POLYETHYLENE GLYCOL METHACRYLATE/DIMETACRYLATE HYDROGELS FOR CONTROLLED RELEASE OF HYDROPHOBIC DRUGS

by

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(Under the Direction of William S. Kisaalita)

ABSTRACT

We report on the release characteristics of a model hydrophobic drug, estradiol, entrapped in polyethylene glycol methacrylate (PEG-MA)/dimethacrylate (PEG-DMA) hydrogels. The crosslinking ratio, temperature, and pH ranged from 10:1 to 10:3, from 33 to 41ºC, 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. Use of high molecular weight polymers resulted in higher hydrogel swelling (300%) in comparison to low molecular weight polymers. Drug size was also found to be a significant factor. Based on the release kinetics of the estradiol drug, the hydrogels displayed a non-Fickian diffusion mechanism, which indicated the media penetration rate was 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 drug release caused complications, attributed to transesterification.

INDEX WORDS: poly(ethylene glycol methacrylate), poly(ethylene glycol dimethacrylate), hydrogels, estradiol, insulin, hydrophobic drug release, ethanol.

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B.S.B.E., University of Georgia, 2002

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTERS OF SCIENCE

ATHENS, GEORGIA

2004

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December, 2004

To my beautiful parents, Pam and John

ACKNOWLEDGEMENTS

These acknowledgements are to extend my sincere thanks and gratitude to those who have provided assistance, support, insight, and knowledge in order to make this research possible. Without their help, I could not have completed this work.

First and foremost, I would like to thank my major advisor Dr. William S. Kisaalita. He has provided wonderful guidance and support through the entire research process, and his encouragement made the project enjoyable and successful. His calming personality and open-door policy was always appreciated and I want to offer him my deepest thanks.

I also wish to thank the other members of my committee, Dr. George Majetich and Dr. Catherine White. They offered excellent guidance and suggestions for my research and I offer them my sincerest thanks for giving their time and energy to the success of my research.

I thank faculty members in the department who provided encouragements and ideas throughout the research process. I would also like to thank Anu, Mike, Clayton, Adam, Javier, Geoff, Sean, Sarah Lee and other colleagues in the department for their help, support, and wonderful graduate lunches.

I also wish to thank Joel Shimkus for his time and energy towards the hydrogel synthesis, as well as his wonderful help with all of my chemistry questions.

I thank Ping Jiang, Dennis Smith, and Dr. Foulger's research team at Clemson University for their invaluable help and insight that made the hydrogel synthesis possible.

I also wish to thank Don Roberts and Dr. John Shields for their time and help with SEM analysis.

In closing, I wish to thank my parents and sister, Joslyn, for all of their love, support, and daily encouragement, without them this work could not have been completed. Finally, I want to thank Barry who gave unending love and support, as well as a wonderful shoulder to lean on.

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CHAPTER 1

INTRODUCTION

Estrogen represents an important hormone to the human body, particularly in females. "Estrogens influence the growth, differentiation, and function of tissues of the female reproductive system, i.e., uterus, ovary, and breast, as well as non-reproductive tissues such as bone and the cardiovascular system" (Parl, 2000). A year after the last menstruation occurs, menopause begins and the ovaries stop production of estrogen, particularly estradiol. After menopause, many physiological changes occur as a result of this loss of estrogen (Lobo, 1997). One of these changes is the experience of hot flushes, which is an increase in peripheral temperature accompanied by a reduction in the core body temperature. A second change is the loss of bone calcium resulting in thin and brittle bones. The loss of calcium in the bones, leads to the process of osteoporosis, which is a disease that causes thinning of the bones leading to an increased risk of bone fractures. A third change is total cholesterol increase accompanied by a decrease in good cholesterol (HDL). This change to the cholesterol results in a higher likelihood of developing coronary artery disease, which subsequently leads to an increased risk of a heart attack. Estrogen also causes a "positive effect on mood and contributes to a sense of well-being. In an estrogen-deficient state, such as occurs after menopause, a higher incidence of depression (clinical or subclinical) is manifest" (Lobo, 1997).

The use of estrogen replacement therapy for post-menopausal women is currently under review due to inconclusive results from experimental studies (Dalton, 2003). Estrogen replacement therapy was found to reverse the changes that occur through the loss of estrogen in

many cases (Lobo, 1997). In these cases, estrogen replacement therapy stopped bone loss and osteoporosis, resulting in reduced hip (25%) and spine (50%) fractures. Estrogen replacement therapy reversed the cholesterol changes by decreasing the total cholesterol and increasing the good cholesterol (HDL), resulting in the decreased likelihood of developing coronary artery disease, thus, estrogen therapy ultimately reduced the risk of dying from a heart attack. Although estrogen replacement therapy appeared to be successful, the effect of long-term use has produced many controversial results (Dalton, 2003), therefore, leaving the ultimate benefits and use of the therapy still to be resolved. The problem with estrogen is the fact that it is a hydrophobic compound, specifically a steroid, which as a drug is very complicated to effectively deliver orally.

Drug delivery technology is constantly progressing through the creation of new techniques and developments that deliver a variety of drugs effectively. These developments benefit numerous patients by achieving a higher compliance and quality of life. The study of hydrogels for drug delivery has been particularly of interest. Hydrogels consist of three-dimensional polymeric networks with excellent water-absorbing capacity and biocompatibility (Pillai and Panchagnula, 2001). 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 studied gels because of their controlled- release characteristics. The release of large protein drugs such as insulin from hydrogels are of great interest. These large molecular weight drugs are too complicated to efficiently deliver into the body, which is also true of steroid drugs. Many protein and steroid drugs exhibit hydrophobicity, as well as instability in environments of extreme pH and proteolytic activity, which cause complications in drug delivery (Pettit and Gombotz, 1998). Therefore, these protein and steroid drugs are often

delivered to the body through injections, which have a low patient compliance. Hydrogels, which have great swelling capacity, can entrap these large molecular weight drugs, and thus release them in a controlled fashion (Pillai and Panchagnula, 2001). Although hydrogels appear to work well with large hydrophilic proteins like insulin, the release of hydrophobic drugs from hydrogels has not been well studied.

AIM

This study focused on hydrophobic drug release from characteristically pH-sensitive hydrogels. A previous study by Yang et al. (2002) 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. (2002) hydrogel synthesis proved to be a challenge. Subsequently, the protocol was altered (Foulger et al., 2001) by removing the poly(glutamic acid) and creating a hydrogel composed only of polyethylene glycol methacrylate [PEG-MA] and polyethylene glycol dimethacrylate [PEG-DMA]. PEG-MA and PEG-DMA reflect the hydrogel's polymer backbone and the ratio of crosslinking between these polymers should alter the drug release characteristics. PEG-MA and PEG-DMA were utilized due to their involvement in the pH-sensitive hydrogels reported in the previous study by Yang et al. (2002), as well as the fact that they are biocompatible. We chose estradiol as a model hydrophobic drug for this study. Estradiol is a steroid, estrogen derivative, which displays important physiological effects in the body and represents an important hormone to the female reproductive system.

Although the use of post-menopausal hormone replacement therapy to provide the missing estradiol due to menopause is still under review, estradiol is necessary for people with other estrogen deficiencies. Estradiol is greatly affected by the digestive enzymes of the GI tract

and could therefore, be utilized with a pH-sensitive hydrogel. A pH-sensitive hydrogel, which characteristically releases at a high pH could protect estrogen in the acidity of the stomach and therefore, release the drug in the more stable and basic environment of the intestine. The aim of this project was to study the hydrophobic drugs pH-controlled release characteristics of PEG-MA/PEG-DMA hydrogels.

The study was carried out through the following specific objectives:

1) Synthesize, characterize, and entrap estradiol in the PEG-MA/PEG-DMA hydrogels
2) Determine the optimum release of estradiol from PEG-MA/PEG-DMA hydrogels with respect to temperature, pH, and crosslinking ratio

Experiments under the first objective constituted the synthesis of the PEG-MA/PEG-DMA hydrogel, which included the determination of the hydrogel's swelling ratio under different pH environments, as well as the hydrogel's entrapment of the model hydrophobic drug, estradiol, and its subsequent release. High and low molecular weight polymers, which provide the structure to the hydrogel (PEG-MA and PEG-DMA), were used in the synthesis in order to determine if the molecular weights are a factor in the entrapment and release of estradiol by the hydrogel. Experiments under the second objective involved statistical evaluation of three variables of pH, temperature, and crosslinking ratio of the hydrogel's backbone polymers, PEG-MA and PEG-DMA, to determine their significance and relative contribution to optimum drug release.

Positive results from this study provide the basis for further development of PEG-MA/PEG-DMA hydrogels for effective drug delivery not only for estrogen, but for other hydrophobic drugs.

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CHAPTER 2

LITERATURE REVIEW

ESTROGEN

Although the use of post-menopausal hormone replacement therapy is under review, estrogen is still needed by those suffering from estrogen deficiencies, particularly young girls who need adequate estrogen levels during the maturation of their reproductive systems (Keiss et al., 2002). The condition of hypogonadism results in the reduced or absent secretion of hormones from the sex glands, which includes the secretion of estrogen. Hypogonadism before puberty causes lack of menstruation and breast development, as well as short height in young girls. After puberty, the occurrence of hypogonadism results in the loss of menstruation, low libido, hot flashes, and the loss of body hair (Keiss et al., 2002). Currently estrogen is taken orally, but the drug is formulated as a derivative of estrogen, because estrogen itself is too rapidly metabolized by the liver through first-pass metabolism. Estrogen is also sensitive to the highly acidic environment of the stomach (Katzung, 2001). The problem with estrogen is the fact that it is a hydrophobic compound, specifically a steroid, which as a drug is very complicated to effectively deliver orally.

PROTEIN AND STEROID DRUG DELIVERY

Protein and steroid drugs are some of the most challenging drugs to deliver into the body. The complications arising with delivering protein drugs are “their large molecular size, their electrical charge and relatively hydrophobic nature (which diminish membrane transport), and their relative instability in environments of extreme pH or proteolytic activity (such as the

stomach and intestine)" (Pettit and Gombotz, 1998). Steroids are normally smaller molecular compounds, but their hydrophobicity, poor absorption, sensitivity to acidic environments (such as the stomach), and deactivation by the liver also cause complications in drug delivery (Katzung, 2001 and Brotherton, 1976). Due to the complications arising with protein and steroid drug delivery, these drugs can often not be delivered orally and have to be delivered through other routes, such as by injection (i.e. insulin), but these other delivery methods have very low patient compliance (Katzung, 2001). Therefore, much research has been conducted to develop better methods for drug delivery of proteins and steroids. Research areas include: delivery methods through the skin, through the lungs, and orally (Langer, 1999).

The delivery through the skin has proven to be relatively inefficient because of the proteins' large size and low electrical charge. The delivery methods through the lungs also have complications through the creation of a disperser for the protein or steroid that will overcome the instability of the protein drug (Langer, 1999). Finally, the oral delivery methods cause problems through the protein and steroid pharmaceuticals' poor permeability through the intestinal epithelium, susceptibility to enzyme attack, rapid post-absorptive clearance, and chemical instability (Pettit and Gombotz, 1998 and Brotherton, 1976). The oral delivery of the protein and steroid drugs provides the most attractive and convenient alternative. A few approaches being developed are the use of microspheres, liposomes, and hydrogels to deliver the large protein drugs. With the microspheres and lipsomes, low uptake usually occurs of the protein drug (Langer, 1999). More promising results have been documented with the hydrogel's drug delivery; therefore, this use of hydrogels for protein and steroid drug delivery is the focus of the research presented in this study.

HYDROGELS FOR CONTROLLED DRUG DELIVERY

Hydrogels are part of a class of synthetic polymeric materials that closely resemble natural living tissue. They have excellent water-absorbing capacity, which allows them to easily swell under certain environmental conditions. Hydrogels have been given the name of 'smart' polymers because of "their ability to undergo structural changes in response to a variety of physical, chemical, and biological stimuli" (Pillai and Panchagnula, 2001, 450). Through various preparations of the hydrogels, a variety of drugs, including proteins, can be entrapped within the hydrogel. As the hydrogel begins to swell, due to various stimuli (see Table 1), the entrapped drug is released.

Table 1: Various stimuli used for triggering drug release from hydrogels (Pillai and Panchagnula, 2001)

Stimulus	Mechanism
pH	pH changes causes swelling and release of drug
Ionic Strength	Change in concentration of ions inside the gel causes swelling and release of drug
Chemical	Formation of charge-transfer complex cause swelling and release of drug
Enzyme-Substrate	Product of enzymatic conversion causes swelling and release of drug
Magnetic	Applied magnetic field causes pore in gel and swelling followed by drug release
Thermal	Change in polymer-polymer and polymer-water interactions causes swelling and drug release
Electrical	Change in charge distribution causes swelling and drug release
Ultrasound irradiation	Temperature increase causes release of drug

Depending on the properties of the drug, different release behaviors will arise. Therefore, when preparing the hydrogel for a specific drug, the synthesis is "tailored considering the physical properties of the drug, loading level, and desired release kinetics" (Kim et al., 1992). Presently,

the trend in hydrogel technology for drug delivery is to develop functional preparations that respond to the environment in terms of swelling or drug release rate (Kim et al., 1992).

There are currently five hydrogels in research and development, including: (1) superporous hydrogel and superporous hydrogel composites, (2) Silk-elastinlike protein polymers, (3) pH/temperature sensitive hydrogels, (4) PEG-MA/PEG-DMA or EGDMA hydrogels, and (5) Varieties of polymer-based hydrogels. One of the newer types of hydrogels is the superporous hydrogel (SPH) and superporous hydrogel composite. These new generations of hydrogels swell and absorb water rapidly, which allows entrapped drugs to be released quickly (Dorkoosh et al., 2000). The SPH can swell quickly, regardless of their size and shape, "due to the presence of open pores forming capillary channels" (Chen and Park, 2000). Through these channels, water is taken up into the dried hydrogels by capillary rise. The fully swollen superporous hydrogel is mechanically weak, thus, their mechanical strength needs to be increased (Chen and Park, 2000). Therefore, the difference between the superporous hydrogel and the superporous hydrogel composite is that the SPH swells faster, but the SPH composite has had some type of composite added to provide the mechanical stability needed.

In order to make a superporous hydrogel, "a monomer, crosslinker, water (if necessary), foam stabilizer, acid, polymerization initiator, initiation catalyst (if any), and foaming agent" are required (Chen et al., 1999). The SPH composite is normally synthesized with the same materials, but with the addition of some type of composite, allowing for more mechanical stability. Ac-Di-Sol, which is cross-linked sodium carboxymethylcellulose, is commonly used as the added composite (Dorkoosh et al., 2000).

Once the SPH or SPH composite polymer solutions are created, they are added to some type of drug solution, normally a protein or peptide. The polymer solutions will "suck up the

total amount of the peptide solution," thus entrapping the drug inside the swollen polymer (Dorkoosh et al., 2002a). The polymers are dried overnight and then ready for drug release studies. One study found that the SPH and SPH composites could entrap and effectively release buserelin, octreotide, and insulin, which represent three peptide drugs of varying molecular weights (Dorkoosh et al., 2002a). Another study found that human insulin could be effectively released via the hydrogels inside live test animals (pigs) (Dorkoosh et al., 2002b). Therefore, SPH and SPH composites are providing very positive results as drug delivery devices for proteins and peptides and might prove to be highly successful as pharmaceutical agents.

A second type of hydrogel is the silk-elastinlike protein polymer. "Silk-elastinlike protein-based polymers (SELP) are genetically engineered biopolymers composed of repeated sequences of amino acid blocks derived from silk (Gly Ala-Gly Ala-Gly Ser) and elastin (Gly Val-Gly Val-Pro)" (Dinerman et al., 2002). The unique property of these hydrogels is their insensitivity to environmental stimuli such as pH, temperature, and ionic strength, which is not characteristic of most other hydrogels. Therefore, the silk-elastinlike hydrogels could prove effective "for localized drug delivery where stimuli-sensitivity is not required" (Dinerman et al., 2002). In another study, the silk-elastin protein compositions, termed Prolastins, were synthesized as water soluble solutions that once injected into the body's physiological conditions would undergo spontaneous gelation and thus, release any mixed compound or drug in a controlled fashion (Cappello et al, 1998). Therefore, the silk-elastin polymers have potential as two different drug delivery methods that could prove to be effective for protein delivery.

A third type of hydrogel is the pH/temperature sensitive hydrogel. These hydrogels are composed of polymers known for their pH and/or temperature sensitivities. Compounds like N-isopropylacrylamide (NIPAAm) and N,N'-diethylaminopropyl methacrylamide (DMAPMAAm)

exhibit a pH-sensitivity around a pH of 7.4 and a temperature-sensitivity around 37°C. Therefore, when these materials are used to synthesize hydrogels, the hydrogels will display pH and temperature-sensitivities. Protein drugs have been studied by entrapping the drugs within these pH/temperature-sensitive hydrogels. Environmental variations will "elicit a change in the pore size of gel matrix, allowing the entrapped protein drug to be released in a stimuli-responsive fashion" (Park, 1999). The environmental variations, particularly the change in pH, will allow for site-specific delivery to specific regions in the gastrointestinal tract. The hydrogels are synthesized with pH-sensitivity that favors swelling under higher pH. Thus, the hydrogels will release the drug in environments of high pH, such as the intestine, and not in environments of low pH, such as the stomach (Yin et al., 2002). One study used these pH/temperature-sensitive hydrogels as delivery devices based on the squeezing hydrogel concept, which utilizes the swelling-deswelling characteristics of the hydrogel's sensitivity to release entrapped drugs in a controlled 'on-off' profile (Gutowska et al., 1997). Protein drugs, such as insulin, have been found to be effectively delivered and released by the pH/temperature-sensitive hydrogels in a controlled rate fashion, warranting further investigation into these hydrogels

A fourth type of common hydrogel is created using polyethylene glycol methacrylate (PEG-MA) and polyethylene, or just ethylene, glycol dimethacrylate (PEG-DMA or EGDMA) as its polymer backbone. This type of hydrogel is commonly found to be pH-responsive, which means the amount of hydrogel swelling increases with increasing pH. One study presented by Park et al. (2003) created hydrogels using PEG-MA, EGDMA, and acrylic acid. This hydrogel was formed under γ-ray irradiation and was able to load insulin into its matrix. The hydrogel easily absorbed and released the insulin, and its release was pH-responsive. A second study created a hydrogel using PEG-MA and PEG-DMA. The final gelation of this hydrogel occurred

under UV-irradiation and it is at this step that materials could become entrapped within the hydrogel. This study mainly focused on embedding a crystalline colloidal array within the hydrogel's matrix during UV-irradiation (Foulger et al., 2001). A study by Markland et al. (1999) worked with PEG-MA and PEG-DMA hydrogels, but added poly(glutamic acid) to the PEG side-chains in order to add a biodegradable property to the gel. The hydrogel was therefore, a poly(glutamic acid)-poly(ethylene glycol) hydrogel, referred to as a PLG-PEG hydrogel. In their first study using the PLG-PEG hydrogel, Markland et al. (1999) found that the enzyme, lysozyme, could be easily loaded into the hydrogel when it was swollen and would only be released through a de-swelling of the hydrogel. The study found that, "variables involved during the synthesis of the PLG-PEG hydrogel, such as PLG concentration, PEG loading, and molecular weight of the PEG, were found to influence significantly the swelling properties of the polypeptide hydrogel" (Markland et al., 1999). In a second study, a new class of PLG-PEG hydrogel was synthesized. This time, the hydrogel drug entrapment and crosslinking was done using UV-induced photopolymerization instead of the swelling/diffusion process that was used before. The UV-induced photopolymerization allowed for a higher amount of high-molecular-weight drug to be loaded into the hydrogel. Again, the hydrogels were found to increase in swelling as the pH increased, but now the release of the entrapped protein drugs, insulin, lysozyme, and albumin, were found to also be released with increasing pH. This new PLG-PEG hydrogel provided very positive results for protein drug loading and release, and by varying the crosslinking density of PLG to PEG, different drug release profiles were obtained (Yang et. al., 2002). Another study by Lowman and Peppas (1999) found that a pH-responsive hydrogel prepared with polyethylene glycol and polymethacrylic acid could absorb a variety of drugs ranging in molecular weights from 238 to 4400. The release of the drugs depended on their

molecular weights with the lowest molecular weight drug, proxyphylline, showing little effect on release due to pH, while pH effect was a much larger factor with the higher molecular weight drugs, vitamin B_{12} and FITC-dextran. Thus, Lowman and Peppas (1999) displayed that drug release is not only affected by changes in pH, but the molecular weight of the drug compared to the pore size of the hydrogel under the various pH-conditions also plays an important role.

The final types of hydrogels are a variety of polymer-based hydrogels ranging from dextran-based to sucrose-based. The dextran-based hydrogels were found to be stable under physiological conditions (pH 7 and 37° C) and had effective protein drug release profiles, of human interleukin-2, that could be varied by altering their crosslink density (Cadee et. al., 2002). The sucrose-containing hydrogels were prepared using sucrose acrylate (SA) as the monomer and PPH as the entrapped model drug. Using these hydrogels, the drug release profiles were studied and found to follow a near zero-order pattern in both low and high pH solutions, which indicated that the hydrogels "can be exploited for oral drug delivery in both stomach and intestinal regions of the GI tract" (Shantha and Harding, 2001). Another type of hydrogel consists of the polymers poly(N-vinyl-2-pyrrolidone) (PVP) and poly(acrylamide-co-itaconic acid) (PAAm-co-IA). This hydrogel can also be loaded with peptide and protein drugs and is ultimately pH dependent. The swelling as well as drug release increases as the pH increases, with the maximum amount of drug released at a pH of 7.4. This property of the hydrogel allows for a useful drug delivery device that is colon-specific because of the colon's known pH of 7.4 (Bajpai and Sonkusley, 2001).

ESTROGEN ANALYSIS

Once estrogen is entrapped within the hydrogel, an appropriate and effective analysis procedure must be used to determine the estrogen release from the hydrogel. As time has passed and technology has advanced, the analysis of steroids, particularly estrogens, has changed. The first approaches to estrogen analysis were very involved, time-consuming, and also required competence in the techniques of chromatography. The methods normally involved a first step of purification of the extracts containing the estrogens. After extraction, the amount of estrogens was determined using the Kober reaction or sulfuric acid-induced fluorescence. The Kober reaction was quite specific for estrogens and allowed the researcher to quantify the amount of estrogens in the final purified product (Dale, 1967).

The second approach to estrogen analysis involved high-performance liquid chromatography (HPLC). In this procedure, the estrogen-containing samples are hydrolyzed, followed by estrogen extraction and purification, accomplished through liquid-liquid extraction or classical absorption chromatography. Once extracted, the estrogens are separated through liquid chromatography. In order to be analyzed by liquid chromatography, the sample must be soluble in the mobile phase. Estrogens are soluble over a "wide range of mobile-phase compositions," which allows for several different types of liquid chromatography separation modes to be employed. The two main types of separations are reverse-phase chromatography and liquid-solid chromatography. Once the estrogens are separated by liquid chromatography, they can be detected and measured either through ultraviolet absorption detectors or fluorescence spectroscopy (Kautsky, 1981).

The third and most recent approach to estrogen analysis is the use of enzyme-linked immunoabsorbent assay (ELISA) that is commercially available (Cayman Chemical, Ann Arbor,

Michigan). ELISA uses the principle of antibody-antibody interaction, which allows for easy visualization of results. First, a 96-well plate is prepared by pre-coating it with a suitable antibody; estrogen analysis uses a mouse monoclonal antibody as its pre-coating antibody. Next, a second antibody is used that binds to the molecule in question, in this case, estrogen, as well as to the pre-coated antibody. For estrogen testing a rabbit antiserum is used for the antibody. The ELISA for estrogen also utilizes an estradiol tracer, which is estradiol linked to an acetylcholinesterase. The concentration of tracer added to the sample is held constant, while the concentration of estrogen in the sample varies. The tracer competes with the estrogen in the sample for the rabbit antiserum's binding sites. After binding occurs to both the rabbit antiserum and thus to the mouse monoclonal antibody, the plate is washed to remove any unbound species and Ellman's Reagent is added to the well. A product is produced from this enzymatic reaction that has a distinct yellow color and absorbs strongly at 412 nm. The intensity of the color is determined spectrophotometrically and is proportional to the amount of estradiol tracer bound to the bottom of the plate. The amount of tracer found is inversely proportional to the amount of free estrogen (estradiol) present in the sample (Gorog, 1989). Therefore, ELISA accurately determines the amount of estrogen in the sample more quickly and less tediously than the previous procedures and can be purchased as a kit (Cayman Chemical, Ann Arbor, Michigan).

A final analysis utilizes spectrophotometry for estrogen analysis. Estradiol can be effectively analyzed by a spectrophotometer at 280 nm under UV light, when dissolved in ethanol (Dawson et al., 1986). This analysis procedure is relatively simple and quick and is also quite inexpensive. As the technology advances in steroid analysis, the tediousness, amount of time required, and competence in several scientific procedures decreases. The newest approach of ELISA for estrogen analysis appears accurate, and direct, but can be very time-consuming and

expensive. Therefore, the use of spectrophotometry for the analysis of estrogen being released from the hydrogels seems efficient and was used in this study.

RELEASE MECHANISM

Hydrogel matrixes are considered swelling-controlled systems, because the drug release is controlled by the inward flux of solvent (Peppas et al. 2000). These swelling-controlled systems are often analyzed with Fickian and non-Fickian diffusional behavior kinetics. Equation 1 displays the simplified expression for Fickian and non-Fickian diffusion that the estradiol release data can be fitted against:

$$M_t/M_\infty = kt^n \quad (1)$$

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 (Brahim et al., 2003). Eq. (1) 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_∞) versus log time plots (Kortesuo et al., 2001). A calculated n equal to 0.5 represents Fickian diffusion, while a calculated n greater than 0.5 represents non-Fickian diffusion. Therefore, using the calculated n value, the diffusional behavior of the hydrogel release can be determined.

PRELIMINARY EXPERIMENTS AND HYPOTHESES

Using the knowledge gained from the current state of hydrogel research, the protein drug release profiles found from the PLG-PEG hydrogels appeared most promising for the release of estrogen drugs. The second study by Yang et al. (2002) on the PLG-PEG hydrogels utilized the crosslinking method of UV irradiation, which seemed efficient for the entrapment of the estrogen drug. Using the irradiation method, no degradation should occur to the drug as the crosslinking

occurs, and a large amount of drug could be trapped within the gel unlike the previous crosslinking swelling/diffusion process (Yang et al., 2002). In our trials, we were not able to replicate the hydrogel synthesis reported by Yang et al. (2002). Subsequently, the hydrogel synthesis was altered to the synthesis procedure presented by Foulger et al. (2001), in which only poly(ethylene glycol) methacrylate and poly(ethylene glycol) dimethacrylate were used. This procedure also utilized UV photopolymerization to complete gelation, which should allow for the entrapment of the estrogen drug within the hydrogel's matrix.

The effect of pH on the PEG-MA/PEG-DMA hydrogel's release of estrogen should follow the previous studies, with little release at low pH and the majority of the release at high pH (Yang et al., 2002). This type of pH-responsive release will create a feasible profile for the hydrogel in the body's physiological conditions. The estrogen drug needs to be released at a higher pH, between 7 to 9, which represents the pH of the intestine. The stomach's acidic pH of 3 will quickly degrade the drug; therefore, if the hydrogel demonstrates little, if any, drug release at a pH of 3, the hydrogel should protect the estrogen drug until it reaches the optimal release environment in the intestine.

The effect of temperature is another important factor to the hydrogel's drug release. Optimum release should occur around 37°C in order for the hydrogel to be effective inside the body's conditions. The PEG-MA/PEG-DMA hydrogel should be mainly pH-responsive and not temperature-responsive, with release profiles favoring 37°C (Yang et al., 2002). This type of hydrogel responsiveness will prove beneficial and effective for estrogen release.

Finally, by altering the PEG-MA:PEG-DMA ratio during crosslinking, the estrogen release profile should change with an increase in the release as the ratio is decreased. By altering the ratio, the most effective estrogen release profile, i.e., efficacious, can be obtained.

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CHAPTER 3

STUDY OF POLYETHYLENE GLYCOL METHACRYLATE/DIMETHACRYLATE HYDROGELS FOR CONTROLLED RELEASE OF HYDROPHOBIC DRUGS[1]

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ABSTRACT

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 (360/550) and high (526/1000) molecular weight polyethylene glycol methacrylate (PEG-MA)/dimethacrylate (PEG-DMA). The crosslinking 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 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. Based on the release kinetics of the estradiol drug, the hydrogels displayed a non-Fickian diffusion mechanism, which indicated 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 (Peppas et al., 2000). 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 (Park, 1999). The release of large protein drugs such as insulin from hydrogels is of great interest because these large molecular weight drugs are normally delivered to the body through injections, with low patient compliance (Katzung, 2001). Hydrogels, which have great swelling capacity, can entrap these large molecular weight drugs, and thus release them in a controlled fashion (Pillai and Panchagnula, 2001). Although hydrogels appear to work well with large hydrophilic proteins like insulin (Yang et al., 2002), 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. (2002) 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. (2002) hydrogel synthesis was unsuccessful. Subsequently, the protocol was altered (Foulger et al., 2001) by removing the poly(glutamic acid) and creating a hydrogel composed only of polyethylene glycol methacrylate [PEG-MA] and polyethylene glycol dimethacrylate [PEG-DMA], hereafter referred to as PEG-MA/PEG-DMA . PEG-MA/PEG-DMA should retain the original pH-dependant release

characteristic and by varying the ratio between these polymers, the resultant crosslinking 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 post-menopausal women has now come under question (Dalton, 2003), estrogen is still needed by patients suffering from estrogen deficiency, e.g., young girls for maturation of their reproductive systems (Kiess et al., 2002).

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 which characteristically 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, two-fold. First, to synthesize, characterize, and entrap estradiol in the PEG-MA/PEG-DMA hydrogels. Second, to determine the optimum release of estradiol from the PEG-MA/PEG-DMA hydrogels with respect to temperature, pH, and crosslinking ratio.

MATERIALS AND METHODS

Materials

Estradiol, insulin, *n*-vinyl pyrrolidinone and 2,2-dimethoxy-2-phenylacetophenone (hydrogel initiator compounds), poly(ethylene glycol) methacrylate (MW360 and MW526), as well as poly(ethylene glycol) dimethacrylate (MW550) were all obtained from Sigma-Aldrich (St. Louis). Poly(ethylene glycol) dimethacrylate with 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 ethanol and 1 mL of 0.01*M* phosphate buffer containing 0.9 wt % sodium chloride was added to the dissolved estradiol (Yang et al., 2002). Next, 200 µL 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 (MW550) was next added (Foulger et al., 2001). 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 (Yang et al., 2002). 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 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 minutes 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 (Yang et al., 2002).

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 1.0N HCl or 1.0N 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

In order 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 30ºC. 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 37ºC in an Innova 4000 temperature-controlled incubator shaker (New Brunswick Scientific). 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 (Yang et al., 2002):

$$SR = M_{wet} / M_{dry} \quad (2)$$

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

Drug Release Procedure

In order 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 deionized water for 24 hours. 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 hours at various temperatures depending on the study. For the final release studies comparing estradiol to insulin release, the temperature was set to 37ºC and the only buffers used were pH 2, 7, and 12. At selected time intervals the 1 mL of buffer was pipetted out and analyzed spectrophotometerically and 1 mL of fresh buffer was added to the sample. The amount of estradiol or insulin 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. For the drug release studies, the fractional amount of drug released over time was calculated based on 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 hours. As shown in Table 2, estradiol losses occurred due to its hydrophobicity.

Drug Release Mechanism

The controlled-swelling characteristic of the hydrogel allows its release kinetics to be analyzed with Fickian and non-Fickian diffusional behavior (Peppas et al., 2000). Equation 3 displays the model which the estradiol release data can be fit to:

$$M_t/M_\infty = kt^n \quad (3)$$

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 (Brahim et al., 2003). Eq. (3) 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_∞) versus log time plots (Kortesuo et al., 2001). 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 crosslinking 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. (1991), more than one factor can be changed at a time allowing for an optimum to be found with a smaller number of experimental runs. The effects of the factor interactions on the response can be obtained that is not available with the one-factor-at-a-time approach. Normally, an unknown response function:

$$Y = f(X_1, X_2, \ldots X_K) \quad (4)$$

exists that relates Y to K factors. Using a low-order polynomial, the function can be estimated in the region on interest. Equation (5) displays the first-order model:

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_K X_K = \beta_o + \sum_i \beta_i X_i \quad (5)$$

and Equation (6) displays the second-order model:

$$Y = \beta_o + \sum_i \beta_i X_i + \sum_i \beta_{ii} X_i^2 + \sum \sum_{i<j} \beta_{ij} X_i X_j \quad (6)$$

where β_o is the y-axis intercept, the β_is are the K first-order coefficients, and the 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 crosslinking 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. (6). 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.

The general linear model (GLM) procedure available in the SAS statistical software package was used to estimate the intercept and coefficients in Eq. (6). GLM uses the method of least squares to fit general linear models and works with both balanced and unbalanced designs (Kisaalita, 1991).

Scanning Electron Microscopy

Freshly prepared 10:1 low and high molecular weight hydrogels were first prepared, following procedures described before, and then freeze-dried overnight. The freeze-dried

samples were kept under vacuum until they were ready for microscopy. Before the samples were scanned, they were mounted onto an aluminum stud and sputter-coated with gold/palladium (Kim and Chu, 2000). The surfaces of the hydrogels were then analyzed with a LEO 982 Field emission scanning electron microscope (FE-SEM, LEO Electron Microscopy, Inc. Thornwood, NY) at 4.0 kV.

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 crosslinkers react under UV-irradiation and allow for gelation of the polymers into a three-dimensional hydrogel network (Yang et al., 2002). Both the high and low molecular weight hydrogels were easily synthesized with various crosslinking ratios of PEG-MA to PEG-DMA. The gelation occurred within 3 minutes of exposure to UV irradiation for the low molecular weight gels and within 5 minutes of exposure for the high molecular weight gels. Both of the low molecular weight crosslinkers have a decreased amount of PEG chains, which apparently accounted for the faster gelation time of these hydrogels as well as their tighter and more compacted structures (Lowman, 2004).

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 hour release experiment. Due to the hydrophobic nature of estradiol, a large amount of drug was lost during synthesis either left in the various pipette tips or in the glass

mixing vessel. A large amount of drug was also found to remain in the gel after a 72 hour release. This was determined by dissolving the gel overnight and measuring the remaining estradiol spectrophotometrically. The hydrogels were loaded with 90 to 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 pipette tips and 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 (Kortesuo et al., 2001). The swelling behavior of both the low and high molecular weight gels were 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 hours, 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 exception to pH 4.0, with a slightly lower ratio ($p < 0.0014$) and pH 7.0, where the swelling ratio was lower by approximately 150 to 200% ($p < 1.75E-6$).

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 hours. 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 exception to those in the basic buffers of pH 10.0 and 12.0, which swelled to 1000 and 900% ratios, respectively ($p < 4.22E-8$).

The addition of ethanol clearly participated in the swelling profile of the hydrogels. Ethanol helped increase the swelling ratios, particularly in the acidic and basic buffers. It is possible the process of transesterification causes the change in the swelling pattern when ethanol comprises 50% of the buffer medium (Wikipedia, 2004). 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 (Wikipedia, 2004).

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. The hydrolysis appears to be base-catalyzed, since the degree of swelling, which ultimately is the degradation of the hydrogels' linkages, is higher in the more basic buffer solutions (Yang et al., 2002). The acidic buffers, pH 2 and 4, show little difference in their swelling ratios compared to the neutral, pH 7, buffer, suggesting that the alteration to the

hydrogel's structure that was synthesized by Yang et al. (2002) through the removal of the acidic side chains of polyglutamic acid, also removed the acidic pH effect to the hydrogel's swelling pattern.

The effect of molecular weight also affects the extent of gel swelling. The low molecular weight gels are only able to swell to about 600 to 700%, compared to the 900 to 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) (Lowman, 2004). The molecular weight of the polymers reflects the number of PEG chains, thus, the low molecular weight polymers create shorter crosslinks and therefore, a more compact network structure. The gels appear to have 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 (Lowman, 2004). Thus, a greater degree of swelling can be achieved in the high molecular weight gels and the effect of pH more readily displayed.

Figure 3 displays the scanning electron microscope images of the 10:1 low and high molecular weight blank hydrogels. The rippled texture of the hydrogel surfaces is rough for the low (A) and smooth for the high (B) molecular weight structures, suggesting a tighter network (shorter PEG chains) for the low molecular weight hydrogel. Also, the tighter nature of the low molecular weight was probably responsible for the cracking of the hydrogel during freezing. These results are consistent with the swelling characteristics in Figures 1 and 2.

Optimum Release of Estradiol

Temperature (T) was found to be the only statistically significant independent variable with fractional release (Fr) of estradiol at 1 hr as the dependant variable. The models are described in equations 7 and 8 for low and high molecular weight formulations, respectively:

$$Fr = 3.8663 - 0.203151\ T + 0.00286\ T^2 \quad (7)$$

$$Fr = 6.15 - 0.3252\ T + 0.0045\ T^2 \quad (8)$$

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 temperatures would increase the degradation by ethanol of the esters involved in the polymer's structure (Wikipedia, 2004). Therefore, the higher the degradation of the hydrogel, the more drug that can be released, which is represented by the dependant variable, Fr. Figure 4 displays the graphs of Equations 7 and 8 compared to the data points collected during the statistical release experiments. The model trend above 36ºC is believable; increasing temperature also increases the process of transesterification and therefore, more drug release should occur. The model trend below 34ºC 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 scatter in the data may be responsible for this anomaly.

The other factors of pH and crosslinking ratio were not found to be significant. The insignificance of pH apparently points back to the alteration to the hydrogel structure prepared by Yang et al. (2002), as described previously. We speculate the insignificance of the crosslinking ratio to be attributed to the small molecular size of estradiol. The degree of

crosslinking, which causes a tightening to the hydrogel's structure, was not large enough to impede the release of estradiol, and therefore, estradiol's small size seems the most accurate reason for no crosslinking ratio significance. Evidence of drug size effect on hydrogel release has been published by Lowman and Peppas (1999).

Drug Release Studies

To test the drug size hypothesis above, we compared the hydrogel release of estradiol and insulin with pH buffers of 2 and 7. Figures 5 and 6 display the release of estradiol and insulin from both low and high molecular weight gels. In Figure 5, 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 6, 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 a pH 7. Thus, the lower release of insulin in the low molecular weight gels and in the acidic, pH 2, buffer, 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 repeated with 50% ethanol buffers and the results are presented in Figure 7. 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 to the insulin drug release.

Release Mechanism

Using the estradiol release data at various pH buffers, temperatures, and crosslinking ratios and fitting it to the Fickian and non-Fickian diffusion Eq. (1), the values for *n*, or the slope of the linear regression lines fitted to the log (M_t/M_∞) 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 (Kortesuo et al., 2001). This finding is confirmed by similarities between our swelling ratio and estradiol release profiles (See Figures 1, 2, 5 and 6).

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 (Chen and Park, 2000), because of its affect 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 (Lowman and Peppas, 1999; Park et al., 2003). 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 towards 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.
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 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) Based on the release kinetics of the estradiol drug, the hydrogels displayed a non-Fickian diffusion mechanism, which indicated the media penetration rate is in the same range as the drug diffusion.

ACKNOWLEDGEMENTS

The authors thank Dr. Catherine White for her time and energy towards making this a successful project. We also thank Dr. Foulger's research team at Clemson University, especially Ping Jiang, for their helpful insight into the PEG-MA/PEG-DMA hydrogel synthesis. Finally, we would like to thank Dr. John Shields and Don Roberts for their SEM work on the hydrogel's surface structures.

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Table 1: Design matrix for optimization of hydrogel drug release.

pH	temperature	crosslinking 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

Table 2. Average estradiol lost during synthesis and within hydrogel's matrix after 72 hour 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%)

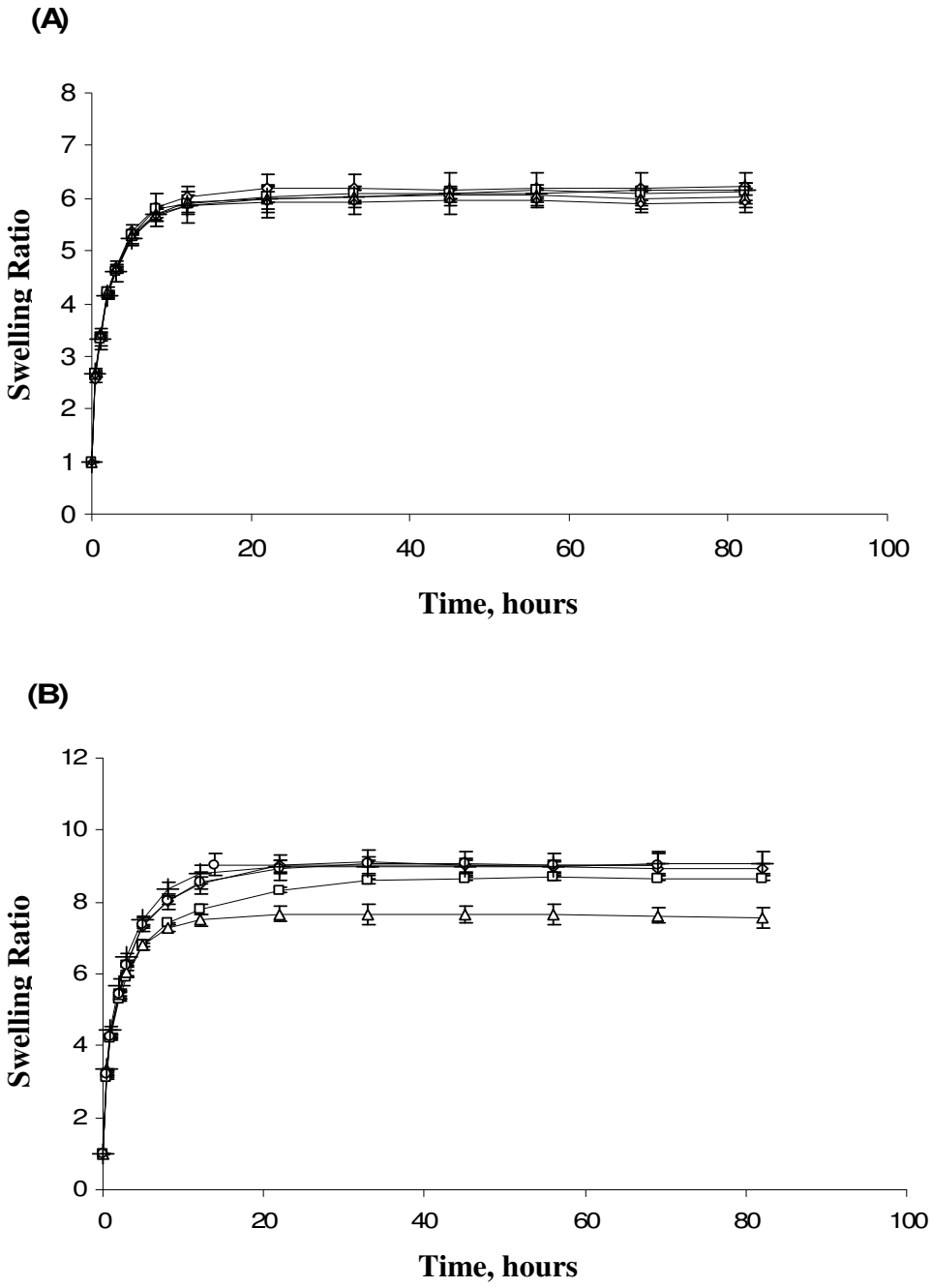


Figure 1. Effects of swelling behavior based on molecular weight, using 50% ethanol buffers of pH 2.0 (◇), pH 4.0 (□), pH 7.0 (△), pH10.0 (○), and pH 12.0 (+). Hydrogels were synthesized with 10:1 crosslinker ratio using (A) low molecular weight crosslinkers and (B) high molecular weight crosslinkers. Error bars represent the standard deviation of triplicate samples. Swelling ratio = ratio of wet hydrogel weight to initial dry hydrogel weight.

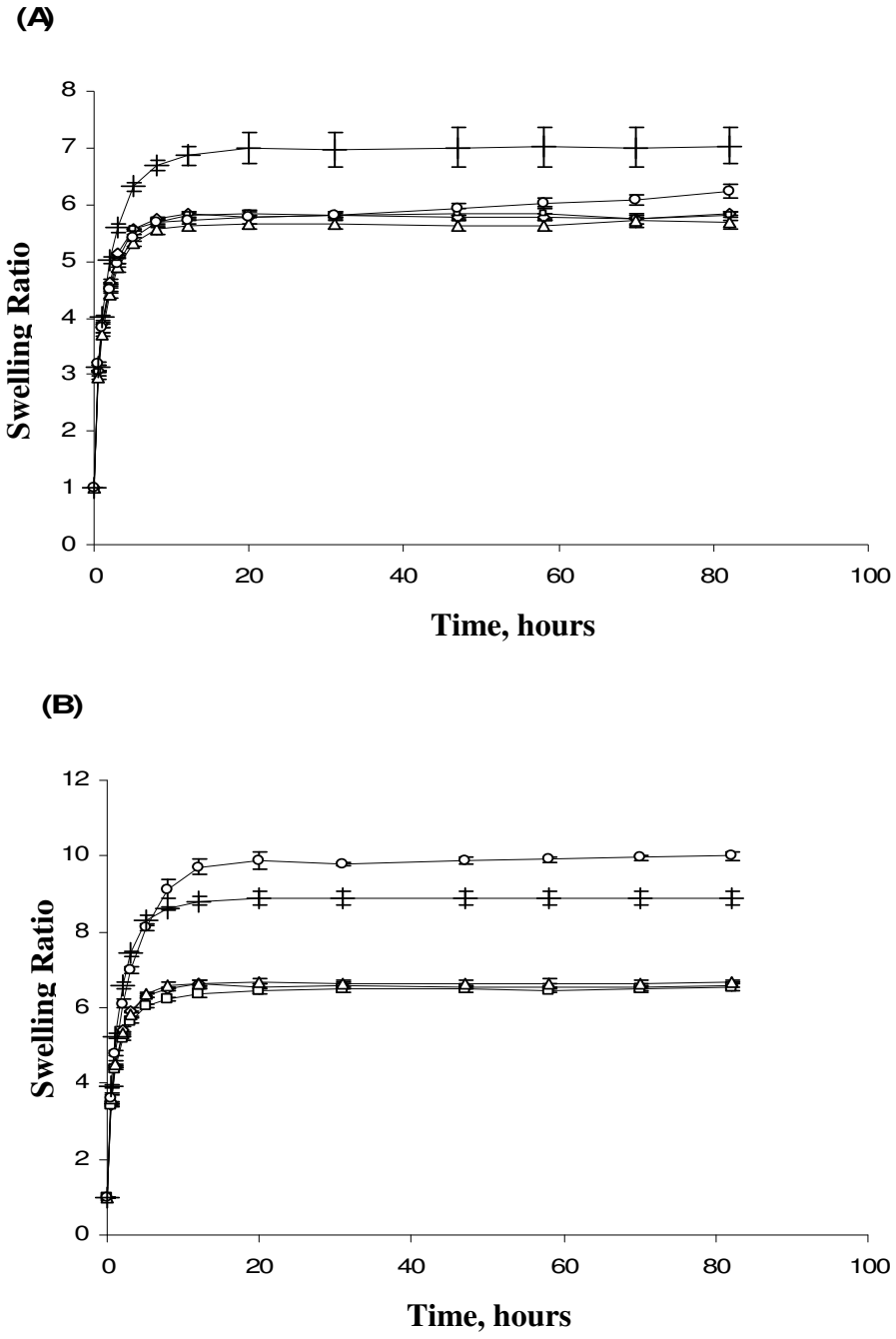
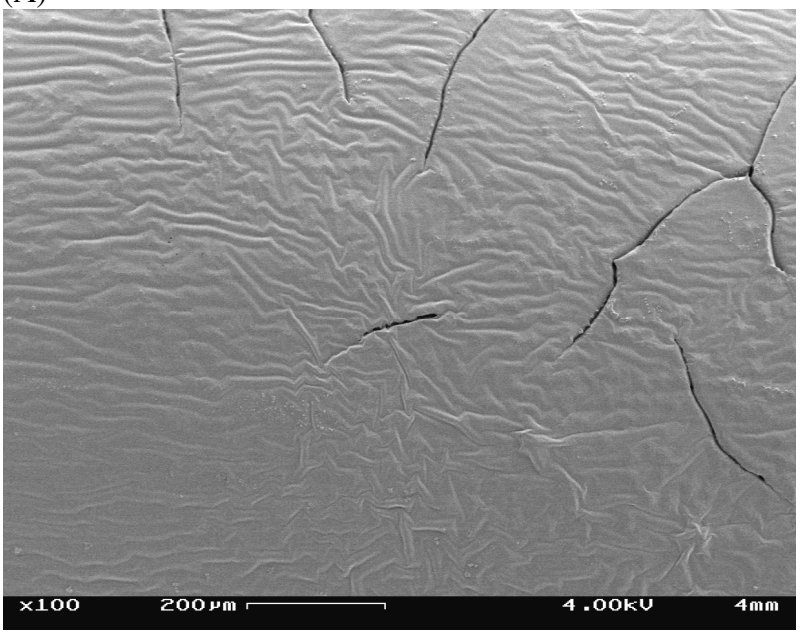


Figure 2. Effects of swelling behavior based on molecular weight, using buffers with no ethanol of pH 2.0 (◇), pH 4.0 (□), pH 7.0 (△), pH10.0 (○), and pH 12.0 (+). Hydrogels were synthesized with 10:1 crosslinker ratio using (A) low molecular weight crosslinkers and (B) high molecular weight crosslinkers. Error bars represent the standard deviation of triplicate samples. Swelling ratio = ratio of wet hydrogel weight to initial dry hydrogel weight.

(A)



(B)

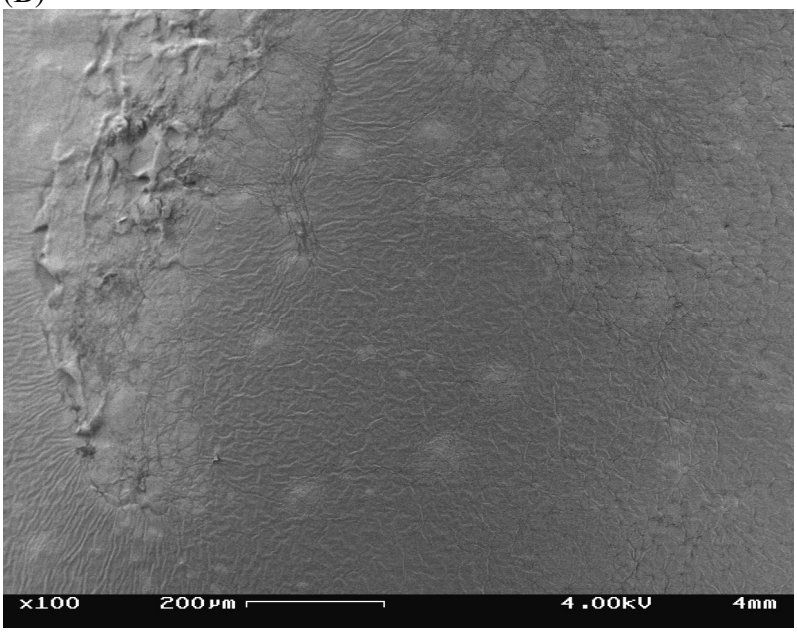


Figure 3. SEM images of 10:1 blank hydrogel surface with (A) low molecular weight polymers and (B) high molecular weight polymers.

Table 3. Statistical model significance of temperature variables (T and T^2), as well as significance of intercept for optimum estradiol release model.

Variables	P values	
	Low MW	High MW
Intercept	0.0047	0.0094
T	0.0061	0.0114
T^2	0.0043	0.0095

Table 4. Average *n* values found using estradiol release data at various temperatures (33, 37, 41°), crosslinking ratios (10:1, 10:2, 10:3), and pH (2, 4, 7, 10, 12). An *n* value greater than 0.5 represents non-Fickian diffusion.

Experimental Conditions	Average *n* values	
	Low MW	High MW
33, 10:1, 7	0.953	0.9834
33, 10:2, 2	0.9624	0.7881
33, 10:2, 4	0.6647	0.7962
33, 10:2, 10	0.7073	1.13
33, 10:2, 12	0.8763	1.4
33, 10:3, 7	1.085	0.9385
37, 10:1, 2	1.14	1.61
37, 10:1, 4	1.007	1.29
37, 10:1, 10	1.373	2.30
37, 10:1, 12	1.005	0.934
37, 10:2, 7	1.0886	1.514
37, 10:3, 2	1.071	0.884
37, 10:3, 4	1.434	1.14
37, 10:3, 10	1.295	1.162
37, 10:3, 12	0.8593	0.7413
41, 10:1, 7	0.9719	1.156
41, 10:2, 2	0.9841	1.11
41, 10:2, 4	0.7915	0.7108
41, 10:2, 10	0.7609	0.7381
41, 10:2, 12	0.978	0.7863
41, 10:3, 7	0.8413	1.031

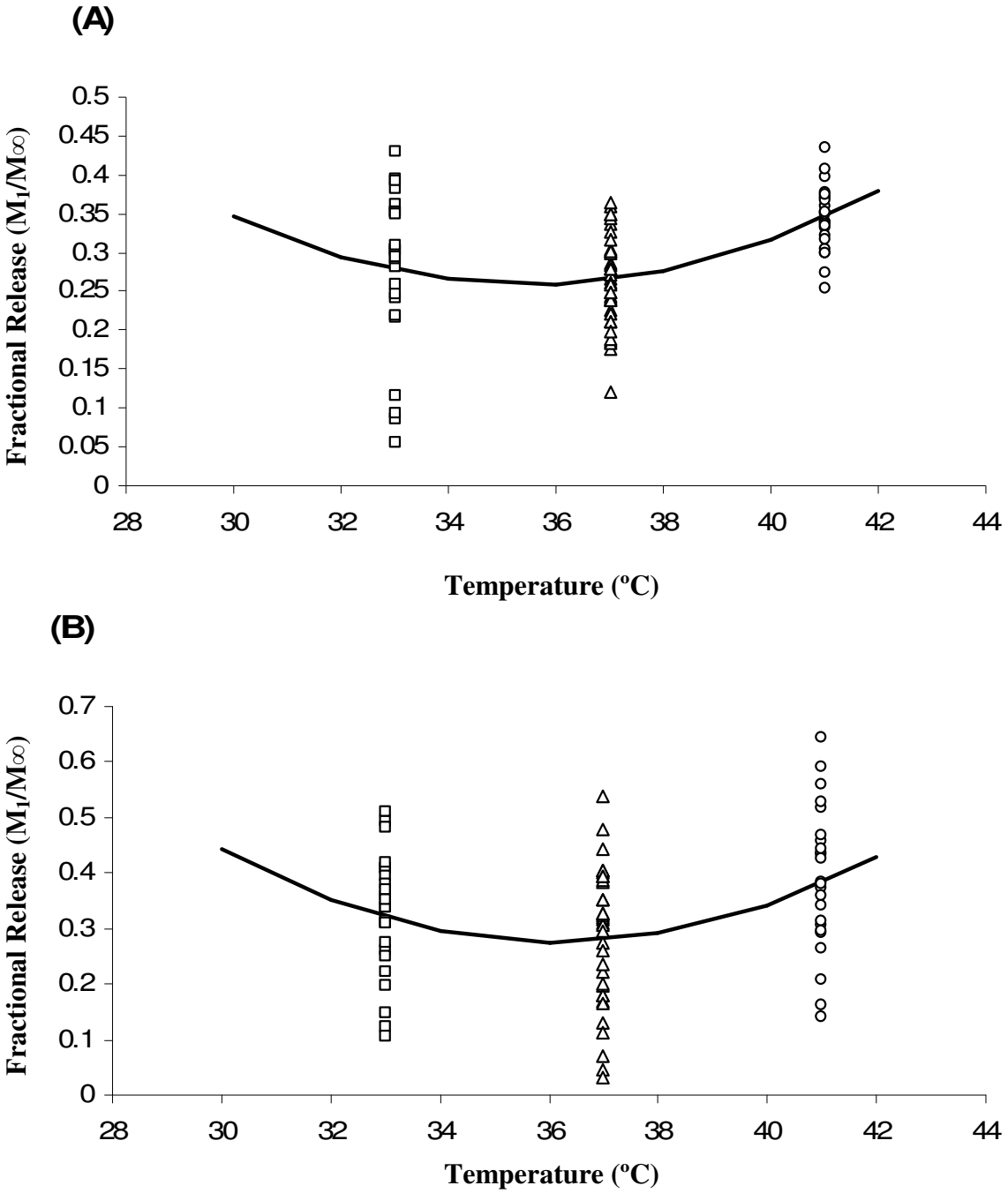


Figure 4. Temperature curves based on equations derived through statistical method for (A) low molecular weight polymers and (B) high molecular weight polymers. Curves compared to data points found during statistical estradiol release experiments at three studied temperatures. Fractional release ($M_1/M\infty$) represents release at 1 hour compared to total estradiol released after 72 hours.

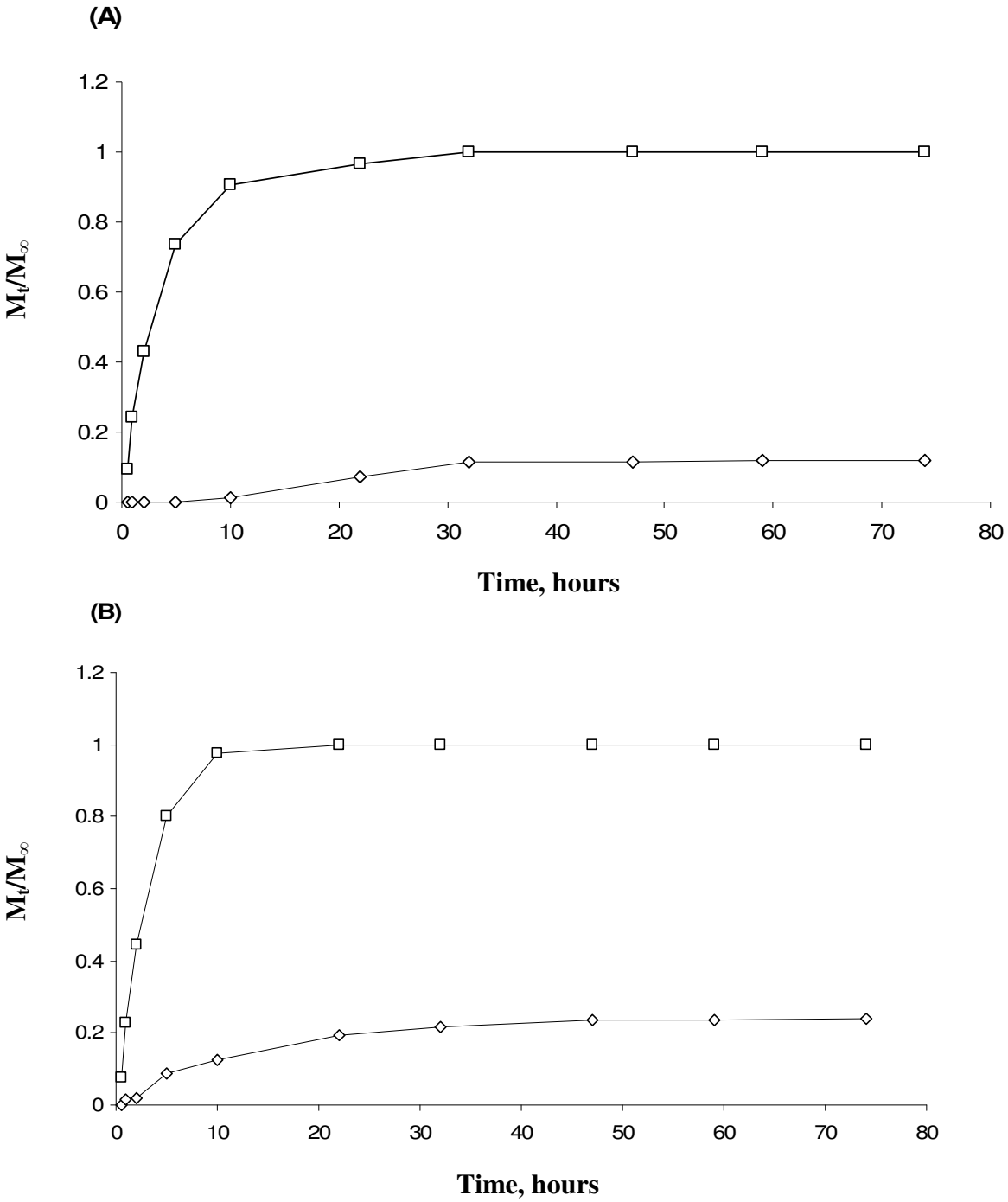


Figure 5. Estradiol (□) and Insulin (◇) release profile. Release buffer for insulin was citric acid-buffered saline at pH 2.0. Release buffer for estradiol was the same with 50% ethanol added. Hydrogels were synthesized with 10:2 crosslink ratio using (A) low molecular weight crosslinkers and (B) high molecular weight crosslinkers. 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.

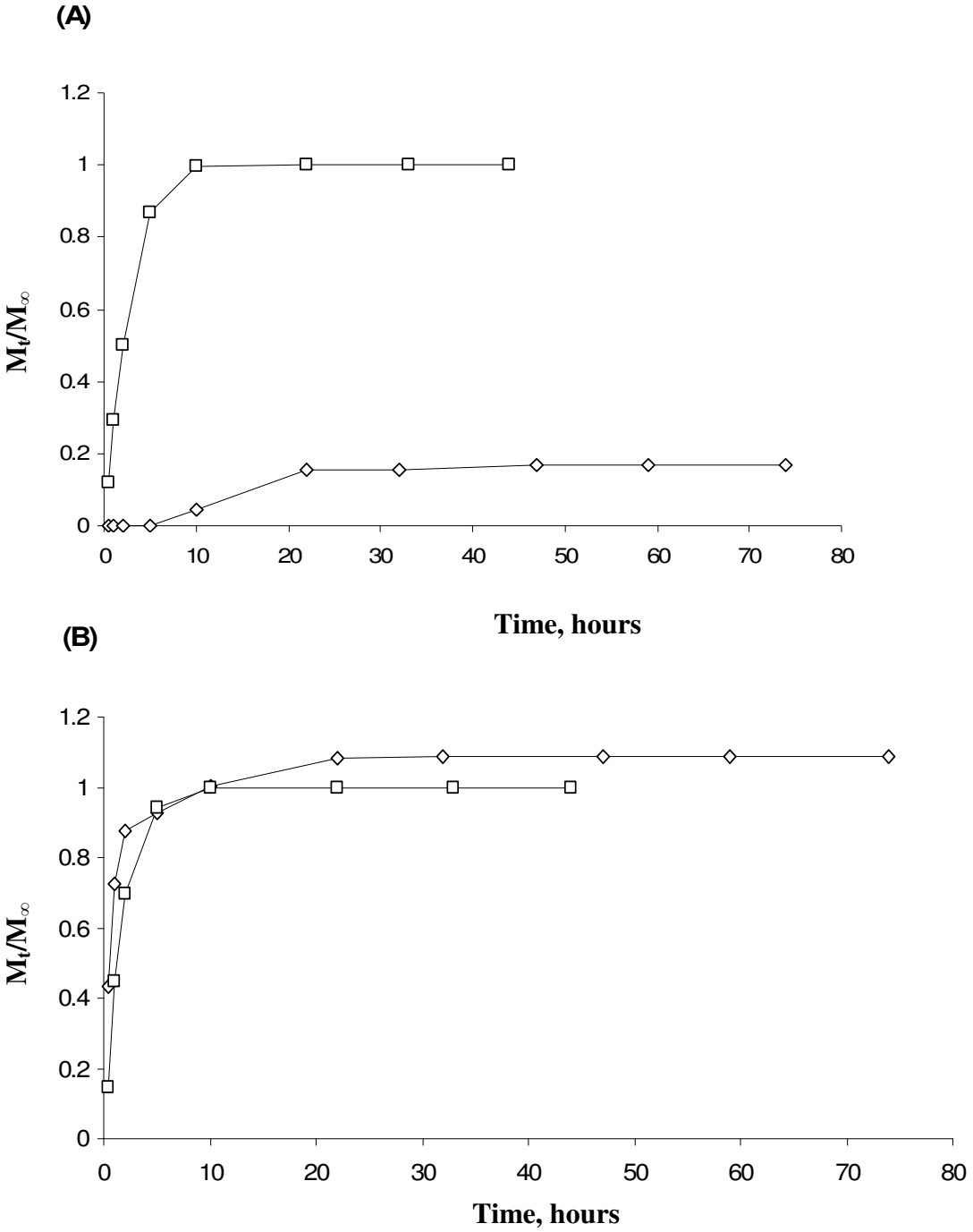


Figure 6. Estradiol(□) and Insulin (◇) release profile. Release buffer for insulin was phosphate-buffered saline at pH 7.0. Release buffer for estradiol was the same with 50% ethanol added. Hydrogels were synthesized with 10:2 crosslink ratio using (A) low molecular weight crosslinkers and (B) high molecular weight crosslinkers. 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.

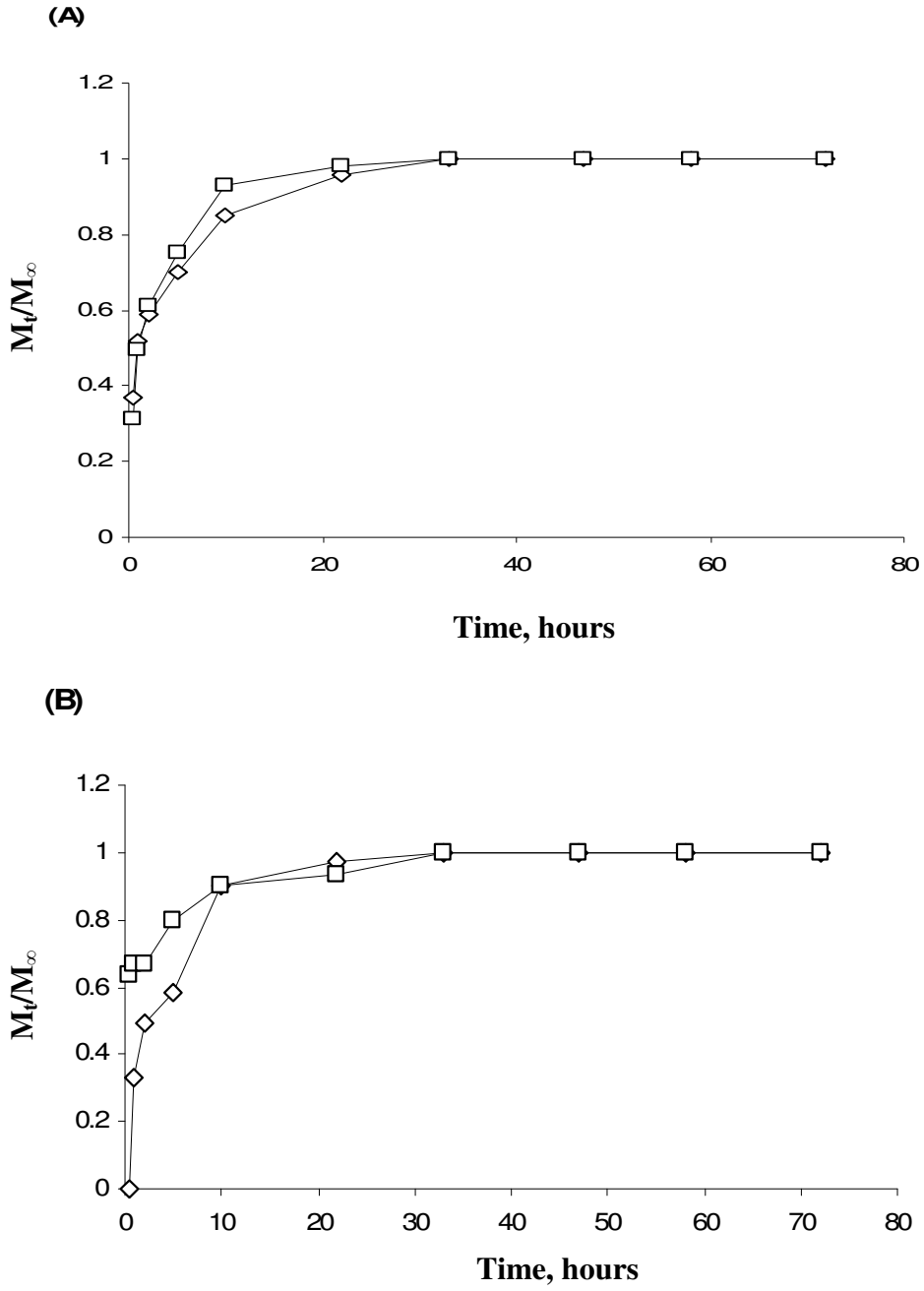


Figure 7. Insulin release based on pH and molecular weight, using 50% ethanol buffers of pH 2.0 (◇) and pH 7.0(□). Hydrogels were synthesized with 10:2 crosslinker ratio using (A) low molecular weight crosslinkers and (B) high molecular weight crosslinkers. Fractional release, M_t/M_∞, is the ratio of 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.

CHAPTER 4

FUTURE DIRECTIONS

The studies conducted in this research introduced a feasible way to entrap and release the model hydrophobic drug, estradiol from PEG-MA/PEG-DMA hydrogels. A large drug loss during loading was found, but this can be easily overcome. Promoting the solubilization of estradiol by addition of ethanol to the release buffer worked, but also caused a problem of increasing the degradation to the hydrogel's linkages. Also, the alteration to the hydrogel synthesis through the removal of acidic side chains in the hydrogel structure caused a loss of pH-sensitivity. To test the optimum release condition idea successfully, the following experimental system can be proposed:

1) Synthesize PEG-MA/PEG-DMA hydrogel with the addition of an acid, such as acrylic or methacrylic acid, in order to return a pH-sensitivity to the hydrogel's swelling and release pattern (Lowman and Peppas, 1999; Park et al., 2003).
2) Try salinization of mixing vessels and pipette tips to prevent excess hydrophobic drug loss during hydrogel synthesis.
3) Select a variety of molecular weight hydrophobic drugs for hydrogel entrapment and release studies, such as estradiol (MW270), chlomiphene (MW600), and cyclosporine A (MW1200) (Sigma-Aldrich).
4) Determine a more efficient composition of buffer medium that does not use ethanol for promotion of hydrophobic drug release, i.e. simulated gastric and intestinal fluids (Chen and Park, 2000).

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APPENDIX A

LABORATORY PROTOCOLS

A1. Hydrogel Synthesis

Materials:

- Estradiol (or insulin)
- Ethanol
- 0.01*M* Phosphate Buffered Saline containing 0.9 wt % sodium chloride
- Poly(ethylene glycol) Methacrylate (MW 360), PEG-MA360
- Poly(ethylene glycol) Methacrylate (MW 526), PEG-MA526
- Poly(ethylene glycol) Dimethacrylate (MW 1000), PEG-DMA1000
- Poly(ethylene glycol) Dimethacrylate (MW 550), PEG-DMA550
- 2,2-dimethoxy-2-phenylacetophenone
- *N*-vinyl-pyrrolidinone
- LWUV Lamp (11.6 mW/cm^2)

Procedure:

1. Preparation of Initiator.
    a) Weigh out 150 mg 2,2-dimethoxy-2-phenylacetophenone into small flask.
    b) Add 0.5 mL of *n*-vinyl-pyrrolidinone to flask and mix well.
    c) Store in refrigerator when not using.
2. Preparation of Hydrogels.
    a) Weigh 1.2 mg of estradiol into mixing vessel.
    b) Add 0.2 mL ethanol to vessel and mix to dissolve estradiol.
    c) For low MW gels: Add 200 μL PEG-MA360 to vessel.

For high MW gels: Add 200 μL PEG-MA526 to vessel.

d) For low MW gels and 10:1 ratio [PEG-MA:PEG-DMA]: Add 20 μL PEG-DMA550 to vessel; 10:2 ratio: Add 40 μL PEG-DMA550 to vessel; 10:3 ratio: Add 60 μL PEG-DMA550 to vessel.

For high MW gels and 10:1 ratio: Add 20 μL PEG- DMA1000 to vessel; 10:2 ratio: Add 40 μL PEG-DMA1000 to vessel; 10:3 ratio: Add 60 μL PEG-DMA1000 to vessel.

e) Add 2.5 μL initiator to vessel and mix thoroughly.

f) By cutting off end of plastic 1 mL syringe, Pipet ~0.8 mL of hydrogel solution into open end of syringe.

g) Clamp syringe upright and expose to UV lamp, irradiating for ~5 min (enclose lamp and syringe with foil).

h) Gently push solid hydrogel from syringe and wash with distilled water.

i) Repeat irradiation with rest of hydrogel solution.

j) Allow hydrogels to dry and then cut into 1 mL cylinders, usually produces ~15 gels.

k) For blank hydrogels, repeat same process but do not add estradiol to vessel in beginning.

l) For insulin-entrapped gels, repeat same process but with 1.2 mg insulin and instead of ethanol, add ~2 drops 0.1*M* HCL.

A2. Preparation of Release Buffers

Materials:

- 2 m*M* citric acid
- 2 m*M* sodium citrate
- 2 m*M* sodium hydroxide (NaOH)
- 2 m*M* boric acid
- 2 m*M* potassium chloride (KCl)
- 200 proof ethanol
- 0.01*M* phosphate buffered saline containing 0.9 wt % sodium chloride

Procedure:

1) pH 2.0 Release Buffer
    a. In flask, combine 1.0 mL NaOH (2 m*M*) with 10 mL citric acid (2 m*M*), dilute to 100 mL with deionized water.
    b. Add 100 mL ethanol, if using for estradiol release.
    c. Stir on stir plate and using pH meter, bring pH down by adding drops of 1N HCl.
2) pH 4.0 Release Buffer
    a. In flask, combine 33.0 mL citric acid (2 m*M*) with 17.0 mL sodium citrate (2 m*M*), dilute to 100 mL deionized water.
    b. Add 100 mL ethanol, if using for estradiol release.
    c. Stir on stir plate and using pH meter, bring pH down by adding drops of 1N HCl.

3) pH 7.0 Release Buffer
    a. In flask, combine 100 mL PBS solution (composition described above) with 400 mL deionized water.
    b. Add 500 mL ethanol, if using for estradiol release.
    c. Stir on stir plate and using pH meter, bring pH down by adding drops of 1N HCl.
4) pH 10.0 Release Buffer
    a. In flask, combine 50 mL boric acid (2 m*M*) with 43.7 mL NaOH (2 m*M*), dilute to 100 mL with deionized water.
    b. Add 100 mL ethanol, if using for estradiol release.
    c. Stir on stir plate and using pH meter, bring pH to 10.0 by adding drops of 1N HCl (to bring pH down) or 1N NaOH (to bring pH up).
5) pH 12.0 Release Buffer
    a. In flask, combine 25 mL KCl (2 m*M*) with 6 mL NaOH (2 m*M*), dilute to 100 mL with deionized water.
    b. Add 100 mL ethanol, if using for estradiol release.
    c. Stir on stir plate and using pH meter, bring pH up by adding drops of 1N NaOH.

A3. Calibration Curves for Release Buffers

Materials:

- pH 2.0, 4.0, 7.0, 10.0, and 12.0 release buffers
- Estradiol or Insulin
- UV-VIS Spectrophotometer

Procedure:

1) For each release buffer, weigh out 1 mg estradiol into mixing vessel and then add 1 mL of specific release buffer to vessel and mix to dissolve estradiol (or insulin).
2) Using 8 separate spectrophotometer cuvettes, prepare following concentrations of estradiol/insulin solution:

Concentration(µg/mL)	Added Release Buffer	Added Estradiol/Insulin Solution
Blank (0 µg/mL)	1000 µL	0
0.5	999.5 µL	0.5 µL
1.0	999 µL	1.0 µL
5.0	995 µL	5.0 µL
10.0	990 µL	10.0 µL
20.0	980 µL	20.0 µL
50.0	950 µL	50.0 µL
100.0	900 µL	100.0 µL

3) Measure spectrophotometer absorbance readings for each cuvette at 280 nm UV (222 nm UV for insulin curves) and record data.
4) Graph Concentration vs. Absorbance and plot linear regression to achieve equation to relate concentration of estradiol/insulin solution to its absorbance. Equation will be used during release experiments to determine concentration of estradiol (or insulin) released from hydrogels at certain time intervals.
5) Repeat procedure for each release buffer.

A4. Hydrogel Swelling Ratio Studies

Materials:

- 10:1 Blank hydrogels
- Release buffers of various pH's (2, 4, 7, 10, 12); 50% ethanol buffers and release buffers with no ethanol added

Procedure:

1) Preparation of Hydrogels
    a. Prepare 10:1 low and high blank hydrogels
    b. Dry hydrogels in dry-air incubator at 30ºC for 2 days
2) Swelling Studies
    a. Label 5 mL plastic tubes to keep track of triplicate gels and various pH buffers
    b. Weigh dried gels before beginning swelling study and record weight
    c. Place dried gels in tubes and add 1 mL of appropriate buffer solution
    d. Place tubes in test-tube holders and put into temperature-controlled shaker at 37ºC and 125 rpms
    e. At selected time intervals pipette out 1 mL of buffer solution from tubes, gently dry gels, and then weigh gels and record weight
    f. Return gels to their respected tubes and add 1 mL of fresh buffer solution; place back into shaker
    g. Repeat steps e-f until end of study (~80 hours)

A5. Drug Release Studies

Materials:

- Blank and estradiol (or insulin)-entrapped hydrogels
- Release buffers of various pH's (depending on study, see **A2**); 50% ethanol buffers and release buffers with no ethanol added for insulin release

Procedure:

1) Preparation of Hydrogels
    a. Prepare needed hydrogels for release study
    b. For any estradiol release studies, place estradiol-entrapped gels and blank gels in 15 mL plastic tubes with 10 mL of deionized water and allow to leak out any unreacted polymers for 24 hours
    c. Allow insulin-entrapped gels to sit overnight instead of in deionized water
2) Hydrogel Release
    a. Label 5 mL plastic tubes to keep track of triplicate gels and various pH's and/or crosslink ratios
    b. After 24 hours, place gels in respected tubes for release and pipette 1 mL of appropriate release buffer
    c. Place tubes in test tube holders and put into temperature-controlled shaker at correct temperature (33ºC, 37ºC, or 41ºC) and shake at 125 rpms.
    d. At various time intervals, pipette out 1 mL release buffer and into 1 mL UV-grade spectrophotometer cuvettes

e. Pipette 1 mL fresh release buffer back into hydrogel tubes and put tubes back into shaker, until next time interval

f. Read spectrophotometer cuvettes at 280 nm for estradiol-entrapped gels and 222 nm for insulin-entrapped gels, and record absorbance readings

g. Repeat steps d-f until study ends (usually ~70-80 hours).

A6. Hydrogel Dissolution Studies.

Materials:

- Hydrogels after 72 hour release experiment (blank and estradiol-entrapped)
- Dichloromethane
- Ethanol
- Coffee filters

Procedure:

1) Dissolution of Gels
    a. Place triplicate gels into 10 mL glass flat-bottom vials with magnetic stir-bars, label vials to keep track of blank or estradiol-entrapped gels
    b. Add 3 mL of a 50/50 dichloromethane/ethanol solution to vials
    c. Place 4 vials onto center of magnetic stirrer and stabilize them
    d. Allow vials to spin overnight
2) Funneling of Gel Particulates
    a. Add 3 mL of a 50/50 dichloromethane/ethanol solution to each vial to dilute gel solution
    b. Funnel gel solution with coffee filters into 15 mL plastic tubes
3) Spectrophotometer Readings
    a. Pipette 1 mL of filtered blank gel solution into 1 mL UV-grade spectrophotometer cuvettes
    b. Use blank gel solution as blank in spectrophotometer readings

c. Pipette 1 mL of filtered estradiol-entrapped gel solution into 1 mL UV-grade spectrophotometer cuvettes and read against blanked gel solution
d. After reading and recording spectrophotometer absorbances, keep filtered blank gel solutions and perform calibration curve as described in **A3**, but use filtered blank gel solution as blank and add known amounts of estradiol to it to create concentration vs. absorbance curve.
e. Linear regression equation that is created through calibration curve will determine concentration of estradiol remaining in gels after 72 hour release through the readings recorded in step c.

A7. Statistical Estradiol Release Experiment Using SAS

Procedure:

1) Estradiol release conducted at various temperatures (33ºC, 37ºC, and 41ºC), crosslink ratios (10:1, 10:2, and 10:3), and in various pH buffers (2, 4, 7, 10,
2) Table 1 displays statistical setup to run experiments, each temperature setting represents 1 batch and the respected pH buffers and crosslinked gels were run in that batch. Drug release protocol is the same at this point.
3) After all three temperature batches were run, the data was collected and the fractional release at 1 hour was determined to be the best statistical variable to use in SAS program as dependent variable.
4) SAS program created and the data needed for program is the temperature (T), pH (Ph), crosslink ratio (R), and the appropriate fractional release (FR) for the respected factors of T, PH, and R. The following is an example printout of SAS program:

```
*SAS with all combined stats;
*LOWmw and fractional release(Fr);

      data expt;
            input T pH R Fr;
            cards;
33 7 1 .381683
33 7 1 .361658
33 7 1 .429944
41 4 2 .337
41 4 2 .349
41 4 2 .3597
37 2 1 .2388
37 2 1 .2432
37 2 1 .30135
     Etc.
run;
proc print;
run;
proc glm;
      model Fr = T Ph R T*T Ph*Ph R*R T*Ph T*R Ph*R;
run;
```

5) After running program for both low and high molecular weight gels, only the temperature variable was determined to be significant. Therefore, the program was run again, but only the variable of temperature was used in the model equation. The model is run as above only the end is changed:

```
proc glm;
      model Fr = T T*T;
run;
```

6) The output created from the program creates the temperature equation that represents the statistical pattern seen with the release data.

APPENDIX B

RAW DATA FOR CHAPTER 3

Table B.1. Swelling Ratio Data for Hydrogels in Various pH Buffers

Column Title	Definition
Dry Weight (g)	Hydrogel weight measured after 2 days of drying
Wet Weight (g)	Hydrogel weight after swelling in particular pH buffer at selected times
Hydrogels	Various hydrogels in each pH buffer (2, 4, 7, 10, 12)
Low 1,2,3	Low molecular weight gels, numbers represent each one of the triplicate run
High 1,2,3	High molecular weight gels, numbers represent each one of the triplicate run
Time (hr)	Time in hours selected to weigh out swelling hydrogels

Table B.1A. Hydrogel weight data for swelling ratios for low and high molecular weight gels in pH 2, 4, 7, 10, and 12 buffers with 50% ethanol added. Data was used to generate Figure 1. All weight in grams (g) and time in hours (hr).

Hydrogels	Dry Weight(g)	Wet Weight	Wet Weight	Wet Weight	Wet Weight	Wet Weight	Wet Weight
	Time (hr)	Time	Time	Time	Time	Time	Time
pH2	0	0.5	1	2	3	5	8
Low 1	0.0235	0.0585	0.0750	0.0969	0.1088	0.1255	0.1361
2	0.0226	0.0596	0.0780	0.0958	0.1072	0.1217	0.1316
3	0.0246	0.0637	0.0815	0.1020	0.1146	0.1330	0.1439
Hi 1	0.0205	0.0627	0.0862	0.1128	0.1286	0.1502	0.1637
2	0.0204	0.0645	0.0858	0.1094	0.1279	0.1491	0.1659
3	0.021	0.0675	0.0908	0.1172	0.1327	0.1531	0.1670
pH4							
Low 1	0.0222	0.0616	0.0781	0.0953	0.1066	0.1223	0.1330
2	0.0228	0.0607	0.0717	0.0966	0.1007	0.1166	0.1240
3	0.0231	0.0601	0.0764	0.0957	0.1057	0.1226	0.1314
Hi 1	0.0201	0.0635	0.0847	0.1059	0.1193	0.1375	0.1496
2	0.0203	0.0637	0.0853	0.1078	0.1199	0.1372	0.1497
3	0.0213	0.0665	0.0902	0.1125	0.1263	0.1460	0.1572
pH7							
Low 1	0.0234	0.0624	0.0794	0.0983	0.1102	0.1255	0.1361
2	0.0225	0.0611	0.0776	0.0946	0.1042	0.1175	0.1250
3	0.0227	0.0605	0.0775	0.0954	0.1058	0.1203	0.1287
Hi 1	0.0196	0.0654	0.0859	0.1057	0.1171	0.1305	0.1405
2	0.0213	0.0715	0.0918	0.1144	0.1276	0.1456	0.1571
3	0.0211	0.0700	0.0930	0.1162	0.1312	0.1457	0.1540
pH10							
Low 1	0.025	0.0652	0.0860	0.1044	0.1165	0.1328	0.1424
2	0.0224	0.0602	0.0753	0.0926	0.1035	0.1173	0.1261
3	0.0245	0.0642	0.0819	0.1012	0.1130	0.1285	0.1381
Hi 1	0.0215	0.0696	0.0925	0.1183	0.1363	0.1616	0.1709
2	0.0212	0.0688	0.0907	0.1162	0.1336	0.1578	0.1751
3	0.022	0.0697	0.0924	0.1160	0.1334	0.1572	0.1727
pH12							
Low 1	0.0236	0.0627	0.0795	0.0984	0.1103	0.1259	0.1363
2	0.0237	0.0620	0.0783	0.0968	0.1087	0.1246	0.1354
3	0.0254	0.0702	0.0850	0.1053	0.1172	0.1300	0.1414
Hi 1	0.0209	0.0697	0.0918	0.1156	0.1321	0.1558	0.1711
2	0.0206	0.0682	0.0932	0.1211	0.1348	0.1528	0.1725
3	0.0217	0.0728	0.0951	0.1224	0..1409	0.1655	0.1854

Table B.1.A continued.

Hydrogels	Wet Weight	Wet Weight	Wet Weight	Wet Weight	Wet Weight	Wet Weight	Wet Weight
	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time(hr)
pH2	12	22	33	45	56	69	82
Low 1	0.1417	0.1452	0.1450	0.1454	0.1452	0.1454	0.1457
2	0.1341	0.1385	0.1394	0.1389	0.1393	0.1398	0.1393
3	0.1502	0.1532	0.1529	0.1516	0.1520	0.1515	0.1547
Hi 1	0.1720	0.1821	0.1851	0.1830	0.1827	0.1821	0.1820
2	0.1772	0.1878	0.1889	0.1882	0.1868	0.1859	0.1859
3	0.1780	0.1875	0.1892	0.1872	0.1867	0.1860	0.1847
pH4							
Low 1	0.1382	0.1431	0.1434	0.1442	0.1440	0.1437	0.1442
2	0.1258	0.1279	0.1297	0.1295	0.1330	0.1306	0.1304
3	0.1364	0.1396	0.1409	0.1409	0.1412	0.1408	0.1413
Hi 1	0.1589	0.1684	0.1741	0.1750	0.1761	0.1758	0.1753
2	0.1584	0.1694	0.1735	0.1750	0.1761	0.1755	0.1764
3	0.1630	0.1762	0.1843	0.1843	0.1835	0.1831	0.1825
pH7							
Low 1	0.1433	0.1458	0.1455	0.1451	0.1471	0.1453	0.1450
2	0.1290	0.1304	0.1311	0.1315	0.1321	0.1305	0.1315
3	0.1347	0.1361	0.1364	0.1382	0.1364	0.1365	0.1362
Hi 1	0.1455	0.1488	0.1487	0.1488	0.1475	0.1465	0.1453
2	0.1636	0.1684	0.1695	0.1688	0.1694	0.1675	0.1682
3	0.1557	0.1583	0.1558	0.1580	0.1571	0.1588	0.1565
pH10							
Low 1	0.1468	0.1484	0.1478	0.1478	0.1501	0.1487	0.1489
2	0.1314	0.1327	0.1326	0.1344	0.1328	0.1322	0.1322
3	0.1439	0.1454	0.1462	0.1458	0.1464	0.1439	0.1450
Hi 1	0.1796	0.1903	0.1978	0.2011	0.2011	0.2011	0.2011
2	0.1886	0.1976	0.1990	0.1938	0.1919	0.1912	0.1912
3	0.1834	0.1904	0.1912	0.1914	0.1914	0.1920	0.1920
pH12							
Low 1	0.1413	0.1459	0.1406	0.1448	0.1450	0.1464	0.1472
2	0.1413	0.1448	0.1463	0.1456	0.1461	0.1470	0.1475
3	0.1436	0.1443	0.1518	0.1526	0.1526	0.1526	0.1536
Hi 1	0.1808	0.1867	0.1876	0.1831	0.1831	0.1835	0.1878
2	0.1774	0.1811	0.1772	0.1788	0.1783	0.1788	0.1790
3	0.1967	0.1991	0.1974	0.1946	0.1946	0.2047	0.2047

Table B.1.B. Hydrogel weight data for swelling ratios for low and high molecular weight gels in pH 2, 4, 7, 10, and 12 buffers with no ethanol added. Data was used to generate Figure 2. All weight in grams (g) and time in hours (hr).

Hydrogels	Dry Weight (g)	Wet Weight	Wet Weight	Wet Weight	Wet Weight	Wet Weight	Wet Weight
	Time(hr)	Time(hr)	Time(hr)	Time(hr)	Time(hr)	Time(hr)	Time(hr)
pH2	0	0.5	1	2	3	5	8
Low 1	0.0231	0.0704	0.0914	0.1076	0.1188	0.1291	0.1334
2	0.0213	0.0657	0.0834	0.0995	0.1097	0.1183	0.1227
3	0.0227	0.0692	0.0864	0.1040	0.1159	0.1256	0.1303
Hi 1	0.0235	0.8080	0.1037	0.1250	0.1376	0.1475	0.1514
2	0.023	0.0788	0.1021	0.1240	0.1362	0.1467	0.1506
3	0.0223	0.0774	0.1012	0.1230	0.1342	0.1414	0.1460
pH4							
Low 1	0.023	0.0704	0.0880	0.1052	0.1159	0.1284	0.1323
2	0.0219	0.0677	0.0858	0.1013	0.1110	0.1211	0.1251
3	0.0217	0.0655	0.0832	0.0978	0.1075	0.1203	0.1209
Hi 1	0.0228	0.0795	0.1007	0.1193	0.1313	0.1392	0.1426
2	0.0223	0.0765	0.0982	0.1149	0.1253	0.1342	0.1393
3	0.0238	0.0799	0.1040	0.1226	0.1327	0.1423	0.1469
pH7							
Low 1	0.0223	0.0670	0.0836	0.0996	0.1101	0.1193	0.1241
2	0.0249	0.0736	0.0931	0.1107	0.1232	0.1347	0.1416
3	0.0247	0.0723	0.0907	0.1072	0.1183	0.1290	0.1347
Hi 1	0.0241	0.0884	0.1112	0.1298	0.1425	0.1528	0.1592
2	0.0223	0.0815	0.1013	0.1188	0.1311	0.1431	0.1486
3	0.0244	0.0854	0.1081	0.1278	0.1397	0.1530	0.1572
pH10							
Low 1	0.0216	0.0684	0.0853	0.0986	0.1093	0.1179	0.1234
2	0.022	0.0711	0.0834	0.1000	0.1102	0.1202	0.1253
3	0.0231	0.0731	0.0860	0.1020	0.1120	0.1227	0.1300
Hi 1	0.0265	0.0926	0.1256	0.1575	0.1820	0.2176	0.2513
2	0.0219	0.0837	0.1069	0.1366	0.1556	0.1793	0.1975
3	0.0239	0.0850	0.1134	0.1465	0.1664	0.1904	0.2128
pH12							
Low 1	0.0231	0.0708	0.0924	0.1141	0.1271	0.1437	0.1520
2	0.025	0.0794	0.1007	0.1264	0.1414	0.1581	0.1687
3	0.025	0.0787	0.1010	0.1263	0.1397	0.1600	0.1691
Hi 1	0.023	0.0895	0.1191	0.1491	0.1692	0.1873	0.1969
2	0.0232	0.0915	0.1225	0.1534	0.1727	0.1968	0.2011
3	0.0226	0.0899	0.1196	0.1505	0.1690	0.1874	0.1953

Table B.1.B continued.

Hydrogels	Wet Weight	Wet Weight	Wet Weight	Wet Weight	Wet Weight	Wet Weight	Wet Weight
	Time(hr)	Time(hr)	Time(hr)	Time(hr)	Time(hr)	Time(hr)	Time(hr)
pH2	12	20	31	47	58	70	82
Low 1	0.1349	0.1340	0.1353	0.1347	0.1351	0.1336	0.1360
2	0.1241	0.1240	0.1228	0.1214	0.1213	0.1223	0.1246
3	0.1331	0.1307	0.1323	0.1311	0.1317	0.1307	0.1311
Hi 1	0.1526	0.1524	0.1513	0.1522	0.1517	0.1516	0.1535
2	0.1530	0.1530	0.1521	0.1525	0.1521	0.1538	0.1517
3	0.1502	0.1458	0.1491	0.1462	0.1470	0.1451	0.1487
pH4							
Low 1	0.1354	0.1355	0.1352	0.1351	0.1369	0.1348	0.1359
2	0.1275	0.1280	0.1275	0.1298	0.1264	0.1261	0.1267
3	0.1248	0.1251	0.1251	0.1240	0.1259	0.1221	0.1236
Hi 1	0.1483	0.1474	0.1512	0.1497	0.1485	0.1498	0.1512
2	0.1426	0.1459	0.1452	0.1449	0.1454	0.1445	0.1479
3	0.1487	0.1512	0.1518	0.1520	0.1518	0.1517	0.1520
pH7							
Low 1	0.1241	0.1289	0.1274	0.1250	0.1243	0.1280	0.1276
2	0.1430	0.1426	0.1426	0.1430	0.1429	0.1449	0.1449
3	0.1371	0.1361	0.1365	0.1360	0.1365	0.1374	0.1369
Hi 1	0.1592	0.1603	0.1582	0.1592	0.1579	0.1592	0.1605
2	0.1505	0.1511	0.1510	0.1503	0.1514	0.1503	0.1506
3	0.1603	0.1621	0.1611	0.1611	0.1582	0.1594	0.1582
pH10							
Low 1	0.1234	0.1275	0.1243	0.1287	0.1320	0.1326	0.1357
2	0.1269	0.1277	0.1302	0.1329	0.1335	0.1351	0.1394
3	0.1302	0.1295	0.1326	0.1336	0.1370	0.1377	0.1400
Hi 1	0.2656	0.2679	0.2577	0.2651	0.2465	0.2473	0.2503
2	0.2095	0.2172	0.2158	0.2033	0.2044	0.2031	0.2035
3	0.2286	0.2288	0.2345	0.2225	0.2205	0.2240	0.2241
pH12							
Low 1	0.1533	0.1552	0.1533	0.1534	0.1534	0.1527	0.1544
2	0.1742	0.1724	0.1718	0.1729	0.1734	0.1731	0.1738
3	0.1751	0.1843	0.1645	0.1668	0.1639	0.1628	0.1660
Hi 1	0.2040	0.2041	0.1896	0.1881	0.1836	0.1839	0.1844
2	0.2011	0.2011	0.1877	0.1942	0.1885	0.1871	0.1881
3	0.2015	0.2065	0.2057	0.2031	0.1990	0.2006	0.2036

Table B.2. Estradiol Losses during synthesis and after 72 hr release experiment. Data used to generate Table 2. Absorbance measured with spectrophotometer @ 280 nm UV.

Column Title	Definition
Blank Absorbance	Spectrophotometer absorbance readings from blank gel
w/Estradiol Absorbance	Spectrophotometer absorbance readings of estradiol release from hydrogel
Estradiol-Blank	Blank absorbance subtracted from estradiol absorbance
Conc. (μg/mL)	Estimated concentration of estradiol using equation created through calibration curve in pH 7
Molecular Weight	High or low molecular weight of hydrogel's polymers
Dilution Correction	Corrected concentration by multiplying by dilution factor
Conc. **	Concentration for estradiol leftover in gel after release has separate equations created through different calibration curves: Low MW Eqn: Absorbance (y) = 0.0102*Concentration (x) + 0.0608 High MW Eqn: Absorbance (y) = 0.0092*Concentration (x) + 0.0623
Total Lost in Whole Gel	Total amount of estradiol lost within all of hydrogel after 72 hour release experiment

Table B.2. Estradiol Losses during synthesis and after 72 hr release experiment. Data used to generate Table 2. Absorbance measured with spectrophotometer @ 280 nm UV.

PIPET TIP				
Blank Absorbance	w/Estradiol Absorbance	Estradiol-Blank	Conc. (µg/mL)	Molecular weight
0.0284	0.1044	0.076	7.2923077	Low
0.205	0.3579	0.1529	19.12307692	High

MIXING VESSEL					
Blank Absorbance	w/Estradiol Absorbance	Estradiol-Blank	Conc. (µg/mL)	Dilution Correction	Average
LOW MW					
0.1932	0.2919	0.0987	10.78461538	32.3538	83.15769
0.1805	0.3819	0.2014	26.58461538	79.7538	
0.346	0.7416	0.3956	56.46153846	169.385	
0.185	0.5567	0.3717	52.78461538	158.354	
0.1348	0.3597	0.2249	30.2	90.6	
0.1055	0.4662	0.3607	51.09230769	153.277	
0.1808	0.42365	0.24285	32.96153846	98.8846	
0.1903	0.2716	0.0813	8.107692308	24.3231	
0.1661	0.2052	0.0391	1.615384615	4.84615	
0.0887	0.1602	0.0715	6.6	19.8	
HIGH MW					
0.474	0.6634	0.1894	24.73846154	74.2154	111.453
0.3538	0.6396	0.2858	39.56923077	118.708	
0.578	0.8551	0.2771	38.23076923	114.692	
0.4074	0.4701	0.0627	5.246153846	15.7385	
0.3624	0.6613	0.2989	41.58461538	124.754	
0.5832	0.660533333	0.077333	7.497435897	22.4923	
0.2537	0.6132	0.3595	50.90769231	152.723	
0.3652	0.8857	0.5205	75.67692308	227.031	
0.2537	0.6132	0.3595	50.90769231	152.723	

PEG DMA			
Estradiol - Blank Absorbance	Conc. (µg/mL)	Dilution Correction	Molecular Weight
0.0531	3.76923	37.6923	LOW
0.076	7.2923	72.923	HIGH

Table B.2.B. Continued.

Estradiol Lost After 72 Hour Release			
Estradiol - Blank Absorbance	Conc.**	Dilution Correction	Total Lost in Whole Gel
LOW MW			
0.1059	4.73913	9.693674889	47.668
0.054	0	0	
0.0753	1.413043	2.890316	
0.2201	17.15217	35.08399	
0.0528	0	0	
HIGH MW			
0.0752	1.411765	2.8877	209.057
0.3799	31.28431	63.990642	
0.5518	48.13725	98.46256684	
0.2788	21.37255	43.71656654	

Table B.3. Estradiol release data from statistical experiments used to calculate *n* in Table 3. Release occurred over 70 hours in 50% ethanol buffers at pH 2, 4, 7, 10, and 12 at various temperatures (33, 37, 41ºC) and crosslinking ratios (10:1, 10:2, 10:3).

Column Title	Definition
Time	Various times that release checked in hours
pH	pH of 50% ethanol buffer solution
MW	High or low molecular weight of hydrogel's polymers
Blank	Spectrophotometer absorbance reading of blank gel
Avg. Blank	Average of blank gel's absorbance reading at each time; used to subtract from estradiol absorbance
w/Estradiol	Estradiol release absorbance reading
Corrected E	Estradiol release absorbance with avg. blank absorbance subtracted
Conc.	Estimated concentration of estradiol released using equation created through calibration curve at each pH pH 2: Absorbance (y) = 0.0064 * Conc. (x) + 0.0042 pH 4: Absorbance (y) = 0.0057 * Conc. (x) + 0.0119 pH 7: Absorbance (y) = 0.0065 * Conc. (x) + 0.0286 pH 10: Absorbance (y) = 0.0056 * Conc. (x) + 0.015 pH 12: Absorbance (y) = 0.006 * Conc. (x) + 0.0062
Total Conc.	Total concentration of estradiol released over 70 hours
Mt/Mo	Fractional estradiol release with concentration at time, t (Mt) over total estradiol released after 70 hrs (Mo)

**All absorbance readings measured at 280 nm UV only.

Table B.3.A. Statistical estradiol release experiment data for Batch 1 at 33ºC with pH 7 and crosslinking ratio 10:1.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	7	low	0.0707	0.075033	0.1855	0.1104667	12.5949	79.51282	0.158401
0.5	7	low	0.0728		0.1494	0.0743667	7.04103	46.65128	0.150929
0.5	7	low	0.0816		0.2171	0.1420667	17.4564	73.12821	0.23871
0.5	7	high	0.1778	0.163333	0.273	0.1096667	12.4718	77.89744	0.160105
0.5	7	high	0.1576		0.2081	0.0447667	2.48718	42.09744	0.059081
0.5	7	high	0.1546		0.2444	0.0810667	8.07179	63.74359	0.126629
1	7	low	0.0815	0.0768	0.2208	0.144	17.7538		0.381683
1	7	low	0.0767		0.1693	0.0925	9.83077		0.361658
1	7	low	0.0722		0.1963	0.1195	13.9846		0.429944
1	7	high	0.118	0.1126	0.2332	0.1206	14.1538		0.341804
1	7	high	0.1294		0.1962	0.0836	8.46154		0.26008
1	7	high	0.0904		0.1802	0.0676	6		0.220756
2	7	low	0.0527	0.0551	0.1829	0.1278	15.2615		0.573621
2	7	low	0.0602		0.1414	0.0863	8.87692		0.55194
2	7	low	0.0524		0.1709	0.1158	13.4154		0.613394
2	7	high	0.0898	0.079067	0.1709	0.0918333	9.72821		0.466689
2	7	high	0.0864		0.15	0.0709333	6.51282		0.414789
2	7	high	0.061		0.1385	0.0594333	4.74359		0.295173
5	7	low	0.0621	0.060167	0.2037	0.1435333	17.6821		0.796001
5	7	low	0.0544		0.2004	0.1402333	17.1744		0.920084
5	7	low	0.064		0.2205	0.1603333	20.2667		0.890533
5	7	high	0.1003	0.085867	0.178	0.0921333	9.77436		0.592166
5	7	high	0.0909		0.1672	0.0813333	8.11282		0.607504
5	7	high	0.0664		0.1942	0.1083333	12.2667		0.487611
10	7	low	0.0403	0.037067	0.1614	0.1243333	14.7282		0.981232
10	7	low	0.0367		0.0899	0.0528333	3.72821		1
10	7	low	0.0342		0.1177	0.0806333	8.00513		1
10	7	high	0.0491	0.047667	0.1284	0.0807333	8.02051		1
10	7	high	0.0456		0.091	0.0433333	2.26667		1
10	7	high	0.0483		0.114	0.0663333	5.80513		1
20	7	low	0.022	0.0228	0.0611	0.0383	1.49231		1
20	7	low	0.0209		0.0368	0.014	0		1
20	7	low	0.0255		0.0484	0.0256	0		1
20	7	high	0.0283	0.028067	0.0504	0.0223333	0		1
20	7	high	0.0297		0.0371	0.0090333	0		1
20	7	high	0.0262		0.0438	0.0157333	0		1
30	7	low	0.0284	0.020567	0.0236	0.0030333	0		1
30	7	low	0.0159		0.02	-0.000567	0		1
30	7	low	0.0174		0.0293	0.0087333	0		1
30	7	high	0.0225	0.020267	0.0232	0.0029333	0		1
30	7	high	0.0206		0.0195	-0.000767	0		1
30	7	high	0.0177		0.0195	-0.000767	0		1
42	7	low	0.0045	0.003633	0.0055	0.0018667	0		1
42	7	low	0.003		0.0033	-0.000333	0		1
42	7	low	0.0034		0.0049	0.0012667	0		1
42	7	high	0.0056	0.0054	0.006	0.0006	0		1
42	7	high	0.006		0.0049	-0.0005	0		1
42	7	high	0.0046		0.0048	-0.0006	0		1
55	7	low	0.0178	0.015133	0.0145	-0.000633	0		1
55	7	low	0.0139		0.0156	0.0004667	0		1
55	7	low	0.0137		0.0146	-0.000533	0		1
55	7	high	0.0176	0.017833	0.0172	-0.000633	0		1
55	7	high	0.0172		0.0167	-0.001133	0		1
55	7	high	0.0187		0.0161	-0.001733	0		1
70	7	low	0	0	0	0	0		1
70	7	low	0		0	0	0		1
70	7	low	0		0	0	0		1
70	7	high	0	0	0	0	0		1
70	7	high	0		0	0	0		1
70	7	high	0		0	0	0		1

Table B.3.A continued.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	7	low	0.042	0.045233	0.1092	0.0639667	5.44103	126.4821	0.043018
0.5	7	low	0.0604		0.0925	0.0472667	2.87179	116.7744	0.024593
0.5	7	low	0.0333		0.1076	0.0623667	5.19487	69.8	0.074425
0.5	7	high	0.1512	0.110233	0.1895	0.0792667	7.79487	79.26667	0.098337
0.5	7	high	0.0843		0.1492	0.0389667	1.59487	33.35897	0.047809
0.5	7	high	0.0952		0.2393	0.1290667	15.4564	72.97949	0.211791
1	7	low	0.0358	0.035967	0.0992	0.0632333	5.32821		0.085144
1	7	low	0.0417		0.1169	0.0809333	8.05128		0.09354
1	7	low	0.0304		0.1408	0.1048333	11.7282		0.242451
1	7	high	0.0903	0.066033	0.1589	0.0928667	9.88718		0.22307
1	7	high	0.0584		0.1162	0.0501667	3.31795		0.147271
1	7	high	0.0494		0.1408	0.0747667	7.10256		0.309114
2	7	low	0.0266	0.040267	0.1243	0.0840333	8.52821		0.152571
2	7	low	0.0731		0.1479	0.1076333	12.159		0.197664
2	7	low	0.0211		0.1577	0.1174333	13.6667		0.438248
2	7	high	0.0797	0.0561	0.1613	0.1052	11.7846		0.371741
2	7	high	0.0457		0.1333	0.0772	7.47692		0.371407
2	7	high	0.0429		0.1937	0.1376	16.7692		0.538894
5	7	low	0.0394	0.030367	0.1997	0.1693333	21.6513		0.323751
5	7	low	0.036		0.1987	0.1683333	21.4974		0.381757
5	7	low	0.0157		0.1993	0.1689333	21.5897		0.747557
5	7	high	0.0925	0.061667	0.1981	0.1364333	16.5897		0.581031
5	7	high	0.0463		0.1615	0.0998333	10.959		0.699923
5	7	high	0.0462		0.2476	0.1859333	24.2051		0.870564
10	7	low	0.0144	0.015767	0.1002	0.0844333	8.58974		0.391664
10	7	low	0.0223		0.1032	0.0874333	9.05128		0.459268
10	7	low	0.0106		0.1204	0.1046333	11.6974		0.915142
10	7	high	0.0452	0.029033	0.1005	0.0714667	6.59487		0.66423
10	7	high	0.0234		0.1227	0.0936667	10.0103		1
10	7	high	0.0185		0.1136	0.0845667	8.61026		0.988546
20	7	low	0.0039	0.004167	0.0281	0.0239333	0		0.391664
20	7	low	0.0076		0.0455	0.0413333	1.95897		0.476044
20	7	low	0.001		0.0527	0.0485333	3.06667		0.959077
20	7	high	0.0198	0.010067	0.0358	0.0257333	0		0.66423
20	7	high	0.0071		0.0319	0.0218333	0		1
20	7	high	0.0033		0.0441	0.0340333	0.8359		1
32	7	low	0.005	0.003433	0.0146	0.0111667	0		0.391664
32	7	low	0.0038		0.0164	0.0129667	0		0.476044
32	7	low	0.0015		0.0265	0.0230667	0		0.959077
32	7	high	0.0094	0.0036	0.0123	0.0087	0		0.66423
32	7	high	0.0004		0.0181	0.0145	0		1
32	7	high	0.001		0.0199	0.0163	0		1
45	7	low	0	0.002233	0.3724	0.3701667	52.5487		0.807128
45	7	low	0		0.4137	0.4114667	58.9026		0.980458
45	7	low	0.0067		0.0249	0.0226667	0		0.959077
45	7	high	0.0295	0.1777	0.3721	0.1944	25.5077		0.986026
45	7	high	0.4267		0.097	-0.0807	0		1
45	7	high	0.0769		0.154	-0.0237	0		1
55	7	low	0.0222	0.030533	0.0354	0.0048667	0		0.807128
55	7	low	0.0256		0.061	0.0304667	0.28718		0.982917
55	7	low	0.0438		0.0427	0.0121667	0		0.959077
55	7	high	0.0227	0.0337	0.0695	0.0358	1.10769		1
55	7	high	0.0386		0.0518	0.0181	0		1
55	7	high	0.0398		0.0308	-0.0029	0		1
70	7	low	0.015	0.025433	0.2126	0.1871667	24.3949		1
70	7	low	0.0305		0.067	0.0415667	1.99487		1
70	7	low	0.0308		0.0726	0.0471667	2.85641		1
70	7	high	0.011	0.013833	0.0361	0.0222667	0		1
70	7	high	0.0124		0.0194	0.0055667	0		1
70	7	high	0.0181		0.0217	0.0078667	0		1

Table B.3.B Statistical estradiol release experiment data for Batch 1 at 33ºC with pH 2, 4, 10, and 12 and crosslinking ratio 10:2.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	2	low	0.0791	0.059833	0.1372	0.0773667	11.4323	57.36979	0.199274
0.5	2	low	0.038		0.158	0.0981667	14.6823	107.9323	0.136032
0.5	2	low	0.0624		0.1003	0.0404667	5.66667	73.88542	0.076695
0.5	2	high	0.1137	0.095733	0.2121	0.1163667	17.526	98.40104	0.178108
0.5	2	high	0.081		0.2439	0.1481667	22.4948	100.8073	0.223146
0.5	2	high	0.0925		0.3156	0.2198667	33.6979	175.5	0.192011
1	2	low	0.0476	0.041933	0.118	0.0760667	11.2292		0.395007
1	2	low	0.0299		0.1618	0.1198667	18.0729		0.303479
1	2	low	0.0483		0.112	0.0700667	10.2917		0.215988
1	2	high	0.0804	0.064033	0.2129	0.1488667	22.6042		0.407823
1	2	high	0.0602		0.187	0.1229667	18.5573		0.407233
1	2	high	0.0515		0.2565	0.1924667	29.4167		0.359627
2	2	low	0.0483	0.043067	0.1128	0.0697333	10.2396		0.573491
2	2	low	0.034		0.17	0.1269333	19.1771		0.481156
2	2	low	0.0469		0.1401	0.0970333	14.5052		0.412308
2	2	high	0.0779	0.0622	0.1751	0.1129	16.9844		0.580427
2	2	high	0.0557		0.2042	0.142	21.5313		0.620821
2	2	high	0.053		0.2931	0.2309	35.4219		0.561461
5	2	low	0.0617	0.0478	0.1579	0.1101	16.5469		0.861916
5	2	low	0.0356		0.2189	0.1711	26.0781		0.722772
5	2	low	0.0461		0.2068	0.159	24.1875		0.739673
5	2	high	0.0734	0.0603	0.1888	0.1285	19.4219		0.777801
5	2	high	0.0537		0.2144	0.1541	23.4219		0.853165
5	2	high	0.0538		0.2793	0.219	33.5625		0.752701
10	2	low	0.0257	0.024633	0.0678	0.0431667	6.08854		0.968044
10	2	low	0.0205		0.175	0.1503667	22.8385		0.934372
10	2	low	0.0277		0.0971	0.0724667	10.6667		0.884041
10	2	high	0.0342	0.028867	0.1092	0.0803333	11.8958		0.898693
10	2	high	0.0271		0.1021	0.0732333	10.7865		0.960165
10	2	high	0.0253		0.1226	0.0937333	13.9896		0.832413
20	2	low	0.0048	0.005633	0.0156	0.0099667	0.90104		0.983749
20	2	low	0.0047		0.0379	0.0322667	4.38542		0.975004
20	2	low	0.0074		0.0345	0.0288667	3.85417		0.936205
20	2	high	0.0273	0.017233	0.0636	0.0463667	6.58854		0.965649
20	2	high	0.0096		0.0359	0.0186667	2.26042		0.982588
20	2	high	0.0148		0.0478	0.0305667	4.11979		0.855888
32	2	low	0	0	0.0007	0.0007	0		0.983749
32	2	low	0		0.0127	0.0127	1.32813		0.987309
32	2	low	0		0.016	0.016	1.84375		0.961159
32	2	high	0.0387	0.138367	0.1642	0.0258333	3.38021		1
32	2	high	0.2642		0.0908	-0.047567	0		0.982588
32	2	high	0.1122		0.0152	-0.123167	0		0.855888
45	2	low	0.0509	0.0641	0.0546	-0.0095	0		0.983749
45	2	low	0.019		0.0726	0.0085	0.67187		0.993534
45	2	low	0.1224		0.0617	-0.0024	0		0.961159
45	2	high	0.0112	0.025	0.0047	-0.0203	0		1
45	2	high	0.0424		0.0296	0.0046	0.0625		0.983208
45	2	high	0.0214		0.015	-0.01	0		0.855888
55	2	low	0.0062	0.011233	0.0214	0.0101667	0.93229		1
55	2	low	0.0155		0.0199	0.0086667	0.69792		1
55	2	low	0.012		0.0338	0.0225667	2.86979		1
55	2	high	0.0201	0.020267	0.0192	-0.001067	0		1
55	2	high	0.0131		0.0167	-0.003567	0		0.983208
55	2	high	0.0276		0.0441	0.0238333	3.06771		0.855888
70	2	low	0.0158	0.0691	0.0128	-0.0563	0		1
70	2	low	0.0915		0.0055	-0.0636	0		1
70	2	low	0.1		0.0207	-0.0484	0		1
70	2	high	0.003	0.010667	0.0076	-0.003067	0		1
70	2	high	0.0076		0.0257	0.0150333	1.69271		1
70	2	high	0.0214		0.1571	0.1464333	22.224		1

Table B.3.B continued.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	4	low	0.0759	0.081433	0.1555	0.0740667	10.9064	55.7076	0.19578
0.5	4	low	0.102		0.1843	0.1028667	15.9591	72.07602	0.22142
0.5	4	low	0.0664		0.1592	0.0777667	11.5556	64.8655	0.178146
0.5	4	high	0.1335	0.1604	0.2527	0.0923	14.1053	85.25731	0.165443
0.5	4	high	0.1543		0.2382	0.0778	11.5614	84.95906	0.136082
0.5	4	high	0.1934		0.3091	0.1487	24	95.48538	0.251347
1	4	low	0.0672	0.072133	0.1334	0.0612667	8.66082		0.351249
1	4	low	0.0874		0.1381	0.0659667	9.48538		0.353022
1	4	low	0.0618		0.1257	0.0535667	7.30994		0.29084
1	4	high	0.0884	0.103567	0.1871	0.0835333	12.5673		0.312847
1	4	high	0.0993		0.1995	0.0959333	14.7427		0.309609
1	4	high	0.123		0.2408	0.1372333	21.9883		0.481627
2	4	low	0.0567	0.058167	0.1286	0.0704333	10.269		0.535587
2	4	low	0.0724		0.1337	0.0755333	11.1637		0.507911
2	4	low	0.0454		0.1292	0.0710333	10.3743		0.450775
2	4	high	0.0721	0.081767	0.1848	0.1030333	15.9883		0.500377
2	4	high	0.0714		0.1786	0.0968333	14.9006		0.484994
2	4	high	0.1018		0.1796	0.0978333	15.076		0.639515
5	4	low	0.0491	0.061933	0.1819	0.1199667	18.9591		0.875919
5	4	low	0.083		0.1757	0.1137667	17.8713		0.755862
5	4	low	0.0537		0.1586	0.0966667	14.8713		0.68004
5	4	high	0.0713	0.0779	0.2357	0.1578	25.5965		0.800604
5	4	high	0.0702		0.2057	0.1278	20.3333		0.724325
5	4	high	0.0922		0.2074	0.1295	20.6316		0.855585
10	4	low	0.0291	0.036967	0.0845	0.0475333	6.25146		0.988138
10	4	low	0.0534		0.1325	0.0955333	14.6725		0.959432
10	4	low	0.0284		0.1445	0.1075333	16.7778		0.938695
10	4	high	0.0438	0.050867	0.1485	0.0976333	15.0409		0.977022
10	4	high	0.0456		0.1727	0.1218333	19.2865		0.951335
10	4	high	0.0632		0.1314	0.0805333	12.0409		0.981688
20	4	low	0.0164	0.017033	0.0327	0.0156667	0.66082		1
20	4	low	0.0212		0.0456	0.0285667	2.92398		1
20	4	low	0.0135		0.0516	0.0345667	3.97661		1
20	4	high	0.0187	0.020333	0.0434	0.0230667	1.95906		1
20	4	high	0.019		0.0558	0.0354667	4.1345		1
20	4	high	0.0233		0.0422	0.0218667	1.74854		1
30	4	low	0.0087	0.0113	0.0127	0.0014	0		1
30	4	low	0.0088		0.0148	0.0035	0		1
30	4	low	0.0164		0.0186	0.0073	0		1
30	4	high	0.0122	0.0126	0.018	0.0054	0		1
30	4	high	0.0116		0.0209	0.0083	0		1
30	4	high	0.014		0.0165	0.0039	0		1
42	4	low	0.0142	0.010767	0.005	-0.005767	0		1
42	4	low	0.0079		0.0075	-0.003267	0		1
42	4	low	0.0102		0.0095	-0.001267	0		1
42	4	high	0.0244	0.015467	0.0114	-0.004067	0		1
42	4	high	0.0152		0.0079	-0.007567	0		1
42	4	high	0.0068		0.0071	-0.008367	0		1
55	4	low	0.0153	0.0189	0.0194	0.0005	0		1
55	4	low	0.0215		0.019	1E-04	0		1
55	4	low	0.0199		0.0189	0	0		1
55	4	high	0.0203	0.0192	0.0177	-0.0015	0		1
55	4	high	0.0188		0.0158	-0.0034	0		1
55	4	high	0.0185		0.0142	-0.005	0		1
70	4	low	0.0386	0.040767	0.0422	0.0014333	0		1
70	4	low	0.0409		0.0405	-0.000267	0		1
70	4	low	0.0428		0.0446	0.0038333	0		1
70	4	high	0.0485	0.050733	0.0589	0.0081667	0		1
70	4	high	0.052		0.0528	0.0020667	0		1
70	4	high	0.0517		0.0519	0.0011667	0		1

Table B.3.B continued.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	10	low	0.079	0.08065	0.1443	0.06365	8.6875	53.00298	0.163906
0.5	10	low	0.0823		0.1634	0.08275	12.0982	79.93155	0.151357
0.5	10	low	no		no			52.65476	0
0.5	10	high	0.2298	0.2109	0.2455	0.0346	3.5	57.1369	0.061256
0.5	10	high	0.1941		0.2557	0.0448	5.32143	61.44465	0.086605
0.5	10	high	0.2088		0.2086	-0.0023	0	43.25386	0
1	10	low	0.053	0.055	0.1109	0.0559	7.30357		0.301701
1	10	low	0.0514		0.1406	0.0856	12.6071		0.309081
1	10	low	0.0606		0.1344	0.0794	11.5		0.218404
1	10	high	0.1475	0.120833	0.1959	0.0750667	10.7262		0.248984
1	10	high	0.1153		0.2	0.0791667	11.4583		0.273087
1	10	high	0.0997		0.161	0.0401667	4.49405		0.103899
2	10	low	0.0466	0.048367	0.1134	0.0650333	8.93452		0.470268
2	10	low	0.0449		0.1378	0.0894333	13.2917		0.47537
2	10	low	0.0536		0.1344	0.0860333	12.6845		0.459304
2	10	high	0.0954	0.088033	0.1549	0.0668667	9.2619		0.411084
2	10	high	0.0867		0.1729	0.0848667	12.4762		0.476135
2	10	high	0.082		0.1529	0.0648667	8.90476		0.309771
5	10	low	0.0536	0.057533	0.1596	0.1020667	15.5476		0.763603
5	10	low	0.0502		0.2112	0.1536667	24.7619		0.785158
5	10	low	0.0688		0.1685	0.1109667	17.1369		0.784761
5	10	high	0.1037	0.089833	0.2012	0.1113667	17.2083		0.712262
5	10	high	0.0877		0.1771	0.0872667	12.9048		0.686158
5	10	high	0.0781		0.1811	0.0912667	13.619		0.624635
10	10	low	0.0206	0.025267	0.1015	0.0762333	10.9345		0.969903
10	10	low	0.0248		0.1147	0.0894333	13.2917		0.951447
10	10	low	0.0304		0.0978	0.0725333	10.2738		0.979878
10	10	high	0.0485	0.041333	0.1405	0.0991667	15.0298		0.97531
10	10	high	0.0378		0.1488	0.1074667	16.7661		0.959022
10	10	high	0.0377		0.1299	0.0885667	13.4503		0.935596
20	10	low	0.0081	0.008567	0.0325	0.0239333	1.59524		1
20	10	low	0.009		0.0453	0.0367333	3.88095		1
20	10	low	0.0086		0.0295	0.0209333	1.05952		1
20	10	high	0.0201	0.0162	0.0391	0.0229	1.41071		1
20	10	high	0.0144		0.0453	0.0291	2.51786		1
20	10	high	0.0141		0.0468	0.0306	2.78571		1
30	10	low	0.0077	0.007333	0.015	0.0076667	0		1
30	10	low	0.0078		0.0176	0.0102667	0		1
30	10	low	0.0065		0.014	0.0066667	0		1
30	10	high	0.0146	0.012567	0.0168	0.0042333	0		1
30	10	high	0.0116		0.0194	0.0068333	0		1
30	10	high	0.0115		0.0222	0.0096333	0		1
42	10	low	0.0122	0.007567	0.0038	-0.003767	0		1
42	10	low	0.0066		0.005	-0.002567	0		1
42	10	low	0.0039		0.0017	-0.005867	0		1
42	10	high	0.0037	0.0038	0.0094	0.0056	0		1
42	10	high	0.0049		0.0066	0.0028	0		1
42	10	high	0.0028		0.0134	0.0096	0		1
55	10	low	0.0101	0.009467	0.0071	-0.002367	0		1
55	10	low	0.0105		0.0069	-0.002567	0		1
55	10	low	0.0078		0.0063	-0.003167	0		1
55	10	high	0.0084	0.007867	0.007	-0.000867	0		1
55	10	high	0.0068		0.0075	-0.000367	0		1
55	10	high	0.0084		0.0079	3.333E-05	0		1
70	10	low	0.0015	0.0005	0.0006	1E-04	0		1
70	10	low	0		0	0	0		1
70	10	low	0		0	0	0		1
70	10	high	0.0038	0.0027	0.001	-0.0017	0		1
70	10	high	0.0024		0.0014	-0.0013	0		1
70	10	high	0.0019		-0.0008	0	0		1

Table B.3.B continued.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	12	low	0.0624	0.0475	0.0963	0.0488	7.1	142.8389	0.049706
0.5	12	low	0.0381		0.118	0.0705	10.7167	80.87222	0.132514
0.5	12	low	0.042		0.1447	0.0972	15.1667	86.94444	0.174441
0.5	12	high	0.1757	0.173533	0.1849	0.0113667	0.86111	54	0.015947
0.5	12	high	0.1285		0.2277	0.0541667	7.99444	50.61667	0.157941
0.5	12	high	0.2164		0.2889	0.1153667	18.1944	107.55	0.169172
1	12	low	0.0362	0.0301	0.0933	0.0632	9.5		0.116215
1	12	low	0.0273		0.0979	0.0678	10.2667		0.259463
1	12	low	0.0268		0.1042	0.0741	11.3167		0.304601
1	12	high	0.147	0.107367	0.172	0.0646333	9.73889		0.196296
1	12	high	0.0824		0.1684	0.0610333	9.13889		0.338492
1	12	high	0.0927		0.2224	0.1150333	18.1389		0.337827
2	12	low	0.031	0.0284	0.1219	0.0935	14.55		0.218078
2	12	low	0.0245		0.1193	0.0909	14.1167		0.434018
2	12	low	0.0297		0.118	0.0896	13.9		0.464473
2	12	high	0.1368	0.095233	0.1728	0.0775667	11.8944		0.416564
2	12	high	0.0615		0.1445	0.0492667	7.17778		0.480299
2	12	high	0.0874		0.2715	0.1762667	28.3444		0.601374
5	12	low	0.0373	0.030433	0.1602	0.1297667	20.5944		0.362257
5	12	low	0.0204		0.153	0.1225667	19.3944		0.673834
5	12	low	0.0336		0.1883	0.1578667	25.2778		0.755208
5	12	high	0.1097	0.087367	0.1731	0.0857333	13.2556		0.662037
5	12	high	0.0586		0.1775	0.0901333	13.9889		0.756668
5	12	high	0.0938		0.2659	0.1785333	28.7222		0.868433
10	12	low	0.0066	0.0022	0.0612	0.059	8.8		0.423865
10	12	low	0		0.0683	0.0661	9.98333		0.79728
10	12	low	0		0.0601	0.0579	8.61667		0.854313
10	12	high	0.0529	0.032867	0.0828	0.0499333	7.28889		0.797016
10	12	high	0.0182		0.0921	0.0592333	8.83889		0.931292
10	12	high	0.0275		0.1016	0.0687333	10.4222		0.965339
20	12	low	0	0	0.0126	0.0126	1.06667		0.431333
20	12	low	0		0.0123	0.0123	1.01667		0.809851
20	12	low	0		0.0079	0.0079	0.28333		0.857572
20	12	high	0.0065	0.004533	0.0381	0.0335667	4.56111		0.881481
20	12	high	0		0.0193	0.0147667	1.42778		0.9595
20	12	high	0.0071		0.0293	0.0247667	3.09444		0.994111
32	12	low	0	0	0	0	0		0.431333
32	12	low	0		0.0126	0.0126	1.06667		0.82304
32	12	low	0		0	0	0		0.857572
32	12	high	0	0	0.0033	0.0033	0		0.881481
32	12	high	0		0	0	0		0.9595
32	12	high	0		0.0041	0.0041	0		0.994111
45	12	low	0	0.003533	0.4971	0.4935667	81.2278		1
45	12	low	0.0106		0.0956	0.0920667	14.3111		1
45	12	low	0		0.0819	0.0783667	12.0278		0.995911
45	12	high	0.0121	0.004267	0	0	0		0.881481
45	12	high	0		0	0	0		0.9595
45	12	high	0.0007		0	0	0		0.994111
55	12	low	0	0.012167	0	0	0		1
55	12	low	0		0.0142	0.0020333	0		1
55	12	low	0.0365		0.0205	0.0083333	0.35556		1
55	12	high	0	0	0.0043	0.0043	0		0.881481
55	12	high	0		0.0125	0.0125	1.05		0.980244
55	12	high	0		0.0054	0.0054	0		0.994111
70	12	low	0	0	0	0	0		1
70	12	low	0		0.006	0.006	0		1
70	12	low	0		0.0021	0.0021	0		1
70	12	high	0	0.0022	0.0468	0.0446	6.4		1
70	12	high	0.0004		0.0144	0.0122	1		1
70	12	high	0.0062		0.0122	0.01	0.63333		1

Table B.3.C. Statistical estradiol release experiment data for Batch 1 at 33ºC with pH 7 and crosslinking ratio 10:3.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	7	low	0.0465	0.055733	0.1564	0.1006667	11.0872	52.06667	0.212942
0.5	7	low	0.0587		0.132	0.0762667	7.33333	45.28205	0.161948
0.5	7	low	0.062		0.1512	0.0954667	10.2872	67.91282	0.151476
0.5	7	high	0.1333	0.115733	0.2066	0.0908667	9.57949	52.69231	0.1818
0.5	7	high	0.0974		0.2316	0.1158667	13.4256	51.81538	0.259105
0.5	7	high	0.1165		0.2325	0.1167667	13.5641	56.78462	0.238869
1	7	low	0.0526	0.048567	0.1231	0.0745333	7.06667		0.348665
1	7	low	0.0453		0.1452	0.0966333	10.4667		0.393092
1	7	low	0.0478		0.1405	0.0919333	9.74359		0.294948
1	7	high	0.0955	0.0916	0.1842	0.0926	9.84615		0.368662
1	7	high	0.0879		0.2051	0.1135	13.0615		0.511184
1	7	high	0.0914		0.1864	0.0948	10.1846		0.418225
2	7	low	0.0388	0.041767	0.1456	0.1038333	11.5744		0.570964
2	7	low	0.0458		0.1024	0.0606333	4.92821		0.501925
2	7	low	0.0407		0.1574	0.1156333	13.3897		0.492109
2	7	high	0.0753	0.0655	0.1609	0.0954	10.2769		0.563698
2	7	high	0.0546		0.1486	0.0831	8.38462		0.673001
2	7	high	0.0666		0.1515	0.086	8.83077		0.573738
5	7	low	0.0507	0.048433	0.1506	0.1021667	11.3179		0.788338
5	7	low	0.0445		0.1621	0.1136667	13.0872		0.79094
5	7	low	0.0501		0.208	0.1595667	20.1487		0.788794
5	7	high	0.0601	0.058867	0.2045	0.1456333	18.0051		0.905401
5	7	high	0.0569		0.1564	0.0975333	10.6051		0.877672
5	7	high	0.0596		0.1594	0.1005333	11.0667		0.768626
10	7	low	0.0286	0.036867	0.1284	0.0915333	9.68205		0.974293
10	7	low	0.052		0.1209	0.0840333	8.52821		0.979275
10	7	low	0.03		0.1388	0.1019333	11.2821		0.95492
10	7	high	0.0425	0.036	0.097	0.061	4.98462		1
10	7	high	0.0311		0.1058	0.0698	6.33846		1
10	7	high	0.0344		0.15	0.114	13.1385		1
20	7	low	0.0176	0.0167	0.054	0.0373	1.33846		1
20	7	low	0.0161		0.0514	0.0347	0.93846		1
20	7	low	0.0164		0.0652	0.0485	3.06154		1
20	7	high	0.0239	0.0832	0.0402	-0.043	0		1
20	7	high	0.0237		0.0409	-0.0423	0		1
20	7	high	0.202		0.0532	-0.03	0		1
30	7	low	0.0059	0.0067	0.0215	0.0148	0		1
30	7	low	0.0083		0.0183	0.0116	0		1
30	7	low	0.0059		0.0189	0.0122	0		1
30	7	high	0.0078	0.008033	0.0123	0.0042667	0		1
30	7	high	0.0086		0.0142	0.0061667	0		1
30	7	high	0.0077		0.0178	0.0097667	0		1
42	7	low	0.0084	0.0081	0.0102	0.0021	0		1
42	7	low	0.0085		0.0094	0.0013	0		1
42	7	low	0.0074		0.0102	0.0021	0		1
42	7	high	0.009	0.009133	0.01	0.0008667	0		1
42	7	high	0.0088		0.01	0.0008667	0		1
42	7	high	0.0096		0.0142	0.0050667	0		1
55	7	low	0.0101	0.0082	0.0083	0.0001	0		1
55	7	low	0.0085		0.007	-0.0012	0		1
55	7	low	0.006		0.0072	-0.001	0		1
55	7	high	0.0064	0.006167	0.0054	-0.000767	0		1
55	7	high	0.0059		0.0062	3.333E-05	0		1
55	7	high	0.0062		0.006	-0.000167	0		1
70	7	low	0.0172	0.0256	0.0315	0.0059	0		1
70	7	low	0.0307		0.0204	-0.0052	0		1
70	7	low	0.0289		0.024	-0.0016	0		1
70	7	high	0.0194	0.0137	0.0061	-0.0076	0		1
70	7	high	0.016		0.0001	-0.0136	0		1
70	7	high	0.0057		0	0	0		1

Table B.3.C continued.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	7	low	0.0697	0.076833	0.1511	0.0742667	7.02564	60.77436	0.115602
0.5	7	low	0.0734		0.0857	0.0088667	0	34.5641	0
0.5	7	low	0.0874		0.1324	0.0555667	4.14872	63.66667	0.065163
0.5	7	high	0.1444	0.143333	0.1906	0.0472667	2.87179	47.48205	0.060482
0.5	7	high	0.1193		0.1881	0.0447667	2.48718	49.95385	0.04979
0.5	7	high	0.1663		0.212	0.0686667	6.1641	40.94872	0.150532
1	7	low	0.0554	0.055767	0.1503	0.0945333	10.1436		0.282508
1	7	low	0.0486		0.0966	0.0408333	1.88205		0.054451
1	7	low	0.0633		0.1589	0.1031333	11.4667		0.245268
1	7	high	0.0658	0.0858	0.176	0.0902	9.47692		0.260071
1	7	high	0.0677		0.1385	0.0527	3.70769		0.124012
1	7	high	0.1239		0.1679	0.0821	8.23077		0.351534
2	7	low	0.0515	0.048933	0.1671	0.1181667	13.7795		0.50924
2	7	low	0.0485		0.1288	0.0798667	7.88718		0.282641
2	7	low	0.0468		0.1866	0.1376667	16.7795		0.50882
2	7	high	0.0931	0.078067	0.1678	0.0897333	9.40513		0.458149
2	7	high	0.0561		0.1462	0.0681333	6.08205		0.245765
2	7	high	0.085		0.1491	0.0710333	6.52821		0.510958
5	7	low	0.0651	0.069533	0.2146	0.1450667	17.9179		0.804067
5	7	low	0.0689		0.1987	0.1291667	15.4718		0.730267
5	7	low	0.0746		0.2465	0.1769667	22.8256		0.867338
5	7	high	0.045	0.0667	0.1781	0.1114	12.7385		0.726428
5	7	high	0.0606		0.1593	0.0926	9.84615		0.44287
5	7	high	0.0945		0.1808	0.1141	13.1538		0.832185
10	7	low	0.0238	0.019667	0.1132	0.0935333	9.98974		0.968441
10	7	low	0.0153		0.0977	0.0780333	7.60513		0.950297
10	7	low	0.0199		0.0968	0.0771333	7.46667		0.984615
10	7	high	0.052	0.036367	0.0945	0.0581333	4.54359		0.822119
10	7	high	0.0229		0.115	0.0786333	7.69744		0.596961
10	7	high	0.0342		0.1074	0.0710333	6.52821		0.991609
20	7	low	0.0022	0.000733	0.0418	0.0410667	1.91795		1
20	7	low	0		0.0405	0.0397667	1.71795		1
20	7	low	0		0.0357	0.0349667	0.97949		1
20	7	high	0.0013	0.008767	0.029	0.0202333	0		0.822119
20	7	high	0.0075		0.0331	0.0243333	0		0.596961
20	7	high	0.0175		0.0396	0.0308333	0.34359		1
32	7	low	0	0.002133	0.0097	0.0075667	0		1
32	7	low	0.006		0.0111	0.0089667	0		1
32	7	low	0.0004		0.029	0.0268667	0		1
32	7	high	0.0021	0.010567	0.0027	-0.007867	0		0.822119
32	7	high	0.0211		0.0118	0.0012333	0		0.596961
32	7	high	0.0085		0.0388	0.0282333	0		1
45	7	low	0.0116	0.013133	0.01	-0.003133	0		1
45	7	low	0		0.0025	-0.010633	0		1
45	7	low	0.0278		0.0323	0.0191667	0		1
45	7	high	0.0007	0.000233	0.0783	0.0780667	7.61026		0.982396
45	7	high	0		0.1597	0.1594667	20.1333		1
45	7	high	0		0.0102	0.0099667	0		1
55	7	low	0.0202	0.023733	0.0318	0.0080667	0		1
55	7	low	0.0214		0.0276	0.0038667	0		1
55	7	low	0.0296		0.0267	0.0029667	0		1
55	7	high	0.0216	0.025867	0.0273	0.0014333	0		0.982396
55	7	high	0.0319		0.0281	0.0022333	0		1
55	7	high	0.0241		0.0331	0.0072333	0		1
70	7	low	0.0096	0.015167	0.0141	-0.001067	0		1
70	7	low	0.0159		0.0164	0.0012333	0		1
70	7	low	0.02		0.0326	0.0174333	0		1
70	7	high	0.0404	0.045867	0.0799	0.0340333	0.8359		1
70	7	high	0.0833		0.0528	0.0069333	0		1
70	7	high	0.0139		0.0319	-0.013967	0		1

Table B.3.D. Statistical estradiol release experiment data for Batch 2 at 37ºC with pH 2, 4, 10, and 12 and crosslinking ratio 10:1.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	2	low	0.0619	0.068267	0.1162	0.0479333	6.83333	78.92188	0.086584
0.5	2	low	0.0844		0.112	0.0437333	6.17708	75	0.082361
0.5	2	low	0.0585		0.1357	0.0674333	9.88021	89.71875	0.110124
0.5	2	high	0.1204	0.164133	0.2153	0.0511667	7.33854	82.22396	0.089251
0.5	2	high	0.1032		0.1853	0.0211667	2.65104	85.90104	0.030862
0.5	2	high	0.2688		0.2159	0.0517667	7.43229	102.4688	0.072532
1	2	low	0.0461	0.0518	0.1329	0.0811	12.0156		0.238831
1	2	low	0.0698		0.1332	0.0814	12.0625		0.243194
1	2	low	0.0395		0.1658	0.114	17.1563		0.301347
1	2	high	0.0791	0.069333	0.2398	0.1704667	25.9792		0.405207
1	2	high	0.0663		0.2367	0.1673667	25.4948		0.327654
1	2	high	0.0626		0.2335	0.1641667	24.9948		0.316458
2	2	low	0.0391	0.0426	0.1357	0.0931	13.8906		0.414835
2	2	low	0.0578		0.1543	0.1117	16.7969		0.467153
2	2	low	0.0309		0.1433	0.1007	15.0781		0.469407
2	2	high	0.0523	0.049567	0.168	0.1184333	17.849		0.622284
2	2	high	0.0455		0.1397	0.0901333	13.4271		0.483963
2	2	high	0.0509		0.2062	0.1566333	23.8177		0.548897
5	2	low	0.0409	0.046467	0.2166	0.1701333	25.9271		0.743351
5	2	low	0.0625		0.2123	0.1658333	25.2552		0.803889
5	2	low	0.036		0.2196	0.1731333	26.3958		0.763613
5	2	high	0.0593	0.055467	0.1838	0.1283333	19.3958		0.858174
5	2	high	0.0539		0.1838	0.1283333	19.3958		0.709756
5	2	high	0.0532		0.2239	0.1684333	25.6615		0.799329
10	2	low	0.0201	0.0255	0.1354	0.1099	16.5156		0.952617
10	2	low	0.0323		0.1055	0.08	11.8438		0.961806
10	2	low	0.0241		0.1322	0.1067	16.0156		0.942122
10	2	high	0.0319	0.032733	0.0982	0.0654667	9.57292		0.974599
10	2	high	0.0268		0.1379	0.1051667	15.776		0.893409
10	2	high	0.0395		0.1394	0.1066667	16.0104		0.955576
22	2	low	0.0029	0.010167	0.0383	0.0281333	3.73958		1
22	2	low	0.0066		0.0327	0.0225333	2.86458		1
22	2	low	0.021		0.0351	0.0249333	3.23958		0.978231
22	2	high	0.0157	0.0139	0.03	0.0161	1.85938		0.997213
22	2	high	0.0108		0.0466	0.0327	4.45313		0.945249
22	2	high	0.0152		0.0447	0.0308	4.15625		0.996137
32	2	low	0.0097	0.006767	0.0057	-0.001067	0		1
32	2	low	0.0106		0.004	-0.002767	0		1
32	2	low	0		0.0116	0.0048333	0.09896		0.979334
32	2	high	0.0111	0.010567	0.0055	-0.005067	0		0.997213
32	2	high	0.0131		0.0368	0.0262333	3.44271		0.985327
32	2	high	0.0075		0.0162	0.0056333	0.22396		0.998323
44	2	low	0	0.000933	0	0	0		1
44	2	low	0		0	0	0		1
44	2	low	0.0028		0.017	0.0160667	1.85417		1
44	2	high	0.0028	0.000933	0.0066	0.0056667	0.22917		1
44	2	high	0		0.0132	0.0122667	1.26042		1
44	2	high	0		0.0049	0.0039667	0		0.998323
58	2	low	0	0.000633	0	0	0		1
58	2	low	0		0	0	0		1
58	2	low	0.0019		0.0007	6.667E-05	0		1
58	2	high	0.0017	0.0083	0	0	0		1
58	2	high	0		0.0041	-0.0042	0		1
58	2	high	0.0232		0.0136	0.0053	0.17188		1
70	2	low	0	0	0	0	0		1
70	2	low	0		0	0	0		1
70	2	low	0		0	0	0		1
70	2	high	0	0	0	0	0		1
70	2	high	0		0	0	0		1
70	2	high	0		0	0	0		1

Table B.3.D continued.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	4	low	0.0991	0.081067	0.1411	0.0600333	4.8359	41.12308	0.117596
0.5	4	low	0.0625		0.1406	0.0595333	4.75897	44.36923	0.107258
0.5	4	low	0.0816		0.1324	0.0513333	3.49744	43.70769	0.080019
0.5	4	high	0.1052	0.113	0.168	0.055	4.06154	37.29231	0.108911
0.5	4	high	0.1076		0.1262	0.0132	0	22.21538	0
0.5	4	high	0.1262		0.1858	0.0728	6.8	49.92308	0.13621
1	4	low	0.0653	0.056267	0.1226	0.0663333	5.80513		0.25876
1	4	low	0.0386		0.1072	0.0509333	3.4359		0.184697
1	4	low	0.0649		0.1252	0.0689333	6.20513		0.221988
1	4	high	0.0892	0.084867	0.131	0.0461333	2.69744		0.181243
1	4	high	0.0535		0.1378	0.0529333	3.74359		0.168513
1	4	high	0.1119		0.1583	0.0734333	6.89744		0.274371
2	4	low	0.0692	0.058433	0.1453	0.0868667	8.9641		0.476743
2	4	low	0.0471		0.1317	0.0732667	6.87179		0.339575
2	4	low	0.059		0.1406	0.0821667	8.24103		0.410536
2	4	high	0.0759	0.0644	0.1821	0.1177	13.7077		0.548817
2	4	high	0.0515		0.1383	0.0739	6.96923		0.482225
2	4	high	0.0658		0.2336	0.1692	21.6308		0.707653
5	4	low	0.0559	0.051	0.166	0.115	13.2923		0.799975
5	4	low	0.0427		0.2153	0.1643	20.8769		0.810102
5	4	low	0.0544		0.205	0.154	19.2923		0.85193
5	4	high	0.0668	0.053267	0.0668	0.0135333	0		0.548817
5	4	high	0.044		0.044	-0.009267	0		0.482225
5	4	high	0.049		0.049	-0.004267	0		0.707653
10	4	low	0.0479	0.046833	0.1289	0.0820667	8.22564		1
10	4	low	0.0487		0.1209	0.0740667	6.99487		0.967753
10	4	low	0.0439		0.1175	0.0706667	6.47179		1
10	4	high	0.0409	0.056533	0.1821	0.1255667	14.9179		0.948845
10	4	high	0.0518		0.1534	0.0968667	10.5026		0.954986
10	4	high	0.0769		0.1766	0.1200667	14.0718		0.989522
20	4	low	0.0184	0.0149	0.0303	0.0154	0		1
20	4	low	0.0125		0.0528	0.0379	1.43077		1
20	4	low	0.0138		0.0257	0.0108	0		1
20	4	high	0.0269	0.0243	0.0653	0.041	1.90769		1
20	4	high	0.0241		0.0594	0.0351	1		1
20	4	high	0.0219		0.0563	0.032	0.52308		1
30	4	low	0.0001	0.001533	0.0059	0.0043667	0		1
30	4	low	0.0013		0.0105	0.0089667	0		1
30	4	low	0.0032		0.0069	0.0053667	0		1
30	4	high	0.0121	0.0099	0.0264	0.0165	0		1
30	4	high	0.0137		0.0148	0.0049	0		1
30	4	high	0.0039		0.0229	0.013	0		1
42	4	low	0.0002	6.67E-05	0	0	0		1
42	4	low	0		0.0005	0.0004333	0		1
42	4	low	0		0	0	0		1
42	4	high	0.002	0.006133	0.0167	0.0105667	0		1
42	4	high	0.0094		0.0056	-0.000533	0		1
42	4	high	0.007		0.0062	6.667E-05	0		1
55	4	low	0	0	0	0	0		1
55	4	low	0		0	0	0		1
55	4	low	0		0.0222	0.0222	0		1
55	4	high	0.0079	0.0042	0	0	0		1
55	4	high	0.0047		0	0	0		1
55	4	high	0		0.0096	0.0054	0		1
70	4	low	0	0.0007	0.0172	0.0165	0		1
70	4	low	0		0	0	0		1
70	4	low	0.0021		0	0	0		1
70	4	high	0	0.001967	0.0073	0.0053333	0		1
70	4	high	0.0059		0	0	0		1
70	4	high	0		0	0	0		1

Table B.3.D. continued.

Time	ph	MW	blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	10	low	0.0565	0.063733	0.1356	0.0718667	6.65641	34.82051	0.191163
0.5	10	low	0.0683		0.0979	0.0341667	0.85641	47.17436	0.018154
0.5	10	low	0.0664		0.1264	0.0626667	5.24103	42.68205	0.122792
0.5	10	high	0.1573	0.1381	0.165	0.0269	0	25.13846	0
0.5	10	high	0.0709		0.175	0.0369	1.27692	55.53846	0.022992
0.5	10	high	0.1861		0.1757	0.0376	1.38462	62.09231	0.022299
1	10	low	0.0825	0.064467	0.129	0.0645333	5.52821		0.349926
1	10	low	0.0453		0.1526	0.0881333	9.15897		0.212306
1	10	low	0.0656		0.1178	0.0533333	3.80513		0.211943
1	10	high	0.1247	0.0921	0.1279	0.0358	1.10769		0.044064
1	10	high	0.0537		0.2062	0.1141	13.1538		0.259834
1	10	high	0.0979		0.2062	0.1141	13.1538		0.234143
2	10	low	0.0815	0.0793	0.1524	0.0731	6.84615		0.546539
2	10	low	0.0606		0.167	0.0877	9.09231		0.405044
2	10	low	0.0958		0.2343	0.155	19.4462		0.667548
2	10	high	0.0886	0.064967	0.1348	0.0698333	6.34359		0.29641
2	10	high	0.0442		0.1618	0.0968333	10.4974		0.448846
2	10	high	0.0621		0.1471	0.0821333	8.2359		0.366782
5	10	low	0.0932	0.063833	0.1811	0.1172667	13.641		0.938292
5	10	low	0.0433		0.2257	0.1618667	20.5026		0.839656
5	10	low	0.055		0.1752	0.1113667	12.7333		0.965878
5	10	high	0.0857	0.0699	0.1818	0.1119	12.8154		0.806202
5	10	high	0.0468		0.2116	0.1417	17.4		0.762142
5	10	high	0.0772		0.2763	0.2064	27.3538		0.807317
10	10	low	0.0672	0.071733	0.1143	0.0425667	2.14872		1
10	10	low	0.0699		0.1495	0.0777667	7.5641		1
10	10	low	0.0781		0.1098	0.0380667	1.45641		1
10	10	high	0.0668	0.067133	0.1274	0.0602667	4.87179		1
10	10	high	0.0679		0.1816	0.1144667	13.2103		1
10	10	high	0.0667		0.1735	0.1063667	11.9641		1
20	10	low	0.0128	0.011833	0.0309	0.0190667	0		1
20	10	low	0.0109		0.0396	0.0277667	0		1
20	10	low	0.0118		0.0247	0.0128667	0		1
20	10	high	0.0181	0.0165	0.0294	0.0129	0		1
20	10	high	0.0138		0.0422	0.0257	0		1
20	10	high	0.0176		0.0391	0.0226	0		1
30	10	low	0.0008	0.002867	0	0	0		1
30	10	low	0		0.011	0.0081333	0		1
30	10	low	0.0078		0	0	0		1
30	10	high	0	0	0.0165	0.0165	0		1
30	10	high	0		0.0094	0.0094	0		1
30	10	high	0		0	0	0		1
42	10	low	0.0259	0.0105	0	0	0		1
42	10	low	0.0056		0.015	0.0045	0		1
42	10	low	0		0	0	0		1
42	10	high	0.0311	0.010367	0	0	0		1
42	10	high	0		0.0237	0.0133333	0		1
42	10	high	0		0.0171	0.0067333	0		1
55	10	low	0.0026	0.000867	0	0	0		1
55	10	low	0		0	0	0		1
55	10	low	0		0	0	0		1
55	10	high	0	6.67E-05	0.0127	0.0126333	0		1
55	10	high	0		0.0002	0.0001333	0		1
55	10	high	0.0002		0.0082	0.0081333	0		1
70	10	low	0	0	0	0	0		1
70	10	low	0		0	0	0		1
70	10	low	0		0	0	0		1
70	10	high	0	0	0	0	0		1
70	10	high	0		0	0	0		1
70	10	high	0		0.0087	0.0087	0		1

Table B.3.D continued.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	12	low	0.0902	0.0886	0.1257	0.0371	5.15	73.41111	0.070153
0.5	12	low	0.0704		0.1293	0.0407	5.75	48.11667	0.119501
0.5	12	low	0.1052		0.1813	0.0927	14.4167	53.92778	0.267333
0.5	12	high	0.2003	0.1631	0.2439	0.0808	12.4333	93.36667	0.133167
0.5	12	high	0.1013		0.3017	0.1386	22.0667	99.00556	0.222883
0.5	12	high	0.1877		0.2334	0.0703	10.6833	108.0278	0.098894
1	12	low	0.07	0.1749	0.3081	0.1332	21.1667		0.358483
1	12	low	0.1929		0.1627	-0.0122	0		0.119501
1	12	low	0.2618		0.1761	0.0012	0		0.267333
1	12	high	0.125	0.1131	0.2593	0.1462	23.3333		0.383077
1	12	high	0.0929		0.2244	0.1113	17.5167		0.399809
1	12	high	0.1214		0.2563	0.1432	22.8333		0.31026
2	12	low	0.0538	0.0582	0.1183	0.0601	8.98333		0.480854
2	12	low	0.0472		0.1386	0.0804	12.3667		0.376515
2	12	low	0.0736		0.11	0.0518	7.6		0.408262
2	12	high	0.0901	0.083667	0.1718	0.0881333	13.6556		0.529335
2	12	high	0.0554		0.1725	0.0888333	13.7722		0.538915
2	12	high	0.1055		0.2131	0.1294333	20.5389		0.500386
5	12	low	0.05	0.055833	0.1765	0.1206667	19.0778		0.74073
5	12	low	0.0566		0.1441	0.0882667	13.6778		0.660778
5	12	low	0.0609		0.1532	0.0973667	15.1944		0.690018
5	12	high	0.0741	0.069733	0.2157	0.1459667	23.2944		0.778829
5	12	high	0.0543		0.2711	0.2013667	32.5278		0.86746
5	12	high	0.0808		0.2262	0.1564667	25.0444		0.732219
10	12	low	0.0307	0.0276	0.0989	0.0713	10.85		0.888527
10	12	low	0.019		0.0933	0.0657	9.91667		0.866874
10	12	low	0.0331		0.1253	0.0977	15.25		0.972803
10	12	high	0.0403	0.035767	0.1311	0.0953333	14.8556		0.937939
10	12	high	0.0262		0.1008	0.0650333	9.80556		0.9665
10	12	high	0.0408		0.1357	0.0999333	15.6222		0.876832
22	12	low	0.008	0.0149	0.0662	0.0513	7.51667		0.990919
22	12	low	0.0203		0.0549	0.04	5.63333		0.983951
22	12	low	0.0164		0.0299	0.015	1.46667		1
22	12	high	0.0236	0.0171	0.0522	0.0351	4.81667		0.989528
22	12	high	0.0111		0.0432	0.0261	3.31667		1
22	12	high	0.0166		0.0537	0.0366	5.06667		0.923734
32	12	low	0.0005	0.000167	0.0092	0.0090333	0.47222		0.997351
32	12	low	0		0.011	0.0108333	0.77222		1
32	12	low	0		0.0045	0.0043333	0		1
32	12	high	0.0068	0.004633	0.0167	0.0120667	0.97778		1
32	12	high	0.0008		0.0102	0.0055667	0		1
32	12	high	0.0063		0.0217	0.0170667	1.81111		0.940499
44	12	low	0.0137	0.005067	0.0035	-0.001567	0		0.997351
44	12	low	0.001		0.0011	-0.003967	0		1
44	12	low	0.0005		0.001	-0.004067	0		1
44	12	high	0.0036	0.0108	0.0082	-0.0026	0		1
44	12	high	0.0105		0.0033	-0.0075	0		1
44	12	high	0.0183		0.0551	0.0443	6.35		0.99928
58	12	low	0.0093	0.0557	0.0246	-0.0311	0		0.997351
58	12	low	0.1197		0.0343	-0.0214	0		1
58	12	low	0.0381		0.0355	-0.0202	0		1
58	12	high	0.0095	0.015733	0.0088	-0.006933	0		1
58	12	high	0.0162		0.0083	-0.007433	0		1
58	12	high	0.0215		0.0224	0.0066667	0.07778		1
70	12	low	0.0037	0.001233	0.0086	0.0073667	0.19444		1
70	12	low	0		0	0	0		1
70	12	low	0		0	0	0		1
70	12	high	0	0	0	0	0		1
70	12	high	0		0.0045	0.0045	0		1
70	12	high	0		0	0	0		1

Table B.3.E. Statistical estradiol release experiment data for Batch 2 at 37ºC with pH 7 and crosslinking ratio 10:2.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	7	low	0.0585	0.049	0.1434	0.0944	10.1231	55.80513	0.1814
0.5	7	low	0.0745		0.0751	0.0261	0	51.06667	0
0.5	7	low	0.0278		0.1094	0.0604	4.89231	52.60513	0.093001
0.5	7	low	0.0315		0.1201	0.0711	6.53846	63.52821	0.102922
0.5	7	low	0.0601		0.1106	0.0616	5.07692	48.51282	0.104651
0.5	7	low	0.0416		0.1424	0.0934	9.96923	95.74513	0.104123
0.5	7	high	0.0931	0.12455	0.1218	-0.00275	0	44.57179	0
0.5	7	high	0.1192		0.1309	0.00635	0	45.01795	0
0.5	7	high	0.1477		0.1119	-0.01265	0	34.72564	0
0.5	7	high	0.14		0.1804	0.05585	4.19231	82.45641	0.050843
0.5	7	high	0.1097		0.1352	0.01065	0	31.04872	0
0.5	7	high	0.1376		0.1016	-0.02295	0	51.20256	0
1	7	low	0.03	0.03655	0.1258	0.08925	9.33077		0.348603
1	7	low	0.0656		0.1262	0.08965	9.39231		0.183922
1	7	low	0.0123		0.115	0.07845	7.66923		0.238789
1	7	low	0.048		0.1385	0.10195	11.2846		0.280554
1	7	low	0.0372		0.1104	0.07385	6.96154		0.24815
1	7	low	0.0262		0.1874	0.15085	18.8077		0.300558
1	7	high	0.0564	0.07315	0.1542	0.08105	8.06923		0.181039
1	7	high	0.0791		0.1501	0.07695	7.43846		0.165233
1	7	high	0.0846		0.1089	0.03575	1.1		0.031677
1	7	high	0.0637		0.232	0.15885	20.0385		0.293862
1	7	high	0.0737		0.1156	0.04245	2.13077		0.068627
1	7	high	0.0814		0.1453	0.07215	6.7		0.130853
2	7	low	0.0367	0.032417	0.1835	0.1510833	18.8436		0.686271
2	7	low	0.061		0.1437	0.1112833	12.7205		0.433019
2	7	low	0.0107		0.1698	0.1373833	16.7359		0.556931
2	7	low	0.0495		0.1532	0.1207833	14.1821		0.503794
2	7	low	0.0115		0.1402	0.1077833	12.1821		0.49926
2	7	low	0.0251		0.2216	0.1891833	24.7051		0.558588
2	7	high	0.0525	0.064683	0.2188	0.1541167	19.3103		0.614278
2	7	high	0.0661		0.1504	0.0857167	8.78718		0.360426
2	7	high	0.0764		0.1628	0.0981167	10.6949		0.339659
2	7	high	0.0688		0.2208	0.1561167	19.6179		0.531781
2	7	high	0.0502		0.1497	0.0850167	8.67949		0.348171
2	7	high	0.0741		0.165	0.1003167	11.0333		0.346337
5	7	low	0.0273	0.033683	0.1513	0.1176167	13.6949		0.931676
5	7	low	0.0539		0.2213	0.1876167	24.4641		0.912081
5	7	low	0.0106		0.1889	0.1552167	19.4795		0.927228
5	7	low	0.0426		0.2305	0.1968167	25.8795		0.911164
5	7	low	0.0311		0.1954	0.1617167	20.4795		0.921406
5	7	low	0.0366		0.27771	0.2440267	33.1426		0.904742
5	7	high	0.0316	0.042	0.1595	0.1175	13.6769		0.92113
5	7	high	0.0327		0.2321	0.1901	24.8462		0.912343
5	7	high	0.0528		0.1529	0.1109	12.6615		0.704275
5	7	high	0.0592		0.2272	0.1852	24.0923		0.823963
5	7	high	0.036		0.1572	0.1152	13.3231		0.777273
5	7	high	0.0397		0.2197	0.1777	22.9385		0.794331
10	7	low	0	0.000517	0.0539	0.0533833	3.81282		1
10	7	low	0		0.0583	0.0577833	4.48974		1
10	7	low	0.0031		0.054	0.0534833	3.82821		1
10	7	low	0		0.0658	0.0652833	5.64359		1
10	7	low	0		0.0539	0.0533833	3.81282		1
10	7	low	0		0.0884	0.0878833	9.12051		1
10	7	high	0	0.00685	0.0583	0.05145	3.51538		1
10	7	high	0.0041		0.0611	0.05425	3.94615		1
10	7	high	0.0147		0.1022	0.09535	10.2692		1
10	7	high	0.0096		0.1167	0.10985	12.5		0.975558
10	7	high	0.0058		0.0804	0.07355	6.91538		1
10	7	high	0.0069		0.1039	0.09705	10.5308		1

Table B.3.E continued.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
20	7	low	0	0	0	0	0		1
20	7	low	0		0	0	0		1
20	7	low	0		0	0	0		1
20	7	low	0		0	0	0		1
20	7	low	0		0	0	0		1
20	7	low	0		0	0	0		1
20	7	high	0	0	0.0059	0.0059	0		1
20	7	high	0		0	0	0		1
20	7	high	0		0	0	0		1
20	7	high	0		0.0193	0.0193	0		1
20	7	high	0		0.0095	0.0095	0		1
20	7	high	0		0.0019	0.0019	0		1
30	7	low	0	0	0	0	0		1
30	7	low	0		0	0	0		1
30	7	low	0		0	0	0		1
30	7	low	0		0	0	0		1
30	7	low	0		0	0	0		1
30	7	low	0		0	0	0		1
30	7	high	0	0	0	0	0		1
30	7	high	0		0	0	0		1
30	7	high	0		0	0	0		1
30	7	high	0		0.0417	0.0417	2.01538		1
30	7	high	0		0	0	0		1
30	7	high	0		0	0	0		1
42	7	low	0	0	0.0105	0.0105	0		1
42	7	low	0		0	0	0		1
42	7	low	0		0	0	0		1
42	7	low	0		0	0	0		1
42	7	low	0		0	0	0		1
42	7	low	0		0	0	0		1
42	7	high	0	0	0	0	0		1
42	7	high	0		0	0	0		1
42	7	high	0		0	0	0		1
42	7	high	0		0	0	0		1
42	7	high	0		0	0	0		1
42	7	high	0		0	0	0		1
55	7	low	0	0	0	0	0		1
55	7	low	0		0	0	0		1
55	7	low	0		0	0	0		1
55	7	low	0		0	0	0		1
55	7	low	0		0	0	0		1
55	7	low	0		0	0	0		1
55	7	high	0	0	0	0	0		1
55	7	high	0		0	0	0		1
55	7	high	0		0	0	0		1
55	7	high	0		0	0	0		1
55	7	high	0		0	0	0		1
55	7	high	0		0	0	0		1
70	7	low	0	0	0	0	0		1
70	7	low	0		0	0	0		1
70	7	low	0		0	0	0		1
70	7	low	0		0	0	0		1
70	7	low	0		0	0	0		1
70	7	low	0		0	0	0		1
70	7	high	0	0	0	0	0		1
70	7	high	0		0	0	0		1
70	7	high	0		0	0	0		1
70	7	high	0		0	0	0		1
70	7	high	0		0	0	0		1
70	7	high	0		0	0	0		1

Table B.3.E continued.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc	Mt/Mo
0.5	7	low	0.0515	0.051217	0.1262	0.0749833	7.1359	64.64615	0.110384
0.5	7	low	0.0431		0.1255	0.0742833	7.02821	52.08462	0.134938
0.5	7	low	0.0587		0.1225	0.0712833	6.56667	93.83846	0.069978
0.5	7	low	0.052		0.1211	0.0698833	6.35128	63.83846	0.09949
0.5	7	low	0.062		0.1327	0.0814833	8.1359	56.08462	0.145065
0.5	7	low	0.04		0.1517	0.1004833	11.059	91.68462	0.12062
0.5	7	high	0.0845	0.139033	0.2092	0.0701667	6.39487	56.59231	0.112999
0.5	7	high	0.1483		0.1625	0.0234667	0	52.21538	0
0.5	7	high	0.126		0.1924	0.0533667	3.81026	45.70513	0.083366
0.5	7	high	0.1192		0.1599	0.0208667	0	42.7359	0
0.5	7	high	0.2133		0.2293	0.0902667	9.48718	50.31282	0.188564
0.5	7	high	0.1429		0.2614	0.1223667	14.4256	78.83846	0.182977
1	7	low	0.0381	0.041933	0.1498	0.1078667	12.1949		0.299024
1	7	low	0.0346		0.1277	0.0857667	8.79487		0.303796
1	7	low	0.046		0.1346	0.0926667	9.85641		0.175014
1	7	low	0.0411		0.1643	0.1223667	14.4256		0.325461
1	7	low	0.0559		0.1214	0.0794667	7.82564		0.284597
1	7	low	0.0359		0.1545	0.1125667	12.9179		0.261515
1	7	high	0.0549	0.0722	0.2022	0.13	15.6		0.388655
1	7	high	0.0679		0.1757	0.1035	11.5231		0.220684
1	7	high	0.0822		0.1674	0.0952	10.2462		0.307546
1	7	high	0.0775		0.1552	0.083	8.36923		0.195836
1	7	high	0.0725		0.171	0.0988	10.8		0.403221
1	7	high	0.0782		0.2089	0.1367	16.6308		0.393925
2	7	low	0.0315	0.032217	0.1568	0.1245833	14.7667		0.527447
2	7	low	0.0236		0.1141	0.0818833	8.19744		0.461182
2	7	low	0.0407		0.151	0.1187833	13.8744		0.322868
2	7	low	0.0216		0.131	0.0987833	10.7974		0.494598
2	7	low	0.0469		0.1296	0.0973833	10.5821		0.473278
2	7	low	0.029		0.1788	0.1465833	18.1513		0.45949
2	7	high	0.0498	0.050167	0.1605	0.1103333	12.5744		0.610847
2	7	high	0.046		0.138	0.0878333	9.11282		0.395207
2	7	high	0.0524		0.1542	0.1040333	11.6051		0.561459
2	7	high	0.0473		0.1464	0.0962333	10.4051		0.439311
2	7	high	0.0405		0.136	0.0858333	8.80513		0.578229
2	7	high	0.065		0.1763	0.1261333	15.0051		0.584252
5	7	low	0.0309	0.050583	0.1849	0.1343167	16.2641		0.779034
5	7	low	0.0377		0.1803	0.1297167	15.5564		0.759858
5	7	low	0.0833		0.2955	0.2449167	33.2795		0.677515
5	7	low	0.0398		0.2118	0.1612167	20.4026		0.814194
5	7	low	0.0563		0.1914	0.1408167	17.2641		0.7811
5	7	low	0.0555		0.303	0.2524167	34.4333		0.835053
5	7	high	0.0422	0.048917	0.1774	0.1284833	15.3667		0.88238
5	7	high	0.0557		0.1731	0.1241833	14.7051		0.676832
5	7	high	0.0454		0.1697	0.1207833	14.1821		0.871753
5	7	high	0.0555		0.1867	0.1377833	16.7974		0.832363
5	7	high	0.0459		0.1464	0.0974833	10.5974		0.788859
5	7	high	0.0488		0.2338	0.1848833	24.0436		0.889225
10	7	low	0.0238	0.02015	0.1416	0.12145	14.2846		1
10	7	low	0.0179		0.1186	0.09845	10.7462		0.966179
10	7	low	0.0145		0.0999	0.07975	7.86923		0.761374
10	7	low	0.0108		0.1004	0.08025	7.94615		0.938667
10	7	low	0.0329		0.1187	0.09855	10.7615		0.97298
10	7	low	0.021		0.1246	0.10445	11.6692		0.962329
10	7	high	0.024	0.023533	0.0954	0.0718667	6.65641		1
10	7	high	0.0252		0.1001	0.0765667	7.37949		0.818159
10	7	high	0.0233		0.0855	0.0619667	5.13333		0.984067
10	7	high	0.0219		0.0987	0.0751667	7.1641		1
10	7	high	0.0219		0.1151	0.0915667	9.68718		0.981398
10	7	high	0.0249		0.1089	0.0853667	8.73333		1

Table B.3.E continued.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc	Mt/Mo
22	7	low	0.0018	0.01775	0.0441	0.02635	0		1
22	7	low	0.0537		0.0578	0.04005	1.76154		1
22	7	low	0.0187		0.1919	0.17415	22.3923		1
22	7	low	0.0073		0.0718	0.05405	3.91538		1
22	7	low	0.0073		0.0562	0.03845	1.51538		1
22	7	low	0.0177		0.0688	0.05105	3.45385		1
22	7	high	0.0195	0.011017	0.0276	0.0165833	0		1
22	7	high	0.0101		0.0625	0.0514833	3.52051		0.885582
22	7	high	0.009		0.0252	0.0141833	0		0.984067
22	7	high	0.0084		0.0325	0.0214833	0		1
22	7	high	0.0089		0.0457	0.0346833	0.9359		1
22	7	high	0.0102		0.0382	0.0271833	0		1
32	7	low	0	0.000867	0.0159	0.0150333	0		1
32	7	low	0		0.0145	0.0136333	0		1
32	7	low	0.0002		0.0082	0.0073333	0		1
32	7	low	0		0.014	0.0131333	0		1
32	7	low	0.0012		0.0129	0.0120333	0		1
32	7	low	0.0038		0.0084	0.0075333	0		1
32	7	high	0.0055	0.006367	0.0058	-0.000567	0		1
32	7	high	0.0057		0.0738	0.0674333	5.97436		1
32	7	high	0.0142		0.0397	0.0333333	0.72821		1
32	7	high	0.0008		0.0131	0.0067333	0		1
32	7	high	0.0095		0.0321	0.0257333	0		1
32	7	high	0.0025		0.01	0.0036333	0		1
44	7	low	0	6.67E-05	0.0042	0.0041333	0		1
44	7	low	0		0.0027	0.0026333	0		1
44	7	low	0		0	0	0		1
44	7	low	0		0.0012	0.0011333	0		1
44	7	low	0.0002		0.0025	0.0024333	0		1
44	7	low	0		0.0019	0.0018333	0		1
44	7	high	0.0063	0.0057	0.003	-0.0027	0		1
44	7	high	0.0012		0.0167	0.011	0		1
44	7	high	0.0021		0.0089	0.0032	0		1
44	7	high	0.0027		0.0061	0.0004	0		1
44	7	high	0.004		0.0111	0.0054	0		1
44	7	high	0.0008		0.0034	-0.0023	0		1
58	7	low	0.0023	0.001733	0.0041	0.0023667	0		1
58	7	low	0.0016		0.0026	0.0008667	0		1
58	7	low	0.0005		0.0131	0.0113667	0		1
58	7	low	0.001		0.0017	-3.33E-05	0		1
58	7	low	0.0038		0.006	0.0042667	0		1
58	7	low	0.0012		0.0075	0.0057667	0		1
58	7	high	0.0031	0.002867	0.0005	-0.002367	0		1
58	7	high	0.003		0.0054	0.0025333	0		1
58	7	high	0.0018		0.0034	0.0005333	0		1
58	7	high	0.0001		0.0054	0.0025333	0		1
58	7	high	0.0044		0.0038	0.0009333	0		1
58	7	high	0.0048		0.0078	0.0049333	0		1
70	7	low	0	0	0	0	0		1
70	7	low	0		0	0	0		1
70	7	low	0		0	0	0		1
70	7	low	0		0	0	0		1
70	7	low	0		0	0	0		1
70	7	low	0		0	0	0		1
70	7	high	0	0	0	0	0		1
70	7	high	0		0	0	0		1
70	7	high	0		0	0	0		1
70	7	high	0		0	0	0		1
70	7	high	0		0	0	0		1
70	7	high	0		0	0	0		1

Table B.3.F. Statistical estradiol release experiment data for Batch 2 at 37ºC with pH 2, 4, 10, and 12 and crosslinking ratio 10:3.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	2	low	0.0532	0.070433	0.1196	0.0491667	7.02604	102.2656	0.068704
0.5	2	low	0.0525		0.1608	0.0903667	13.4635	97.93229	0.137478
0.5	2	low	0.1056		0.1397	0.0692667	10.1667	97.67708	0.104084
0.5	2	high	0.1209	0.1258	0.2085	0.0827	12.2656	80.38021	0.152595
0.5	2	high	0.1511		0.1871	0.0613	8.92188	82.77083	0.10779
0.5	2	high	0.1054		0.3108	0.185	28.25	101.5677	0.27814
1	2	low	0.0316	0.069167	0.1859	0.1167333	17.5833		0.240642
1	2	low	0.1123		0.1575	0.0883333	13.1458		0.271712
1	2	low	0.0636		0.1503	0.0811333	12.0208		0.227152
1	2	high	0.0931	0.090667	0.1806	0.0899333	13.3958		0.319251
1	2	high	0.102		0.2238	0.1331333	20.1458		0.351183
1	2	high	0.0769		0.2027	0.1120333	16.849		0.444029
2	2	low	0.0307	0.037667	0.1553	0.1176333	17.724		0.413955
2	2	low	0.0337		0.1828	0.1451333	22.0208		0.49657
2	2	low	0.0486		0.1567	0.1190333	17.9427		0.410846
2	2	high	0.0613	0.066733	0.1919	0.1251667	18.901		0.554396
2	2	high	0.0801		0.1654	0.0986667	14.7604		0.529512
2	2	high	0.0588		0.1826	0.1158667	17.4479		0.615815
5	2	low	0.0294	0.040833	0.2873	0.2464667	37.8542		0.78411
5	2	low	0.0384		0.2536	0.2127667	32.5885		0.829336
5	2	low	0.0547		0.2583	0.2174667	33.3229		0.752
5	2	high	0.0618	0.0673	0.2012	0.1339	20.2656		0.806518
5	2	high	0.0835		0.2037	0.1364	20.6563		0.779071
5	2	high	0.0566		0.2217	0.1544	23.4688		0.84688
10	2	low	0.0211	0.025	0.1207	0.0957	14.2969		0.923911
10	2	low	0.0165		0.1107	0.0857	12.7344		0.959368
10	2	low	0.0374		0.142	0.117	17.625		0.932441
10	2	high	0.0282	0.0376	0.1141	0.0765	11.2969		0.947061
10	2	high	0.0403		0.1389	0.1013	15.1719		0.962371
10	2	high	0.0443		0.1119	0.0743	10.9531		0.95472
22	2	low	0.0035	0.005533	0.0352	0.0296667	3.97917		0.962821
22	2	low	0.0051		0.0352	0.0296667	3.97917		1
22	2	low	0.008		0.0437	0.0381667	5.30729		0.986776
22	2	high	0.0129	0.013167	0.0398	0.0266333	3.50521		0.990669
22	2	high	0.0147		0.0365	0.0233333	2.98958		0.99849
22	2	high	0.0119		0.0365	0.0233333	2.98958		0.984155
32	2	low	0.0055	0.001833	0.0059	0.0040667	0		0.962821
32	2	low	0		0.0056	0.0037667	0		1
32	2	low	0		0.0143	0.0124667	1.29167		1
32	2	high	0.0009	0.0022	0.0112	0.009	0.75		1
32	2	high	0.0019		0.0072	0.005	0.125		1
32	2	high	0.0038		0.0167	0.0145	1.60938		1
44	2	low	0	0.023367	0.0519	0.0285333	3.80208		1
44	2	low	0.0243		0.0053	-0.018067	0		1
44	2	low	0.0458		0.0085	-0.014867	0		1
44	2	high	0.0129	0.0043	0	0	0		1
44	2	high	0		0	0	0		1
44	2	high	0		0.0021	-0.0022	0		1
58	2	low	0	0.001033	0.0002	-0.000833	0		1
58	2	low	0		0	0	0		1
58	2	low	0.0031		0.0047	0.0036667	0		1
58	2	high	0.0035	0.001167	0.0006	-0.000567	0		1
58	2	high	0		0	0	0		1
58	2	high	0		0.0046	0.0034333	0		1
70	2	low	0	0	0	0	0		1
70	2	low	0		0	0	0		1
70	2	low	0		0	0	0		1
70	2	high	0	0	0	0	0		1
70	2	high	0		0.0017	0.0017	0		1
70	2	high	0		0	0	0		1

Table B.3.F continued.

Time	ph	MW	blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	4	low	0.0768	0.0778	0.1115	0.0337	0.78462	32.65128	0.02403
0.5	4	low	0.0937		0.1858	0.108	12.2154	75.42051	0.161964
0.5	4	low	0.0629		0.1273	0.0495	3.21538	55.06667	0.058391
0.5	4	high	0.1364	0.134533	0.2281	0.0935667	9.99487	59.24103	0.168715
0.5	4	high	0.1403		0.1567	0.0221667	0	20.89231	0
0.5	4	high	0.1269		0.2639	0.1293667	15.5026	60.84103	0.254804
1	4	low	0.048	0.058833	0.1225	0.0636667	5.39487		0.189257
1	4	low	0.0741		0.1629	0.1040667	11.6103		0.315904
1	4	low	0.0544		0.1553	0.0964667	10.441		0.247998
1	4	high	0.13	0.099133	0.1886	0.0894667	9.3641		0.326783
1	4	high	0.0834		0.1431	0.0439667	2.3641		0.113157
1	4	high	0.084		0.2395	0.1403667	17.1949		0.537424
2	4	low	0.0427	0.058133	0.1193	0.0611667	5.01026		0.342705
2	4	low	0.0864		0.2038	0.1456667	18.0103		0.554702
2	4	low	0.0453		0.1642	0.1060667	11.9179		0.464425
2	4	high	0.1268	0.0932	0.2226	0.1294	15.5077		0.588556
2	4	high	0.077		0.1601	0.0669	5.89231		0.395189
2	4	high	0.0758		0.1789	0.0857	8.78462		0.681811
5	4	low	0.0473	0.059767	0.171	0.1112333	12.7128		0.732056
5	4	low	0.0827		0.2456	0.1858333	24.1897		0.875433
5	4	low	0.0493		0.2024	0.1426333	17.5436		0.783014
5	4	high	0.137	0.085733	0.1994	0.1136667	13.0872		0.80947
5	4	high	0.0648		0.1499	0.0641667	5.47179		0.657094
5	4	high	0.0554		0.2	0.1142667	13.1795		0.898432
10	4	low	0.0492	0.053433	0.1389	0.0854667	8.74872		1
10	4	low	0.0516		0.1431	0.0896667	9.39487		1
10	4	low	0.0595		0.1597	0.1062667	11.9487		1
10	4	high	0.0783	0.070133	0.1721	0.1019667	11.2872		1
10	4	high	0.0743		0.1453	0.0751667	7.1641		1
10	4	high	0.0578		0.1389	0.0687667	6.17949		1
20	4	low	0.0125	0.014	0.0366	0.0226	0		1
20	4	low	0.0175		0.041	0.027	0		1
20	4	low	0.012		0.0418	0.0278	0		1
20	4	high	0.0258	0.022833	0.037	0.0141667	0		1
20	4	high	0.0191		0.0421	0.0192667	0		1
20	4	high	0.0236		0.041	0.0181667	0		1
30	4	low	0.007	0.003533	0.0059	0.0023667	0		1
30	4	low	0		0.0098	0.0062667	0		1
30	4	low	0.0036		0.0108	0.0072667	0		1
30	4	high	0.0033	0.004833	0.0102	0.0053667	0		1
30	4	high	0.0074		0.012	0.0071667	0		1
30	4	high	0.0038		0.0109	0.0060667	0		1
42	4	low	0	0.000833	0.0006	-0.000233	0		1
42	4	low	0		0.0002	-0.000633	0		1
42	4	low	0.0025		0.0028	0.0019667	0		1
42	4	high	0.0111	0.004433	0.007	0.0025667	0		1
42	4	high	0.0021		0.0049	0.0004667	0		1
42	4	high	0.0001		0.0034	-0.001033	0		1
55	4	low	0.0013	0.000433	0	0	0		1
55	4	low	0		0	0	0		1
55	4	low	0		0	0	0		1
55	4	high	0	0	0	0	0		1
55	4	high	0		0	0	0		1
55	4	high	0		0	0	0		1
70	4	low	0	0.001433	0	0	0		1
70	4	low	0.0043		0.0036	0.0021667	0		1
70	4	low	0		0	0	0		1
70	4	high	0	0	0	0	0		1
70	4	high	0		0	0	0		1
70	4	high	0		0	0	0		1

Table B.3.F continued.

Time	ph	MW	blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	10	low	0.0714	0.064467	0.0772	0.0127333	0	28.21538	0
0.5	10	low	0.0557		0.1447	0.0802333	7.94359	35.68718	0.222589
0.5	10	low	0.0663		0.1058	0.0413333	1.95897	62.74359	0.031222
0.5	10	high	0.2144	0.205967	0.1948	-0.011167	0	17.90769	0
0.5	10	high	0.1605		0.2522	0.0462333	2.71282	46.35897	0.058518
0.5	10	high	0.243		0.2711	0.0651333	5.62051	31.94359	0.175951
1	10	low	0.0773	0.0701	0.15	0.0799	7.89231		0.279716
1	10	low	0.0373		0.1315	0.0614	5.04615		0.363989
1	10	low	0.0957		0.1671	0.097	10.5231		0.198937
1	10	high	0.158	0.1279	0.18	0.0521	3.61538		0.20189
1	10	high	0.1068		0.2579	0.13	15.6		0.395022
1	10	high	0.1189		0.2194	0.0915	9.67692		0.478889
2	10	low	0.0697	0.062333	0.113	0.0506667	3.39487		0.400036
2	10	low	0.0518		0.1325	0.0701667	6.39487		0.543181
2	10	low	0.0655		0.2074	0.1450667	17.9179		0.484512
2	10	high	0.1596	0.1312	0.1773	0.0461	2.69231		0.352234
2	10	high	0.1171		0.2356	0.1044	11.6615		0.646571
2	10	high	0.1169		0.1968	0.0656	5.69231		0.657088
5	10	low	0.0516	0.0742	0.1918	0.1176	13.6923		0.885314
5	10	low	0.0489		0.1866	0.1124	12.8923		0.90444
5	10	low	0.1221		0.2375	0.1633	20.7231		0.814794
5	10	high	0.1483	0.117533	0.2203	0.1027667	11.4103		0.989404
5	10	high	0.0927		0.2232	0.1056667	11.8564		0.902323
5	10	high	0.1116		0.2058	0.0882667	9.17949		0.944453
10	10	low	0.0669	0.060767	0.1104	0.0496333	3.2359		1
10	10	low	0.0649		0.1112	0.0504333	3.35897		0.998563
10	10	low	0.0505		0.1365	0.0757333	7.25128		0.930364
10	10	high	0.0816	0.077167	0.107	0.0298333	0.18974		1
10	10	high	0.0714		0.1352	0.0580333	4.52821		1
10	10	high	0.0785		0.1173	0.0401333	1.77436		1
20	10	low	0.0202	0.020067	0.0288	0.0087333	0		1
20	10	low	0.018		0.049	0.0289333	0.05128		1
20	10	low	0.022		0.041	0.0209333	0		0.930364
20	10	high	0.031	0.0269	0.0319	0.005	0		1
20	10	high	0.0252		0.0424	0.0155	0		1
20	10	high	0.0245		0.0539	0.027	0		1
30	10	low	0	0	0.0436	0.0436	0		1
30	10	low	0		0	0	0		1
30	10	low	0		0.0823	0.0823	8.26154		1
30	10	high	0	0	0.0006	0.006	0		1
30	10	high	0		0.0015	0.0015	0		1
30	10	high	0		0.0022	0.0022	0		1
42	10	low	0	0	0	0	0		1
42	10	low	0		0	0	0		1
42	10	low	0		0	0	0		1
42	10	high	0.0037	0.001233	0	0	0		1
42	10	high	0		0	0	0		1
42	10	high	0		0.0005	-0.000733	0		1
55	10	low	0	0	0	0	0		1
55	10	low	0		0	0	0		1
55	10	low	0		0	0	0		1
55	10	high	0	0	0	0	0		1
55	10	high	0		0	0	0		1
55	10	high	0		0	0	0		1
70	10	low	0	0	0	0	0		1
70	10	low	0		0	0	0		1
70	10	low	0		0	0	0		1
70	10	high	0	0	0	0	0		1
70	10	high	0		0	0	0		1
70	10	high	0		0	0	0		1

Table B.3.F continued.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	12	low	0.0522	0.050567	0.1587	0.1081333	16.9889	133.4944	0.127263
0.5	12	low	0.0515		0.1505	0.0999333	15.6222	96.67778	0.161591
0.5	12	low	0.048		0.1398	0.0892333	13.8389	91.52222	0.151208
0.5	12	high	0.2364	0.239733	0.3025	0.0627667	9.42778	52.84444	0.178406
0.5	12	high	0.2332		0.2043	-0.035433	0	18.18889	0
0.5	12	high	0.2496		0.3289	0.0891667	13.8278	65.72778	0.21038
1	12	low	0.0363	0.040233	0.2148	0.1745667	28.0611		0.337467
1	12	low	0.0475		0.1516	0.1113667	17.5278		0.342892
1	12	low	0.0369		0.12	0.0797667	12.2611		0.285177
1	12	high	0.1441	0.186133	0.2385	0.0523667	7.69444		0.324012
1	12	high	0.1946		0.1923	0.0061667	0		0
1	12	high	0.2197		0.2487	0.0625667	9.39444		0.353309
2	12	low	0.0338	0.0348	0.1755	0.1407	22.4167		0.505389
2	12	low	0.0396		0.1268	0.092	14.3		0.490806
2	12	low	0.031		0.1298	0.095	14.8		0.446886
2	12	high	0.1135	0.117167	0.2027	0.0855333	13.2222		0.574222
2	12	high	0.1197		0.1478	0.0306333	4.07222		0.223885
2	12	high	0.1183		0.1853	0.0681333	10.3222		0.510354
5	12	low	0.0306	0.0345	0.2658	0.2313	37.5167		0.786425
5	12	low	0.0394		0.1702	0.1357	21.5833		0.714056
5	12	low	0.0335		0.1905	0.156	24.9667		0.719679
5	12	high	0.1265	0.112133	0.2222	0.1100667	17.3111		0.901808
5	12	high	0.1039		0.1676	0.0554667	8.21111		0.675321
5	12	high	0.106		0.2536	0.1414667	22.5444		0.853351
10	12	low	0.0117	0.013133	0.1531	0.1399667	22.2944		0.953431
10	12	low	0.0164		0.1354	0.1222667	19.3444		0.914148
10	12	low	0.0113		0.0917	0.0785667	12.0611		0.851463
10	12	high	0.0513	0.0409	0.068	0.0271	3.48333		0.967725
10	12	high	0.0408		0.0807	0.0398	5.6		0.983201
10	12	high	0.0306		0.0962	0.0553	8.18333		0.977855
22	12	low	0.0023	0.003767	0.0448	0.0410333	5.80556		0.99692
22	12	low	0.0027		0.0487	0.0449333	6.45556		0.980922
22	12	low	0.0063		0.0237	0.0199333	2.28889		0.876472
22	12	high	0.0171	0.017767	0.0202	0.0024333	0		0.967725
22	12	high	0.0174		0.0258	0.0080333	0.30556		1
22	12	high	0.0188		0.0317	0.0139333	1.28889		0.997464
32	12	low	0	0.006933	0.0076	0.0006667	0		0.99692
32	12	low	0.0119		0.0096	0.0026667	0		0.980922
32	12	low	0.0089		0.0803	0.0733667	11.1944		0.998786
32	12	high	0.0142	0.008367	0.0248	0.0164333	1.70556		1
32	12	high	0.0075		0.0135	0.0051333	0		1
32	12	high	0.0034		0.0128	0.0044333	0		0.997464
44	12	low	0.0042	0.001933	0.0106	0.0086667	0.41111		1
44	12	low	0		0.0192	0.0172667	1.84444		1
44	12	low	0.0016		0.0088	0.0068667	0.11111		1
44	12	high	0.0075	0.0073	0.0095	0.0022	0		1
44	12	high	0.0089		0.0077	0.0004	0		1
44	12	high	0.0055		0.011	0.0037	0		0.997464
58	12	low	0.0086	0.006233	0.005	-0.001233	0		1
58	12	low	0.0021		0.0049	-0.001333	0		1
58	12	low	0.008		0.0091	0.0028667	0		1
58	12	high	0.015	0.0145	0.0068	-0.0077	0		1
58	12	high	0.0136		0.0091	-0.0054	0		1
58	12	high	0.0149		0.0217	0.0072	0.16667		1
70	12	low	0	0	0	0	0		1
70	12	low	0		0	0	0		1
70	12	low	0		0	0	0		1
70	12	high	0.0177	0.010833	0.0007	-0.010133	0		1
70	12	high	0		0.0001	-0.010733	0		1
70	12	high	0.0148		0.0073	-0.003533	0		1

Table B.3.G. Statistical estradiol release experiment data for Batch 3 at 41ºC with pH 7 and crosslinking ratio 10:1.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	7	low	0.0229	0.023133	0.1319	0.1087667	12.3333	51.41026	0.2399
0.5	7	low	0.0281		0.193	0.1698667	21.7333	85.61026	0.253864
0.5	7	low	0.0184		0.116	0.0928667	9.88718	49.85641	0.198313
0.5	7	high	0.0461	0.0662	0.102	0.0358	1.10769	33.52821	0.033038
0.5	7	high	0.0833		0.091	0.0248	0	25.85128	0
0.5	7	high	0.0692		0.16	0.0938	10.0308	48.32821	0.207555
1	7	low	0.009	0.0082	0.0804	0.0722	6.70769		0.370374
1	7	low	0.0083		0.1371	0.1289	15.4308		0.434108
1	7	low	0.0073		0.0821	0.0739	6.96923		0.338099
1	7	high	0.0251	0.0317	0.1109	0.0792	7.78462		0.265219
1	7	high	0.0341		0.0876	0.0559	4.2		0.162468
1	7	high	0.0359		0.1143	0.0826	8.30769		0.379457
2	7	low	0.0074	0.0055	0.1058	0.1003	11.0308		0.584938
2	7	low	0.0061		0.1425	0.137	16.6769		0.628909
2	7	low	0.003		0.104	0.0985	10.7538		0.553796
2	7	high	0.0228	0.024833	0.1074	0.0825667	8.30256		0.512848
2	7	high	0.0287		0.1106	0.0857667	8.79487		0.502678
2	7	high	0.023		0.12	0.0951667	10.241		0.591362
5	7	low	0.0061	0.006333	0.1203	0.1139667	13.1333		0.840399
5	7	low	0.0094		0.1626	0.1562667	19.641		0.858332
5	7	low	0.0035		0.1232	0.1168667	13.5795		0.826167
5	7	high	0.0133	0.0218	0.1133	0.0915	9.67692		0.816152
5	7	high	0.0268		0.1042	0.0824	8.27692		0.822853
5	7	high	0.0253		0.1337	0.1119	12.8154		0.856537
10	7	low	0.0048	0.005767	0.0877	0.0819333	8.20513		1
10	7	low	0.007		0.1132	0.1074333	12.1282		1
10	7	low	0.0055		0.0907	0.0849333	8.66667		1
10	7	high	0.0121	0.015333	0.0872	0.0718667	6.65641		1
10	7	high	0.0166		0.0737	0.0583667	4.57949		1
10	7	high	0.0173		0.089	0.0736667	6.93333		1
20	7	low	0	0	0.0118	0.0118	0		1
20	7	low	0		0.0122	0.0122	0		1
20	7	low	0		0.0116	0.0116	0		1
20	7	high	0	0	0.0146	0.0146	0		1
20	7	high	0		0.0118	0.0118	0		1
20	7	high	0		0.0281	0.0281	0		1
30	7	low	0	0	0	0	0		1
30	7	low	0		0	0	0		1
30	7	low	0		0	0	0		1
30	7	high	0	0	0	0	0		1
30	7	high	0		0	0	0		1
30	7	high	0		0	0	0		1
42	7	low	0	0	0	0	0		1
42	7	low	0		0	0	0		1
42	7	low	0		0	0	0		1
42	7	high	0	0.0084	0	0	0		1
42	7	high	0		0	0	0		1
42	7	high	0.0252		0	0	0		1
55	7	low	0	0	0	0	0		1
55	7	low	0		0	0	0		1
55	7	low	0		0	0	0		1
55	7	high	0	0	0	0	0		1
55	7	high	0		0	0	0		1
55	7	high	0		0	0	0		1
70	7	low	0	0	0	0	0		1
70	7	low	0		0	0	0		1
70	7	low	0		0	0	0		1
70	7	high	0	0	0	0	0		1
70	7	high	0		0	0	0		1
70	7	high	0		0	0	0		1

Table B.3.G continued.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	7	low	0.0888	0.088733	0.1579	0.0691667	6.24103	56.08205	0.111284
0.5	7	low	0.0996		0.148	0.0592667	4.71795	80.60513	0.058532
0.5	7	low	0.0778		0.1539	0.0651667	5.62564	49.02051	0.114761
0.5	7	high	0.146	0.116667	0.1827	0.0660333	5.75897	41.38462	0.139157
0.5	7	high	0.1214		0.2085	0.0918333	9.72821	36.46154	0.266807
0.5	7	high	0.0826		0.2604	0.1437333	17.7128	67.81538	0.261192
1	7	low	0.0729	0.062467	0.1712	0.1087333	12.3282		0.331108
1	7	low	0.0604		0.1936	0.1311333	15.7744		0.254231
1	7	low	0.0541		0.1498	0.0873333	9.0359		0.29909
1	7	high	0.0851	0.075133	0.183	0.1078667	12.1949		0.433829
1	7	high	0.0864		0.1629	0.0877667	9.10256		0.516456
1	7	high	0.0539		0.2207	0.1455667	17.9949		0.526543
2	7	low	0.0734	0.055533	0.1695	0.1139667	13.1333		0.565289
2	7	low	0.0535		0.2048	0.1492667	18.5641		0.48454
2	7	low	0.0397		0.1559	0.1003667	11.041		0.524323
2	7	high	0.0597	0.053167	0.1403	0.0871333	9.00513		0.651425
2	7	high	0.0678		0.1425	0.0893333	9.34359		0.772714
2	7	high	0.032		0.1507	0.0975333	10.6051		0.682925
5	7	low	0.0555	0.0507	0.1976	0.1469	18.2		0.889813
5	7	low	0.0487		0.3009	0.2502	34.0923		0.907495
5	7	low	0.0479		0.2089	0.1582	19.9385		0.93106
5	7	high	0.0408	0.043467	0.129	0.0855333	8.75897		0.863073
5	7	high	0.0533		0.1124	0.0689333	6.20513		0.942897
5	7	high	0.0363		0.1873	0.1438333	17.7282		0.944344
10	7	low	0.0261	0.016733	0.0802	0.0634667	5.3641		0.985461
10	7	low	0.012		0.0938	0.0770667	7.45641		1
10	7	low	0.0121		0.0673	0.0505667	3.37949		1
10	7	high	0.0192	0.016067	0.0815	0.0654333	5.66667		1
10	7	high	0.0188		0.0582	0.0421333	2.08205		1
10	7	high	0.0102		0.0692	0.0531333	3.77436		1
22	7	low	0.0063	0.0044	0.0383	0.0339	0.81538		1
22	7	low	0.0005		0.0229	0.0185	0		1
22	7	low	0.0064		0.0143	0.0099	0		1
22	7	high	0.0042	0.007267	0.0229	0.0156333	0		1
22	7	high	0.0119		0.0255	0.0182333	0		1
22	7	high	0.0057		0.022	0.0147333	0		1
32	7	low	0.0012	0.000567	0.0018	0.0012333	0		1
32	7	low	0.0005		0.0039	0.0033333	0		1
32	7	low	0		0.0046	0.0040333	0		1
32	7	high	0.0031	0.004233	0.0037	-0.000533	0		1
32	7	high	0.0044		0.0038	-0.000433	0		1
32	7	high	0.0052		0.0101	0.0058667	0		1
46	7	low	0	0	0	0	0		1
46	7	low	0		0	0	0		1
46	7	low	0		0	0	0		1
46	7	high	0.0003	0.000333	0	0	0		1
46	7	high	0.0007		0	0	0		1
46	7	high	0		0	0	0		1
58	7	low	0	0	0	0	0		1
58	7	low	0		0	0	0		1
58	7	low	0		0	0	0		1
58	7	high	0	0	0	0	0		1
58	7	high	0		0	0	0		1
58	7	high	0		0	0	0		1
70	7	low	0		0	0	0		1
70	7	low	0	0	0	0	0		1
70	7	low	0		0	0	0		1
70	7	high	0	0.000867	0	0	0		1
70	7	high	0.0026		0	0	0		1
70	7	high	0		0	0	0		1

Table B.3.H. Statistical estradiol release experiment data for Batch 3 at 41ºC with pH 2, 4, 10, and 12 and crosslinking ratio 10:2.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	2	low	0.0457	0.0535	0.1137	0.0602	8.75	69.13021	0.126573
0.5	2	low	0.0594		0.107	0.0535	7.70313	64.47396	0.119477
0.5	2	low	0.0554		0.1371	0.0836	12.4063	79.34896	0.156351
0.5	2	high	0.1331	0.1435	0.1079	-0.0356	0	25.55208	0
0.5	2	high	0.1607		0.1772	0.0337	4.60938	44.76042	0.102979
0.5	2	high	0.1367		0.1974	0.0539	7.76563	50.95833	0.152392
1	2	low	0.0342	0.047467	0.1377	0.0902333	13.4427		0.321028
1	2	low	0.0484		0.1156	0.0681333	9.98958		0.274416
1	2	low	0.0598		0.1439	0.0964333	14.4115		0.337972
1	2	high	0.088	0.097467	0.1247	0.0272333	3.59896		0.140848
1	2	high	0.1173		0.1788	0.0813333	12.0521		0.372236
1	2	high	0.0871		0.1472	0.0497333	7.11458		0.292007
2	2	low	0.0506	0.0456	0.1483	0.1027	15.3906		0.54366
2	2	low	0.0443		0.1438	0.0982	14.6875		0.502222
2	2	low	0.0419		0.1416	0.096	14.3438		0.51874
2	2	high	0.0655	0.079433	0.1179	0.0384667	5.35417		0.350387
2	2	high	0.097		0.1684	0.0889667	13.2448		0.668141
2	2	high	0.0758		0.1762	0.0967667	14.4635		0.575838
5	2	low	0.0377	0.039767	0.1956	0.1558333	23.6927		0.886386
5	2	low	0.0357		0.1873	0.1475333	22.3958		0.849584
5	2	low	0.0459		0.2168	0.1770333	27.0052		0.859074
5	2	high	0.0581	0.075433	0.1437	0.0682667	10.0104		0.742152
5	2	high	0.0889		0.163	0.0875667	13.026		0.959158
5	2	high	0.0793		0.1924	0.1169667	17.6198		0.921607
10	2	low	0.0154	0.0158	0.0654	0.0496	7.09375		0.989
10	2	low	0.0141		0.0791	0.0633	9.23438		0.99281
10	2	low	0.0179		0.069	0.0532	7.65625		0.955563
10	2	high	0.0292	0.036	0.0722	0.0362	5		0.937831
10	2	high	0.0315		0.0519	0.0159	1.82813		1
10	2	high	0.0473		0.0645	0.0285	3.79688		0.996116
22	2	low	0.0157	0.008733	0.0178	0.0090667	0.76042		1
22	2	low	0.004		0.0159	0.0071667	0.46354		1
22	2	low	0.0065		0.0348	0.0260667	3.41667		0.998622
22	2	high	0.0147	0.019433	0.0338	0.0143667	1.58854		1
22	2	high	0.0184		0.0179	-0.001533	0		1
22	2	high	0.0252		0.0249	0.0054667	0.19792		1
32	2	low	0.0058	0.0027	0.0004	-0.0023	0		1
32	2	low	0		0	0	0		1
32	2	low	0.0023		0.0012	-0.0015	0		0.998622
32	2	high	0.0045	0.008467	0.0084	-6.67E-05	0		1
32	2	high	0.009		0.0045	-0.003967	0		1
32	2	high	0.0119		0.007	-0.001467	0		1
46	2	low	0.0081	0.0097	0.0066	-0.0031	0		1
46	2	low	0.0072		0.0035	-0.0062	0		1
46	2	low	0.0138		0.0146	0.0049	0.10938		1
46	2	high	0.0032	0.001733	0.0006	-0.001133	0		1
46	2	high	0.0015		0	0	0		1
46	2	high	0.0005		0.0035	0.0017667	0		1
58	2	low	0.0014	0.001867	0.0027	0.0008333	0		1
58	2	low	0		0	0	0		1
58	2	low	0.0042		0	0	0		1
58	2	high	0	0.005967	0.0011	-0.004867	0		1
58	2	high	0.0009		0	0	0		1
58	2	high	0.017		0.0037	-0.002267	0		1
70	2	low	0	0	0	0	0		1
70	2	low	0		0	0	0		1
70	2	low	0		0	0	0		1
70	2	high	0	0.003367	0	0	0		1
70	2	high	0		0	0	0		1
70	2	high	0.0101		0	0	0		1

Table B.3.H continued.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	4	low	0.0346	0.044533	0.1173	0.0727667	6.79487	38.1641	0.178044
0.5	4	low	0.0508		0.1341	0.0895667	9.37949	48.24103	0.19443
0.5	4	low	0.0482		0.1574	0.1128667	12.9641	71.73333	0.180726
0.5	4	high	0.1002	0.081	0.1921	0.1111	12.6923	36.87692	0.34418
0.5	4	high	0.0775		0.2106	0.1296	15.5385	47.95385	0.32403
0.5	4	high	0.0653		0.1669	0.0859	8.81538	52.44615	0.168084
1	4	low	0.0195	0.024367	0.0924	0.0680333	6.06667		0.337006
1	4	low	0.0241		0.1014	0.0770333	7.45128		0.348889
1	4	low	0.0295		0.1364	0.1120333	12.8359		0.359665
1	4	high	0.052	0.046333	0.1468	0.1004667	11.0564		0.643999
1	4	high	0.047		0.1577	0.1113667	12.7333		0.589563
1	4	high	0.04		0.1632	0.1168667	13.5795		0.427007
2	4	low	0.014	0.0223	0.0972	0.0749	7.12308		0.52365
2	4	low	0.0257		0.1251	0.1028	11.4154		0.585521
2	4	low	0.0272		0.1398	0.1175	13.6769		0.550329
2	4	high	0.0495	0.0371	0.097	0.0599	4.81538		0.774579
2	4	high	0.0325		0.1112	0.0741	7		0.735536
2	4	high	0.0293		0.1364	0.0993	10.8769		0.634399
5	4	low	0.0204	0.025333	0.1369	0.1115667	12.7641		0.858103
5	4	low	0.0256		0.1364	0.1110667	12.6872		0.848517
5	4	low	0.03		0.2216	0.1962667	25.7949		0.909923
5	4	high	0.0352	0.034367	0.1069	0.0725333	6.75897		0.957864
5	4	high	0.0383		0.1331	0.0987333	10.7897		0.960539
5	4	high	0.0296		0.1462	0.1118333	12.8051		0.878557
10	4	low	0.0104	0.012	0.0553	0.0433	2.26154		0.917361
10	4	low	0.0112		0.0728	0.0608	4.95385		0.951207
10	4	low	0.0144		0.0826	0.0706	6.46154		1
10	4	high	0.0222	0.0209	0.0596	0.0387	1.55385		1
10	4	high	0.021		0.0618	0.0409	1.89231		1
10	4	high	0.0195		0.0909	0.07	6.36923		1
20	4	low	0.0052	0.008767	0.0141	0.0053333	0		0.917361
20	4	low	0.0064		0.0391	0.0303333	0.26667		0.956734
20	4	low	0.0147		0.037	0.0282333	0		1
20	4	high	0.0156	0.028367	0.0402	0.0118333	0		1
20	4	high	0.0543		0.0238	-0.004567	0		1
20	4	high	0.0152		0.0436	0.0152333	0		1
30	4	low	0.0085	0.007	0.0153	0.0083	0		0.917361
30	4	low	0.006		0.0243	0.0173	0		0.956734
30	4	low	0.0065		0.0067	-0.0003	0		1
30	4	high	0.0108	0.016967	0.0206	0.0036333	0		1
30	4	high	0.0291		0.0067	-0.010267	0		1
30	4	high	0.011		0.0311	0.0141333	0		1
42	4	low	0.0058	0.011733	0.0194	0.0076667	0		0.917361
42	4	low	0.0076		0.0539	0.0421667	2.08718		1
42	4	low	0.0218		0.0188	0.0070667	0		1
42	4	high	0.0071	0.029733	0.0146	-0.015133	0		1
42	4	high	0.0434		0.0027	-0.027033	0		1
42	4	high	0.0387		0.0112	-0.018533	0		1
55	4	low	0	0	0	0	0		1
55	4	low	0		0.0036	0.0036	0		1
55	4	low	0		0	0	0		1
55	4	high	0	0	0	0	0		1
55	4	high	0		0	0	0		1
55	4	high	0		0.0022	0.0022	0		1
70	4	low	0	0	0.0491	0.0491	3.15385		1
70	4	low	0		0.0036	0.0036	0		1
70	4	low	0		0	0	0		1
70	4	high	0	0	0	0	0		1
70	4	high	0		0	0	0		1
70	4	high	0		0	0	0		1

Table B.3.H continued.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	10	low	0.0595	0.0478	0.1171	0.0693	6.26154	33.12308	0.189039
0.5	10	low	0.0419		0.1125	0.0647	5.55385	36.8	0.15092
0.5	10	low	0.042		0.1171	0.0693	6.26154	28.26154	0.221557
0.5	10	high	0.0596	0.083667	0.2218	0.1381333	16.8513	60.85128	0.276926
0.5	10	high	0.0881		0.2017	0.1180333	13.759	49.71282	0.276769
0.5	10	high	0.1033		0.2198	0.1361333	16.5436	95.62051	0.173013
1	10	low	0.0388	0.028933	0.0956	0.0666667	5.85641		0.365846
1	10	low	0.0276		0.0972	0.0682667	6.10256		0.31675
1	10	low	0.0204		0.0856	0.0566667	4.31795		0.374342
1	10	high	0.0279	0.041033	0.1453	0.1042667	11.641		0.468229
1	10	high	0.0479		0.1609	0.1198667	14.041		0.559212
1	10	high	0.0473		0.2383	0.1972667	25.9487		0.444385
2	10	low	0.0324	0.0245	0.0874	0.0629	5.27692		0.525159
2	10	low	0.0226		0.107	0.0825	8.29231		0.542085
2	10	low	0.0185		0.0809	0.0564	4.27692		0.525676
2	10	high	0.0193	0.027867	0.1229	0.0950333	10.2205		0.636187
2	10	high	0.0315		0.1051	0.0772333	7.48205		0.709717
2	10	high	0.0328		0.1733	0.1454333	17.9744		0.632361
5	10	low	0.0315	0.024067	0.1383	0.1142333	13.1744		0.922898
5	10	low	0.0224		0.1395	0.1154333	13.359		0.9051
5	10	low	0.0183		0.1321	0.1080333	12.2205		0.958084
5	10	high	0.0224	0.029667	0.1747	0.1450333	17.9128		0.930558
5	10	high	0.0329		0.1112	0.0815333	8.14359		0.87353
5	10	high	0.0337		0.2343	0.2046333	27.0821		0.915585
10	10	low	0.0142	0.0105	0.0557	0.0452	2.55385		1
10	10	low	0.0105		0.0618	0.0513	3.49231		1
10	10	low	0.0068		0.0468	0.0363	1.18462		1
10	10	high	0.0104	0.013633	0.0697	0.0560667	4.22564		1
10	10	high	0.0109		0.0624	0.0487667	3.10256		0.93594
10	10	high	0.0196		0.0848	0.0711667	6.54872		0.984072
20	10	low	0.0071	0.008367	0.0168	0.0084333	0		1
20	10	low	0.0064		0.0157	0.0073333	0		1
20	10	low	0.0116		0.015	0.0066333	0		1
20	10	high		0.009			0		1
20	10	high	0.009		0.0583	0.0493	3.18462		1
20	10	high			0.0475	0.0385	1.52308		1
30	10	low	0.0054	0.0047	0.0027	-0.002	0		1
30	10	low	0.004		0.0087	0.004	0		1
30	10	low	0.0047		0.0046	-0.0001	0		1
30	10	high	0.0186	0.010733	0.0153	0.0045667	0		1
30	10	high	0.0063		0.0232	0.0124667	0		1
30	10	high	0.0073		0.0387	0.0279667	0		1
42	10	low	0.006	0.006	0.0037	-0.0023	0		1
42	10	low	0.0051		0.0016	-0.0044	0		1
42	10	low	0.0069		0.001	-0.005	0		1
42	10	high	0.0041	0.0048	0.0282	0.0234	0		1
42	10	high	0.004		0.002	-0.0028	0		1
42	10	high	0.0063		0.0153	0.0105	0		1
55	10	low	0	0	0	0	0		1
55	10	low	0		0	0	0		1
55	10	low	0		0	0	0		1
55	10	high	0	0	0	0	0		1
55	10	high	0		0	0	0		1
55	10	high	0		0	0	0		1
70	10	low	0.0036	0.0046	0.002	-0.0026	0		1
70	10	low	0.0007		0.0154	0.0108	0		1
70	10	low	0.0095		0	0	0		1
70	10	high	0.0036	0.003133	0.0218	0.0186667	0		1
70	10	high	0.0001		0.0154	0.0122667	0		1
70	10	high	0.0057		0.0038	0.0006667	0		1

Table B.3.H continued.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	12	low	0.0879	0.070733	0.124	0.0532667	7.84444	79.84444	0.098247
0.5	12	low	0.047		0.1457	0.0749667	11.4611	70.31667	0.162993
0.5	12	low	0.0773		0.1305	0.0597667	8.92778	54.04444	0.165193
0.5	12	high	0.161	0.1878	0.254	0.0662	10	79.63889	0.125567
0.5	12	high	0.2082		0.2465	0.0587	8.75	48.35	0.180972
0.5	12	high	0.1942		0.2994	0.1116	17.5667	79.01667	0.222316
1	12	low	0.0749	0.054367	0.1592	0.1048333	16.4389		0.304133
1	12	low	0.038		0.1592	0.1048333	16.4389		0.396776
1	12	low	0.0502		0.1208	0.0664333	10.0389		0.350946
1	12	high	0.118	0.118267	0.2113	0.0930333	14.4722		0.30729
1	12	high	0.1377		0.1627	0.0444333	6.37222		0.312766
1	12	high	0.0991		0.2013	0.0830333	12.8056		0.384377
2	12	low	0.0771	0.0534	0.1521	0.0987	15.4167		0.497217
2	12	low	0.0278		0.1536	0.1002	15.6667		0.619578
2	12	low	0.0553		0.1084	0.055	8.13333		0.501439
2	12	high	0.0821	0.081267	0.1919	0.1106333	17.4056		0.525846
2	12	high	0.0919		0.1328	0.0515333	7.55556		0.469034
2	12	high	0.0698		0.1608	0.0795333	12.2222		0.539056
5	12	low	0.08	0.061133	0.1975	0.1363667	21.6944		0.768926
5	12	low	0.035		0.1962	0.1350667	21.4778		0.925022
5	12	low	0.0684		0.1765	0.1153667	18.1944		0.838096
5	12	high	0.0781	0.0741	0.2097	0.1356	21.5667		0.796652
5	12	high	0.0811		0.193	0.1189	18.7833		0.85752
5	12	high	0.0631		0.193	0.1189	18.7833		0.77677
10	12	low	0.0218	0.0206	0.0955	0.0749	11.45		0.91233
10	12	low	0.0208		0.0545	0.0339	4.61667		0.990677
10	12	low	0.0192		0.0571	0.0365	5.05		0.931538
10	12	high	0.0244	0.026567	0.086	0.0594333	8.87222		0.908057
10	12	high	0.0265		0.0619	0.0353333	4.85556		0.957946
10	12	high	0.0288		0.0626	0.0360333	4.97222		0.839696
22	12	low	0.0079	0.0062	0.0257	0.0195	2.21667		0.940092
22	12	low	0.0022		0.0141	0.0079	0.28333		0.994706
22	12	low	0.0085		0.0241	0.0179	1.95		0.967619
22	12	high	0.0139	0.0147	0.0429	0.0282	3.66667		0.954098
22	12	high	0.0162		0.0331	0.0184	2.03333		1
22	12	high	0.014		0.0482	0.0335	4.55		0.897279
32	12	low	0.003	0.002567	0.0373	0.0347333	4.75556		0.999652
32	12	low	0.0001		0.011	0.0084333	0.37222		1
32	12	low	0.0046		0.0169	0.0143333	1.35556		0.992701
32	12	high	0.0115	0.023467	0.031	0.0075333	0.22222		0.956889
32	12	high	0.0428		0.0246	0.0011333	0		1
32	12	high	0.0161		0.0223	-0.001167	0		0.897279
46	12	low	0.0034	0.002833	0.0092	0.0063667	0.02778		1
46	12	low	0.0027		0.0074	0.0045667	0		1
46	12	low	0.0024		0.0114	0.0085667	0.39444		1
46	12	high	0.0241	0.0191	0.0459	0.0268	3.43333		1
46	12	high	0.0164		0.0245	0.0054	0		1
46	12	high	0.0168		0.074	0.0549	8.11667		1
58	12	low	0	0.009467	0.0004	-0.009067	0		1
58	12	low	0.0005		0.015	0.0055333	0		1
58	12	low	0.0279		0.0029	-0.006567	0		1
58	12	high	0.0085	0.015567	0.0124	-0.003167	0		1
58	12	high	0.0156		0.0067	-0.008867	0		1
58	12	high	0.0226		0.0171	0.0015333	0		1
70	12	low	0.0018	0.0006	0.001	0.0004	0		1
70	12	low	0		0	0	0		1
70	12	low	0		0	0	0		1
70	12	high	0.0144	0.010333	0.0029	-0.007433	0		1
70	12	high	0.0076		0.0046	-0.005733	0		1
70	12	high	0.009		0.0063	-0.004033	0		1

Table B.3.I. Statistical estradiol release experiment data for Batch 3 at 41ºC with pH 7 and crosslinking ratio 10:3.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	7	low	0.0505	0.043267	0.1304	0.0871333	9.00513	43.71795	0.205982
0.5	7	low	0.0486		0.1166	0.0733333	6.88205	33.57949	0.204948
0.5	7	low	0.0307		0.135	0.0917333	9.71282	36.13333	0.268805
0.5	7	high	0.0631	0.099633	0.1462	0.0465667	2.7641	30.81026	0.089714
0.5	7	high	0.1077		0.185	0.0853667	8.73333	46.44103	0.188052
0.5	7	high	0.1281		0.1046	0.0049667	0	15.16923	0
1	7	low	0.0272	0.021533	0.0869	0.0653667	5.65641		0.335367
1	7	low	0.0205		0.0825	0.0609667	4.97949		0.353238
1	7	low	0.0169		0.0826	0.0610667	4.99487		0.407039
1	7	high	0.0278	0.048	0.1178	0.0698	6.33846		0.295439
1	7	high	0.0499		0.1225	0.0745	7.06154		0.340106
1	7	high	0.0663		0.0971	0.0491	3.15385		0.207911
2	7	low	0.0201	0.0206	0.106	0.0854	8.73846		0.535249
2	7	low	0.0255		0.1002	0.0796	7.84615		0.586897
2	7	low	0.0162		0.0928	0.0722	6.70769		0.592677
2	7	high	0.0262	0.0367	0.1125	0.0758	7.26154		0.531125
2	7	high	0.0332		0.1547	0.118	13.7538		0.636263
2	7	high	0.0507		0.1013	0.0646	5.53846		0.573022
5	7	low	0.0267	0.0213	0.1625	0.1412	17.3231		0.931496
5	7	low	0.0206		0.1363	0.115	13.2923		0.982743
5	7	low	0.0166		0.1325	0.1112	12.7077		0.944366
5	7	high	0.025	0.039033	0.1549	0.1158667	13.4256		0.966877
5	7	high	0.0383		0.1487	0.1096667	12.4718		0.904814
5	7	high	0.0538		0.0972	0.0581667	4.54872		0.872887
10	7	low	0.0028	0.000933	0.049	0.0480667	2.99487		1
10	7	low	0		0.0333	0.0323667	0.57949		1
10	7	low	0		0.0426	0.0416667	2.01026		1
10	7	high	0	0.004867	0.0401	0.0352333	1.02051		1
10	7	high	0.0042		0.0622	0.0573333	4.42051		1
10	7	high	0.0104		0.046	0.0411333	1.92821		1
20	7	low	0	0	0.002	0.002	0		1
20	7	low	0		0	0	0		1
20	7	low	0		0	0	0		1
20	7	high	0	0	0	0	0		1
20	7	high	0		0.0059	0.0059	0		1
20	7	high	0		0.0137	0.0137	0		1
30	7	low	0	0	0	0	0		1
30	7	low	0		0	0	0		1
30	7	low	0		0	0	0		1
30	7	high	0	0	0	0	0		1
30	7	high	0		0	0	0		1
30	7	high	0		0	0	0		1
42	7	low	0	0	0	0	0		1
42	7	low	0		0	0	0		1
42	7	low	0		0	0	0		1
42	7	high	0	0	0	0	0		1
42	7	high	0		0	0	0		1
42	7	high	0		0	0	0		1
55	7	low	0	0	0	0	0		1
55	7	low	0		0	0	0		1
55	7	low	0		0	0	0		1
55	7	high	0	0	0	0	0		1
55	7	high	0		0	0	0		1
55	7	high	0		0	0	0		1
70	7	low	0	0	0	0	0		1
70	7	low	0		0	0	0		1
70	7	low	0		0	0	0		1
70	7	high	0	0	0	0	0		1
70	7	high	0		0	0	0		1
70	7	high	0		0	0	0		1

Table B.3.I continued.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.	Total Conc.	Mt/Mo
0.5	7	low	0.0373	0.051533	0.1384	0.0868667	8.9641	68.61538	0.130643
0.5	7	low	0.0393		0.1232	0.0716667	6.62564	52.30769	0.126667
0.5	7	low	0.078		0.1303	0.0787667	7.71795	44.53846	0.173287
0.5	7	high	0.1583	0.163467	0.2369	0.0734333	6.89744	43.60513	0.158179
0.5	7	high	0.1372		0.2809	0.1174333	13.6667	62.6359	0.218192
0.5	7	high	0.1949		0.3551	0.1916333	25.0821	121.2	0.206948
1	7	low	0.0346	0.0376	0.1734	0.1358	16.4923		0.371001
1	7	low	0.028		0.1245	0.0869	8.96923		0.298137
1	7	low	0.0502		0.1249	0.0873	9.03077		0.376051
1	7	high	0.1223	0.107467	0.1932	0.0857333	8.78974		0.359755
1	7	high	0.0848		0.2334	0.1259333	14.9744		0.457262
1	7	high	0.1153		0.3157	0.2082333	27.6359		0.434967
2	7	low	0.0281	0.037667	0.1792	0.1415333	17.3744		0.624215
2	7	low	0.029		0.1412	0.1035333	11.5282		0.518529
2	7	low	0.0559		0.1285	0.0908333	9.57436		0.591019
2	7	high	0.0795	0.080033	0.1805	0.1004667	11.0564		0.613313
2	7	high	0.0707		0.1813	0.1012667	11.1795		0.635746
2	7	high	0.0899		0.2678	0.1877667	24.4872		0.637006
5	7	low	0.0217	0.028133	0.2009	0.1727667	22.1795		0.947459
5	7	low	0.0217		0.1706	0.1424667	17.5179		0.853431
5	7	low	0.041		0.1573	0.1291667	15.4718		0.9384
5	7	high	0.0841	0.0796	0.1766	0.097	10.5231		0.85464
5	7	high	0.0834		0.2382	0.1586	20		0.955052
5	7	high	0.0713		0.3012	0.2216	29.6923		0.881992
10	7	low	0.0068	0.009867	0.0619	0.0520333	3.60513		1
10	7	low	0.0133		0.0883	0.0784333	7.66667		1
10	7	low	0.0095		0.0563	0.0464333	2.74359		1
10	7	high	0.0267	0.0247	0.0945	0.0698	6.33846		1
10	7	high	0.0238		0.0716	0.0469	2.81538		1
10	7	high	0.0236		0.112	0.0873	9.03077		0.956503
22	7	low	0.0039	0.011333	0.0111	-0.000233	0		1
22	7	low	0.0003		0.0268	0.0154667	0		1
22	7	low	0.0298		0.0078	-0.003533	0		1
22	7	high	0.0125	0.008867	0.0324	0.0235333	0		1
22	7	high	0.0081		0.0242	0.0153333	0		1
22	7	high	0.006		0.0339	0.0250333	0		0.956503
32	7	low	0	0	0.0005	0.0005	0		1
32	7	low	0		0.0035	0.0035	0		1
32	7	low	0		0	0	0		1
32	7	high	0	0.001767	0.0016	-0.000167	0		1
32	7	high	0.0042		0.0057	0.0039333	0		1
32	7	high	0.0011		0.0205	0.0187333	0		0.956503
46	7	low	0	0	0	0	0		1
46	7	low	0		0	0	0		1
46	7	low	0		0	0	0		1
46	7	high	0.0122	0.004433	0	0	0		1
46	7	high	0		0	0	0		1
46	7	high	0.0011		0.0673	0.0628667	5.27179		1
58	7	low	0	0	0	0	0		1
58	7	low	0		0	0	0		1
58	7	low	0		0	0	0		1
58	7	high	0	0	0	0	0		1
58	7	high	0		0	0	0		1
58	7	high	0		0	0	0		1
70	7	low	0	0	0	0	0		1
70	7	low	0		0	0	0		1
70	7	low	0		0	0	0		1
70	7	high	0	0	0	0	0		1
70	7	high	0		0	0	0		1
70	7	high	0		0	0	0		1

Table B.4. Estradiol release data for Figures 5 and 6. Release occurred over 44-70 hours in 50% ethanol buffers at pH 2 and 7 at 37ºC.

Column Title	Definition
Time	Various times that release checked in hours
pH	pH of 50% ethanol buffer solution
MW	High or low molecular weight of hydrogel's polymers
Blank	Spectrophotometer absorbance reading of blank gel
Avg. Blank	Average of blank gel's absorbance reading at each time; used to subtract from estradiol absorbance
w/Estradiol	Estradiol release absorbance reading
Corrected E	Estradiol release absorbance with avg. blank absorbance subtracted
Conc.	Estimated concentration of estradiol released using equation created through calibration curve at each pH pH 2: Absorbance (y) = 0.0064 * Conc. (x) + 0.0042 pH 7: Absorbance (y) = 0.0065 * Conc. (x) + 0.0286

**All absorbance readings measured at 280 nm UV only.

Table B.4. Estradiol release data for Figures 5 and 6. Release occurred over 44-70 hours in 50% ethanol buffers at pH 2 and 7 at 37ºC.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.
0.5	2	low	0.0732	0.071067	0.1082	0.0371333	5.145833
0.5	2	low	0.0805		0.1554	0.0843333	12.52083
0.5	2	low	0.0595		0.1139	0.0428333	6.036458
0.5	2	high	0.124	0.1358	0.1476	0.0118	1.1875
0.5	2	high	0.102		0.2083	0.0725	10.67188
0.5	2	high	0.1814		0.1557	0.0199	2.453125
1	2	low	0.0591	0.053867	0.1324	0.0785333	11.61458
1	2	low	0.0561		0.1534	0.0995333	14.89583
1	2	low	0.0464		0.1305	0.0766333	11.31771
1	2	high	0.0879	0.093967	0.1351	0.0411333	5.770833
1	2	high	0.0717		0.1746	0.0806333	11.94271
1	2	high	0.1223		0.1658	0.0718333	10.56771
2	2	low	0.0635	0.055933	0.1432	0.0872667	12.97917
2	2	low	0.0595		0.1884	0.1324667	20.04167
2	2	low	0.0448		0.1509	0.0949667	14.18229
2	2	high	0.0834	0.090933	0.1658	0.0748667	11.04167
2	2	high	0.0636		0.175	0.0840667	12.47917
2	2	high	0.1258		0.2071	0.1161667	17.49479
5	2	low	0.0645	0.049633	0.1981	0.1484667	22.54167
5	2	low	0.0455		0.239	0.1893667	28.93229
5	2	low	0.0389		0.2216	0.1719667	26.21354
5	2	high	0.0821	0.0816	0.2039	0.1223	18.45313
5	2	high	0.0639		0.2277	0.1461	22.17188
5	2	high	0.0988		0.2506	0.169	25.75
12	2	low	0.0177	0.021333	0.1074	0.0860667	12.79167
12	2	low	0.0362		0.1144	0.0930667	13.88542
12	2	low	0.0101		0.1311	0.1097667	16.49479
12	2	high	0.0323	0.038367	0.094	0.0556333	8.036458
12	2	high	0.026		0.1279	0.0895333	13.33333
12	2	high	0.0568		0.1157	0.0773333	11.42708
22	2	low	0.0022	0.003533	0.0296	0.0260667	3.416667
22	2	low	0.003		0.0322	0.0286667	3.822917
22	2	low	0.0054		0.062	0.0584667	8.479167
22	2	high	0.0222	0.0258	0.0321	0.0063	0.328125
22	2	high	0.0178		0.0422	0.0164	1.90625
22	2	high	0.0374		0.0462	0.0204	2.53125
33	2	low	0.0006	0.0005	0.0461	0.0456	6.46875
33	2	low	0		0.0079	0.0074	0.5
33	2	low	0.0009		0.0128	0.0123	1.265625
33	2	high	0.0058	0.008067	0.0082	0.0001333	0
33	2	high	0.003		0.0124	0.0043333	0.020833
33	2	high	0.0154		0.0305	0.0224333	0
45	2	low	0	0.000733	0.0028	0.0020667	0
45	2	low	0		0.0003	-0.000433	0
45	2	low	0.0022		0.0079	0.0071667	0
45	2	high	0.0103	0.01	0.009	-0.001	0
45	2	high	0.0026		0.0014	-0.0086	0
45	2	high	0.0171		0.0056	-0.0044	0
56	2	low	0.003	0.001	0.0054	0.0044	0.03125
56	2	low	0		0	-0.001	0
56	2	low	0		0.0037	0.0027	0
56	2	high	0.0141	0.006933	0.006	-0.000933	0
56	2	high	0.0023		0.0019	-0.005033	0
56	2	high	0.0044		0.0095	0.0025667	0
70	2	low	0	0.001267	0.0006	-0.000667	0
70	2	low	0		0.0049	0.0036333	0
70	2	low	0.0038		0	-0.001267	0
70	2	high	0.001	0.000333	0.0047	0.0043667	0.026042
70	2	high	0		0	-0.000333	0
70	2	high	0		0.001	0.0006667	0

Table B.4. continued.

Time	pH	MW	Blank	Avg. Blank	w/Estradiol	Corrected E	Conc.
0.5	7	low	0.0678	0.049767	0.1012	0.0514333	4.614583
0.5	7	low	0.0538		0.1338	0.0840333	9.708333
0.5	7	low	0.0277		0.1167	0.0669333	7.036458
0.5	7	high	0.1153	0.1392	0.2242	0.085	9.859375
0.5	7	high	0.1588		0.1699	0.0307	1.375
0.5	7	high	0.1435		0.1976	0.0584	5.703125
1	7	low	0.0565	0.040133	0.1415	0.1013667	12.41667
1	7	low	0.0403		0.1326	0.0924667	11.02604
1	7	low	0.0236		0.1151	0.0749667	8.291667
1	7	high	0.0887	0.086033	0.1918	0.1057667	13.10417
1	7	high	0.0955		0.1667	0.0806667	9.182292
1	7	high	0.0739		0.1932	0.1071667	13.32292
2	7	low	0.0474	0.035233	0.1467	0.1114667	13.99479
2	7	low	0.0397		0.1442	0.1089667	13.60417
2	7	low	0.0186		0.1225	0.0872667	10.21354
2	7	high	0.0552	0.062867	0.149	0.0861333	10.03646
2	7	high	0.0684		0.1275	0.0646333	6.677083
2	7	high	0.065		0.1665	0.1036333	12.77083
5	7	low	0.0381	0.029533	0.2016	0.1720667	23.46354
5	7	low	0.0356		0.1664	0.1368667	17.96354
5	7	low	0.0149		0.2138	0.1842667	25.36979
5	7	high	0.0544	0.063	0.1263	0.0633	6.46875
5	7	high	0.0718		0.1396	0.0766	8.546875
5	7	high	0.0628		0.174	0.111	13.92188
10	7	low	0.0063	0.005633	0.0751	0.0694667	7.432292
10	7	low	0.0106		0.0738	0.0681667	7.229167
10	7	low	0		0.0839	0.0782667	8.807292
10	7	high	0.0192	0.022567	0.0474	0.0248333	0.458333
10	7	high	0.0239		0.076	0.0534333	4.927083
10	7	high	0.0246		0.055	0.0324333	1.645833
22	7	low	0	0.0048	0.0213	0.0165	0
22	7	low	0.0085		0.018	0.0132	0
22	7	low	0.0059		0.0313	0.0265	0.71875
22	7	high	0.013	0.012067	0.0147	0.0026333	0
22	7	high	0.0074		0.0234	0.0113333	0
22	7	high	0.0158		0.0151	0.0030333	0
33	7	low	0	0	0.004	0.004	0
33	7	low	0		0.0001	0.0001	0
33	7	low	0		0.0066	0.0066	0
33	7	high	0.0021	0.002533	0.0041	0.0015667	0
33	7	high	0		0.0045	0.0019667	0
33	7	high	0.0055		0.003	0.0004667	0
44	7	low	0.0017	0.000567	0	0	0
44	7	low	0		0	0	0
44	7	low	0		0.0061	0.0055333	0
44	7	high	0	0	0	0	0
44	7	high	0		0	0	0
44	7	high	0		0	0	0

Table B.5. Insulin Release data for Figures 5 and 6. Release occurred over 70-74 hours in buffers with no ethanol added at pH 2 and 7 at 37ºC.

Column Title	Definition
Time	Various times that release checked in hours
pH	pH of buffer solution
MW	High or low molecular weight of hydrogel's polymers
Blank	Spectrophotometer absorbance reading of blank gel
Avg. Blank	Average of blank gel's absorbance reading at each time; used to subtract from insulin absorbance
w/Insulin	Insulin release absorbance reading
Corrected I	Insulin release absorbance with avg. blank absorbance subtracted
Conc.	Estimated concentration of insulin released using equation created through calibration curve at each pH pH 2: Absorbance (y) = 0.0107 * Conc. (x) + 0.0134 pH 7: Absorbance (y) = 0.0101 * Conc. (x) + 0.0141

**All absorbance readings measured at 222 nm UV only.

Table B.5. Insulin Release data for Figures 5 and 6. Release occurred over 70-74 hours in buffers with no ethanol added at pH 2 and 7 at 37ºC.

Time	pH	MW	Blank	Avg. Blank	w/Insulin	Corrected I	Conc.
0.5	2	low	1.1945	1.222133	1.8084	0.5862667	53.53894
0.5	2	low	1.1716		1.0999	-0.122233	0
0.5	2	low	1.3003		1.1292	-0.092933	0
0.5	2	high	1.6835	1.6738	1.7213	0.0475	6.373832
0.5	2	high	1.6166		1.6487	-0.0251	0
0.5	2	high	1.7213		1.7627	0.0889	14.11215
1	2	low	0.3906	0.743033	1.4486	0.7055667	64.68847
1	2	low	0.2981		0.6809	-0.062133	0
1	2	low	1.5404		0.8364	0.0933667	7.47352
1	2	high	1.6247	1.649667	1.6247	-0.024967	0
1	2	high	1.5948		1.6247	-0.024967	0
1	2	high	1.7295		1.7709	0.1212333	10.07788
2	2	low	0.2615	0.563767	0.11673	-0.447037	0
2	2	low	0.2837		0.6455	0.0817333	6.386293
2	2	low	1.1461		0.5239	-0.039867	0
2	2	high	1.6232	1.573667	1.6554	0.0817333	6.386293
2	2	high	1.4076		1.427	-0.146667	0
2	2	high	1.6902		1.728	0.1543333	13.17134
5	2	low	0.2393	0.384567	0.7972	0.4126333	37.31153
5	2	low	0.2525		0.4962	0.1116333	9.180685
5	2	low	0.6619		0.4232	0.0386333	2.358255
5	2	high	1.0216	1.2115	1.323	0.1115	9.168224
5	2	high	1.1529		1.2098	-0.0017	0
5	2	high	1.46		1.2516	0.0401	2.495327
10	2	low	0.1317	0.171033	0.3108	0.1397667	11.80997
10	2	low	0.1139		0.258	0.0869667	6.875389
10	2	low	0.2675		0.2607	0.0896667	7.127726
10	2	high	0.3675	0.412467	0.6058	0.1933333	16.8162
10	2	high	0.3815		0.447	0.0345333	1.975078
10	2	high	0.4884		0.4512	0.0387333	2.367601
22	2	low	0.0601	0.089167	0.2331	0.1439333	12.19938
22	2	low	0.0715		0.1864	0.0972333	7.834891
22	2	low	0.1359		0.1649	0.0757333	5.825545
22	2	high	0.1507	0.207067	0.2557	0.0486333	3.292835
22	2	high	0.2476		0.2191	0.0120333	0
22	2	high	0.2229		0.2254	0.0183333	0.461059
32	2	low	0.0522	0.763767	0.3056	-0.458167	0
32	2	low	0.7589		0.1028	-0.660967	0
32	2	low	1.4802		0.0915	-0.672267	0
32	2	high	0.1009	0.114567	0.1086	-0.005967	0
32	2	high	0.1204		0.0943	-0.020267	0
32	2	high	0.1224		0.1057	-0.008867	0
47	2	low	0.058	0.069367	0.0911	0.0217333	0.778816
47	2	low	0.0419		0.118	0.0486333	3.292835
47	2	low	0.1082		0.0948	0.0254333	1.124611
47	2	high	0.0856	0.090867	0.0537	-0.037167	0
47	2	high	0.0846		0.0419	-0.048967	0
47	2	high	0.1024		0.0774	-0.013467	0
59	2	low	0.1605	0.124133	0.0801	-0.044033	0
59	2	low	0.1189		0.0828	-0.041333	0
59	2	low	0.093		0.052	-0.072133	0
59	2	high	0.0765	0.096767	0.0606	-0.036167	0
59	2	high	0.0828		0.0503	-0.046467	0
59	2	high	0.131		0.0537	-0.043067	0
74	2	low	0.1327	0.065967	0.0685	0.0025333	0
74	2	low	0.0222		0.0413	-0.024667	0
74	2	low	0.043		0.0362	-0.029767	0
74	2	high	0.0622	0.066267	0.0388	-0.027467	0
74	2	high	0.0543		0.0214	-0.044867	0
74	2	high	0.0823		0.0405	-0.025767	0

Table B.5. continued.

Time	pH	MW	Blank	Avg. Blank	w/Insulin	Corrected I	Conc.
0.5	7	low	1.4807	1.508567	1.3194	-0.189167	0
0.5	7	low	1.4393		1.2136	-0.294967	0
0.5	7	low	1.6057		1.3668	-0.141767	0
0.5	7	high	2.0828	1.956067	2.0828	0.1267333	22.30363
0.5	7	high	2.0036		2.1797	0.2236333	41.49175
0.5	7	high	1.7818		2.3046	0.3485333	66.22442
1	7	low	1.0835	0.958233	0.5978	-0.360433	0
1	7	low	0.5272		0.6271	-0.331133	0
1	7	low	1.264		0.6787	-0.279533	0
1	7	high	1.5037	1.4131	1.5272	0.1141	19.80198
1	7	high	1.3511		1.6685	0.2554	47.78218
1	7	high	1.3845		1.5272	0.1141	19.80198
2	7	low	0.8177	0.6809	0.3875	-0.2934	0
2	7	low	0.2996		0.4093	-0.2716	0
2	7	low	0.9254		0.5049	-0.176	0
2	7	high	1.8225	1.6098	1.7767	0.1669	15.12871
2	7	high	1.5726		1.9316	0.3218	30.46535
2	7	high	1.4343		1.5726	-0.0372	0
5	7	low	0.5119	0.456567	0.4362	-0.020367	0
5	7	low	0.2364		0.4545	-0.002067	0
5	7	low	0.6214		0.4545	-0.002067	0
5	7	high	1.3965	1.228667	1.3295	0.1008333	8.587459
5	7	high	1.2204		1.1139	-0.114767	0
5	7	high	1.0691		1.3143	0.0856333	7.082508
10	7	low	0.2304	0.1963	0.2547	0.0584	4.386139
10	7	low	0.133		0.2469	0.0506	3.613861
10	7	low	0.2255		0.264	0.0677	5.306931
10	7	high	0.4502	0.473933	0.6162	0.1422667	12.68977
10	7	high	0.6284		0.4502	-0.023733	0
10	7	high	0.3432		0.5786	0.1046667	8.966997
22	7	low	0.1126	0.0889	0.2165	0.1276	11.23762
22	7	low	0.0709		0.1952	0.1063	9.128713
22	7	low	0.0832		0.2313	0.1424	12.70297
22	7	high	0.1941	0.205267	0.3533	0.1480333	13.26073
22	7	high	0.2401		0.2363	0.0310333	1.676568
22	7	high	0.1816		0.3137	0.1084333	9.339934
32	7	low	0.2539	0.112667	0.0951	-0.017567	0
32	7	low	0.0437		0.1016	-0.011067	0
32	7	low	0.0404		0.1149	0.0022333	0
32	7	high	0.1082	0.108667	0.1327	0.0240333	0.983498
32	7	high	0.1227		0.0805	-0.028167	0
32	7	high	0.0951		0.112	0.0033333	0
47	7	low	0.0429	0.0301	0.0273	-0.0028	0
47	7	low	0.0241		0.0652	0.0351	2.079208
47	7	low	0.0233		0.0705	0.0404	2.60396
47	7	high	0.0705	0.072	0.0488	-0.0232	0
47	7	high	0.0777		0.0297	-0.0423	0
47	7	high	0.0678		0.0479	-0.0241	0
59	7	low	0.0217	0.2546	0.1275	-0.1271	0
59	7	low	0.563		0.1184	-0.1362	0
59	7	low	0.1791		0.1214	-0.1332	0
59	7	high	0.1757	0.143467	0.0388	-0.104667	0
59	7	high	0.1096		0.0281	-0.115367	0
59	7	high	0.1451		0.0289	-0.114567	0
74	7	low	0.021	0.0168	0.0077	-0.0091	0
74	7	low	0.0147		0.0242	0.0074	0
74	7	low	0.0147		0.0139	-0.0029	0
74	7	high	0.0315	0.034233	0.0178	-0.016433	0
74	7	high	0.0389		0.0115	-0.022733	0
74	7	high	0.0323		0.0162	-0.018033	0

Table B.6. Insulin Release data for Figure 7. Release occurred over 72 hours in 50% ethanol buffers at pH 2 and 7 at 37ºC.

Column Title	Definition
Time	Various times that release checked in hours
pH	pH of 50% ethanol buffer solution
MW	High or low molecular weight of hydrogel's polymers
Blank	Spectrophotometer absorbance reading of blank gel
Avg. Blank	Average of blank gel's absorbance reading at each time; used to subtract from insulin absorbance
w/Insulin	Insulin release absorbance reading
Corrected I	Insulin release absorbance with avg. blank absorbance subtracted
Conc.	Estimated concentration of insulin released using equation created through calibration curve at each pH pH 2: Absorbance (y) = 0.0096 * Conc. (x) + 0.019 pH 7: Absorbance (y) = 0.0094 * Conc. (x) – 0.0143

**All absorbance readings measured at 222 nm UV only.

Table B.6. Insulin Release data for Figure 7. Release occurred over 72 hours in 50% ethanol buffers at pH 2 and 7 at 37ºC.

Time	pH	MW	Blank	Avg. Blank	w/Insulin	Corrected I	Conc.
0.5	2	low	0.6378	0.663367	1.3579	0.6945333	70.36806
0.5	2	low	0.7145		1.0357	0.3723333	36.80556
0.5	2	low	0.6378		1.1275	0.4641333	46.36806
0.5	2	high	1.659	1.865567	2.1818	0.3162333	0
0.5	2	high	1.8808		2.3579	0.4923333	0
0.5	2	high	2.0569		2.3579	0.4923333	0
1	2	low	0.3864	0.395167	0.7754	0.3802333	37.62847
1	2	low	0.3621		0.5213	0.1261333	11.15972
1	2	low	0.437		0.5535	0.1583333	14.51389
1	2	high	1.3664	1.585633	1.7132	0.1275667	22.61806
1	2	high	1.6261		1.7132	0.1275667	22.61806
1	2	high	1.7644		1.8224	0.2367667	45.36806
2	2	low	0.3124	0.3449	0.5082	0.1633	15.03125
2	2	low	0.3633		0.4543	0.1094	9.416667
2	2	low	0.359		0.4041	0.0592	4.1875
2	2	high	1.7123	1.788033	1.7634	-0.024633	0
2	2	high	1.7634		1.9675	0.1794667	16.71528
2	2	high	1.8884		1.9675	0.1794667	16.71528
5	2	low	0.231	0.240333	0.4561	0.2157667	20.49653
5	2	low	0.231		0.3602	0.1198667	10.50694
5	2	low	0.259		0.4079	0.1675667	15.47569
5	2	high	1.2676	1.4162	1.3232	-0.093	0
5	2	high	1.4615		1.5864	0.1702	15.75
5	2	high	1.5195		1.4615	0.0453	2.739583
10	2	low	0.0832	0.089733	0.3616	0.2718667	26.34028
10	2	low	0.0752		0.2312	0.1414667	12.75694
10	2	low	0.1108		0.3339	0.2441667	23.45486
10	2	high	0.6876	0.947633	0.9401	-0.007533	0
10	2	high	1.112		1.3551	0.4074667	40.46528
10	2	high	1.0433		1.2089	0.2612667	25.23611
22	2	low	0.0226	0.022333	0.1922	0.1698667	15.71528
22	2	low	0.0298		0.1747	0.1523667	13.89236
22	2	low	0.0146		0.1804	0.1580667	14.48611
22	2	high	0.3059	0.335733	0.3572	0.0214667	0.256944
22	2	high	0.4466		0.4081	0.0723667	5.559028
22	2	high	0.2547		0.4413	0.1055667	9.017361
33	2	low	0.0117	0.007467	0.0945	0.0870333	7.086806
33	2	low	0		0.07	0.0625333	4.534722
33	2	low	0.0107		0.0945	0.0870333	7.086806
33	2	high	0.0533	0.081633	0.1067	0.0250667	0.631944
33	2	high	0.1205		0.123	0.0413667	2.329861
33	2	high	0.0711		0.1334	0.0517667	3.413194
47	2	low	0	0.005567	0.0208	0.0152333	0
47	2	low	0		0.0098	0.0042333	0
47	2	low	0.0167		0.0197	0.0141333	0
47	2	high	0	0.006233	0.0137	0.0074667	0
47	2	high	0.0187		0.0088	0.0025667	0
47	2	high	0		0.03	0.0237667	0.496528
59	2	low	0	0	0.001	0.001	0
59	2	low	0		0	0	0
59	2	low	0		0.004	0.004	0
59	2	high	0	0.000667	0.009	0.0083333	0
59	2	high	0.002		0.006	0.0053333	0
59	2	high	0		0.0131	0.0124333	0
72	2	low	0	0	0	0	0
72	2	low	0		0	0	0
72	2	low	0		0	0	0
72	2	high	0	0	0	0	0
72	2	high	0		0	0	0
72	2	high	0		0	0	0

Table B.6. continued.

Time	pH	MW	Blank	Avg. Blank	w/Insulin	Corrected I	Conc.
0.5	7	low	0.545	0.8021	0.9067	0.1046	12.64894
0.5	7	low	1.2699		1.3046	0.5025	54.97872
0.5	7	low	0.5914		1.0828	0.2807	31.38298
0.5	7	high	1.4187	1.543767	1.6739	0.1301333	46.09574
0.5	7	high	1.6739		1.5948	0.0510333	20.85106
0.5	7	high	1.5387		1.8958	0.3520333	116.9149
1	7	low	0.3318	0.5912	0.6235	0.0323	4.957447
1	7	low	1.0268		0.9221	0.3309	36.7234
1	7	low	0.415		0.7258	0.1346	15.84043
1	7	high	1.4661	1.5271	1.5241	-0.003	1.202128
1	7	high	1.5241		1.5241	-0.003	1.202128
1	7	high	1.5911		1.5911	0.064	8.329787
2	7	low	0.2454	0.510467	0.4942	-0.016267	0
2	7	low	0.9542		0.7782	0.2677333	30.00355
2	7	low	0.3318		0.563	0.0525333	7.109929
2	7	high	1.6702	1.602167	1.5241	-0.078067	0
2	7	high	1.6702		1.5241	-0.078067	0
2	7	high	1.4661		1.5241	-0.078067	0
5	7	low	0.1929	0.404867	0.3925	-0.012367	0.205674
5	7	low	0.7837		0.7837	0.3788333	41.8227
5	7	low	0.238		0.411	0.0061333	2.173759
5	7	high	1.2286	1.274267	1.3747	0.1004333	12.20567
5	7	high	1.3334		1.3747	0.1004333	12.20567
5	7	high	1.2608		1.3747	0.1004333	12.20567
10	7	low	0.0847	0.153233	0.249	0.0957667	11.70922
10	7	low	0.309		0.3834	0.2301667	26.00709
10	7	low	0.066		0.313	0.1597667	18.51773
10	7	high	0.7984	0.851967	1.0414	0.1894333	21.67376
10	7	high	1.0872		0.8511	-0.000867	1.429078
10	7	high	0.6703		0.8953	0.0433333	6.131206
22	7	low	0	0	0.004	0.004	1.946809
22	7	low	0		0.0307	0.0307	4.787234
22	7	low	0		0.0729	0.0729	9.276596
22	7	high	0.2025	0.233567	0.2705	0.0369333	5.450355
22	7	high	0.3171		0.2669	0.0333333	5.067376
22	7	high	0.1811		0.2186	-0.014967	0
33	7	low	0	0.003533	0	0	1.521277
33	7	low	0.0106		0	0	1.521277
33	7	low	0		0.0171	0.0135667	2.964539
33	7	high	0	0	0.0488	0.0488	6.712766
33	7	high	0		0.0327	0.0327	5
33	7	high	0		0.0535	0.0535	7.212766
47	7	low	0	0	0	0	0
47	7	low	0		0	0	0
47	7	low	0		0	0	0
47	7	high	0	0	0	0	0
47	7	high	0		0	0	0
47	7	high	0		0	0	0
59	7	low	0	0	0	0	0
59	7	low	0		0	0	0
59	7	low	0		0	0	0
59	7	high	0	0	0	0	0
59	7	high	0		0	0	0
59	7	high	0		0	0	0
72	7	low	0	0	0	0	0
72	7	low	0		0	0	0
72	7	low	0		0	0	0
72	7	high	0	0	0	0	0
72	7	high	0		0	0	0
72	7	high	0		0	0	0

Table and Figure 7. Calibration curve data and graphs of release buffers with and without 50% ethanol added at various pHs and with various estradiol or insulin concentrations added.

Column Title	Definition
Conc. (μg/mL)	Added Estradiol/Insulin Concentration.
Abs	Spectrophotometer absorbance reading

Table and Figure B.7.A. Calibration Curve of Release Buffer with 50% Ethanol at pH 2 and various estradiol concentrations. Table displays data for estradiol concentration vs. measured spectrophotometer absorbance readings. Curve compares estradiol concentration to spectrophotometer absorbance. Linear regression line gives equation which was used to determine concentration of estradiol release in hydrogel release experiments. Absorbencies measured at 280 nm UV.

Conc.	Abs
0	0
0	0
0	0
0.5	0.005
0.5	0.005
0.5	0.006
1	0.01
1	0.009
1	0.012
5	0.035
5	0.037
5	0.038
10	0.066
10	0.069
10	0.065
20	0.138
20	0.155
20	0.131
50	0.323
50	0.323
50	0.332
100	0.647
100	0.645
100	0.643

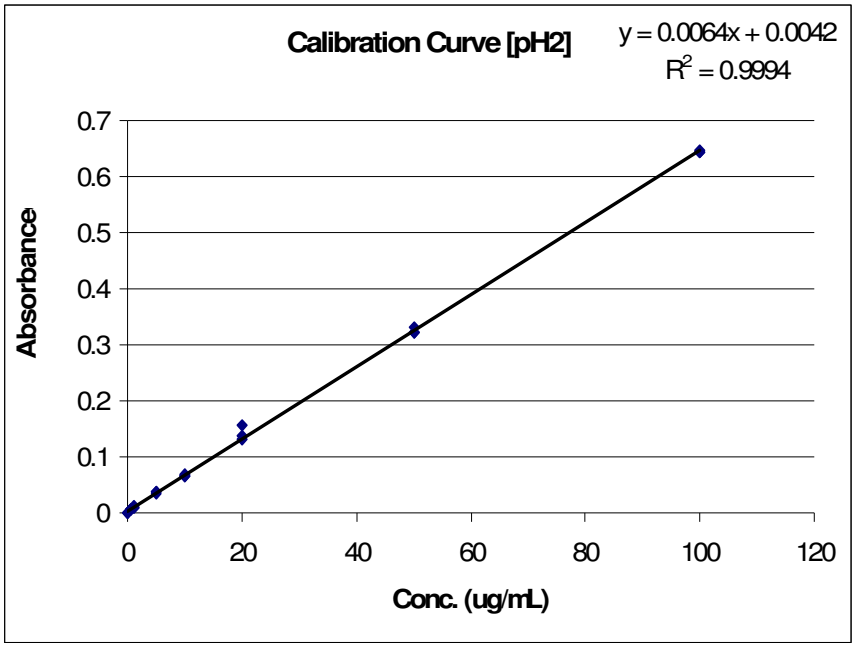

Table and Figure B.7.B. Calibration Curve of Release Buffer with 50% Ethanol at pH 4 and various estradiol concentrations. Table displays data for estradiol concentration vs. measured spectrophotometer absorbance readings. Curve compares estradiol concentration to spectrophotometer absorbance. Linear regression line gives equation which was used to determine concentration of estradiol release in hydrogel release experiments. Absorbencies measured at 280 nm UV.

Conc.	Abs.
0.5	0.022
0.5	0.006
0.5	0.01
1	0.022
1	0.018
1	0.015
5	0.034
5	0.028
5	0.033
10	0.06
10	0.057
10	0.076
20	0.165
20	0.145
20	0.133
50	0.316
50	0.313
50	0.261
100	0.599
100	0.544
100	0.54
250	1.5
250	1.408
250	1.382

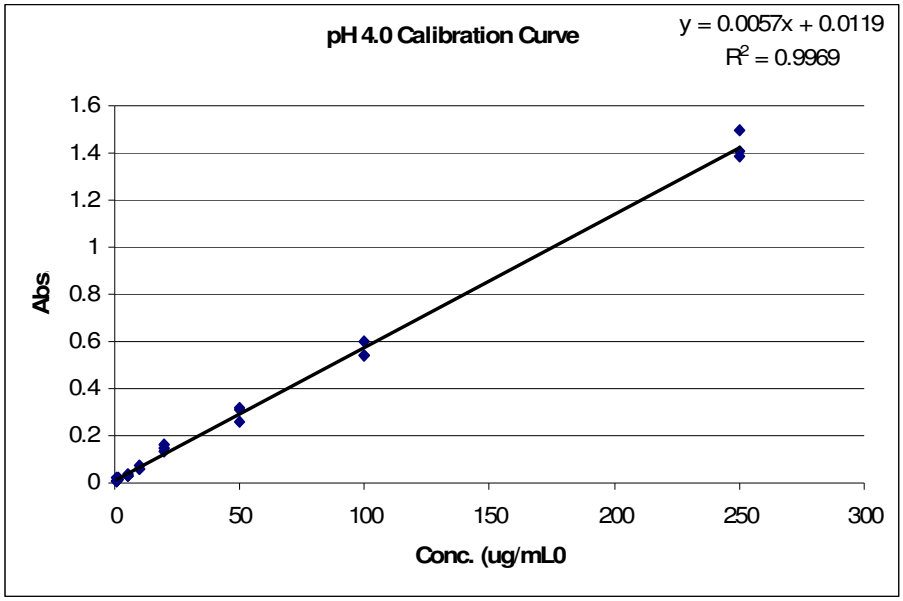

Table and Figure B.7.C. Calibration Curve of Release Buffer with 50% Ethanol at pH 7 and various estradiol concentrations. Table displays data for estradiol concentration vs. measured spectrophotometer absorbance readings. Curve compares estradiol concentration to spectrophotometer absorbance. Linear regression line gives equation which was used to determine concentration of estradiol release in hydrogel release experiments. Absorbencies measured at 280 nm UV.

Conc.	Abs.
0.5	0.006
0.5	0.008
0.5	0.01
1	0.01
1	0.015
1	0.012
5	0.052
5	0.03
5	0.057
10	0.073
10	0.066
10	0.08
20	0.127
20	0.162
20	0.181
50	0.369
50	0.297
50	0.447
100	0.705
100	0.914
100	0.731
250	1.607
250	1.858
250	1.322

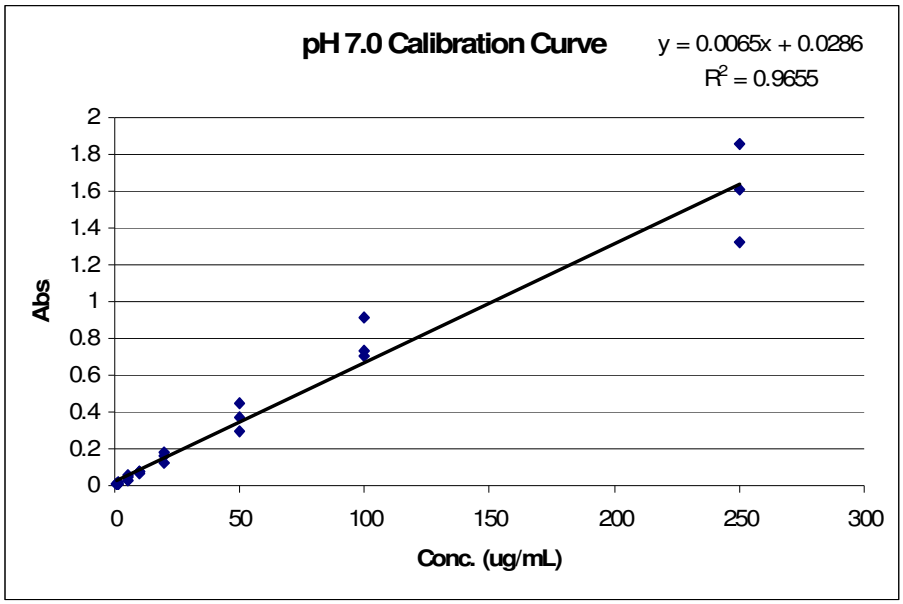

Table and Figure B.7.D. Calibration Curve of Release Buffer with 50% Ethanol at pH 10 and various estradiol concentrations. Table displays data for estradiol concentration vs. measured spectrophotometer absorbance readings. Curve compares estradiol concentration to spectrophotometer absorbance. Linear regression line gives equation which was used to determine concentration of estradiol release in hydrogel release experiments. Absorbencies measured at 280 nm UV.

Conc.	Abs.
0.5	0.009
0.5	0.003
0.5	0.006
1	0.024
1	0.023
1	0.013
5	0.036
5	0.029
5	0.029
10	0.078
10	0.06
10	0.06
20	0.134
20	0.221
20	0.139
50	0.393
50	0.296
50	0.31
100	0.56
100	0.56
100	0.447
250	1.589
250	1.351
250	1.369

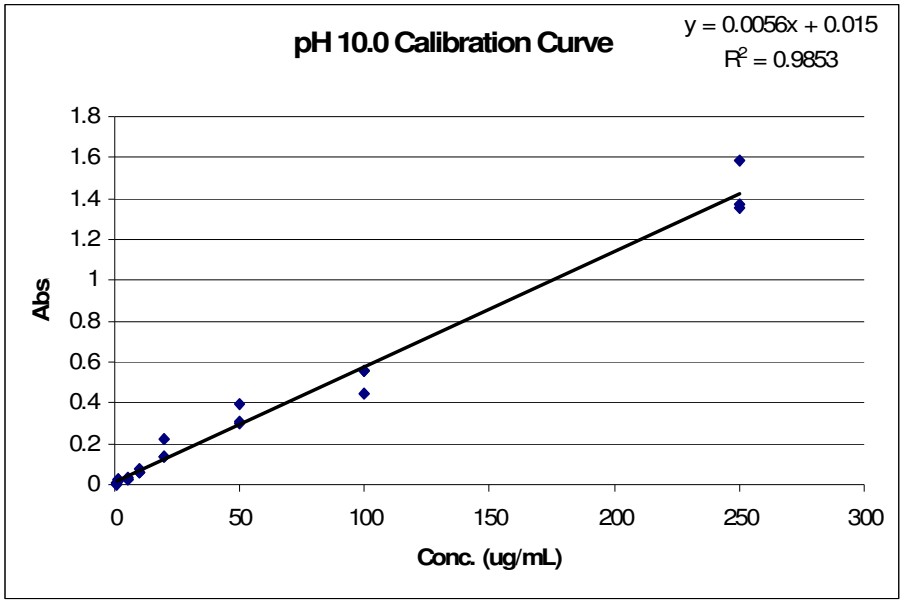

Table and Figure B.7.E. Calibration Curve of Release Buffer with 50% Ethanol at pH 12 and various estradiol concentrations. Table displays data for estradiol concentration vs. measured spectrophotometer absorbance readings. Curve compares estradiol concentration to spectrophotometer absorbance. Linear regression line gives equation which was used to determine concentration of estradiol release in hydrogel release experiments. Absorbencies measured at 280 nm UV.

Conc.	ABS
0	0
0	0
0	0
0.5	0.006
0.5	0.005
0.5	0.01
1	0.015
1	0.021
1	0.012
5	0.033
5	0.047
5	0.037
10	0.065
10	0.062
10	0.062
20	0.128
20	0.132
20	0.136
50	0.311
50	0.298
50	0.309
100	0.587
100	0.606
100	0.613

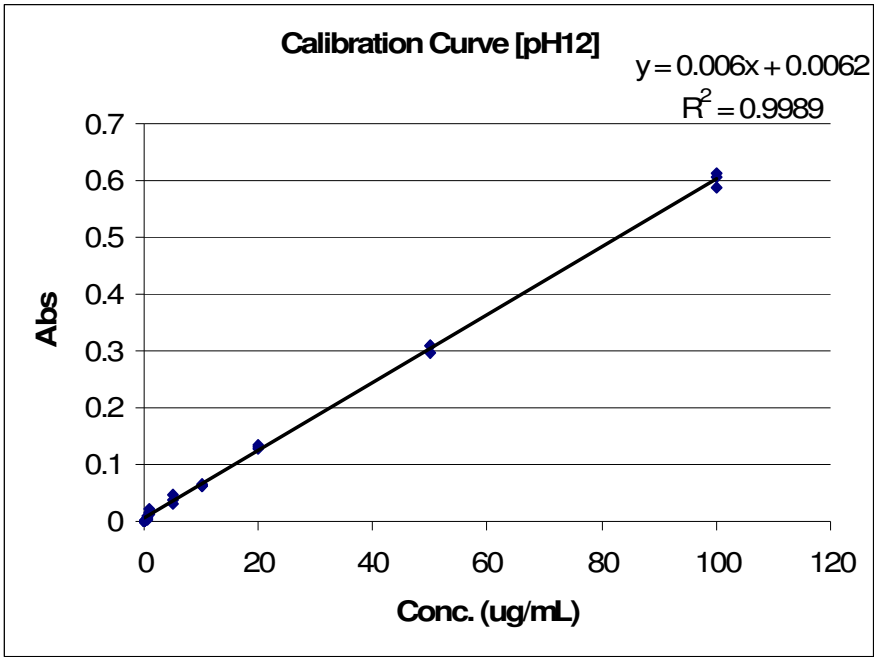

Table and Figure B.7.F. Calibration Curve of Release Buffer with no ethanol added at pH 2 and various insulin concentrations. Table displays data for insulin concentration vs. measured spectrophotometer absorbance readings. Curve compares insulin concentration to spectrophotometer absorbance. Linear regression line gives equation which was used to determine concentration of insulin release in hydrogel release experiments. Absorbencies measured at 222 nm UV.

Conc.	ABS
0	0
0	0
0	0
0.5	0.019
0.5	0.007
0.5	0.009
1	0.026
1	0.03
1	0.029
5	0.067
5	0.07
5	0.068
10	0.123
10	0.148
10	0.123
20	0.23
20	0.24
20	0.195
50	0.542
50	0.589
50	0.592
100	1.044
100	1.097
100	1.088

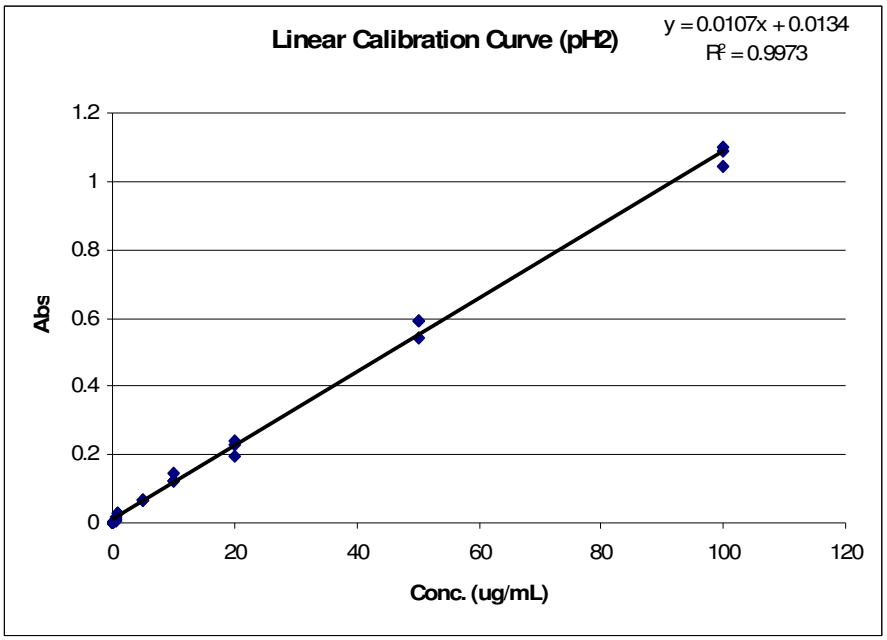

Table and Figure B.7.G. Calibration Curve of Release Buffer with no ethanol added at pH 7 and various insulin concentrations. Table displays data for insulin concentration vs. measured spectrophotometer absorbance readings. Curve compares insulin concentration to spectrophotometer absorbance. Linear regression line gives equation which was used to determine concentration of insulin release in hydrogel release experiments. Absorbencies measured at 222 nm UV.

Conc.	ABS
0	0
0	0
0	0
0.5	0.014
0.5	0.007
0.5	0.008
1	0.02
1	0.023
1	0.026
5	0.059
5	0.064
5	0.052
10	0.111
10	0.119
10	0.119
20	0.23
20	0.245
20	0.248
50	0.511
50	0.537
50	0.571
100	0.972
100	1.037
100	1.022

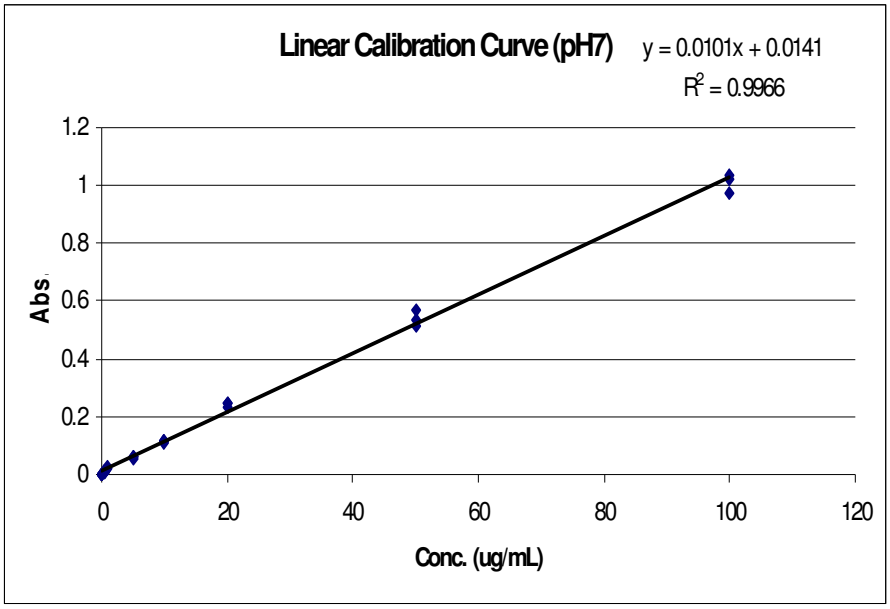

Table and Figure B.7.H. Calibration Curve of Release Buffer with 50% ethanol added at pH 2 and various insulin concentrations. Table displays data for insulin concentration vs. measured spectrophotometer absorbance readings. Curve compares insulin concentration to spectrophotometer absorbance. Linear regression line gives equation which was used to determine concentration of insulin release in hydrogel release experiments. Absorbencies measured at 222 nm UV.

Conc.	Abs
5	0.034
5	0.031
5	0.028
10	0.103
10	0.091
10	0.111
20	0.297
20	0.237
20	0.236
50	0.513
50	0.513
50	0.545
100	0.9
100	1.016

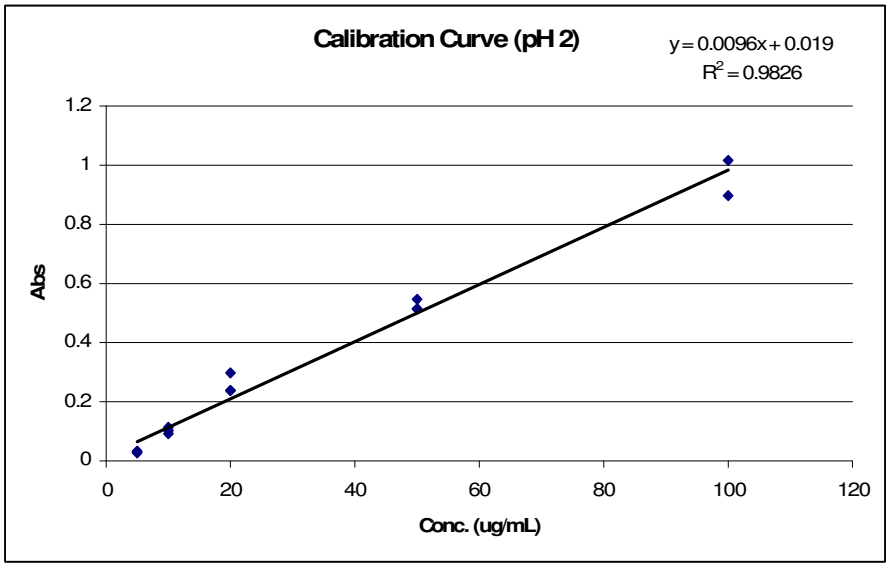

Table and Figure B.7.I. Calibration Curve of Release Buffer with 50% ethanol added at pH 7 and various insulin concentrations. Table displays data for insulin concentration vs. measured spectrophotometer absorbance readings. Curve compares insulin concentration to spectrophotometer absorbance. Linear regression line gives equation which was used to determine concentration of insulin release in hydrogel release experiments. Absorbencies measured at 222 nm UV.

Conc.	Abs
5	0.04
5	0.076
10	0.076
10	0.08
10	0.065
20	0.187
20	0.179
20	0.152
50	0.421
50	0.456
50	0.44
100	0.898
100	0.883
100	1.032