2 mg) was first dissolved in 0.2 mL of ethanol, and 1 mL of 0.01 M phosphate buffer containing 0.9 wt % sodium chloride was added to the dissolved estradiol (*5*). Next, 200 μL of PEG-MA was added; the PEG-MA with a molecular weight of 360 was used for low molecular weight gels. For a 10:1 PEG-MA:PEG-DMA ratio low molecular weight hydrogel, 20 μL of PEG-DMA (MW 550) was next added (*6*). Finally, 2.5 μL of the reaction initiator was then added. The reaction initiator consisted of 150 mg of 2,2-dimethoxy-2-phenylacetophenone dissolved in 0.5 mL *n*-vinyl pyrrolidinone (*5*). The solution was stirred prior to irradiation. The same procedure was followed for the high molecular weight gels, with the exception that PEG-MA and PEG-DMA of molecular weights of 526 and 1000 were used, respectively.

To perform the UV irradiation, 1 mL plastic syringe tips were cut off in order to hold and expel the hydrogel mixture. The hydrogel mixture (0.7 mL) was pipetted into the plastic syringe, and the syringe was clamped upright and exposed to irradiation at 365 nm using a B-100SP Model LWUV lamp (Fisher Scientific). Gelation usually occurred within 4−5 min of irradiation. The gel was then gently pushed out of the syringe, washed with distilled water, and allowed to air-dry. The rest of the mixture was then irradiated, washed, and also allowed to air-dry. Next, the gels were cut into cylinders of ~0.1 mL and stored at room temperature until ready for use (*5*).

Blank gels were prepared using the same procedure, but without the 1.2 mg of estradiol. Insulin-entrapped gels also used the same hydrogel procedure, but without the use of ethanol to dissolve the drug. Therefore, 1.2 mg of insulin was dissolved in the 1 mL of phosphate buffer by adding ~2 drops of 0.1 N HCL to bring the pH down, which allowed the insulin to be fully soluble in the mixture. The rest of the hydrogel constituents were then added in the same process, and irradiation was performed.

Release Buffer Solutions. The following buffers were used for the swelling and drug release experiments: citric acid-buffered saline, pH 2; citrate-buffered saline, pH 4; phosphate-buffered saline, pH 7; boric acid-buffered saline, pH 10; and potassium chloride-buffered saline, pH 12. To overcome the hydrophobicity of estradiol, the release buffers for estradiol were modified by adding ethanol (50%, v/v), because estradiol is highly soluble in ethanol. The pH change due to the addition of ethanol was corrected with either 1.0 N HCl or 1.0 N NaOH. In vivo, due to its low solubility, estradiol is transported bound to carriers, specifically albumin, and therefore the use of ethanol in the release buffers was necessary to simulate the solubilizing effect of the protein carriers. Ethanol addition was not necessary for insulin release since insulin is hydrophilic.

Hydrogel Swelling. To determine if the PEG-MA/PEG-DMA hydrogel exhibited pH sensitivity, the swelling of the hydrogel was studied under various pH buffers. In each case, swelling studies were conducted, with and without ethanol. First, blank hydrogels were freshly made and then dried in an incubator for 2 days at 30 °C. The swelling studies were carried out in triplicate by placing the dried blank gels in 5 mL plastic vials containing 1 mL of the appropriate buffer solution. The vials were shaken at 100 rpm at 37 °C in an Innova 4000 temperature-controlled incubator shaker (New Brunswick Scientific, Edison, NJ). At various time intervals, the gels were removed, gently dried with a kim-wipe and weighed, and then returned to the vials with 1 mL of fresh buffer solution. The swelling ratio (SR) was estimated by comparing the ratio of the wet hydrogel weight (M_{wet}), which was measured at the various time intervals, to the initial dry hydrogel weight (M_{dry}), which was measured before the swelling study began (*5*):

$$SR = M_{wet}/M_{dry} \quad (1)$$

Statistical evaluation of the swelling profiles was conducted using *t*-tests from the data analysis package located in Microsoft Excel.

Drug Release Procedure. To remove a factor (such as an unreacted polymer) that was interfering with the estradiol absorbance analytical procedure from the prepared hydrogels, the gels were rinsed in 15 mL plastic tubes each with 10 mL of deionized water for 24 h. This step was not necessary for the insulin-entrapped gels. The drug release studies were then performed in triplicate by placing both blank and drug-entrapped hydrogels into 5 mL capped plastic tubes with 1 mL of the appropriate buffer. The samples were shaken continuously for 72 h at various temperatures depending on the study. For the final release studies comparing estradiol to insulin release, the temperature was set to 37 °C and the only buffers used were pH 2, 7, and 12. At selected time intervals, 1 mL of buffer was pipetted out and analyzed spectrophotometrically and 1 mL of fresh buffer was added to the sample. The amount of estradiol (280 nm) or insulin (222 nm) released at the various time intervals was determined with a Beckman Spectrophotometer 640B. The actual amount of drug released was determined by subtracting the average of the blank absorbances that were measured to account for any additional unreacted polymers that were being released. To convert the absorbance values into drug concentrations, standard calibration curves were created for both estradiol and insulin prior to the drug release studies. A series of estradiol and insulin solutions ranging from 0.5 to 500 μg mL^{-1} in concentration were used to create the calibration curves at 280 nm for estradiol and 222 nm for insulin. For the drug release studies, the fractional amount of drug released over time was calculated on the basis of the amount of drug entrapped within the hydrogel during synthesis. For the statistical methods, the fractional amount of drug released was based on the total amount of drug released after the 72 h. As shown in Table 2, estradiol losses occurred due to its hydrophobicity.

Table 1. Design Matrix for Optimization of Hydrogel Drug Release

pH	temp (°C)	cross-linking ratio	X_1	X_2	X_3
4.0	33	10:2	−1	−1	0
10.0	33	10:2	1	−1	0
4.0	41	10:2	−1	1	0
10.0	41	10:2	1	1	0
4.0	37	10:1	−1	0	−1
10.0	37	10:1	1	0	−1
4.0	37	10:3	−1	0	1
10.0	37	10:3	1	0	1
7.0	33	10:1	0	−1	−1
7.0	41	10:1	0	1	−1
7.0	33	10:3	0	−1	1
7.0	41	10:3	0	1	1
7.0	37	10:2	0	0	0
7.0	37	10:2	0	0	0
7.0	37	10:2	0	0	0
7.0	37	10:2	0	0	0
7.0	37	10:2	0	0	0

Drug Release Mechanism. The controlled-swelling characteristic of the hydrogel allows its release kinetics to be analyzed for Fickian and non-Fickian diffusional behavior (*1*). Equation 2 displays the model to which the estradiol release data can be fit:

$$M_t/M_\infty = kt^n \tag{2}$$

Where M_t and M_∞ represent drug release at time t and at equilibrium, respectively, k is the rate constant characteristic of the system, and n is the diffusional exponent (*9*). Equation 2 can only be applied to the first 60% of drug release. The diffusional exponent (n) is calculated as the slope, and the rate constant (k) is calculated as the intercept of linear regression lines fitted to the $\log(M_t/M_\infty)$ versus log time plots (*10*). Ultimately, the value of n determines if the hydrogel release represents Fickian ($n = 0.5$) or non-Fickian ($n > 0.5$) diffusion.

Statistical Analysis. It was hypothesized that an optimum release profile exists at a specific combination of the three variables of pH, temperature, and cross-linking ratio of PEG-MA to PEG-DMA. Thus, a statistical design must be developed to find the optimum release. Using the experimental design presented by Kisaalita et al. (*11*) allows more than one factor to be changed at a time, allowing for an optimum to be found with a smaller number of experimental runs. Effects of factor interactions on the response can be obtained that are not available with the one-factor-at-a-time approach. Normally, an unknown response function

$$Y = f(X_1, X_2, \ldots X_K) \tag{3}$$

exists that relates Y to K factors. Using a low-order polynomial allows the function to be estimated in the region of interest. Equation 4 displays the first-order model:

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_K X_K = \beta_o + \sum_i \beta_i X_i \tag{4}$$

and eq 5 displays the second-order model:

$$Y = \beta_o + \sum_i \beta_i X_i + \sum_i \beta_{ii} X_i^2 + \sum_{i<j} \Sigma \beta_{ij} X_i X_j \tag{5}$$

where β_o is the y-axis intercept, the β_is are the K first-order coefficients, and β_{ji}s are the $K(K-1)/2$ cross-product or interaction coefficients for the models written in terms of x_i. For this experiment, $K = 3$ for the three variables of pH, temperature, and cross-linking ratio. The matrix for $K = 3$ factors is a cuboctahedronal design requiring a minimum of 17 runs/experiment in order to evaluate the second-order coefficients from eq 5. Table 1 displays the relative location of experimental points. The zero level in the design is the average of the low (−1) and high (+1) levels. The chosen zero level is at the conditions that are considered to be the optimum for the estrogen release from the hydrogels. X_1, X_2, and X_3 are the scaled design variables, where the step size for each of the variables is linear but large enough to reveal any effects.

Table 2. Average Estradiol Lost during Synthesis and within the Hydrogel's Matrix after 72 h Release Experiment for Both Low and High Molecular Weight Polymers

estradiol losses	low MW	high MW
pipet tip	1.215385 μg/gel (1.4%)	3.18179 μg/gel (3.2%)
mixing vessel	13.85692 μg/gel (15.4%)	18.5755 μg/gel (18.6%)
PEG-DMA	11.21893 μg/gel (12.5%)	6.282051 μg/gel (6.3%)
after release	7.944663 μg/gel (8.8%)	34.83333 μg/gel (35%)
total loss	34.2386 μg/gel (38%)	62.87806 μg/gel (63%)

The general linear model (GLM) procedure available in the SAS statistical software package was used to estimate the intercept and coefficients in eq 5. GLM uses the method of least squares to fit general linear models and works with both balanced and unbalanced designs (*11*).

Results and Discussion

Synthesis of Hydrogels. The gelation of the hydrogels occurred due to the interaction of alkene bonds. PEG-DMA contains alkene bonds on both ends of the PEG chain, while PEG-MA contains an alkene bond on one end of its PEG chain and a hydroxyl group on the other. The alkene bonds of the two cross-linkers react under UV irradiation and allow for gelation of the polymers into a three-dimensional hydrogel network (*5*). Both the high and low molecular weight hydrogels were easily synthesized with various cross-linking ratios of PEG-MA to PEG-DMA. The gelation occurred within 3 min of exposure to UV irradiation for the low molecular weight gels and within 5 min of exposure for the high molecular weight gels. Both of the low molecular weight cross-linkers have a decreased amount of PEG, which apparently accounted for the faster gelation time of these hydrogels as well as their tighter and more compacted structures (*13*).

A separate experiment was conducted to determine the amount of estradiol lost during hydrogel synthesis. Table 2 displays the various amounts of estradiol lost during hydrogel synthesis and after a 72 h release experiment. Due to the hydrophobic nature of estradiol, a large amount of drug was lost during synthesis, either left in the various pipet tips or in the glass mixing vessel. A large amount of drug was also found to remain in the gel after a 72 h release. This was determined by dissolving the gel overnight and measuring the remaining estradiol spectrophotometrically. The hydrogels were loaded with 90−100 μg of estradiol. As shown in Table 2, approximately 60% was found in the low molecular weight gels, and about 40% was found in the high molecular weight gels. A lower amount of drug was entrapped within the high molecular weight gels, possibly due to the large polymers' thickness, which caused these polymers to attach more readily to the pipet tips and

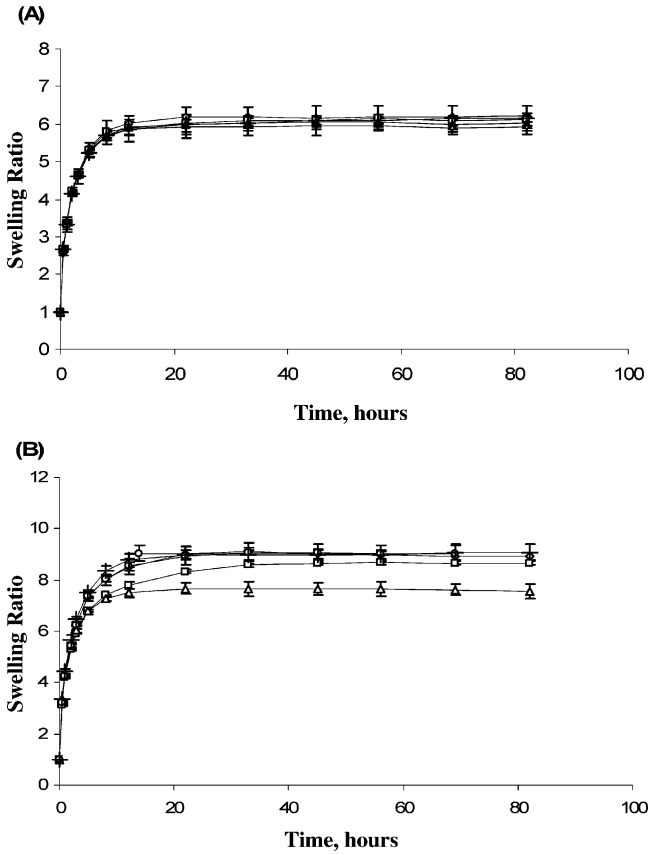

Figure 1. Effects of swelling behavior based on molecular weight, using 50% ethanol buffers of pH 2.0 (◇), pH 4.0 (□), pH 7.0 (△), pH 10.0 (○), and pH 12.0 (+). Hydrogels were synthesized with a 10:1 cross-linker ratio using (A) low molecular weight cross-linkers and (B) high molecular weight cross-linkers. Error bars represent the standard deviation of triplicate samples. Swelling ratio = ratio of wet hydrogel weight to initial dry hydrogel weight.

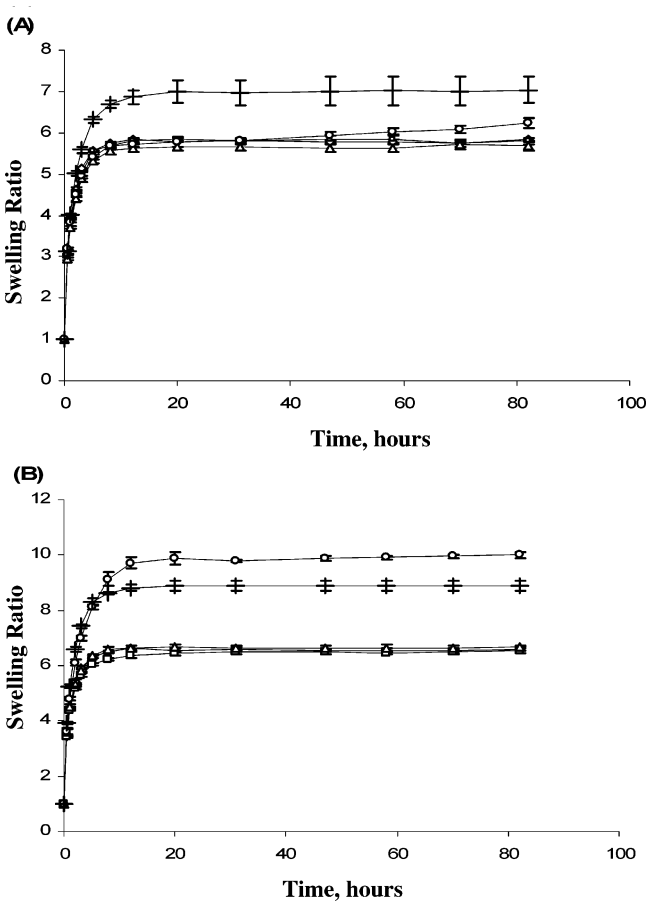

Figure 2. Effects of swelling behavior based on molecular weight, using buffers with no ethanol of pH 2.0 (◇), pH 4.0 (□), pH 7.0 (△), pH 10.0 (○), and pH 12.0 (+). Hydrogels were synthesized with a 10:1 cross-linker ratio using (A) low molecular weight cross-linkers and (B) high molecular weight cross-linkers. Error bars represent the standard deviation of triplicate samples. Swelling ratio = ratio of wet hydrogel weight to initial dry hydrogel weight.

mixing vessels. These polymers are extremely lipophilic and easily attract the estradiol, perhaps explaining the discrepancies in the hydrogel's drug entrapment. Thus, the hydrophobicity of estradiol is a major factor in hydrogel synthesis that must be taken into account during formulation.

Swelling Studies. The hydrogel swelling studies give insight into the balance between the media penetration rate and the drug diffusion (*10*). The swelling behavior of both the low and high molecular weight gels was studied under various buffers of pH 2.0, 4.0, 7.0, 10.0, and 12.0. In the first study, the buffers were comprised of 50% ethanol. Figure 1 displays the hydrogel swelling for both the low and high molecular weight gels. In both sets there was a rapid initial hydration of the gels followed by a constant increase until around 20 h, when the increase in gel hydration appeared to level off. The low molecular weight gels all swelled to a similar ratio of approximately 600% ($0.08 < p < 0.758$). The high molecular weight gels were also close in their final swelling ratio of approximately 900% ($0.06 < p < 0.763$), with the exception of pH 4.0, which had a slightly lower ratio ($p < 0.0014$), and pH 7.0, where the swelling ratio was lower by approximately 150−200% ($p < 1.75 \times 10^{-6}$).

Figure 2 displays the second swelling study where ethanol was not added to the various pH buffer solutions. Similarly, there was an initial rapid hydration followed by a leveling off around 20 h. In this group, the low molecular weight gels all had swelling ratios around 600% ($0.056 < p < 0.1934$), except for those in the pH 12.0 buffer, which swelled to approximately 700% ($p < 0.006$). The high molecular weight gels also had close ratios, which were slightly higher than 600% ($p = 0.353$), with the exception of those in the basic buffers of pH 10.0 and 12.0, which swelled to 1000 and 900% ratios, respectively ($p < 4.22 \times 10^{-8}$).

The addition of ethanol clearly participated in the swelling profile of the hydrogels. As seen in Figure 1B, ethanol helped increase the swelling ratios, particularly in the acidic and basic buffers. It is possible that the process of transesterification causes the change in the swelling pattern when ethanol comprises 50% of the buffer medium (*14*). Through transesterification, the ethanol reacts with the esters involved in both structures of PEG-MA and PEG-DMA. The more acidic or basic the solution, the greater the degradation of the esters that can occur in the presence of ethanol, thus resulting in the higher degree of hydrogel swelling (or breakup of the hydrogel structures) in the acidic and basic buffers. This explains the loss of pH effect on the hydrogel's swelling in the 50% ethanol buffers, as well as the lower degree of swelling at neutral pH 7 (*14*).

The swelling pattern of the high molecular weight gels that occurred with the buffers not containing ethanol appears to result from the hydrolysis of the ester bonds in the structures of the PEG-MA and PEG-DMA, as seen in Figure 2B. The hydrolysis appears to be base-catalyzed, since the degree of swelling, which ultimately

Table 3. Statistical Model Significance of Temperature Variables (T and T^2) and Significance of Intercept for Optimum Estradiol Release Model

	p values	
variables	low MW	high MW
intercept	0.0047	0.0094
T	0.0061	0.0114
T^2	0.0043	0.0095

is the degradation of the hydrogels' linkages, is higher in the more basic buffer solutions (*5*). The acidic buffers, pH 2 and 4, show little difference in their swelling ratios compared to the neutral buffer, pH 7, suggesting that the alteration to the hydrogel's structure that was synthesized by Yang et al. (*5*) through the removal of the acidic side chains of polyglutamic acid also removed the acidic pH effect on the hydrogel's swelling pattern.

The molecular weight also affects the extent of gel swelling. As seen in Figures 1A and 2A, the low molecular weight gels are only able to swell to about 600−700%, compared to the 900−1000% swelling ratio of the high molecular weight hydrogels. The low molecular weight gels were impeded by the effect of pH, due to their tight structures (shorter PEG chains) (*13*). The molecular weight of the polymers reflects the number of PEG chains; thus, the low molecular weight polymers create shorter cross-links and, therefore, a more compact network structure. The gels appear to have a limit to their swelling and can only swell to a certain extent. The tight network caused by the lower number of PEG chains apparently stops any further swelling, regardless of the pH. The high molecular weight gels have a higher number of PEG chains and, therefore, a greater ability to expand their network structure (*13*). Thus, a greater degree of swelling can be achieved in the high molecular weight gels, and the effect of pH is more readily displayed.

Optimum Release of Estradiol. Temperature (T) was found to be the only statistically significant independent variable with fractional release (Fr) of estradiol at 1 h as the dependent variable. The models are described in eqs 6 and 7 for low and high molecular weight formulations, respectively:

$$\mathrm{Fr} = 3.8663 - 0.203151T + 0.00286T^2 \qquad (6)$$

$$\mathrm{Fr} = 6.15 - 0.3252T + 0.0045T^2 \qquad (7)$$

Table 3 displays the statistical significance of the factors of T, T^2, and the intercept.

The statistical significance of temperature relates back to the process of transesterification: increasing the temperature would increase the degradation by ethanol of the esters involved in the polymer's structure (*14*). Therefore, the higher the degradation of the hydrogel, the more drug that can be released, which is represented by the dependent variable, Fr. Figure 3 displays the graphs of eqs 6 and 7 compared to the data points collected during the statistical release experiments. The model trend above 36 °C is believable; increasing the temperature also increases the process of transesterification, and therefore more drug release should occur. The model trend below 34 °C is not in agreement with the expected decrease in drug release with temperature. While no explanation is available for the contradictory trend, we believe that the high degree of scatter in the data may be responsible for this anomaly.

The other factors of pH and cross-linking ratio were not found to be significant. The insignificance of pH

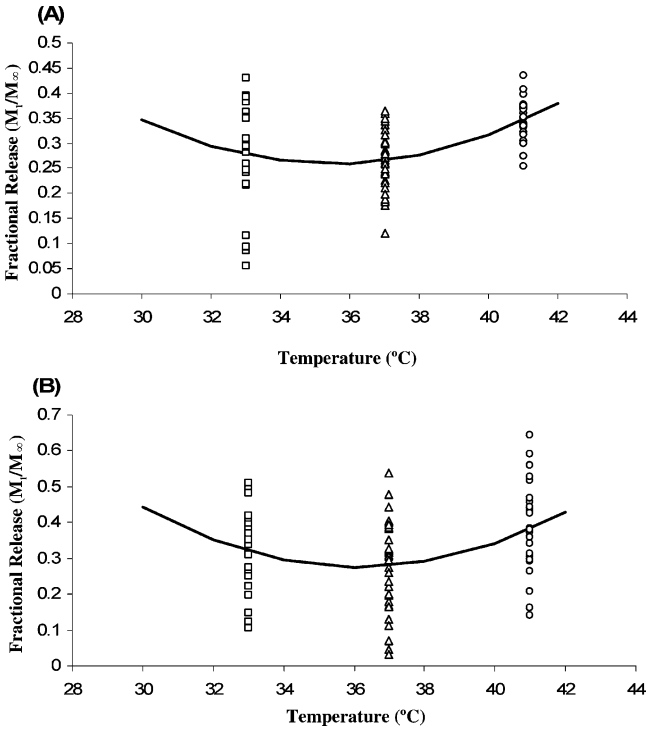

Figure 3. Temperature curves based on equations derived through statistical method for (A) low molecular weight polymers and (B) high molecular weight polymers. Curves compared to data points were found during statistical estradiol release experiments at three studied temperatures. Fractional release (M_1/M_∞) represents release at 1 h compared to total estradiol released after 72 h.

apparently points back to the alteration to the hydrogel structure prepared by Yang et al. (*5*), as described previously. We speculate that the insignificance of the cross-linking ratio can be attributed to the small molecular size of estradiol. The degree of cross-linking, which causes a tightening of the hydrogel's structure, was not large enough to impede the release of estradiol; therefore, estradiol's small size seems to be the most accurate reason for the lack of cross-linking ratio significance. Evidence of the effect of drug size on hydrogel release has been published by Lowman and Peppas (*15*).

Drug Release Studies. To test the drug size hypothesis above, we compared the hydrogel release of estradiol and insulin with pH buffers of pH 2 and 7. Figures 4 and 5 display the release of estradiol and insulin from both low and high molecular weight gels. In Figure 4, the release of estradiol is higher at pH 2 in both low and high molecular weight gels compared to insulin, with 88 and 76% higher fractional releases, respectively. In Figure 5, estradiol release at pH 7 is higher than insulin release in the low molecular weight gels by 83%. Both insulin and estradiol were able to reach 100% fractional release in the high molecular weight gels at pH 7. Thus, the lower release of insulin in the low molecular weight gels and in the acidic buffer, pH 2, suggests that molecular drug size does indeed affect the hydrogel release profile. The low molecular weight gels reached their swelling limit, which apparently does not create a large enough pore-size for most of the insulin to be released. Also, the acidic buffer does not allow much insulin to be released in either molecular weight gels, which suggests that the acid is affecting the release of insulin in a way that does not affect the estradiol release.

Ethanol was not initially used in the release buffers for insulin release; therefore, the experiment was re-

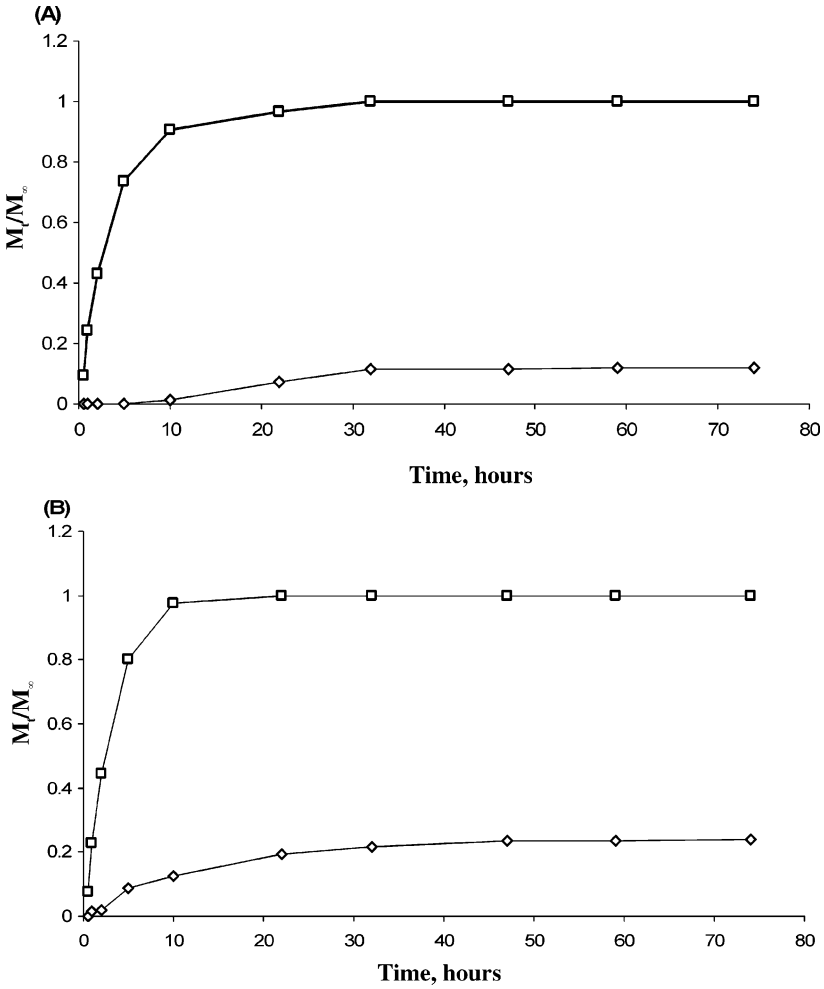

Figure 4. Estradiol (□) and insulin (◇) release profile. Release buffer for insulin was citric acid-buffered saline at pH 2.0. Release buffer for estradiol was the same with 50% ethanol added. Hydrogels were synthesized with a 10:2 cross-link ratio using (A) low molecular weight cross-linkers and (B) high molecular weight cross-linkers. Fractional release, M_t/M_∞, is the ratio of release at time t (M_t) compared to the total amount of estradiol or insulin entrapped within hydrogel (M_∞). Data points represent the averages of triplicate samples.

peated with 50% ethanol buffers, and the results are presented in Figure 6. As shown, no difference in insulin release was observed at both pH 2.0 and 7.0. Again, transesterification of the hydrogel appears to be affecting the swelling and, thus, the drug release of the hydrogels. By increasing the degradation of the gel linkages and, therefore, the hydrogel swelling, transesterification caused higher insulin release in both molecular weight gels and in the pH 2 buffer than was seen previously. Ultimately, ethanol removed any pH, polymer weight, or drug size effects on the insulin drug release.

Release Mechanism. Using the estradiol release data at various pH buffers, temperatures, and cross-linking ratios and fitting it to the Fickian and non-Fickian diffusion eq 2, the values for n, or the slope of the linear regression lines fitted to the $\log(M_t/M_\infty)$ versus log time plots, all resulted in values greater than 0.5 (Table 4), suggesting non-Fickian diffusion. Non-Fickian diffusion is desirable, as it indicates that the media penetration rate is in the same range as drug diffusion (*10*). This finding is confirmed by similarities between our swelling ratio and estradiol release profiles (See Figures 1, 2, 4, and 5).

Through the observations made in this study, the use of hydrogels represents a potentially effective delivery device for hydrophobic drugs. The challenge of drug loss during the synthesis of the hydrogel can be easily surpassed during formulation, but the use of ethanol in the buffer medium to promote drug release needs to be changed, possibly to simulated gastric and intestinal fluids (*16*), because of its effect on the hydrogel drug release. The pH-responsive characteristic of the hydrogel can be easily restored through the addition of an acid such as acrylic or methacrylic acid into the synthesis procedure (*15*, *17*). Therefore, through slight modifications, PEG-MA/PEG-DMA hydrogels may prove to be a beneficial alternative delivery vehicle for a variety of important pharmaceuticals.

Conclusion

This research is a first step toward finding an efficient drug delivery system for hydrophobic drugs. The experiments explored the use of hydrogels for hydrophobic drug release by analyzing the synthesis and pH-responsive characteristics of the PEG-MA/PEG-DMA hydrogel, the ability to promote estradiol release into the surrounding environment, and the significant factors involved in optimal drug release. The results from these experiments support the following conclusions:

(1) PEG-MA/PEG-DMA hydrogels were easily synthesized, and estradiol was able to become entrapped through UV irradiation, although 40−60% of the estradiol added to the synthesis solution was lost due to the hydrophobic nature of the drug.

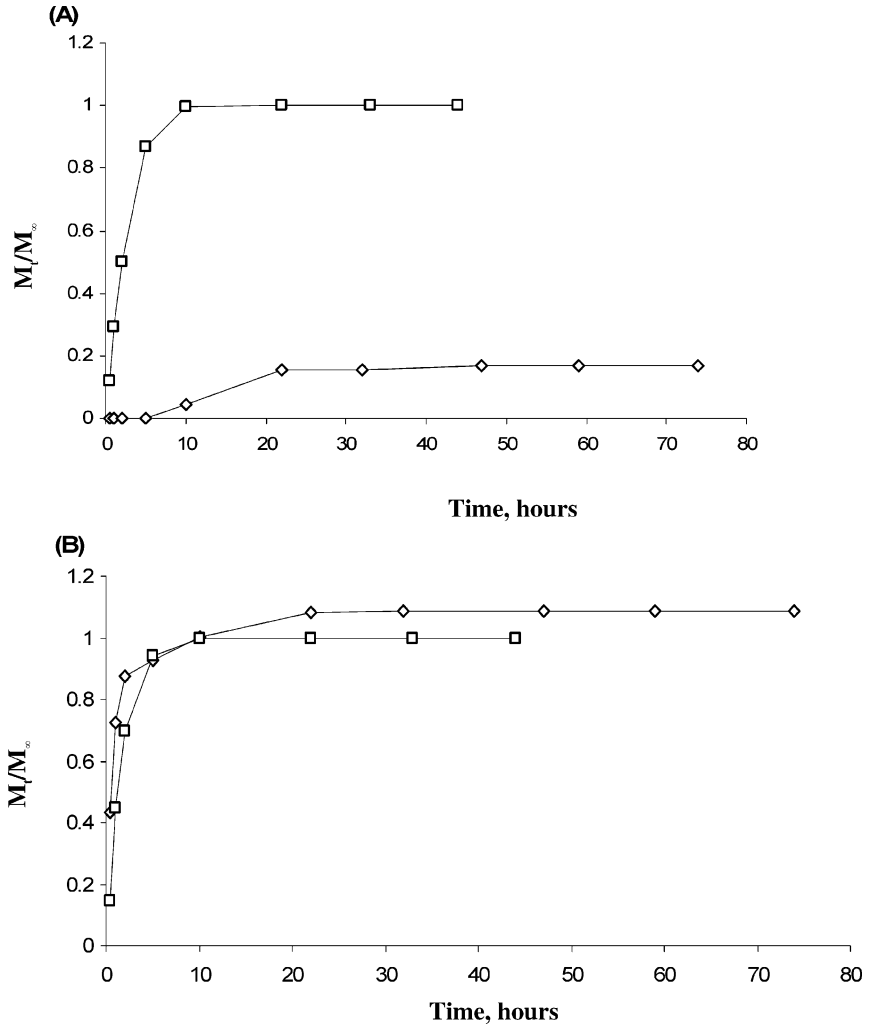

Figure 5. Estradiol (□) and insulin (◇) release profile. Release buffer for insulin was phosphate-buffered saline at pH 7.0. Release buffer for estradiol was the same with 50% ethanol added. Hydrogels were synthesized with a 10:2 cross-link ratio using (A) low molecular weight cross-linkers and (B) high molecular weight cross-linkers. Fractional release, M_t/M_∞, is the ratio of release at time t (M_t) compared to the total amount of estradiol or insulin entrapped within hydrogel (M_∞). Data points represent the averages of triplicate samples.

Table 4. Average *n* Values Found Using Estradiol Release Data at Various Temperatures (33, 37, 41 °C), Crosslinking Ratios (10:1, 10:2, 10:3), and pH (2, 4, 7, 10, 12)[a]

experimental conditions (temp (°C), crosslinking ratio, pH)	average *n* values		experimental conditions (temp (°C), crosslinking ratio, pH)	average *n* values	
	low MW	high MW		low MW	high MW
33, 10:1, 7	0.953	0.9834	37, 10:3, 2	1.071	0.884
33, 10:2, 2	0.9624	0.7881	37, 10:3, 4	1.434	1.14
33, 10:2, 4	0.6647	0.7962	37, 10:3, 10	1.295	1.162
33, 10:2, 10	0.7073	1.13	37, 10:3, 12	0.8593	0.7413
33, 10:2, 12	0.8763	1.4	41, 10:1, 7	0.9719	1.156
33, 10:3, 7	1.085	0.9385	41, 10:2, 2	0.9841	1.11
37, 10:1, 2	1.14	1.61	41, 10:2, 4	0.7915	0.7108
37, 10:1, 4	1.007	1.29	41, 10:2, 10	0.7609	0.7381
37, 10:1, 10	1.373	2.30	41, 10:2, 12	0.978	0.7863
37, 10:1, 12	1.005	0.934	41, 10:3, 7	0.8413	1.031
37, 10:2, 7	1.0886	1.514			

[a] Values of n greater than 0.5 represent non-Fickian diffusion.

(2) The use of ethanol in the release buffer promoted the release of estradiol from the hydrogels but also altered the swelling and release characteristics of the hydrogel, attributed to the transesterification reaction.

(3) The removal of poly(glutamic acid) from the hydrogel synthesis removed the acidic side chains in the hydrogel's structure and, thus, removed the pH-responsive characteristics of the PEG-MA/PEG-DMA gel.

(4) The molecular weight of the polymers used in the hydrogel synthesis, which altered the network structures that were created, also affected the swelling characteristics of the gel: The high molecular weight swelling ratios were 300% higher than the low molecular weight swelling ratios.

(5) Both insulin and estradiol could be effectively entrapped and released from the PEG-MA/PEG-DMA

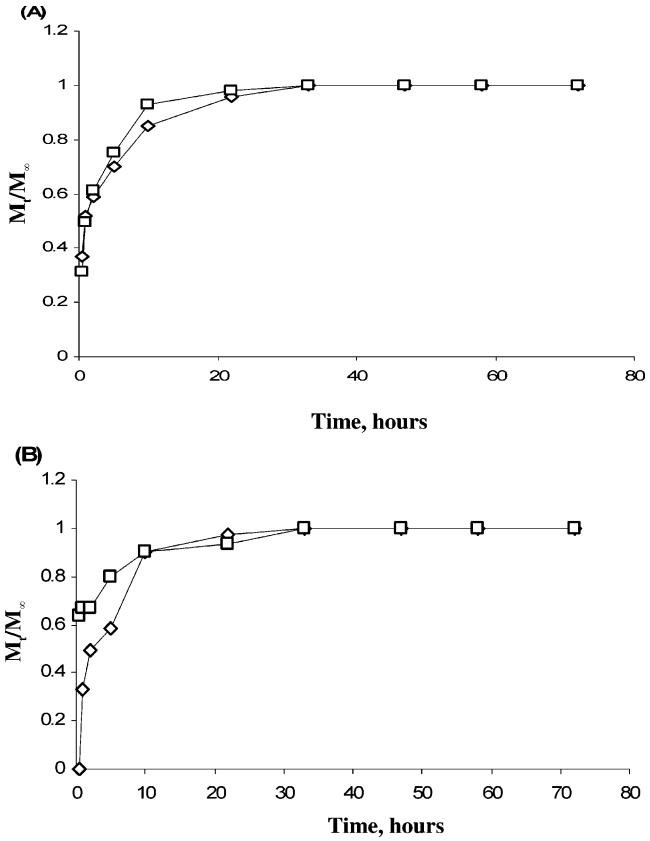

Figure 6. Insulin release based on pH and molecular weight, using 50% ethanol buffers of pH 2.0 (◇) and pH 7.0 (□). Hydrogels were synthesized with a 10:2 cross-linker ratio using (A) low molecular weight cross-linkers and (B) high molecular weight cross-linkers. Fractional release, M_t/M_∞, is the ratio of release at time t (M_t) compared to the total amount of insulin entrapped within hydrogel (M_∞). Data points represent the averages of triplicate samples.

hydrogels, but the size of the insulin drug was a factor. In comparison to 100% estradiol (MW 272) release, the fractional release of insulin (MW 5733) was 12 and 24% in low and high molecular weight gels at pH 2, respectively, and 17% in low molecular weight gels at pH 7.

6) On the basis of the release kinetics of the estradiol drug, the hydrogels displayed a non-Fickian diffusion mechanism, which indicated that the media penetration rate is in the same range as the drug diffusion.

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