Evaluation of Biomedical Hydrogel Parameters for Biomolecular Diffusion in Controlled Drug Delivery

BY

Mohammad Rahman
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THESIS

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Defense Committee:

Richard Gemeinhart, Chair and Advisor
G. Ali Mansoori
David Eddington

DEDICATION

This thesis is decdicated to my family, for without whom it would have never been achieved.

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LIST ABBREVIATIONS

MMP-2	Matrix Metalloprotease-2
ECM	Extracellular Matrix
Ig	Immunoglobulin
MMP	Matrix Metalloprotease
EPR	Enhanced Permeation and Retention
NIR	Near –Infrared
BSA	Bovine Serum Albumin
FRET	Fluorescence Resonance Energy Transfer
DMSO	Dimethyl Sulfoxide
PBS	Phosphate Buffered Saline
NMR	Nuclear Magnetic Resonance
ATR-FTIR	Attenuated Total Reflectance Fourier Transfer Infrared
PEGDA	Poly(ethylene glycol) Diacrylate
mPEGMA	Methoxy poly(ethylene glycol) Monoacrylate
P(MAA-g-EG)	Poly(methacrylic acid-g-ethylene glycol)
pHEMA	Poly(2-hydroxyethyl methacrylate)
EDMA	Ethylene dimethacrylate
TEMED	*N,N,N′N′*-tetramethylene-diamine
APS	Ammonium persulfate
DCM	Dichloromethane
3-D	Three Dimensional

SUMMARY

Hydrogels made of non-toxic poly(ethylene glycol) diacrylate (PEGDA) provide a suitable platform for a variety of different applications in the medical field including controlled drug delivery. Their biomimetic and immunological properties make them ideal for *in situ* applications. Also, the wide range of available fabrication techniques permits hydrogels to be tailored to a given application. These factors have contributed to their use in multitude of devices cleared by the Food and Drug Administration.

Hydrogels are three-dimensional (3-D) constructs composed of an insoluble crosslinked network. PEGDA hydrogels, in particular, are hydrophilic, self-crosslinking, water-swollen polymer networks. This water swollen hydrophilic nature permits the interaction and, even encapsulation of biomolecules such as proteins, without the fear of denaturing one would see in their hydrophobic polymer counterparts. This permits for the study of biomolecules in their native states. This is critical for the examination of how a system will affect the biological entities' properties, such as diffusivity. Diffusivity of a molecule in a system is paramount for understanding and controlling delivery and the subsequent release of a drug. The effects of the state of the system on the diffusion of a given molecule can be easily observed through varying the components of the system. In the PEGDA hydrogel system, the molecular weight of the PEGDA monomer that composes the polymeric backbone and crosslinker used were varied. This manipulation alters the intrinsic properties that influence the diffusivity of molecules into and throughout the hydrogel. Also, this manipulation of the intrinsic properties manifests itself in changes to microstructural properties. These changes in the microstructure of the hydrogels were deduced by the comparative analysis of equilibrium swelling information. The various hydrogels were quantifiably compared using calculated mesh sizes derived from the equilibrium swelling.

The effects of varying the molecular weight of the monomers on the structural state of the system were elucidated through analysis of hydrogel mesh size. The results of the of the mesh size analysis appear to conclude that increase in the molecular weight of the monomer caused a directly proportional increase in mesh size of the hydrogels.

Additionally, tethers made from methoxy poly(ethylene glycol) monoacrylate (mPEGMA) were incorporated into the hydrogel. The tether length was varied through using different mPEGMA molecular weights. This contributed another layer of complexity to the hydrogels, which could be manipulated in order to tailor them to a desired diffusion profile for a specific molecule or biomolecule. In order to further elucidate the effects of varying the different components on the diffusion of small molecules, diffusion was studied using 7-amino-4-methylcoumarin (AMC). The diffusion of AMC through hydrogels was measured using fluorescence spectroscopy in conjunction with a Franz cell apparatus. Also, to examine the effect of these manipulations on the diffusion of biomolecules, bovine serum albumin (BSA) was used in a second diffusion study. The BSA diffusion study was also accomplished by using fluorescence spectroscopy in conjunction with Franz cell apparatus. The BSA and AMC studies with mesh size analysis allowed for a clear understanding of the dynamic between the hydrogel structure and its diffusion properties. The rate of diffusion is directly proportional to the increase in monomer molecular weight and mesh size, but while it is inversely proportional to tether length. The effect of the tether length was seen in the higher molecular weights of PEGDA hydrogel due to proportionally small volumes occupied by the polymer's unattached ends in comparison to the relatively large void volume. Because the lower molecular weight hydrogels had large portion of the of void volume assumed by the mPEGMA, a structural change could

affect molecular diffusion. Also, the smaller void volume aided in creating physical crosslinking (polymer chain entanglement) due to the shorter distance between polymer tethers and their neighboring polymer chains. The entanglement effectively decreases the mesh size negating any increase that could be seen by its presence. Furthermore, the system was still able effectively alter the diffusion of a small molecule such as AMC, while permitting the diffusion of a large biomolecule. This implies the possibility of using two mechanisms for controlling the release of a drug. One a possible mechanism could use an enzyme, such one found in the matrix metalloprotease family, to initiate the initial release of drug. Another mechanism could use a structural element of the hydrogel, such as incorporation of tethers with varying length paired cooperatively and antagonistically with the monomer to achieve a desired drug release profile.

I. INTRODUCTION

1.1 Background

Illness and disease have been present throughout the history of mankind, and mankind, in turn, has continuously sought out means with which to combat illness and disease. Currently, and historically, the goal has been the elimination and treatment of disease to improve the lives of the inflicted. As our knowledge and technological base has increased throughout the years, the plethora of skills and tools to heal the sick has increased as well. In the past, people had to resort to mysticism. Now, we have derived scientific theory from objective observations and evidence based proofs, driving the frontiers of human knowledge.

One component within this vast scientific frontier is hydrogel-based technologies. Hydrogel technologies, since the middle of the past century, have developed and entered into many fields. These fields span a diverse range of applications from those in food products to tissue engineering, cosmetics and medicine (Qiu and Park, 2001; Li, Neoh et al., 2004; Zhang et al., 2009). The use of hydrogels in these diverse areas is due to their hydrophilic 3-D network. This provides many desirable physiochemical properties for various uses in biomedical fields. One of these uses is the encapsulation of biomacromolecules, such as proteins, which remain stable because of the hydrophilic properties of hydrogels. The proteins in this hydrophilic environment of the hydrogel remain in their native states, which are usually more stable and biologically relevant. This hydrophilic environment is desirable because in comparison to their hydrophobic polymer network counterparts, made of common materials such as poly(lactide-co-glycolide) (PLGA), they are better able to maintain the biologically relevant conformations of encapsulated biomacromolecules (Lin and Metters, 2006).

Additionally, hydrogels, in general, are biomechanically compatible and support cellular activities, making them applicable for use in medical fields. Typically, hydrogels are synthesized under conditions that are relatively gentle compared to those of hydrophobic polymers. This gentler polymerization is more suited to in situ systems than that of its hydrophobic counterpart. This quality has expanded the opportunities for the applications of the hydrogel in the medical field. Furthermore, the lack of need for organic solvent and fact that the polymerizing process can be modified to tailor the mechanical properties of the hydrogel make the range of medical applications even broader. Also, the physical material and chemical properties of the hydrogel system can be altered to suite a given application (Lin and Metters, 2006; Kloxin et al., 2009). The above attributes have given the hydrogel a prominent place in the growing range of interdisciplinary research that allows for the integration of hydrogels with newly emerging technologies. Applications of hydrogels will most likely, during the foreseeable future, continue to grow as technology and diverse disciplines continue to converge.

II. HYDROGELS

2.1 Introduction

It has been more than a half century since Wichterle and Lim first explored hydrogels (Wichterle and Lim, 1960). Hydrogels are crosslinked polymeric networks that have the ability to absorb and retain large quantities of water when in their swollen states (Peppas et al., 2000). The crosslinking of the polymer chains during formation of the network also prevents the network from dissolving. The crosslinking can occur via chemical or physical mechanisms. The crosslinked nature of the hydrogel allows it to maintain a defined shape where relative dimensional proportions remain constant, even in the fully swollen state. The mechanism of polymerization and type of polymer used to form the hydrogel system is often dictated by the needs of a given application. The correct combination of polymer and polymerization technique is especially critical in the controlled drug release field to obtain a desired release profile. Also, the type of polymer and the polymerization technique serve as a means with which to classify hydrogels.

2.2 Classification

2.2.1 Charge State

The presence of charged groups in the hydrogel can be used to when categorizing hydrogels. The ionic or neutral charge state of the pendent groups, for example, is often used to differentiate hydrogels. Hydrogels that have ionizable pendent groups tend to have a greater the ability to exert a force on solvent than those with without ionizable group pendent groups. This interactive force can allow for increases in swelling of the hydrogels. The ionization effect has been incorporated in many biomedical hydrogels and these hydrogels make use of it in several

ways. An example of one use in particular, is the controlled release of a biomolecule using a release profile that has been tailored to that specific molecule using ionic reaction (Peppas et al., 2000).

2.2.2 Porosity

The porosity of hydrogels can be used to distinguish and further classify them. Hydrogel porosity can range from nonporous to porous. Nonporous hydrogels, as the name implies, lack a clear and defined pathway through which a molecule is able to move in a relatively unhampered manner. Porous hydrogels are typically polymerized using the similar mechanisms as nonporous, which will be discussed in a later chapter. The difference is that the porous hydrogel synthesis requires additional procedures and agents to develop the pores, which are formed by gas bubbles or voids in the material caused during, or just after, formation of the hydrogel. Salt leaching and particulate leaching techniques are examples of some of pore creation techniques. The salt, or particulate, is incorporated into the hydrogel before polymerization. After polymerization has occurred, a solvent is used to dissolve the salt or particulate, leaving a void volume behind. The use of solvent can also have disadvantages, however, especially in a biological setting. (Shastri et al., 2000). This technique that can be used to make porous hydrogels, can also be used to makehydrogels that are sometimes referred to as macroporous. Macroporous hydrogels can be further classified as superporous hydrogels (SPH). SPH have interconnected pores in the range of hundreds of microns and can be made reliably by gas foaming technique (Gemeinhart et al., 2000).

2.2.3 Dimensional Scale

Just as pore size is used to classify hydrogels, the dimensional scale of hydrogels can also serve to categorize them. The bulk, or the visible macro scale, is typically what is referred to

when discussing hydrogels. However, hydrogels are often created and analyzed at the micro scale, and then the term microgel appears. Microgels is a term that was first coined by Baker in the 1950s (Baker, 1949). These hydogels have dimensions of 0.1 to 100 μm. Microgels' dimensional ranges at one time entered deep into the nano scale. Now, hydrogels in the nano scale are, not surprisingly, called nanogels. Conventionally, the range for the nanogel dimensions is from 1 to 100 nanometers (Baker, 1949; Oh et al., 2008; Scott et al., 2008). These microgels, and especially nanogels, will continue to develop rapidly as nanotechnology expands. As nanotechnologies become more refined and sophisticated, these hydrogels will become more dynamically sophisticated. This has been seen already in "smart" hydogels. These hydrogels are able interact with their surrounding environment, including direct responses to stimuli within the environment. Also, due to the constantly changing dynamic of biological settings, these hydogels can be considered highly bioactive (LaVan et al., 2003). Additionally, hydrogels are classified by the materials and methods used to synthesize them. The materials and methods will be discussed later in this chapter.

2.3 Properties of Hydrogels

2.3.1 Bulk Properties of Hydrogels

No matter the material or method used to fabricate hydrogels, they share common properties. They are composed of crosslinked polymeric chain networks. This crosslinking can be achieved via physical or chemical interactions, which will be discussed in more detail later (Qiu and Park, 2001). This provides them with a defined 3-D shape that can, in turn, provide a physical support for biological structures with defined interfaces. This is an important feature when creating a composite utilizing hydrogels. Uniformity can also be seen with a hydrogel when swollen; it will maintain its proportional dimensions and shape. For example, if the

hydrogel was a cube before swollen, it will remain a cube shape and simply display a greater volume when swollen. The ability to maintain shape, similar to how solids maintain shape, is observed at the macro-scale.

Contrarily to what is observed at the macro-scale, at the micro-scale hydrogels perform similarly to solvents. Diffusants travel throughout the hydrogels at rates based on their respective diffusion coefficients, as is seen in solvents. This allows hydrogels to be used to separate different molecules based on their size. Furthermore, this permits for the use of hydrogels as a platform from which to transfer nutrients and support gas exchange. This is crucial for the survival of biological entities such as cells. Also, due to these characteristics, the hydrogel readily lends itself to the diffusive controlled release of soluble molecules and proteins. Later, in Section 2.5 this and other applications will be examined in more detail. Meanwhile, nanoscopic hydrogel particles' role in the nano-realm is that of the diffusant, a complete role reversal from that of which is played in the microscopic and macroscopic realms (Hern and Hubbell, 1998; Hubbell, 1999; Prokop et al., 2002; Lutolf and Hubbell, 2005; Tanaka et al., 2005).

2.3.2 Mechanical Properties

Hydrogels have viscoelastic mechanical behavior (Xu et al., 2008). Viscoelastic materials display mechanical behavior such as strain response in time dependence. Dynamic mechanical analysis (DMA) is a method that can be used to determine time and temperature relationship. DMA operates, typically, by applying an oscillating force to impose a sinusoidal stress on a sample. This, in turn, causes a sinusoidal strain response in the sample out of phase with the stress input (Figure 1). This allows for the determination of material properties, such as Young's modulus (G), shear storage modulus (G'), and shear loss modulus (G"). A given modulus

provides a quantitative measurement to assess the mechanical compatibility for a given application. The following expression demonstrated how the modulus is calculated:

$$G' = \left(\frac{\sigma}{\gamma}\right)\cos\delta \qquad (1)$$

$$G'' = \left(\frac{\sigma}{\gamma}\right)\sin\delta \qquad (2)$$

$$\frac{G''}{G'} = \tan\delta \qquad (3)$$

Where σ is the maximum stress, γ is the maximum strain and δ lag phase angle.

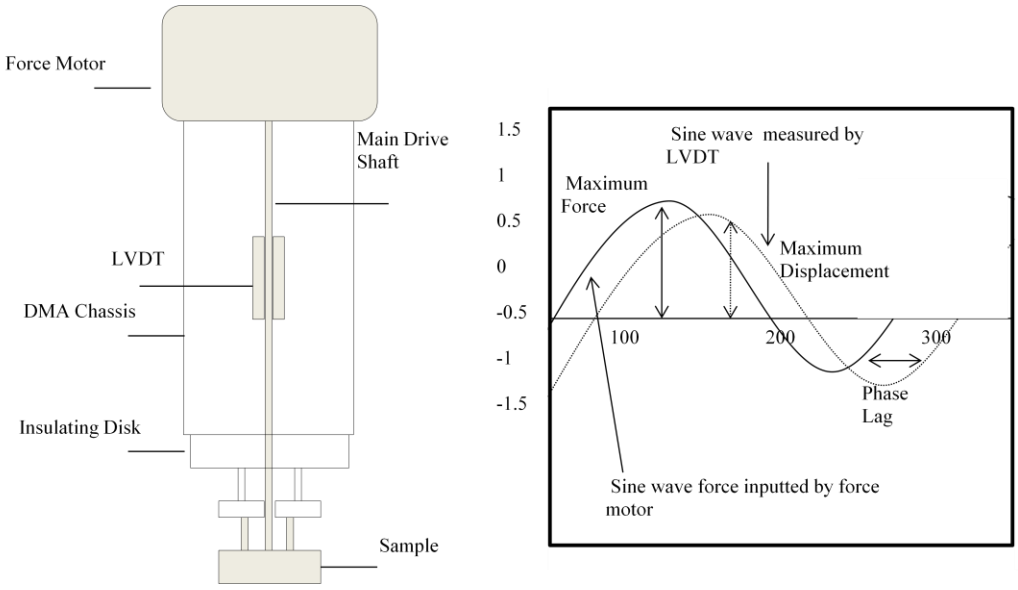

Figure 1. Schematic of PerkinElmer DMA 8000 (on the left). On the right is the graphical representation of sinusoidal force input and sinusoidal strain output detected by linear variable differential transducer (LVDT). Adapted from Menard, 2008.

Additionally, the mechanical responses can be modeled using a physical analog with springs and dashpots. The springs and dashpots can be placed in series, parallel or a combination of both as seen in Figure 2. The generalized Maxwell and Voigt are expressed in equations (4) and (5), respectively. The expression below describes the relationship where η_i is the viscosity of the liquid and τ_i is the relaxation constant.

$$G_i = \frac{\sigma(t)}{\gamma} = G_i e^{-t/\tau_i},\ \eta_i/Gi = \tau_i \qquad (4)$$

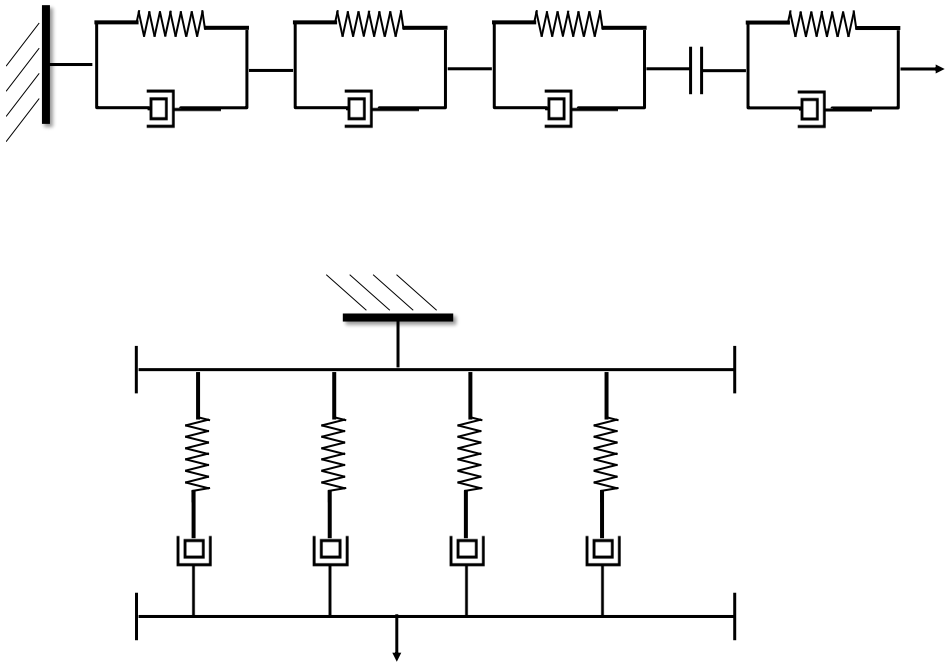

Figure 2. Schematic of Maxwell model (top) and Voigt model (bottom) uses physical elements composed of spring dashpot in series and parallel combinations as an analog to simulate the viscoelastic system behavior. Adapted from Ferry, 1980.

2.4 Methods and Materials Used for the Synthesis of Hydrogel

The are many ways to to create hydrogels and there are many factors that influence which method is chosen to create them. Certainly, one of these factors is the material that will be used when manufacturing hydrogels. The choice of materials can limit the synthesis method of the hydrogel to a few, or even a couple, techniques. Also, the mechical and immunological compatibility needs of an application can act as a guiding force when selecting a synthesis process. This is exemplified by injectable biodegradable systems using photopolymerization to repair small bone defects and allowing for healing bone tissue to undergo less physical stress throughout the duration of the healing process. Also, the polymerization conditions used in this example are typically mild and will not significantly harm surrounding tissue (Ifkovits and

Burdick, 2007). Additionally, the type of crosslinking that the hydrogel undergoes, be it physical or chemical crosslinking, is another factor to be considered in this process. Crossliking provides a means with which to catagorize hydrogels based on general type and method of creation. In the following section the methods of creating hydrogels will be discussed. However, the manner the cosslinking is accomplished the general hydrogel form is the same. The polymer chains interact with each to form a network, which is infuse and surround with water molecules. This can be seen in Figure 3. The polymer chains in the left part of the figure are surrounded with water molecules reprensented by solid black circles. Then after the polymer chains are crossliked the water molecule still surround the polymer chains in network,on the right side of the Figure 3.

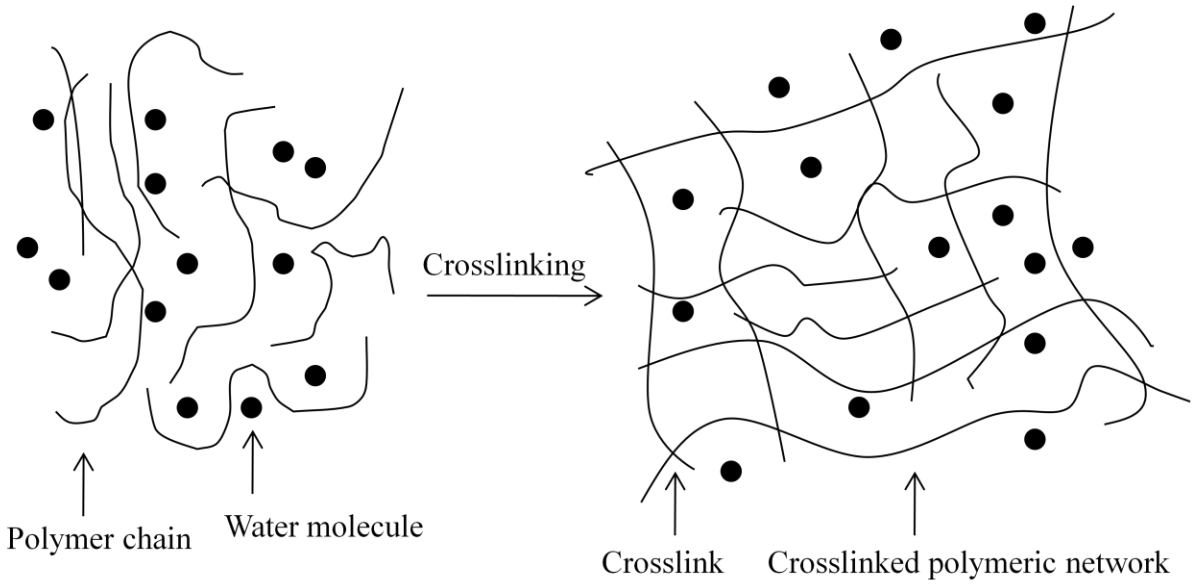

Figure 3. Schematic description of hydrogel formation by chemical (covalent bond) or physical (ionic bond) cross-linking of polymers. Solid dots represents water molecules surround polymer before (left side) and polymers after crosslinking (right side). Adapted from Varghese and Elisseeff, 2006.

2.4.1 Methods

2.4.1.1 Physical Crosslinking

2.4.1.1.1 Polymer Chain Entanglements

Physical polymer chain entanglement shares some common advantages with other physical crosslinking methods. An important advantage is that it does not use initiators and catalysts, which are often toxic. This method also allows for the possible reversal of the process. Physical entanglement can be accomplished by simply applying heat to a monomer. People have shown that heating N-carboxymethylchitosan (CMC) leads to hydrogel formation (Hennink and van Nostrum, 2002; Di Colo et al., 2006).

2.4.1.1.2 Crosslinking by Crystallization

The introduction of crystals along with monomer material allows for sites of physical crosslinking. Crystallization is produced using a freeze-thaw process. This involves the repetitive cyclic freezing and thawing of the base materials. This method allows for hydrogel property optimization through altering synthesis conditions (Stauffer and Peppas, 1992; Cappello et al., 1998; Liu et al., 2009). A good example is poly(vinyl alcohol) (PVA) hydrogels, made using the freeze-thaw method. Under optimum conditions, these hydrogels are stable and display greater mechanical properties than PVA hydrogels synthesized at room temperature (Yokoyama et al., 1986; Hassan and Peppas, 2000; Hennink and van Nostrum, 2002).

2.4.1.1.3 Ionic Interactions

Ionic interactions can serve as a means to create crosslinked hydrogels. One of the common types of hydrogels that use this crosslinking method is alginate hydrogels. Alginate hydrogels have mannuronic and glucuronic acid residues that are exploited during the crosslinking process. Calcium ions interact with these residues to create the network. Metal ions are usually involved in crosslinking, but ionic groups are not always needed in polymers

(Gacesa, 1988; Rowleyn et al., 1999; Drury and Mooney, 2003). The addition of an ion can stabilize a structure by incorporating itself into the 3-D structure, thus making it energetically stable. This was described by Watanabe et al. with the interaction of potassium and glucose polymer, resulting in a stable microstructure (Watanabe et al., 1996).

2.4.1.1.4 Hydrogen Bonding

Hydrogen bonding forces can induce the formation of hydrogels. This can be seen when mixing poly(2-methacryloyloxethyl phosphorylcholine-co-methacrylic acid) (PMA) and poly(methacryloyloxethyl phosphorycholine-co-n-butyl methacrylate) (PMB), which results in hydrogel formation at room temperature due to hydrogen bonding (Kimura et al., 2004; Kimura et al., 2005). Also, the hydrogen bond has been implicated in the swelling properties of poly(methacrylic acid-g-ethylene glycol) P(MAA-g-EG). The carboxylic groups form hydrogen bonds when they are protonated. They are protonated at a given pH range, thus allowing the hydrogel to respond to it surrounding conditions. This property can be manipulated to allow for dynamic controlled drug release (Bell and Peppas, 1996; Hassan et al., 1997; Robinson and Peppas, 2002; Kim and Peppas, 2003).

2.4.1.1.5 Protein Interaction

Natural and genetically engineered proteins have been used to create hydrogels. Genetic engineering permits for a high level of control over mechanical and chemical properties at the amino acid level. Cappello and colleagues produced a hydrogel by repeating silk-like and elastine-like blocks so that they would arrange in a aligned insoluble beta sheets (Joseph et al., 1990; Cappello et al., 1998).

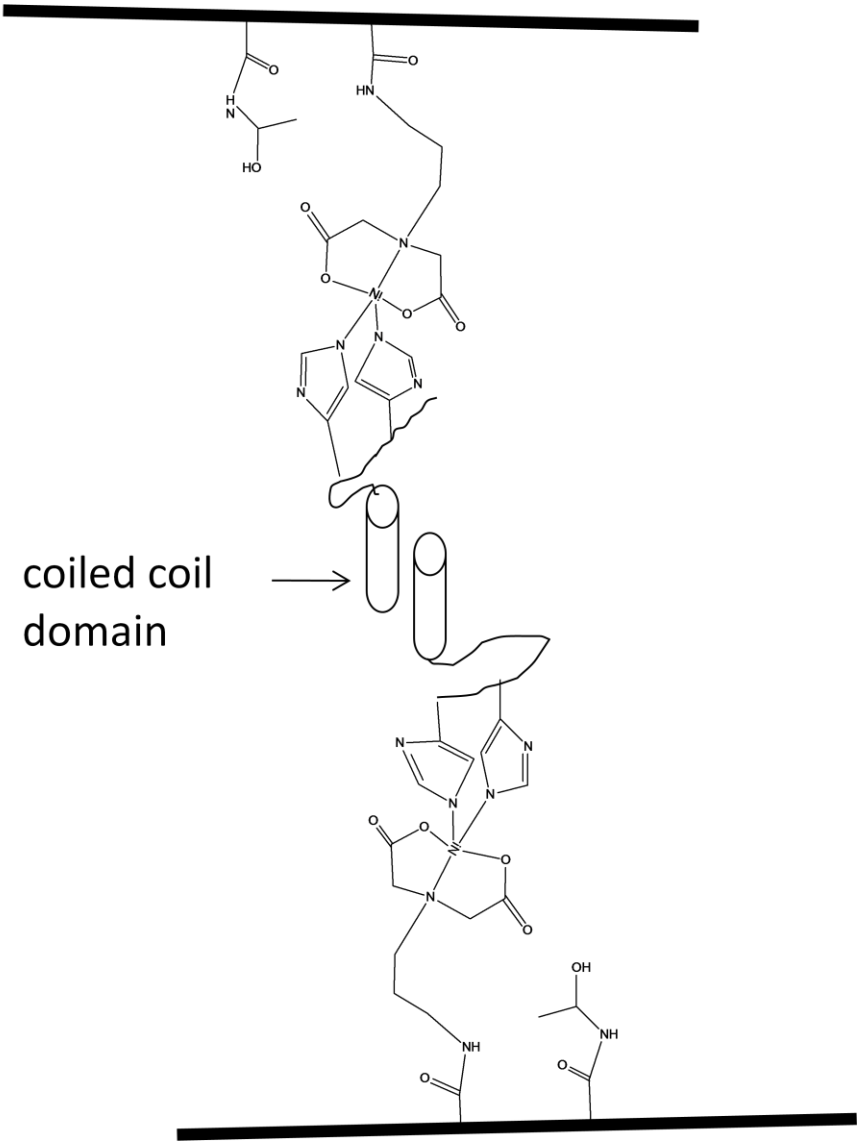

Figure 4. Design of hybrid hydrogels. Schematic illustration of a hybrid hydrogel system that utilizes genetically engineered coiled-coil protein and a synthetic water-soluble polymer crosslinker (copolymer of HPMA and N-(N,-N dicarboxymethylamino-propyl) methacrylamide). Divalent transition metal ions are shown to form complexes with nitrogen–oxygen donor ligands on the synthetic, and t terminal histidine residues of the protein. A tetrameric coiled-coil (not drawn to scale), consisting of antiparallel dimmers. Adapted from Wang et al., 1999; Jindich and Jiyuan, 2007.

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Also, Tirell used natural proteins that form coiled coils to construct hydrogels. These protein hydrogels had the ability to change confirmation based on stimuli such as temperature

and pH. Increasing temperature and pH would turn the hydrogel into a thick solution (Yoshikawa et al., 1994; Petka et al., 1998). In Figure 4, a schematic of a hybrid hydrogel that includes a protein and an synthetic polymer in shown. The protein used in the hybrid hydrogel system was engineered to have a coiled-coil domain to allow for interaction between the proteins. The configuration of the interaction of the two proteins is dependent on the thermal and chemical of the surrounding. Figure 4 shows dimmers interacting in an antiparallel configuration to form tetrameric coiled-coil with the copolymer crosslinker. The interaction is dependent on the proprieties of the surrounding environment and may be reverse by changing the proprieties of the surrounding environment.

2.4.1.2 Chemical crosslinking

Unlike physically crosslinked hydrogels, chemically crosslinked hydrogels involve covalently bonded polymer chains. Typically, chemically crosslinked hydrogels are achieved through the use of chemical initiators, catalysts, crosslinking agents and exposure to high energy. Below, the methods are described in more detail.

2.4.1.2.1 Radical Polymerization and Crosslinking

Historically, low molecular weight vinyl monomers, when reacted with crosslinking agents, have utilized radical chemical processes for hydrogel formation. Later, the same process was expanded to create branched, multivalent, multiarmed polymers and copolymers. These expansions lead to a subsequent growth in crosslinking agents used. In Table I is a list of some common crosslinkers that typically that have an unsaturated ends. The unsaturated ends allow for the molecule to react with reactive groups of two monomers and crosslink those monomers. The crosslinking is usually accomplished via a free radical reaction such as with hydrogels made

from a commonly used crosslinker with ethylene dimethacrylate (EDMA) and a common monomer (2-hydroxyethyl methacrylate) (HEMA) (Figure 5) (Mabilleau et al., 2006; Jindich and Jiyuan, 2007).

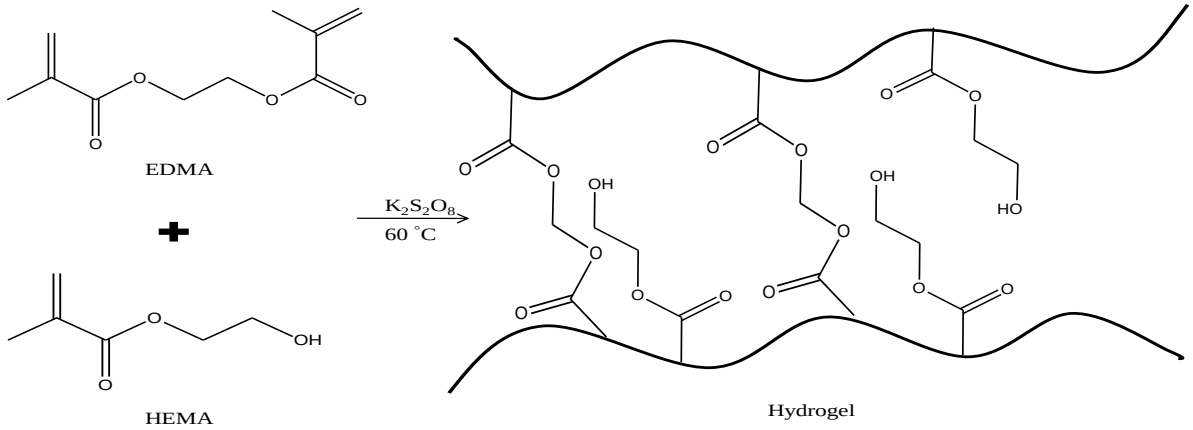
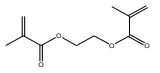
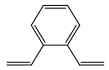
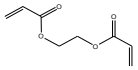
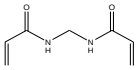


Figure 5. Schematic of Hydrogels prepared by crosslinking copolymerization of 2-hydroxyethyl methacrylate (HEMA) and ethylene dimethacrylate (EDMA) Adapted from Mabilleau et al., 2006.

Table I. Names and structures of some commonly use crosslinkers. Adapted from [a] Mabilleau et al., 2006; [b] Jindich and Jiyuan, 2007.

Structure of Monomer	Monomer Name
 ab	Ethylene glycol dimethacrylate [ab]
 a	Divinyl benzene [a]
 a	Ethylene glycol diacrylate [a]
 b	Methylenebisacrylamide [b]

2.4.1.2.2 Photopolymerization

Radicals can be generated using a light source, usually in the ultraviolet spectrum range, and a photoinitiator. The photopolymerization system typically radicalizes unsaturated groups such as vinyl groups. This system can be used to form hydrogels in situ, which is a great advantage. Other advantages are that the hydrogel can be made with designed patterns and physical properties using well established lithography techniques (Hubbell, 1996; Lee et al., 2008; Liu et al., 2009). Moreover, this system has been used to create biodegradable hydrogels (Metters et al., 2000; Martens et al., 2002). The formation of a degradable polyvinyl alcohol-based hydrogel is depicted (Figure 6). The hydrogel was created by photopolymerization and is degraded by a hydrolysis.

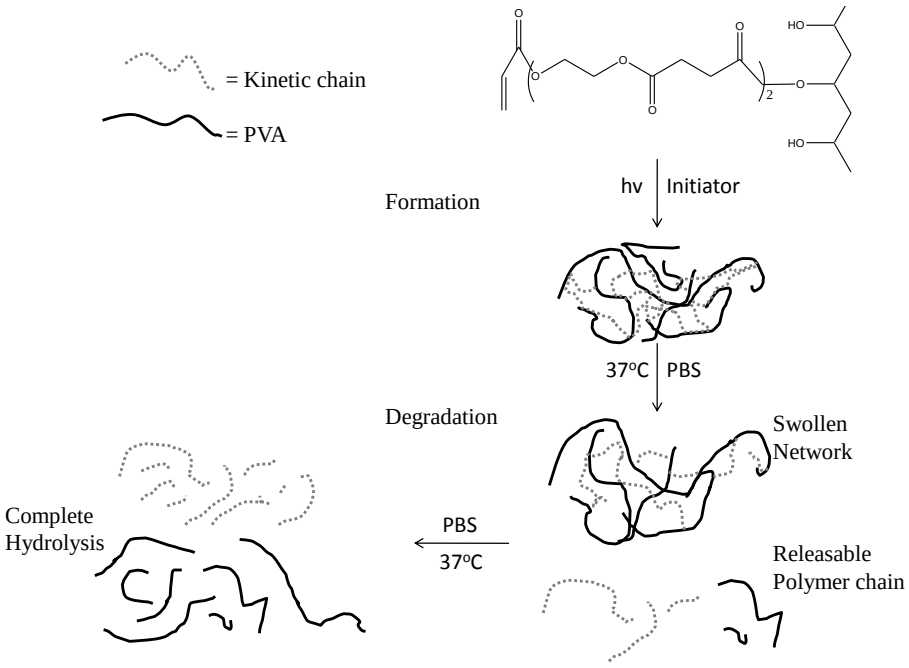


Figure 6. Schematic of the formation of hydrogel networks from multifunctional PVA chains, and the subsequent degradation process and products. Adapted from Martens et al., 2002.

Scientists, such as Hubbell, were able to use 2,2-dimethoxy-2-phenylacetophenone, a photointiator, in an aqueous solution containing poly(ethylene glycol)-co-poly(α-hydroxy acid) diacrylate. The resulting hydrogels' degradation rates could be varied as desired (Sawhney, 1993).

2.4.1.2.3 Paired Initiators

Another system makes use of paired initiators, peroxydisulfate and *N,N,N′N′*-tetramethylene-diamine (TEMED) to polymerize water-soluble polymers with vinyl groups. The system can create a hydrogel in a matter of seconds under ambient temperature (Kurisawa and Yui, 1998). Unfortunately, these initiators can be toxic to biological entities such as proteins. Even with this limitation, hydrogels, after being rinsed to remove excess initiators, can be used in biological situations. Furthermore, by pairing of TEMED with ammonium persulfate (APS), a

redox initiator, has allowed for the use of hydrogels in biological systems (Hennink et al., 1997; vanDijkWolthuis et al., 1997; Franssen and Hennink, 1998). Cells were able to survive in the presence of hydrogels made using this system (Keskar et al., 2009).

2.4.1.2.4 High Energy Irradiation

High-energy radiation sources have been used to create hydrogels. The energy source is usually in the form of an electron beam or gamma rays. The radiation forms free radicals in two ways. First, it can free a hydrogen molecule by hemolytic scission of a carbon hydrogen bond. Another way to form free radicals is the creation of hydroxyl radicals. These radicals can interact with the polymer and cause it to be radicalized (Peppas and Mikos, 1986). The network can grow by hemolytic scission or hydroxyl radials, but the process is typically done in an inert atmosphere to prevent oxidation (Nikolic et al. 2007; Krkljes and Kacarevic-Popovic, 2008; El-Din et al., 2009).

2.4.2 Natural Material

In general, polymers derived from natural materials are stable under physiological conditions. On the other hand, polymers derived from natural material have a tendency to be more difficult to process than their synthetic counterparts. Also, there are issues with immunogenicity. Another possible concern, if the material was extracted from nonhuman animal species, is human immune recognition of these materials as foreign entities (Chung and Park, 2007).

Some of these naturally derived materials are collagen, fibrin, silk alginate and hyaluronic acid. Animal derived collagen has been used to create porous networks that are used in skin replacement (Pieper et al., 2002). Fibrin is an important component in blood clotting. It

has, for that reason, been used in hydrogels that are formed in situ (Sakiyama-Elbert and Hubbell, 2000; Sakiyama-Elbert and Hubbell, 2000; Ye et al., 2000). Alginate, on the other hand, is a polysaccharide obtained from brown algae and, as discussed earlier, polymerizes due to interaction with divalent chemical species. Below is a list of these polymers, and other nature polymers, used for the synthesis of hydrogels (Table II).

Table II. Table of polymers from natural origins that are used in hydrogels. Compiled from [a] Lin and Metters, 2006; [b] Lee and Shin, 2007.

Polymers from Natural Origins	
Collagen [a]	Chitosan [a]
Gelatin [a b]	Alginate [a]
Silk [b]	Hyaluronic acid [a b]
Fibrin [a]	Chondroitin sulfate
Agarose [b]	Dextran [a]

2.4.3 Synthetic Material

Typically hydrogels made from synthetic sources provide more mechanical support than those made from natural polymer sources. Unlike natural polymers, synthetic polymers do not have moieties that can be recognized by biological entities such as cells. This limits their bioactive properties and their applications (Peppas et al., 2000; Lin and Metters, 2006; Chung and Park, 2007). This can be overcome by including a recognizable motif (Benton et al., 2009; Salinas and Anseth, 2008). The addition of functional groups and bioresponsive motifs is relatively straightforward, due to the well-described structure of synthetic polymers. A commonly used modification is the addition of an arginine-glycine-aspartic acid (RGD) peptide

sequence. This peptide sequence can be recognized by cells and helps aid in their survival (Lin and Metters, 2006; Bentons et al., 2009; Salinas and Anseth, 2008).

Table III. Table of synthetic polymers that are used in the synthesis of hydrogels. Adapted from Peppas et al., 2000.

Abbreviation of Monomer	Monomer Name
HEEMA	Hydroxyethoxyethyl methacrylate
HDEEMA	Hydroxydiethoxyethyl methacrylate
MEMA	Methoxyethyl methacrylate
MEEMA	Methoxyethoxyethyl methacrylate
MDEEMA	Methoxydiethoxyethyl methacrylate
EGDMA	Ethylene glycol dimethacrylate
NVP	N-vinyl-2-pyrrolidone
NIPAAm	N-isopropyl Acrylamide
Vac	Vinyl acetate
AA	Acrylic acid
HPMA	N-(2-hydroxypropyl) methacrylamide
EG	Ethylene glycol
PEGA	PEG acrylate
PEGMA	PEG methacrylate
PEGDA	PEG diacrylate

2.4.4 Poly(Ethylene Glycol) and Derivatives

Poly(ethylene glycol) (PEG), or what is sometime referred to poly(ethylene oxide) (PEO) for high molecular weight, is a synthetically derived material. It is synthesized with the use of anionic or cationic polymerization of ethylene oxide. It has been used in a great number of FDA approved medical applications. This is because it expresses low toxicity and biocompatibility due to low immunological or inflammatory response (Lee and Yuk, 2007;

Zalipsky, 1995). PEG's ability to fly under the radar unrecognized by the immune system has caused it to be described as having "stealth" properties or as being a stealthy molecule (Peppas et al., 2000). This allows it to increase the half-life and decrease immunogenicity of asparaginase and other high molecular weight substances (Burnham, 1994). Additionally, PEG has hydroxyl groups at both ends of the molecule that can be used for adding different functional groups and derivatives.

There are numerous derivatives of PEG that are achieved by adding reactive functional groups such as the acryloyl group. Addition, as a result of having two hydroxyl groups, allows the molecule to be heterofunctionalized or homofunctionalized, such as with poly(ethylene glycol) diacrylate (PEGDA). PEGDA is synthesized using an acrylation process with acryloyl chloride as source of the acryloyl group. The vinyl hydrogen of the PEGDA allows it to be used as a crosslinker for polymerization into hydrogels. The synthesis of PEGDA and its use as a monomer for the fabrication of hydrogel is covered in more detail, Section 3.2. Another member of the PEG family is methoxy poly(ethylene glycol) monoacrylate (mPEGMA) or poly(ethylene glycol) methyl ether acrylate. mPEGMA's single functionality permits it to be tethered to a network at one end and free at the other end. The tethering of mPEGMA to the hydrogel alters the physical properties of the hydrogel. The shear modulus of a mPEGMA-co-PEGDA can be modulated significantly by changing the concentration of mPEGMA in the hydrogel. Additionally, the swelling properties of the hydrogel can be controlled through variation in the amount of mPEGMA incorporated (Beamish et al., 2010).

2.5 Applications

2.5.1 Biomedical Devices

The field of biomedical devices has significantly incorporated hydrogels. This incorporation has been accomplished because many of the current fabrication techniques, such as photolithography and molding, can be used to make medical devices. One of the current focuses is to use these techniques toward the creation of biomedical microdevices and components such as valves. Many microdevices use microfluidic channels, which need valves to control their flow. Valves made from pH sensing hydrogels have been demonstrated to work effectively. The PEG-based hydrogel valve is actuated by changing in swelling resulting from changes in pH, which can be seen below (Beebe et al., 2000; Liu et al., 2002).

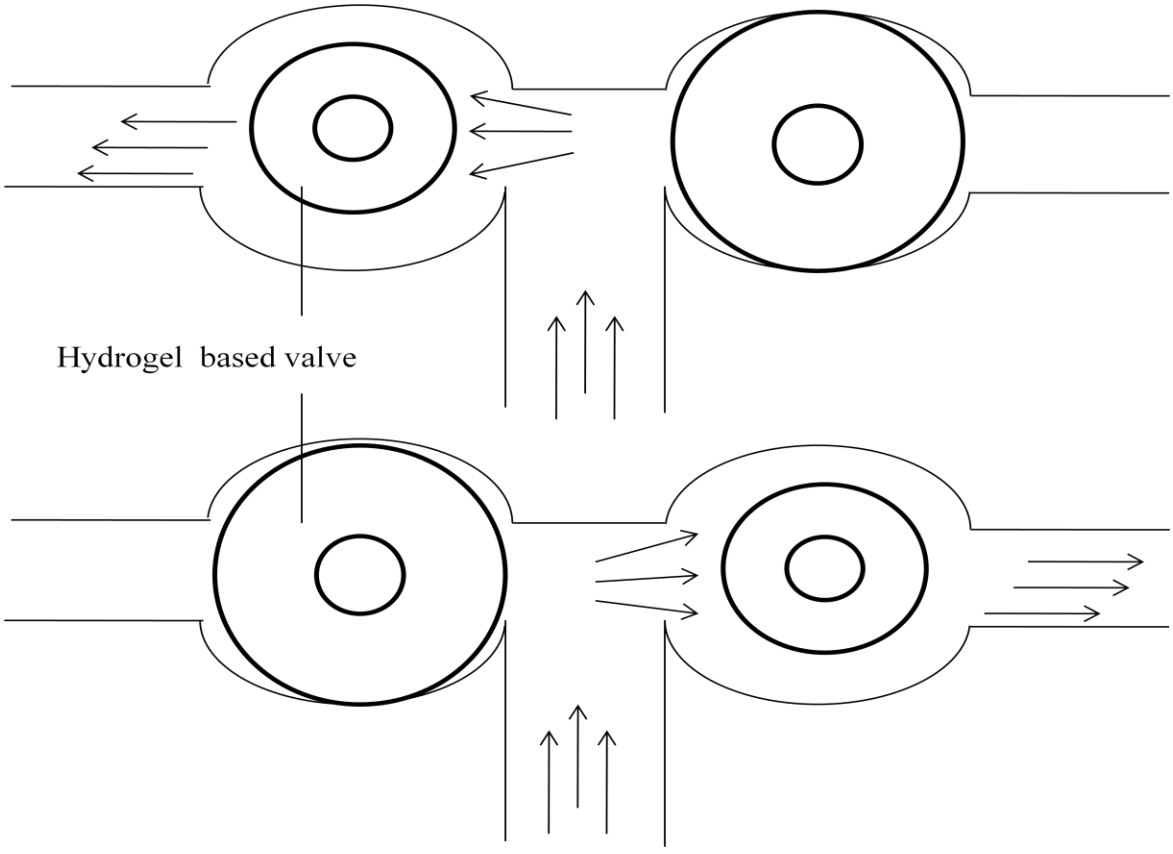


Figure 7. A carton schematic of fluid flow controlled within microdice channels by swelling of the hydrogels. The hydrogels act as environmentally responsive valves. The hydrogel senses the change in pH and swells accordantly. Adapted from Sershen et al., 2005.

Additionally, microelectromechanical systems (MEMS) based biosensors have been developed using hydrogels. The bioMEMS sensors measure pH, CO_2 level and other biological conditions (Van der Linden et al., 2002; Herber et al., 2003). These devices allow for the monitoring of biological conditions, but there is a diagnostic aspect that hydrogel-based systems have addressed. For example, an iron oxide based magnetic resonance image contrast agent is surrounded by a poly(N-(2-aminoethyl) methacrylamide) (AEM) hydrogel shell. The shell has targeted peptides attached to help the contrast agent reach the target cells at desired concentrations (Gong et al., 2009). This same increase in efficacy can be seen with other nanogels. This targeting approach has also been used with near-infrared fluorescence (NIRF) dyes. In this case, hydroxyethyl methacrylate and divinylbenzene create the nanogel system. Also, some nanogel systems do not have any targeting peptide but yet still increase efficacy of the agent (Yang et al., 2009).

2.5.2 Tissue Engineering

The goal in tissue engineering revolves around the regeneration or repair of tissue and organ system function. Materials used in tissue engineering need to mimic natural properties of the tissue or organ they are replacing. Hydrogels have biomimetic properties that make them appropriate for use as scaffolds in tissue engineering. Scaffolds made from PEG, and/or other monomer-based hydrogels with encapsulated cells, have been used for their ability to provide protection against the host's immune response (Burdick and Anseth, 2002; Nguyen and West, 2002). These scaffolds have been used for the engineering of blood vessels, nerves, skin and other organs (Leor et al., 2009; Liu et al., 2009; Shen et al., 2009). The complexity of these scaffolds is continuously growing due to the incorporation of peptide, cells and released biomolecules such as growth factors. The hydrogel system currently incorporates natural and

synthetic materials as well as degradable components, which add further complexity to the system. The degradability is important because degradability allows for the matching of the mechanical properties as the tissue regenerates (Peppas et al., 2006).

2.5.3 Drug Delivery

The same properties that have made the hydrogel ideal for tissue engineering make it useful in the delivery of drugs. The chemical and physical properties of the hydrogel can be optimized to obtain specific characteristics such as porosity, biodegradability and surface functionality. The engineering of these hydrogels is accomplished at the molecular level to control which specific properties are present (Peppas et al., 2006; Lin and Metters, 2006; Chung and Park, 2007; Lee and Shin, 2007; Burdick and Anseth, 2002). Altering the permeability of the hydrogel can achieve sustained release of an agent. Furthermore, altering enviro-responsivity of the hydrogels can facilitate pulsatile release. The use of PEGylation, or the modification of a surface with PEG, allows for stealth release. Additionally, the inclusion of a biorecognition site has been utilized for targeted release (Hassan et al., 1997; Peppas, 1997). The approaches to controlled drug delivery and the subsequent release of a therapeutic agent through the use of hydrogels is depicted in the Figure 8. The mechanisms for controlled release of therapeutic agents include a stimulus-response hydrogel system that can react to changes in pH, temperature or other local environment conditions. Also, degradation and swelling provide a mechanism for controlled release of a therapeutic agent.

Researchers have demonstrated that the swelling nature of hydrogels can be used to dictate the release of a drug from within the system. The swelling nature of the PVA and PEG hydrogels has been manipulated by changing the polymer molecular weight, composition and

other factors. This manipulation varies the crosslinking density, which influences the swelling (Peppas and Langer, 2004; Stringer and Peppas 1996). Thus, control over the release of a drug from a hydrogel delivery system can be obtained by simply altering the properties of the hydrogel.

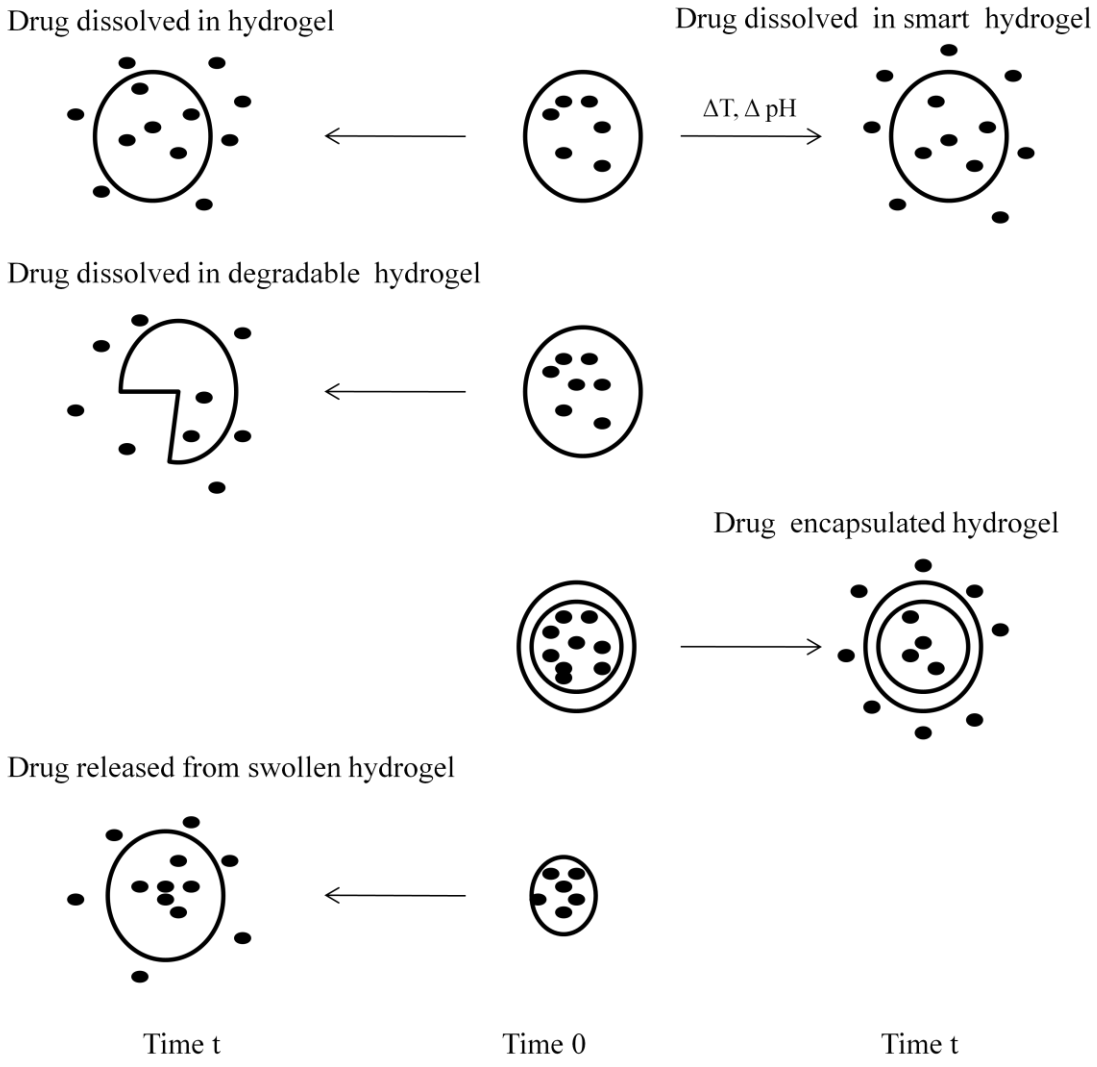


Figure 8. Schematic of hydrogel-based therapeutic system delivery and release mechanisms. Adapted from Peppas et al., 2006.

2.6 Conclusion

Hydrogels are a viable platform for controlled drug delivery and release due to their ability to be modulated in order to obtain a desired release profile. Hydrogel systems allow for modulation of physical and chemical properties to trigger the release of therapeutic agents, and obtain a desired controlled release profile. Monomer and/or crosslinker concentration, relative ratios, molecule weight and other factors can be easily changed to obtain the overall change in the hydrogel's properties. PEG-based hydrogels are especially useful for this drug delivery and controlled release because of their of nontoxic and low immune and inflammatory response properties. Additionally, because the United States Food and Drug Administration has approved other applications using PEGDA, the relative safety of the material is ensured due to the extensive testing needed to obtain this approval. PEG-based hydrogels are stealthy and are created through a versatile array of fabrication methods. They can be made using different crosslinkers and polymerization methods such as photopolymerization, high-energy polymerization (e.g., electron beam) or chemical initiators. This versatility allows for their use in a multitude of applications ranging from injectable *in situ* polymerization to implantable systems.

III. SYNTHESIS AND CHARACTERIZATION OF MONOMERS

3.1 Introduction

The synthesis of derivatives of poly(ethylene glycol) diacrylate (PEGDA) and methoxy poly(ethylene glycol) monoacrylate (mPEGMA) was accomplished by the acrylation of poly(ethylene glycol) (PEG) and methoxy poly(ethylene glycol) (mPEG), respectively (Geetha, Mandal et al. 1993; Hern and Hubbell 1998). These derivatives served as the monomer and comonomer for the synthesis of hydrogels. The process focused on overall refinement of the polymer to a high degree of acrylation and purity. To reach a high degree of acrylation and purity, an engineering approach was taken and all possible sources of contamination were identified and eliminated from the process. Later in this chapter, in Section 3.4, a summary of these contaminations is provided. Also, the synthesis was designed in three different stages. These stages are referred to as preparatory, acryloylation, and purification.

3.2 Materials and Methods

Poly(ethylene glycol) of varying molecular weight was purchased from Fluka Biochemika (Buchs, Switzerland) and is represented as PEG(X), where X represents the average molecular weight of the poly(ethylene glycol), e.g. PEG(4000) for poly(ethylene glycol) with an average molecular weight of 4000 Da. Acyloyl chloride (96%) and molecular sieves were purchased from Aldrich (St. Louis, MO). Triethylamine (99%), anhydrous ethyl ether and dichloromethane were obtained from ThermoFisher Scientific (Waltham, MA). Also, anhydrous toluene was purchased from Acros Organics (Geel, Belgium). Additionally, all dialysis membranes were obtained from Spectrum Laboratories, Inc (Rancho Dominguez, California). These materials were used without additional purification unless otherwise specified.

3.2.1 Poly(Ethylene Glycol) Diacrylate Synthesis

The synthesis of PEGDA begins with the preparatory stage, where, as the name implies, chemicals and material are prepared for the synthesis. First, molecular sieves were activated. The activation was achieved by heating the sieves while under vacuum and then allowing them to cool in a vacuum oven. Next, sufficient quantities of TEA and toluene were dried over the molecular sieves overnight. Additionally, 20 grams of PEG was azeotropically distilled after being dissolved in toluene. The azeotropic distillation utilized a three neck round bottom flask where the PEG solution was present. Also, a Dean-Stark trap, nitrogen supply and condenser were connected to the different necks of the flask. The trap allowed for the monitoring and removal of water from the reactant. This was done with all of the various molecular weight PEGs.

After the PEG was dried, dichloromethane (DCM) was added to the PEG until the solution became clear. This clarity marked the beginning of the acryloylation stage of the synthesis. An addition funnel was attached to one of the necks of the flask and the system was purged with nitrogen gas. The funnel and the rest of the system apparatus are depicted Figure 9. All subsequent steps in this stage were conducted under nitrogen atmosphere. Placing the flask in an ice bath cooled the system. Next, in a drop-wise manner, a three molar excess of acryloyl chloride for each mole of hydroxyl group present in the PEG was added while in the presence of TEA with the same molar excess. The reaction was kept shielded from light to prevent any photopolymerization of the monomer and was allowed to continue overnight. Also, throughout the reaction, the system was chilled using an ice bath.

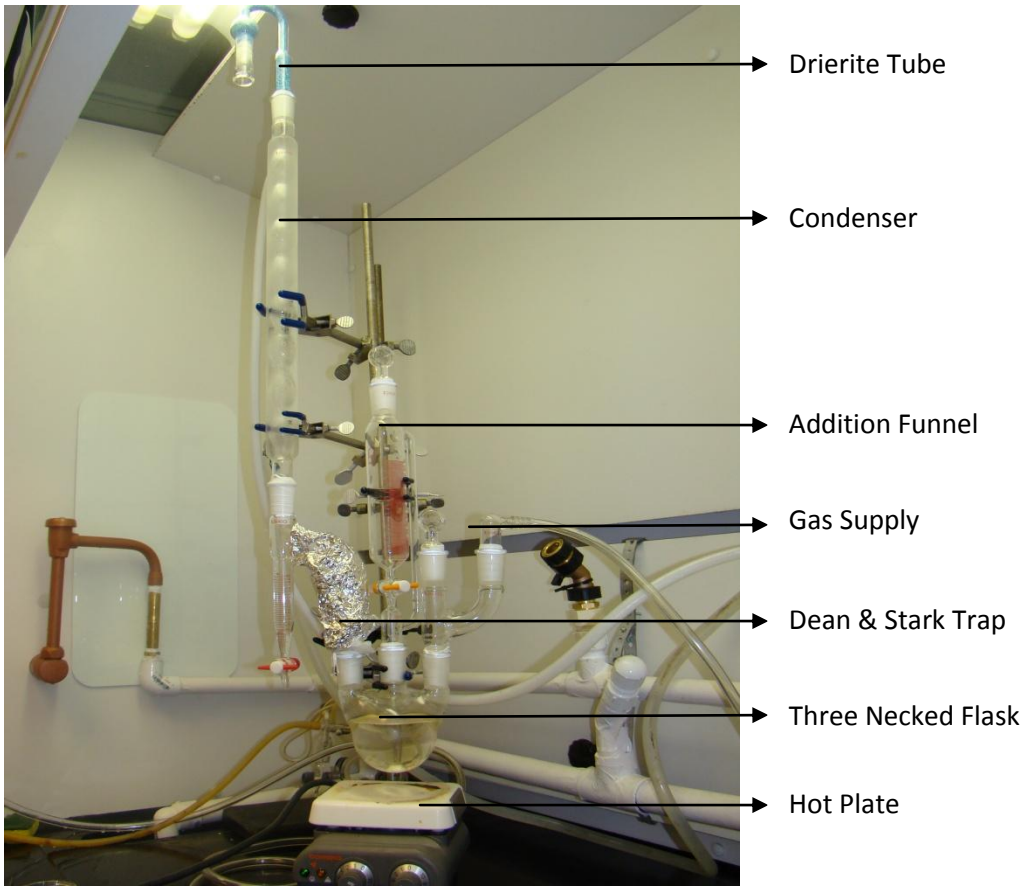


Figure 9. An image of the PEGDA synthesis apparatus.

At the completion of the reaction, the reaction vessel was placed under vacuum to concentrate the product. This step was adopted from recent work reported by Anseth (Salinas and Anseth, 2008). After the product was concentrated, the product was purified. The overall chemical schematic of the PEGDA synthesis is outlined in Figure 10.

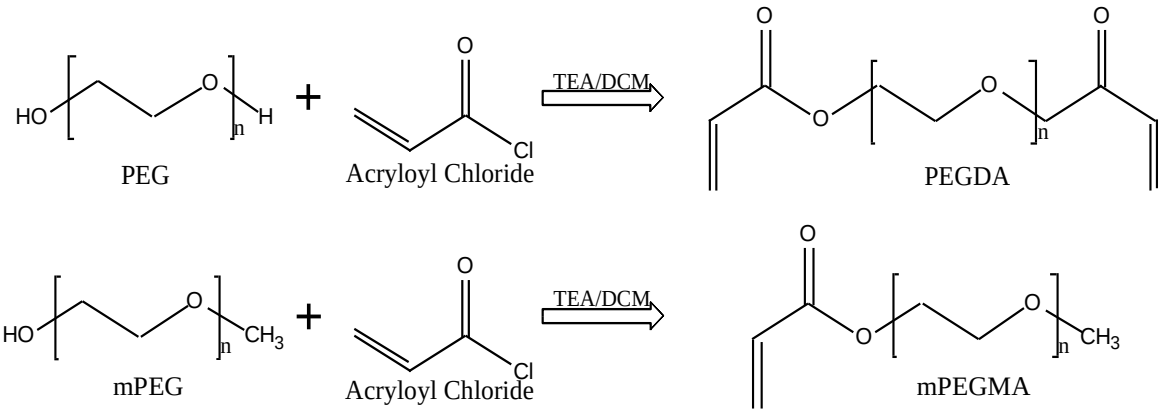


Figure 10. This is a schematic of the synthesis of poly(ethylene glycol) diacrylated (top) and methoxy poly(ethylene glycol) monoacrylate(bottom).

The concentrated PEGDA solution was initially vacuum-filtered with a coarse filter to remove the excess TEA-derived salts and begin the purification process. Cold ethyl ether was added to the filtrate to precipitate the PEGDA. The product was then filtered to separate the DCM and ethyl ether solution from the PEGDA. Sufficient amount of DCM was then added to the dissolve the PEGDA. The previous precipitation and filtering steps were then repeated with a finer filter than was used in the previous filtering. The product was placed in a low-pressure moisture-free atmosphere overnight to allow the remaining solvent to evaporate. The product at this point has an off white to yellowish color which can be seen in the left side of Figure 11.

Figure 11. On the left is a picture PEG(4000)DA after the initial purification without dialysis. On the right is PEG(4000)DA after undergoing further purification, including dialysis.

The product was next dissolved in deionized (DI) water and dialyzed. The solution was placed in dialysis tubes with the appropriate molecular weight cutoff for the given PEGDA molecular weight being purified. The dialysis tubing was then placed in DI water for dialysis. The water was exchanged several times over the course a twenty-four hour time period. The product at this has a white appearance as seen in the right side of Figure 11. Finally, the product was lyophilized and characterized using attenuated total reflectance Fourier transfer infrared (ATR–FTIR) and nuclear magnetic resonance (NMR) spectroscopy.

3.2.2 Methoxy Poly(Ethylene Glycol) Monoacrylate Synthesis

All materials a used were identical to those state in Section 3.2 except for poly(ethylene glycol)s that were replaced with methoxy poly(ethylene glycol)s (mPEGs). Methoxy poly(ethylene glycol)s were purchased from Flucka Biochemika (Buchs, Switzerland). Also, methoxy poly(ethylene glycol) is represented mPEG(X), where X represents the average molecular weight of the methoxy poly(ethylene glycol), e.g. mPEG(4000) for methoxy poly(ethylene glycol) with an average molecular weight of 4000 Da. The synthesis mPEGMA is

the same process as the PEGDA. The synthesis schematic can be seen in the lower portion on Figure 10.

3.3 Characterization

3.3.1 Attenuated Total Reflectance Fourier Transfer Infrared Spectroscopy

ATR-FTIR spectroscopy (Nexus 870 FT-IR, Thermo Electron Corporation, Madison, WI) was used to analyze the products of the respective mPEGMA and PEGDA syntheses. Through analyzing the absorbance and transmittance of energy at different wavelengths it was ascertained that mPEGMA and PEGDA were produced from mPEGMA and PEGDA syntheses, respectively. The absorption of the carbon-carbon double bond (C=C) at 1633 cm^{-1}, and carbonyl ester group O-C=O at 1723 cm^{-1} in ATR-FTIR spectrum of the products were compared to those of the precursors to confirm the presence of the intended products (Dacosta et al., 1994).

3.3.2 Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy was conducted, using a 400 MHz Bruker DPX-400 NMR, (Bruker BioSpin Corporation Newark, DE) and the product of the acrylation reaction. Proton NMR (^{1}H NMR) mode was used to evaluated the presence of TEA-derived impurities (Imani et al., 2007). Also, the degree of acrylation was evaluated by comparing the acrylate hydrogen integration to that of the ethylene oxide backbone hydrogen (Geetha et al., 1993; Shu, Liu et al., 2004). Degree of acrylation was described by the percent of acrylation of the product and is summarized in the expressions below.

$$\left[\frac{(PEO\ \text{Intergral}/(\text{number of PEO Hydrogen} - 4))}{(\text{Vinyl Integal}/2)}\right] \times 100 = \%\ \text{acrylation} \qquad (5)$$

$$\left\{\left[\frac{(\text{molecular weight of PEG} - 18)}{44}\right] \times 4\right\} - 4 = \text{Total number of PEO Hydrogens} \qquad (6)$$

TEA-derived impurities were present before dialysis was conducted on the product. Their presence corresponds to the peak near 1 ppm in ^{1}H NMR spectrum (Imani et al., 2007). After the products were dialyzed the peak at 1ppm decreased or no longer could be seen (Figure 30, Appendix B). All samples that were tested were dissolved in deuterated chloroform.

3.4 Results

There was an increase in absorption of the carbon-carbon double bond (C=C) at 1633 cm^{-1} in the spectrums of the products compared to those of the precursors. Also, there was an increase in absorbance in the peak around 1723 cm^{-1} which is indicative of carbonyl ester group O-C=O (Dacosta et al., 1994). The comparison of the peaks was done after normalizing them with respect to the CH_2 peak around 2870-2890 cm^{-1} (Table IV). This ATR-FTIR spectral analysis was used to elucidate the chemical structures of the products and indicated that the MPEGs and PEGs were acrylated (Appendix A).

Table IV. A list of common chemical substituents and their correlating infrared spectral absorbances. Compiled from [a]Imani et al., 2007; [b]Geetha et al., 1993; [c]Dacosta et al., 1994.

Band	Wavenumber (cm^{-1})
C=C [a]	1630-1640 [a]
-C=O [a b]	1720-1730 [a b]
O-C=O [a c]	1723/1724 [a c]
OCH_2-CH_2 [a]	1100-1120 [a]
CH_2 [a]	2870-2890 [a]

The purification of the product was successful in removed the impurities, such as residual TEA. This was due to the fact that the lower molecular weight impurities' diffusion out of the dialysis material could be obtained while retention of the larger desired product was obtained. Thus, a separation by size was achieved leaving the desired product within the dialysis tubing. The synthesis of these acrylated products was improved and is pictured in a systematic analysis of all procedures and components of the process to remove any source of impurities and contaminates. This is shown below in Table V.

Table V. A list of contaminates and impurities.

Contaminates & Impurities / Sources	**Resolution**
Silicone sealed joints	Teflon sealed joints
Molecular sieve not absorbing water	Activating molecular sieves
Condensation in apparatus	Preheat apparatus before reaction.
Incomplete dehydration of PEGs and MPEGs	Azeotropic distillation with a Dean & Stark Trap.
TEA impurities in product	Dialyze product Repeat filtering and progressively finer fillers.

The average percentage of acrylation for the products synthesized was 86.77 percent or greater. The product yield was typically low due to the extensive purification process. The yields were as low as 56.34 percent, but the degree of acrylation for these syntheses was relatively high. The relatively low yield product did not affect the subsequent experiments because the amount needed was produced by a single individual synthesis of a particular product.

Table VI. List of synthesized acrylated products with their average percentage of acrylation and yield (n = 3, mean ± stdev).

Product	**Acrylation(%)**	**Yield (%)**
PEG(4000)DA	87.75 ± 2.11	98.18
PEG(6000)DA	88.90 ± 2.45	62.12
PEG(8000)DA	98.23 ± 7.89	78.93
PEG(20000)DA	86.77 ± 1.58	57.22
mPEG(2000)MA	87.64 ± 1.26	56.34
mPEG(5000)MA	97.87 ± 3.41	79.14

3.5 Conclusion

The various molecular weights of acrylated polymer molecules needed to study the effects of changing the structural parameters of the hydrogel construct were successfully synthesized. ^{1}HNMR and ATR-FTIR spectroscopy were successful in confirming the structure, purity and degree of acrylation of the product. The relatively high acrylation and purity will ensure that observed changes in the hydrogels are to due to the changes in the material used to construct the hydrogels and not due to unknown quantities of impurities.

IV. HYDROGEL STRUCTURAL NETWORK AND CHARACTERIZATION

4.1 Introduction

The versatility of the hydrogel in medical and biopharmaceutical fields is derived from the ability to tailor its properties by controlling its structure. This control is achieved by the monomers, crosslinkers, and methods used to create the hydrogel. Since the structure controls the properties of the hydrogel, it is logical to use a property such as the swelling property to deduce the actual structure of the hydrogel. Characterizing the swelling behavior of the material and relating this behavior to its structural parameters provides the means with which to predict how molecules (i.e. biomolecules) would interact with it. Also, comparing structural parameters and swelling characteristics provides the ability to quantify and predict the effect of componential change, such as the relationship between polymer chain length and solute diffusion through the hydrogel. This is very important for controlled drug release applications. Additionally, many theories have been developed to describe the swelling behavior and network structure of hydrogels. They incorporate mechanistic and statistical approaches to predict the relationship of network parameters to observable swelling characteristics. These theories allow for the intelligent design of hydrogel-based controlled drug release (Peppas and Reinhart, 1983; Peppas et al., 2000; Peppas et al., 2000; Peppas et al., 2006).

4.2 Network Structure

Hydrogels are crosslinked three-dimensional networks in the swollen state. Theories used to describe them typically treat these networks as a homogenous framework. They usually do not deal with network defects, interpenetrating networks or regions of non-uniformly crosslinked

polymers. Flaws in the real network may take one or many forms, such as chain entanglements or loops. These flaws may be caused by end-linking and, in the case of interpenetrating hydrogels, crosslinking of previously crosslinked hydrogel (Peppas, 1986). Theories that do not account for network defects are still relevant for the characterization of the structure of hydrogels used in medical applications, such as drug delivery, because the system still works in the given framework (Flory and Rehner, 1943). Flory derived the rubber elastic theory that was applied to hydrogels. It states that they will have an elastic response to an externally applied stress. This theory specifically states that the crosslinked network obtains an equilibrium strain during a constantly applied stress (Flory, 1979). The Flory-Rehner theory was developed to describe solid state crosslinked hydrogels (Flory and Rehner, 1943). This model assumes that the polymer chain lengths follow a Gaussian distribution and that there is tetrafunctional crosslinking at crosslinked junctions.

$$\frac{1}{\bar{M}_c} = \frac{2}{\bar{M}_n} - \frac{\left(\frac{v}{V_1}\right)\left[\ln\left(1-v_{2,s}\right)+v_{2,s}+\chi_1 {v_{2,s}}^2\right]}{v_{2,s}^{1/3}-\frac{v_{2,s}}{2}} \qquad (7)$$

The number average molecular weight between crosslinks is represented by $\bar{M}_c$. The next variable, $\bar{M}_n$, is number average molecular weight of polymer before crosslinking and v stands for specific volume of PEG. Also, V_1 represents molar volume of the swelling agent and $v_{2,s}$ is polymer volume fraction in the swollen state. Finally, X_1 is Flory polymer solvent interaction parameter (Equation 7).

The Flory- Rehner theory was further modified by Peppas and Merrill in 1977 for hydrogels synthesized in a solution. Where $v_{2,r}$ is polymer volume fraction in the relaxed state

(Peppas and Merrill, 1977), v for PEG is .893cm^3/g , V_1 of water is 18.1cm^3/mol and χ_1 is 0.426 for water interacting with PEG (Mellott et al., 2001; Revzin et al., 2001).

$$\frac{1}{\bar{M}_c} = \frac{2}{\bar{M}_n} - \frac{\left(\frac{v}{V_1}\right)\left[\ln\left(1-v_{2,s}\right)+v_{2,s}+\chi_1 {v_{2,s}}^2\right]}{v_{2,r}\left[\left(\frac{v_{2,s}}{v_{2,r}}\right)^{1/3}-\left(\frac{v_{2,s}}{2v_{2,r}}\right)\right]} \qquad (8)$$

The parameters, such as $\bar{M}_c$ and the polymer volume fraction in both perturbed (swollen state) state and relaxed state give an indication of the structure of the hydrogel (Equation 8). They can be used to further describe the microstructure or even the nanostructure of the hydrogel. They can also be used to determine the mesh size (ξ). This factor characterizes the space between crosslinked junctions. This will be further explained in the following sections.

4.3 Methods

4.3.1 Synthesis of Poly(Ethylene Glycol) Diacrylate Hydrogels

The following procedures were conducted on the various molecular weight PEGDAs. First, 0.3 g of PEGDA was dissolved in 1.84 mL of double deionized water. Stock solutions of APS (20% w/v) and TEMED (20% w/v) with 0.2 g/mL concentration were prepared. 0.07 mL of APS stock solution and 0.09 mL of TEMED stock solution were added to PEGDA solution. Using a syringe, the solution was injected into a 6 cm x 2.5 cm x 0.3 cm mold. The mold then was placed in an oven at 37° C for 30 minutes. The mold consisted of two glass plates separated by a rubber gasket. Three spring clips held this mold together. The hydrogels were then removed from the mold upon deconstruction of the mold.

4.3.2 Synthesis of Methoxy Poly(Ethylene Glycol) Monoacrylate Modified Poly (Ethylene Glycol) Diacrylate Hydrogels

The synthesis of hydrogels that incorporated MPEG with PEGDA was similar to the previously described protocol. The only modification to the protocol was the addition of various mPEGMAs to the PEGDA in a 1 to 10 molar ratio, respectively. The amount of PEGDA was kept constant at 0.3 g. Also, the same mold used for unmodified hydrogels was used (Figure 12). The synthesis was achieved with free radical polymerization of PEGDA via the creation of a radical on the of unsaturated PEGDA end group. Also, mPEGMA was incorporated in the PEGDA hydrogel via free radical polymerization (Beamish et al., 2010). This synthesis is depicted in Figure 13.

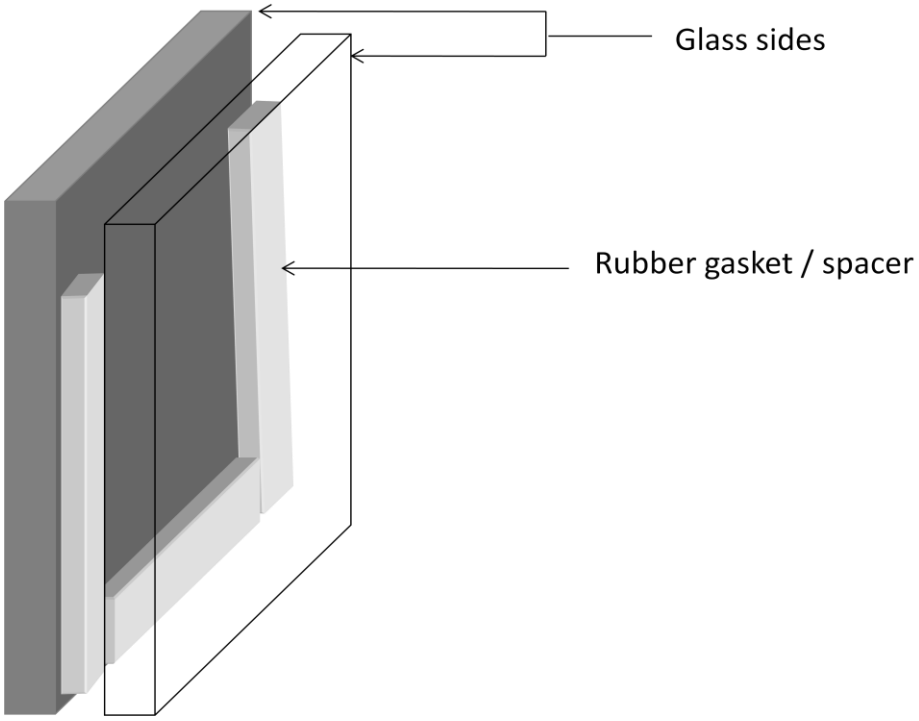


Figure 12. Schematic of the mold used for hydrogel synthesis.

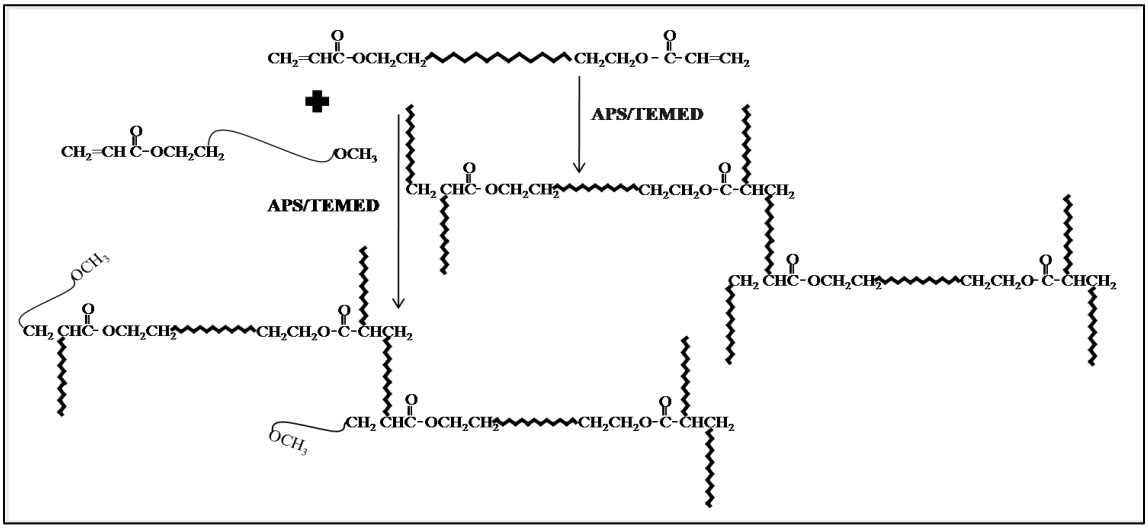


Figure 13. Schematic of hydrogel synthesis: On the right hand side is the synthesis of the hydrogel achieved with free radical polymerization of PEGDA via the creation of a radical on the of unsaturated PEGDA end group. On the left hand side, the PEGDA hydrogel is synthesis with the incorporation of mPEGMA via free radical polymerization. Adapted from Beamish et al., 2010

4.3.3 Swelling Analysis

Swelling experiments were conducted to analyze the swelling behavior and collect information to be used in conjunction with the Peppas and Merrill equation (Equation 8). The analysis was conducted on PEGDA hydrogels and mPEGMA modified hydrogels, synthesized as previously described. The samples of the various hydrogels were weighed after polymerization. The samples were then swollen in deionized water and allowed to reach equilibrium swelling before being weighed again. Equilibrium was determined by repeatedly weighing each sample until the weight did not change more than five percent from the previous weight. The samples were then dried and their weight was recorded again. Next each sample was dried using a dehydrator until no change in weight was detected. The sample was weighed in air and butanol, an organic non-solvent. The information gained from comparing the weight in air and butanol

with the use of Archimedes's buoyancy principle was used to provide a multitude of volumetric parameters. These include the volumetric swelling ratio (Q), Equation 9, the volume of the swelling with respect to the dry volume and the mass swelling ratio (q), Equation10.

$$Q = \frac{\left[\frac{(W_{s,a} - W_{s,b})}{\rho}\right]}{\left[\frac{(W_{d,a} - W_{d,b})}{\rho}\right]} \qquad (9)$$

$$q = \frac{(W_{s,a})}{(W_{d,a})} \qquad (10)$$

The variable $W_{s,a}$ stands for the weight of the hydrogel sample in the air after swelling.The weight of the hydrogel sample in butanol after swelling is $W_{s,b}$. The weight of the hydrogel sample in butanol is represented by $W_{d,b}$ and $W_{d,a}$, the weight of the hydrogel sample in air after being dried. Finally, ρ is the specific density of butanol with the value of 0.81g / mL.

4.3.4 Mesh Size Signifcance and Analysis

The mesh size (ξ) for a non-degradable non-porous hydrogel is a parameter that helps when understanding the movement of solute in the matrix when in a presence of solvent. The mesh size is characterized by the degree of swelling that occurs, until equilibrium is reached, when in the presence of a solvent. A larger the mesh size allows, more solvent will enter. Also, more solute can diffuse through the hydrogel in a swollen state. Once equilibrium swelling is reached, mesh size can prevent movement of solute if it that mesh size is smaller than the size of that particular solute (Figure 14).

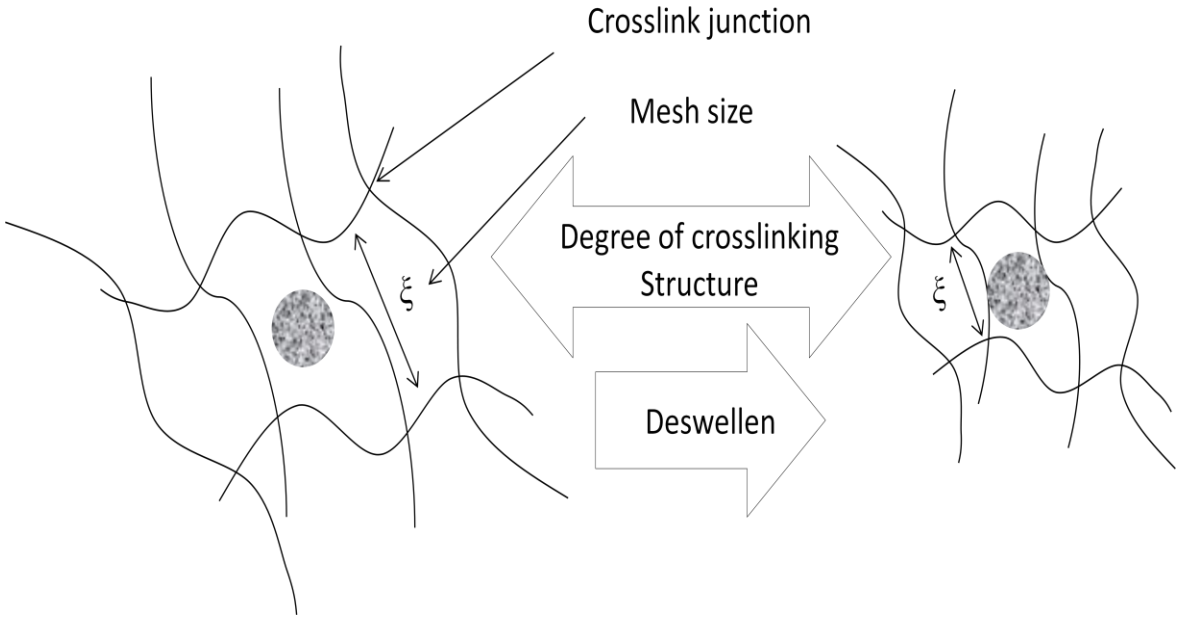


Figure 14. Schematic of mesh size (ξ) in hydrogels in the deswollen state and swollen state. Adapted from Metters et al., 2000.

The mesh size is a parameter that can control the diffusion of solute, or even prevent diffusion of solute in some instances. The nanostructure's (i.e. mesh size) interaction with molecules such as BSA influences their diffusivity. Swelling experiments can determine the mesh size and allow for tailoring of a hydrogel to fit the diffusion profile of a target molecule. Using the Peppas and Merrill modified equilibrium swelling model, mesh size can be calculated Equation 11, (Cruise et al., 1998).

$$\xi = v_{2,s}^{-1/3}[C_n\, 2M_c/M_r]^{1/2} l \qquad (11)$$

Mesh is calculated using the equation above, where C_n is the Flory characteristic ratio, which is 4 for PEG. M_c is the molecular weight of the PEG repeating units, which is 44 g/mol. Also l is the bond length along the polymer backbone, equaling 0.15nm (Cruise et al., 1998).

4.4 Controlled Release System

The drug delivery applications for the hydrogel usually obtain control over drug release through chemical, enzymatic, swelling and diffusion mechanisms (Sakiyann-Elbert and Hubbell, 2000; Peppas et al., 1997; Qiu and Park, 2001; Prokop etal., 2002). Chemically controlled release systems achieve control over drug release by chemical reactions occurring inside the matrix. The reactions are typically hydrolysis reactions which hydrolytic cleavage of a tethered drug or polymer occurs. On the other hand, enzyme controlled release incorporates biologically recognizable motifs with which enzymes can reactor that can be degraded to release an agent. However, the enzyme binding affinity and other enzyme interactions are typically difficult to predict and regulate, thus limiting the design of the system. Another system is a swelling-controlled release system. In this system, the active agent is enclosed within the hydrogel while in the unperturbed state and, as the hydrogel swells and enters a rubbery state, the agent diffuses out relatively rapidly. Finally, diffusion controlled systems can regulate the release of a molecule by changing its diffusivity through the mesh of the system. The structure of the hydrogel is modulated to obtain a desired release. Lastly, diffusion characteristics of a molecule and how they are influenced by the structure can be seen in the expression below, Equation 12, developed by Peppas and Lustig (Lustig and Peppas, 1988).

$$\frac{D_e}{D_o} = \left(1 - \frac{r_s}{\xi}\right) exp\left(-Y\left(\frac{v_{2,s}}{1-v_{2,s}}\right)\right) \qquad (12)$$

D_e is the effective diffusion coefficient and D_o stands for the diffusion coefficient of the molecule in solvent. The constant r_s represents the size of the molecule. Y is the critical volume needed for translational movement of the molecule into the free volume of the system with respect to the molecules per unit of solvent. Y can be approximated in a system where the

polymer volume faction is relatively constant throughout the hydrogel. The expression can be simplified to approximate the effect of the mesh size and effective diffusion, Equation 13. Here K is a scaling factor based on the function of the degree of swelling.

$$\frac{D_e}{D_o} \cong K\left(1 - \frac{r_s}{\xi}\right) \qquad (13)$$

This provides an approximate starting point for choosing the structural modifications to obtain the release of a particular molecule. Since a factor, such as polymer chain length, that controls the structure can easily be adjusted to gain the appropriate release profile, and because adjustments to the system and their direct effect on the structure can be ascertained and tracked via equilibrium swelling analysis, these features allow for the optimization of the system to a variety of biomolecules.

4.5 Results

Swelling behavior of the hydrogel has presented some observable trends. The swelling ratios (q) increased in nonlinear proportional manner to the increase in the molecular weight of the PEGDA monomer (Figure 15, 16). Additionally, the monomer's molecular weight is also proportional to the polymer chain length, Figure 17. A longer polymer chain length is associated with a larger molecular weight PEGDA. Thus, an increase the polymer chain length increased q.

In addition, the mesh size expressed a similar trend. The mesh size increased as the molecular weight of the PEGDA monomer increased (Table VII and Figure 18). The hydrogels with larger mesh size had higher q value, Figure 19. This result was expected because is larger mesh size results in an increase in the void volume within the hydrogel allowing for more space to be occupied by water (Metters et al., 2000).

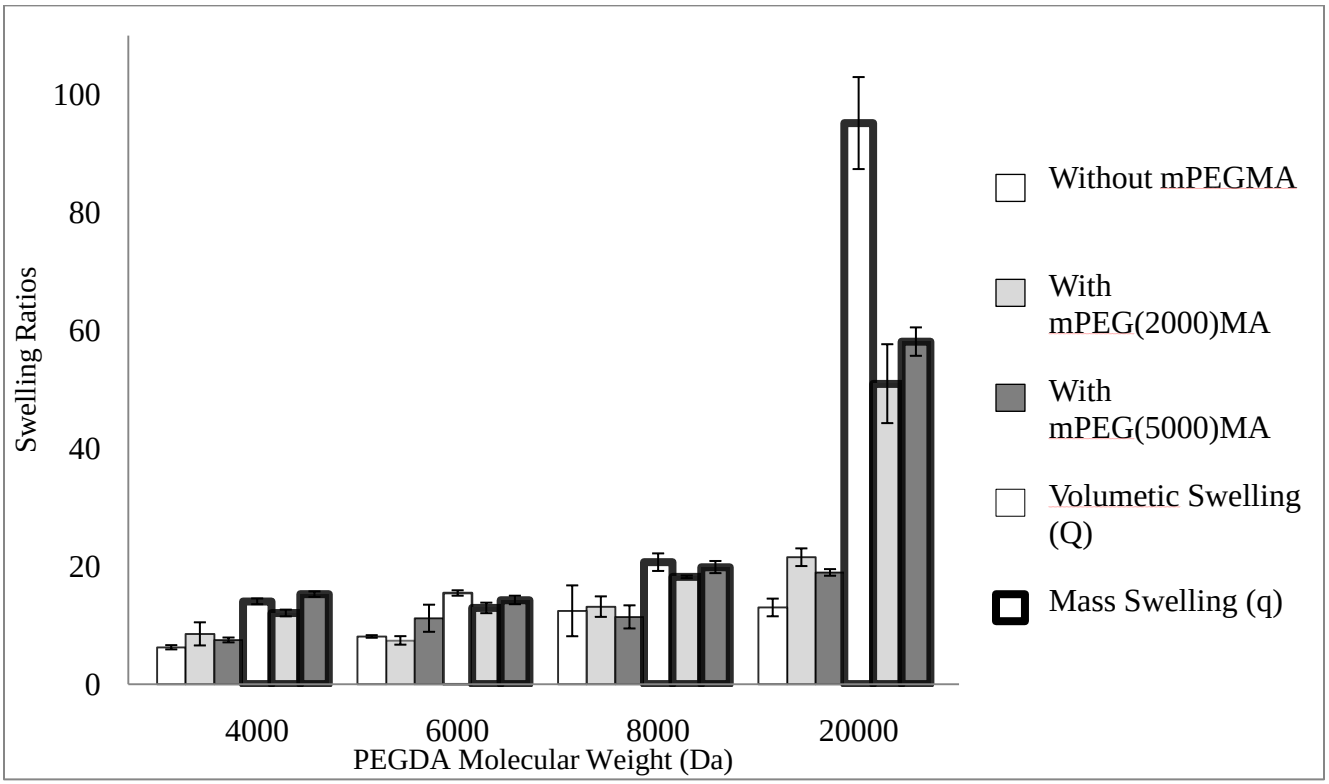


Figure 15. Volumetric swelling ratios (Q), and the mass swelling ratios (q) of various hydrogels synthesized. (n = 3, mean ± stdev)

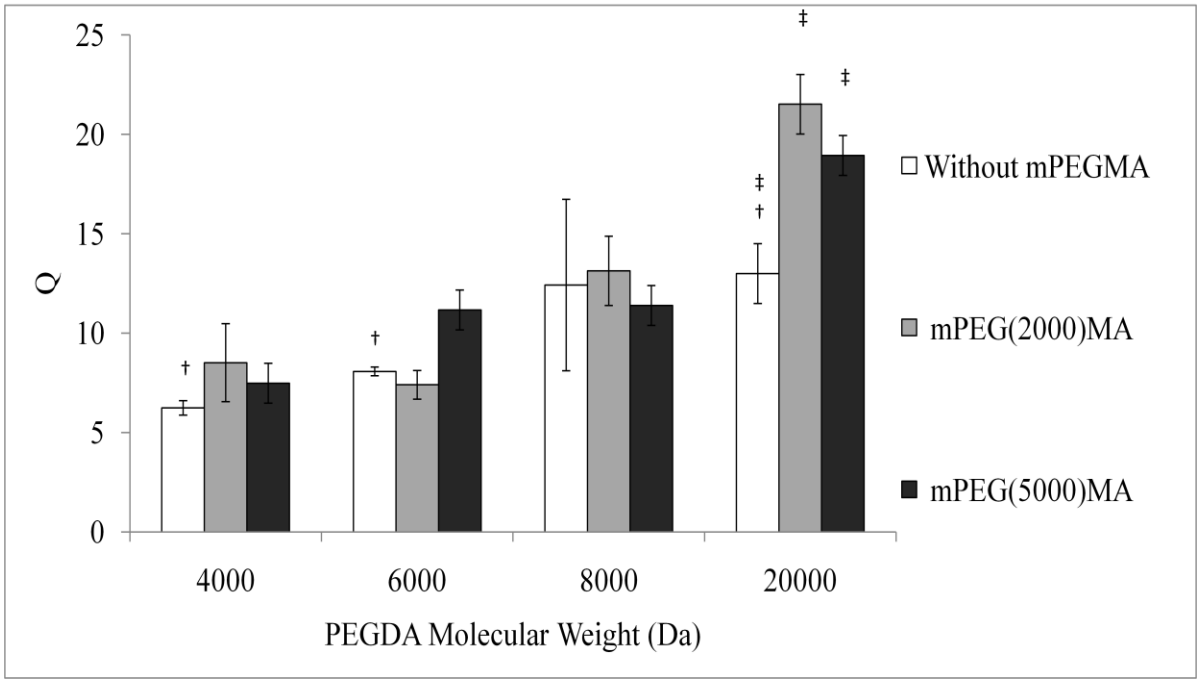


Figure 16. Volumetric swelling ratio (Q) of hydrogels composed of varies poly(ethylene glycol) (PEGDA) molecular weights monomers. Here, mPEG(2000)MA (graybar) and mPEG(5000)MA (black bar) were added to the hydrogels of the various PEGDA molecular weight monomers. († $p \leq 0.05$ Q values of hydrogels composed of PEGDAs derived from PEGs with molecular weights of 4000 Da, 6000 Da, 8000 Da, and 20000 Da were compared to each other, ‡ $p \leq 0.05$ PEG(20000)DA hydrogels with mPEG(2000)MA or mPEG(5000)MA were compared to PEG(20000)DA hydrogels unincorporated without mPEGMA, n=3, mean ± stdev)

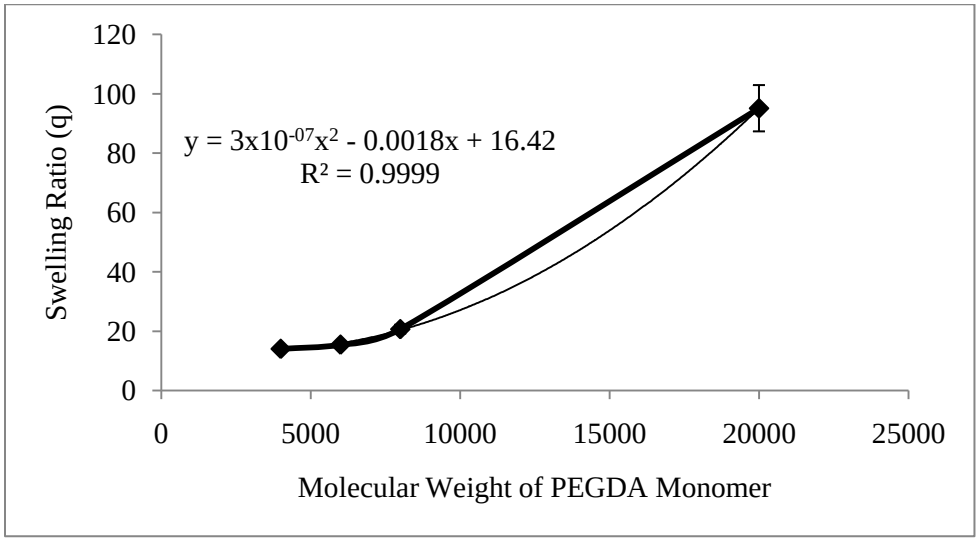


Figure 17. A graph of the relationship of the molecular weight of the PEGDA monomer used to synthesize hydrogels and the respective mass swelling in ratio (q) of the hydrogels.(n = 3, mean ± stdev)

Table VII. A list of calculated mesh sizes of various hydrogels synthesized. The hydrogel are listed by monomer or monomers used in their synthesis. (n = 3, mean ± stdev)

PEGDA(g/mol)	mPEGMA(g/mol)	Mesh Size (nm)
4000		6.5 ± 0.33
4000	2000	8.87 ± 1.79
4000	5000	6.89 ± 0.26
6000		8.58 ±0.39
6000	2000	8.1 ± 1.02
6000	5000	9.4 ± 0.85
8000		12.38 ± 5.34
8000	2000	13.34 ± 0.69
8000	5000	11.97 ± 1.6
20000		14 ± 0.89
20000	2000	22.35±.38
20000	5000	21.06±.44

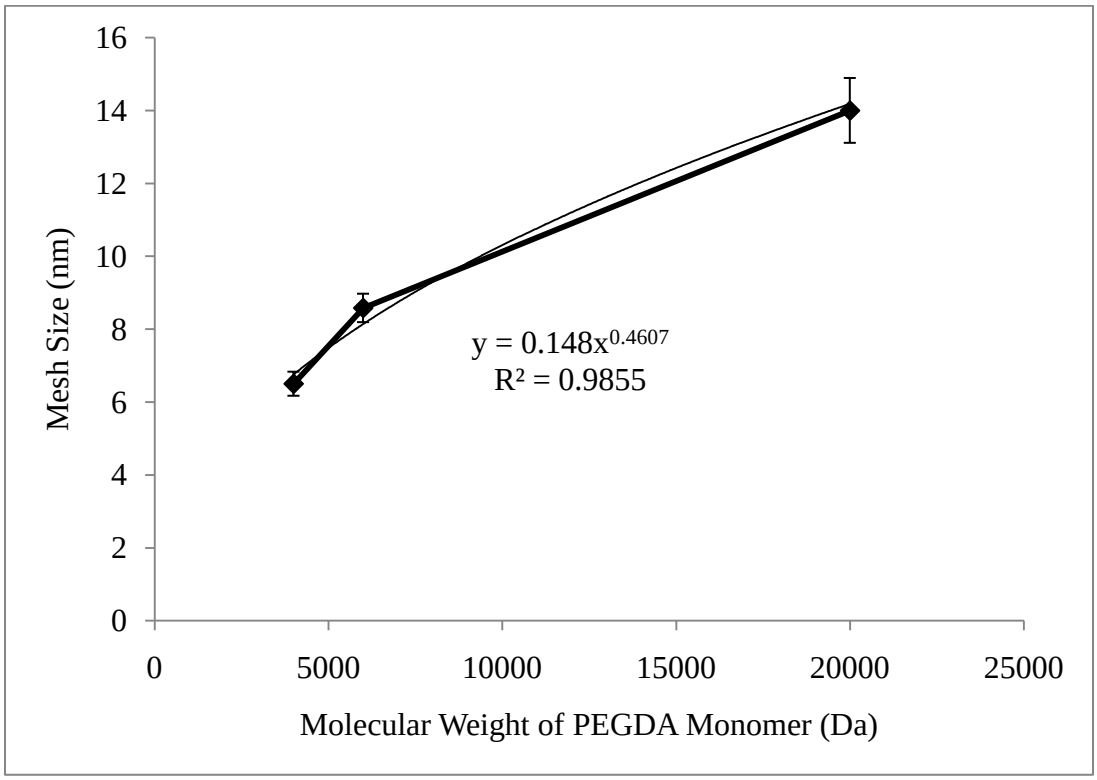


Figure 18. A graph of the relationship of the molecular weight of the PEGDA monomer used to synthesize hydrogels and mesh size (nm) of those hydogels. (n = 3, mean ± stdev)

The incorporation of mPEG(2000)MA or mPEG(5000)MA did not appear to affect the volumetric swelling ratio (Q), Figure 15 and 16. The exception were hydrogels made with PEG(20000)DA, which showed an increased volumetric swelling ratio. Additionally, the PEG(20000)DA with MPEG(2000)MA hydrogels had a greater Q value than those made PEG(20000)DA and mPEG(5000)MA (Figure 15). The mPEGMA's lengths allowed them to entangle with the PEGDA network when it was made using lower molecular weight monomers. Also, untangled mPEGMAs occupied void volume preventing water from entering those spaces. These factors counter the increase in void space that would be expected due to increase in mesh size that typically would be predicted due to the loss of crosslinking sites from the presence of mPEGMA. Furthermore, hydrogels made from highest molecular weight monomer had an inherently large void volume and the space occupied by the mPEGMA was comparatively quite

small. The inability of the relatively short mPEGMAs to span the void and crosslink reinforces the theory that entanglement and mPEGMAs polymer volumes limited their effects on hydrogel properties. The data was compared using ANOVA followed by Tukey's post-hoc.

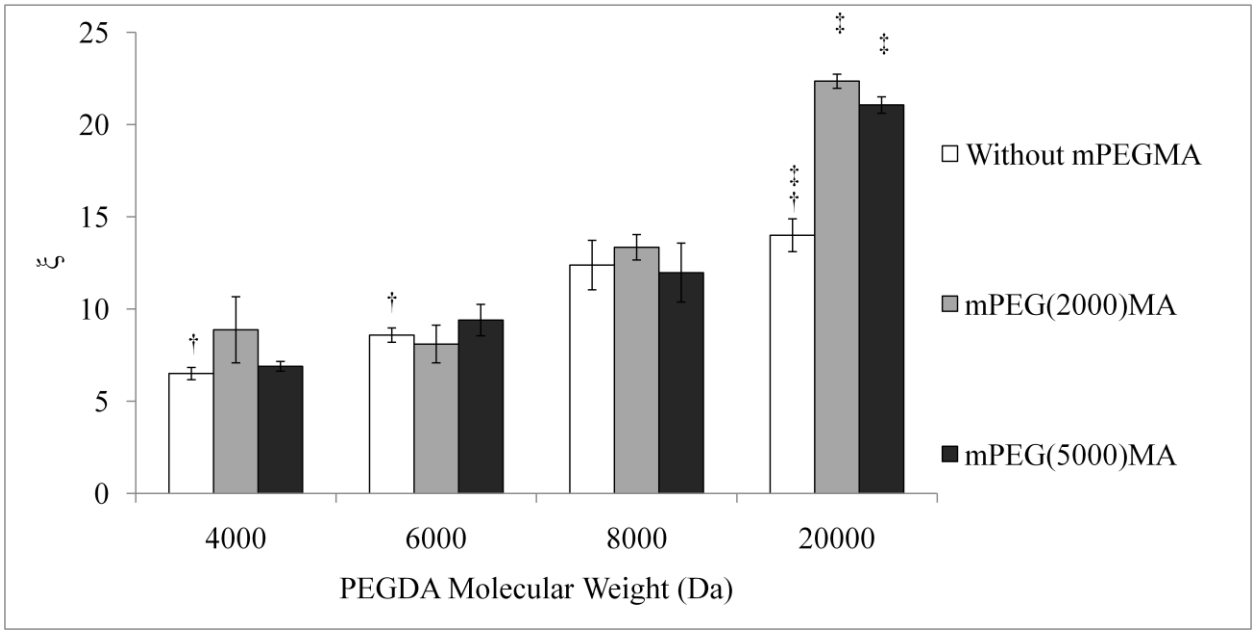


Figure 19. Mesh size (ξ) of hydrogels composed of various poly(ethylene glycol) (PEGDA) molecular weight monomers. mPEG(2000)MA(gray bar) and mPEG(5000)MA (black bar) were added to the hydrogels of the varies PEGDA molecular weight monomers. († $p \leq 0.05$ ξ values of hydrogels composed of PEGDAs derived from PEGs with molecular weights of 4000 Da, 6000 Da, 8000 Da, and 20000 Da were compared to without mPEGMA each other, ‡ $p \leq 0.05$ PEG(20000)DA hydrogels with mPEG(2000)MA or mPEG(5000)MA were compared to PEG(200000) hydrogels without mPEGMA, n=3, mean ± stdev)

4.6 Conclusion

Hydrogel with higher molecular weight PEGDA tend to have larger Q, q, and ξ values. The incorporation of mPEGMA had negotiable effect on hydrogel synthesized with monomer molecular less than PEG(20000)DA. However Q, q, and ξ values differed with the incorporation mPEGMA in hydrogel synthesized with PEG(20000)DA. This resulted from the large difference in molecular weight between mPEGMAs and PEG(20000)DA compared to the other PEGDAs.

V. STUDY OF DIFFUSION THROUGH HYDROGELS

5.1 Introduction

It is essential to understand how molecules move through the hydrogel if it is to be used as a vehicle for drug delivery and release. Examining the changes in diffusivity of active agents and biomolecules that resulted from varying the componential structure of the hydrogel system can lead to better understanding of this parameter and its affect on drug release. Exploration of the diffusivity of an active agent was accomplished by using a small fluorescent molecule (i.e. AMC) as a model agent. Quantitative measurements of the molecule were taken using fluorescence spectroscopy analysis. Similarly, fluorescently labeled BSA was used to model a larger class of biomolecules similar to MMP-2, an enzyme that has been implicated in many disease states. Furthermore, diffusion of AMC and BSA in the presences of mPEGMA, changes in PEGDA monomer molecular weight and changes in the mPEGMA molecular weight was studied. The responses to these variables provide an insight as to the optimum componential structure for controlled delivery and release.

5.2 Materials and Methods

5.2.1 Preparation of Hydrogels

The PEGDA hydrogels and PEGDA hydrogels modified with mPEGMA used in the diffusion experiment were those previously described in 4.3.1 and 4.3.2, respectively. After synthesis, the hydrogels were swollen in PBS for 48 hours. Circular 6 mm diameter samples were taken after swelling was complete. The samples were then used in Franz cells to conduct the diffusion study.

5.2.2 Franz Cell Diffusion

Horizontal Franz cell apparatus, Figure 20, was used to conduct the diffusion experiments. The hydrogel samples were gently placed in between two diffusion chambers, making sure that the diffusion ports in the centers of these chambers were covered. The two chambers were then clamped together and a seal between the two chambers was made using silicone. One chamber (the receptor chamber) was filled with PBS solution and the other (the donor chamber) with a solution of known concentration containing the fluorescent molecule of interest. The molecules will be discussed in greater detail in the following sections. Next, the apparatus was protected from light to prevent photobleaching of the fluorescent molecules. Also, the system was kept at 25° C by circulating water through the outer jacket. Samples were taken from the donor chamber on constant time intervals. The chamber was then replenished with PBS solution after each sample was removed. The samples were analyzed using fluorescence spectroscopy.

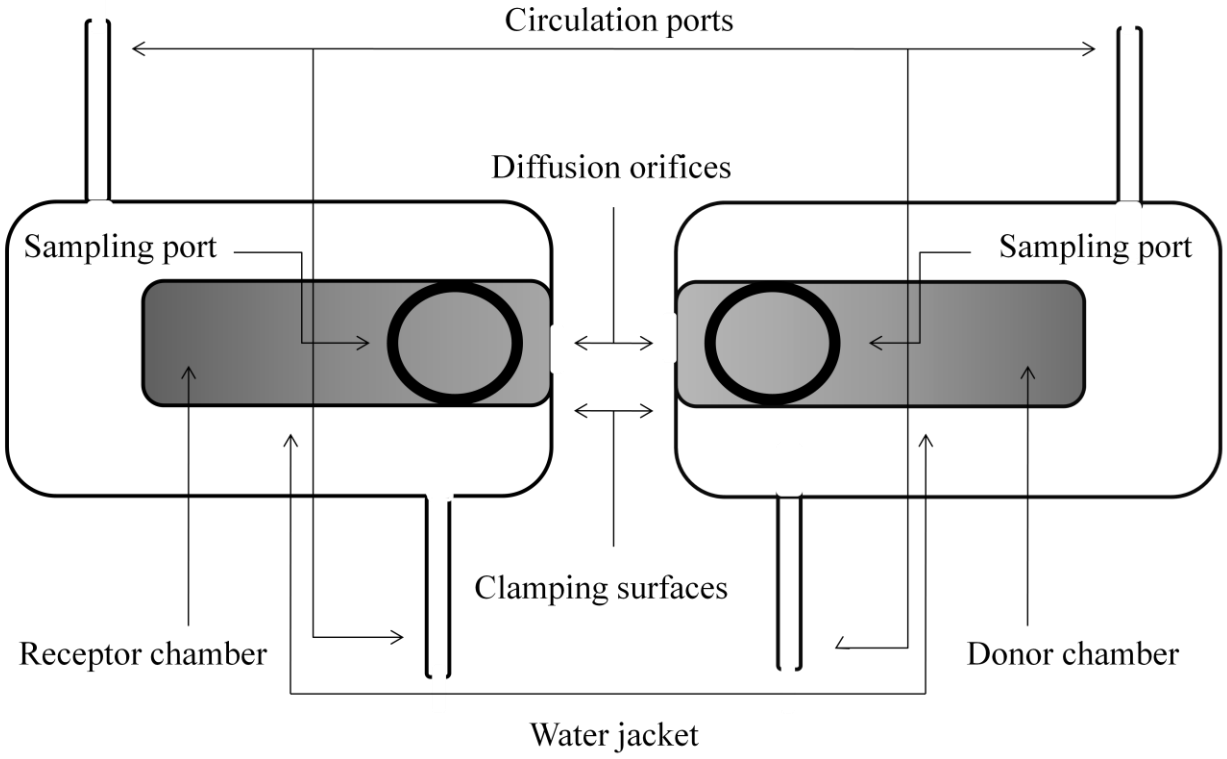


Figure 20. Overhead schematic view of Franz cell apparatus used in the diffusion experiments.

5.2.3 Fluorescence Spectroscopy

Fluorescence measurements were taken using SpectraMax Gemini XS plate reader from Molecular Devices (Sunnyvale, CA). The fluorescence measurements taken were converted to concentrations through analytical comparison to standards prepared from each fluorescent molecule.

5.2.3.1 Bovine Serum Albumin

Bovine serum albumin labeled with Alexa Fluor 488 was purchased from Invitrogen (Carlsbad, California). BSA was chosen because it has a hydrodynamic radius 3.6 nm, which is

similar to that of enzymes (MMP-2 and MMP-9) found in the matrix metalloprotease family whose members are associated with some cancers (Radomsky et al., 1990; Liabakk et al., 1996; Tcherkasskaya et al., 2003). The significance of this relationship will be further discussed later. Stock solution of BSA at 0.1 (mg/mL) concentrations was created to fill the donor chamber. The samples from the receptor chamber were excited at 497 nm and emission was recorded at 520nm for fluorescence spectroscopy analysis (Weaver, Durack et al. 1997; Weaver and Voss 1998).

5.2.3.2 7-Amino-4-Methylcoumarin

The 7-amino– 4-methylcoumarin (AMC), Figure 21, was purchased from AnaSpec, Inc. (Fremont, CA). Stock solution of 0.15625 mg/mL concentration of was used to conduct diffusion studies on all hydrogels. The samples for the receptor chamber were excited of 342nm and emission was recorded at 441nm for fluorescence spectroscopy analysis. Then data was statistical analyzed using ANOVA followed by Tukey's post-hoc analysis.

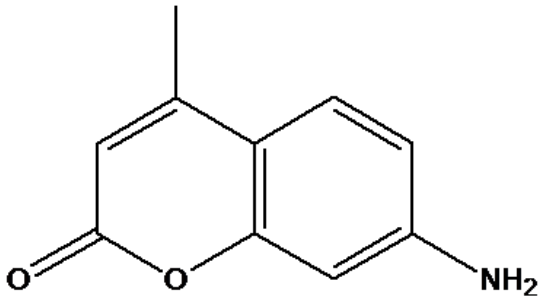


Figure 21. Chemical structure 7-Amino–4–Methylcoumarin

5.2.4 Calculating Diffusion Coefficient

The diffusion coefficient was calculated from the lag time (t_L). The lag time is the amount of time needed to establish a constant gradient across the hydrogel. The lag time was extrapolated, Figure 22, from the steady state region of a mass verses time graph using equation, Equation14, where D is the diffusion coefficient, and h is the thickness of the hydrogel (Komatsu and Suzuki, 1979).

$$D = \frac{h^2}{6t_L} \qquad (14)$$

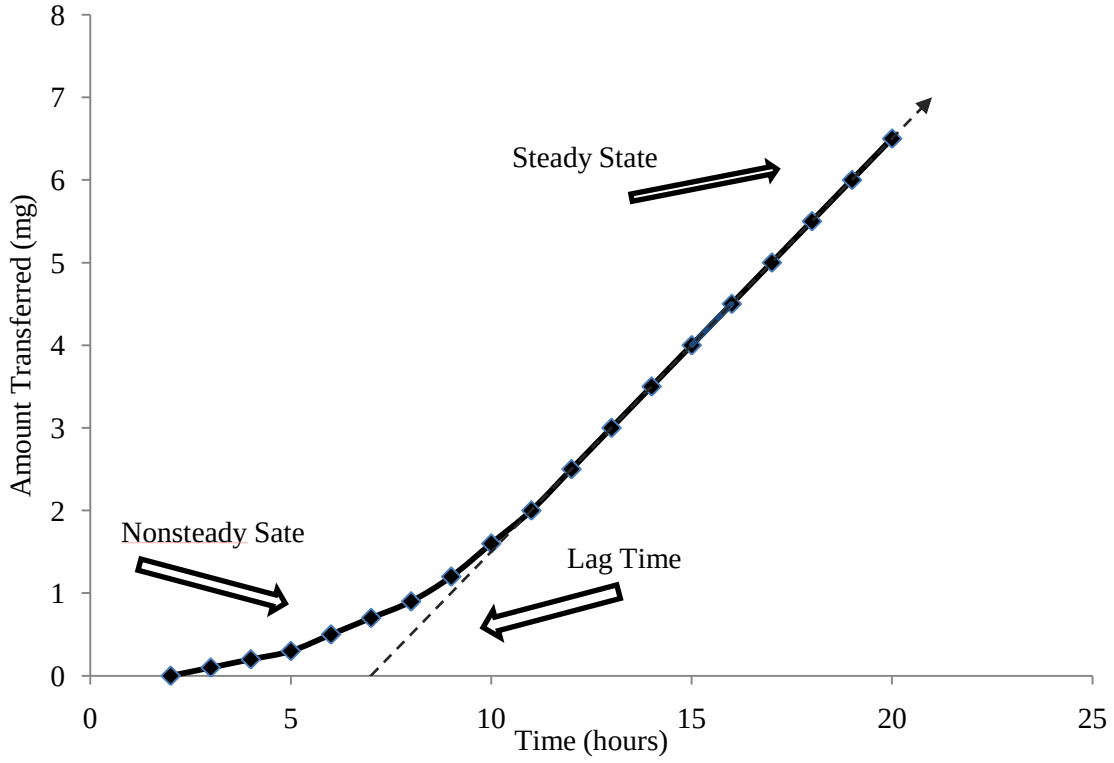


Figure 22. A graph of the mass transfer versus time of butyl paraben difusion through skin. Based on data from Komatsu and Suzuki, 1979.

5.3 Results

After the diffusion of the molecules reaches a constant rate, state-state condition is achieved and the diffusion proceeds in a linear manner. This allows for the extrapolation of diffusion coefficient. The rate of diffusion of AMC increased as the molecular weight of the PEGDA monomer used to create the hydrogel increased. This rate of increase is expressed as a linear equation. This increase was seen in the increase in diffusion coefficient (Figure 23.). This trend was also observed in hydrogels modified with mPEGMAs. The inclusion of mPEG(2000)MA increased the diffusion coefficient in hydrogels with high molecular weight PEGDA, but not in hydrogels with the lower molecular weight PEGDA monomers. There was a similar trend with MPEG(5000)MA. In addition, PEG(8000)DA and PEG(20000)DA hydrogels with mPEG(2000)MA had higher diffusion coefficients than those with mPEG(5000)MA (Figure 24.). This trend continues to be demonstrated with BSA diffusion in hydrogels made with PEG(20000)DA monomer (Figure 35).

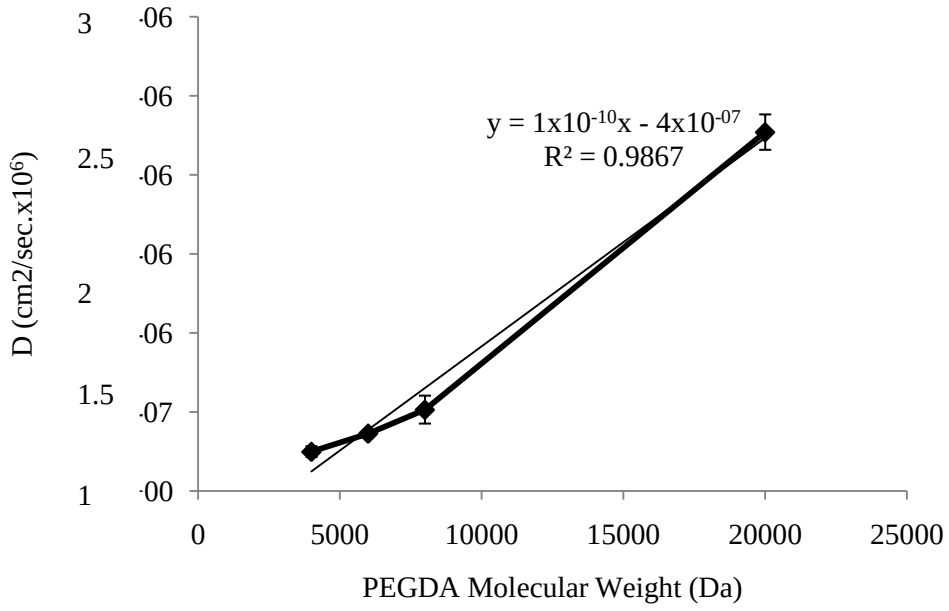


Figure 23. AMC diffusion coefficients in hydrogels made from various molecular weights of PEGDA monomers. (n = 3, mean ± stdev)

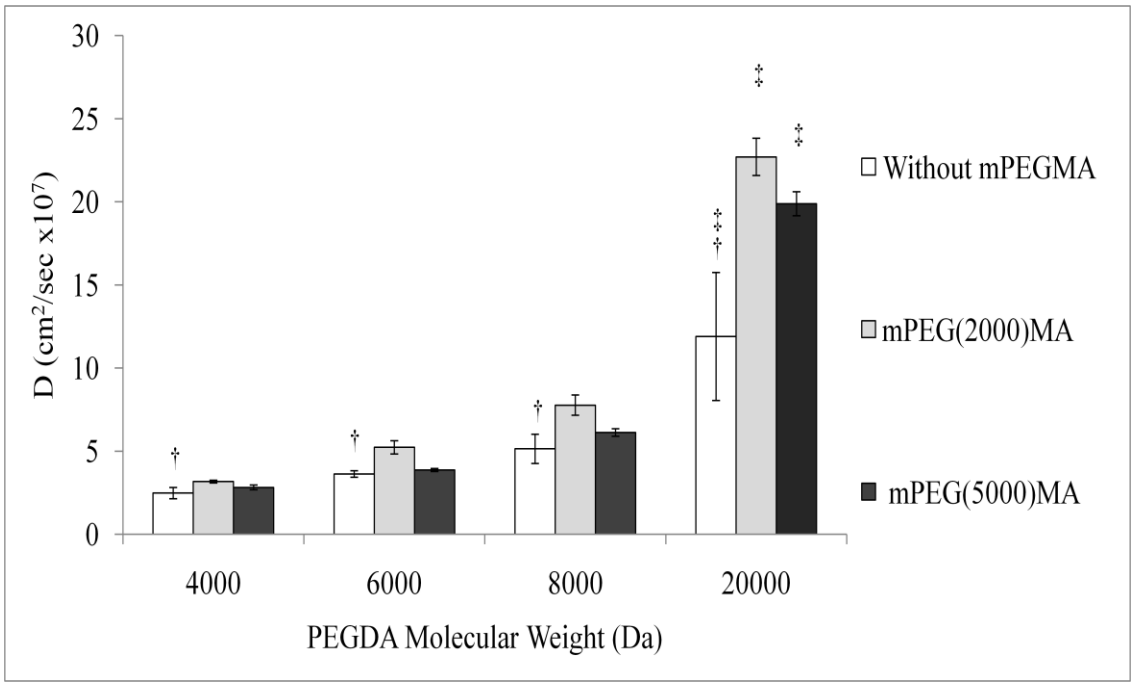


Figure 24. The diffusion coefficients (D) of 7-amino-4-methylcoumarin (AMC) through hydrogels composed of various poly(ethylene glycol) (PEGDA) molecular weights monomers. mPEG(2000)MA(gray bar) and mPEG(5000)MA (black bar) were add to the hydrogels of the various PEGDA molecular weight monomers . († $p \leq 0.05$ D values of hydrogels composed of PEGDAs derived from PEGs with molecular weights of 4000 Da, 6000 Da, 8000 Da, and 20000 Da were compared to each other, ‡ $p \leq 0.05$ PEG(20000)DA hydrogels with mPEG(2000)MA or mPEG(5000)MA add to them were compared to PEG(20000)DA hydrogels without mPEGMAs, n=3, mean ± stdev)

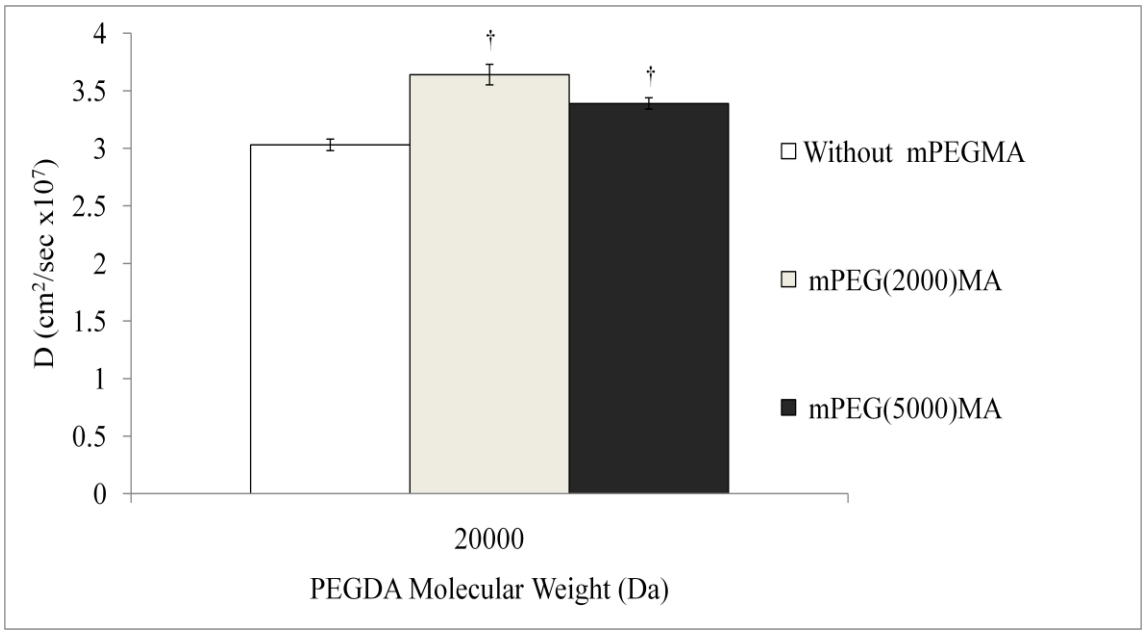


Figure 25. The diffusion coefficients (D) of Bovine Serum Albumin Bovine Serma Albumin (BSA) through hydrogels composed of poly(ethylene glycol) (PEGDA) monomer derived from PEG with molecular weight of 20000 Da. mPEG(2000)MA(gray bar) and mPEG(5000)MA (black bar) was add to hydrogels composed from PEGDA monomer derived from PEG with molecular weight of 20000 Da. PEG(20000)DA. PEG(20000)DA without mPEGMA is represented by the white bar. († $p \leq 0.05$ D of PEG(20000)DA hydrogels with mPEG(2000)MA or mPEG(5000)MA to each other)

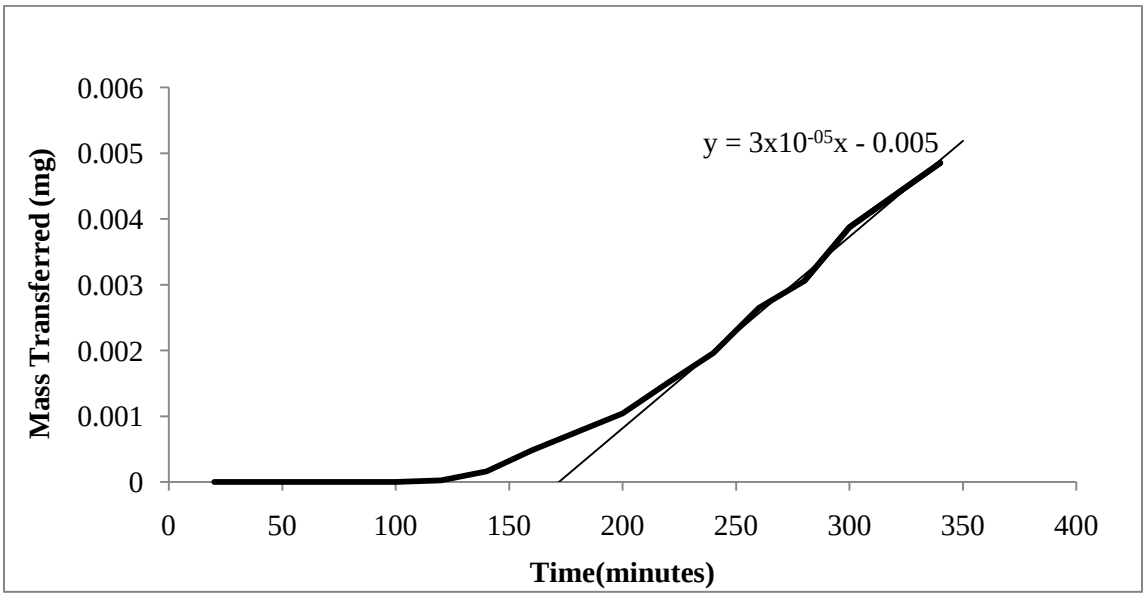


Figure 26. A graph of the mass transfer versus time of 7-Amino–4–Methylcoumarin (AMC) difussion through PEG(20000)DA hydrogel.

5.4 Discussion

The diffusivity trends of AMC through the various hydrogels followed the general prediction of the Peppas and Lustig expression relating nanostructure (i.e. mesh size) and effective diffusion. The AMC moved more rapidly and established a constant gradient across the hydrogels earlier as the mesh size was increased in the various hydrogels. Increasing the molecular weight of the monomers from which the hydrogels were comprised modulated the increase in mesh size. This resulted in a shorter lag time and, consequently, higher effective diffusion coefficients in hydrogels with higher molecular weight PEGDA monomers. Furthermore, the addition of mPEGMAs to the higher molecular weight PEGDA hydrogels increased the rate of diffusion due to the increase in the average mesh size of the hydrogels. This increase of the average mesh size was probably due to space occupied by the untethered ends of the polymer chains within the hydrogels. There was a decrease in crosslinking density which, in turn, increased the average mesh size. Also, a different average diffusion coefficient was calculated in hydrogels with a different molecular weight of mPEGMA. These results reinforce the concept that small active drug molecules' diffusivity can be modulated by varying the chain length of monomers, or the tethered chain length, even in the higher molecular weight PEGDA hydrogels tested.

As was seen with AMC, the different mPEGMAs mixed with the PEG(2000)DA hydrogels appear to have similar effects on the diffusion coefficients of the respective hydrogels. Thus, hydrogels can be made with large enough mesh size to allow BSA to enter and, yet, these hydrogels can still be modulated to control the diffusion of AMC. This proves that relatively large biomolecules, such as those found in MMP family of enzymes, can be used to trigger the release of a drug and, from that point onward, its release can be controlled by altering physical

properties of the hydrogel. Also, this implies that employing a similar strategy to that which was applied to regulate the diffusion of the triggering biomolecules can control the release.

VI. CONCLUSIONS AND FUTURE WORK

6.1 Conclusions

Poly(ethyl glycol) diacrylate (PEGDA) and methoxy poly(ethyl glycol) monoacrylated (mPEGMA) based hydrogels provide a robust biomimetic platform for controlled drug delivery and release. The physical properties of the hydrogels that govern the release of a drug can be easily tailored to suite a given release profile by simple alterations of structural makeup. This release profile can be achieved by varying the PEGDA polymer chain length or even the mPEGMA chain length, a process that alters the structure of the hydrogels at the nano scale. These alterations can be quantified and analyzed by equilibrium swelling processes, which provide quantification of the mesh size.

In turn, this information facilitates the ability to intelligently predict how drug release from a given platform will behave. Many of the models using this information are good at first approximation but fail when hydrogels do not behave as perfect networks. Highly crosslinked structures and defects in the hydrogel can cause them to deviate from predicted behavior. These flaws can be controlled. Even when these imperfections are predicted, the behavior is typically close enough to begin choosing the componential structure required for optimum release profiles based upon the size of the target agent to be released.

The PEGDA-based system utilized in this work confirmed that the incorporation of mPEGDA can be used to modify the hydrogel structural properties and change the ability of molecules to diffuse in and out of the hydrogel. The system was robust enough to allow a large biomolecule to enter the hydrogel and still retain sensitive control over the diffusion of a small molecule. This may open the door for future work that can be continued by other.

6.2 Future work

The hydrogel systems in this work can be manipulated to permit the entry of relatively large biomolecules (i.e. BSA) and have also indicated the ability to regulate diffusion of small molecules such as AMC. Based on this, some aspects of system might be interesting to explore. These include (1) the degree of control over the small molecule diffusion with hydrogels having a mesh size large enough to accommodate BSA, (2) the effect of mechanical properties on diffusion, (3) molecular selectivity of the system and (4) addition of attachments to the tethers in order to observe how they might affect the hydrogel's diffusive properties. (Beamish et al., 2010; Drury and Mooney, 2003)

Additionally, further refinement of the system should be made to permit it to be used as a targeting drug delivery system. To facilitate this, drug and enzymatic cleavable peptide sequence complexes can be conjugated to the tethers. These peptides could be cleaved by a MMP family enzyme, which is highly up regulated in the disease state being addressed. After this has been accomplished, structural and diffusive properties should be analyzed similar to what has been done in this work. Moreover, the system should be probed to elucidate enzymatic activity and the role played by the diffusive properties in the release of any different drugs.

The hydrogel based therapeutic system will have to be optimized for the particular disease state, such as Glioblastoma with MMP-6 and MMP-9 overexpression. In a disease, such as glioblastoma, the hydrogel based therapeutic system has to be designed specifically for the disease's natural pathology. The pathology will dictate if the therapeutic system should be placed in one central location or many different locations. Also, the pathology of the disease determines the drug release profile needed to rid the patient of the cancer (Rao et al., 1996; Sawaya et al.,

1996; Holland, 2001; Visted et al., 2003; Tauro and Gemeinhart, 2005; Nieder, 2007; Nieder et al., 2007). The primary pathology determinants in the drug release profile are the MMP activity and MMP concentration. MMP activity and concentration in the present hydrogel therapeutic system has yet to be studied.

Exploration of the hydrogel therapeutic device's therapeutic effect in combination with current treatment also has yet to be explored. The different treatments such as surgery, chemotherapy and radiation have to be evaluated in a combination with the hydrogel device to observe if there is a measurable improvement in patients' health. Further, evaluation has to be conducted in the most effective and efficient manner to incorporate the hydrogel therapeutic device. This can only take place after carrying out more extensive *in vitro* experiments followed by *in situ* experiments to determine toxicity and efficacy of the therapeutic system.

Also, it will be important to study the hydrogel based therapeutic device's ability to treat other diseases. The examination of the hydrogel therapeutic device for treating other diseases must begin with incorporating different therapeutic agents into the device. Each therapeutic agent has to be attached to the hydrogel in a unique chemical matter to make a complete therapeutic device for a given disease. Also, the release of the therapeutic agent must be assessed and optimized for the effective treatment of the given disease.

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APPENDICES

APPENDIX PAGE

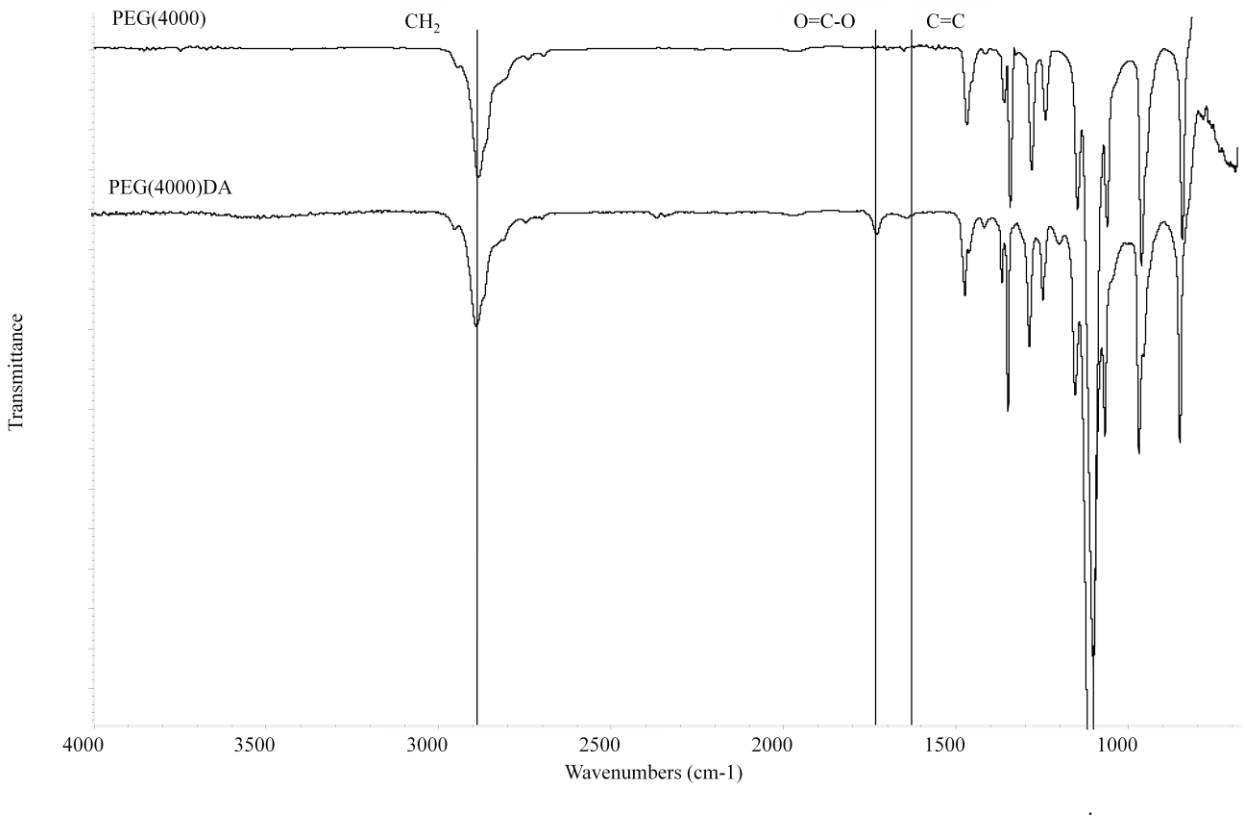

Figure 27. Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectrum of the product PEG(4000)DA and the precursor PEG(4000).

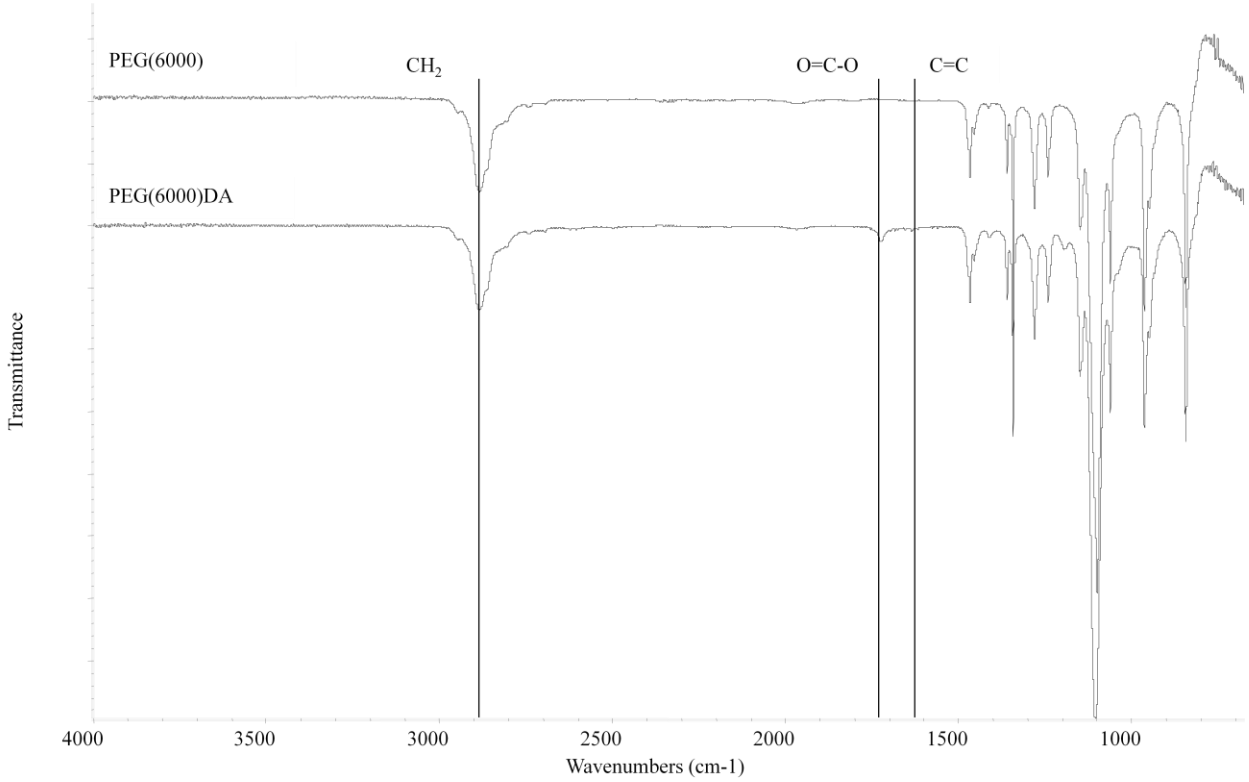

Figure 28. Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectrum of the product PEG(6000)DA and the precursor PEG(6000).

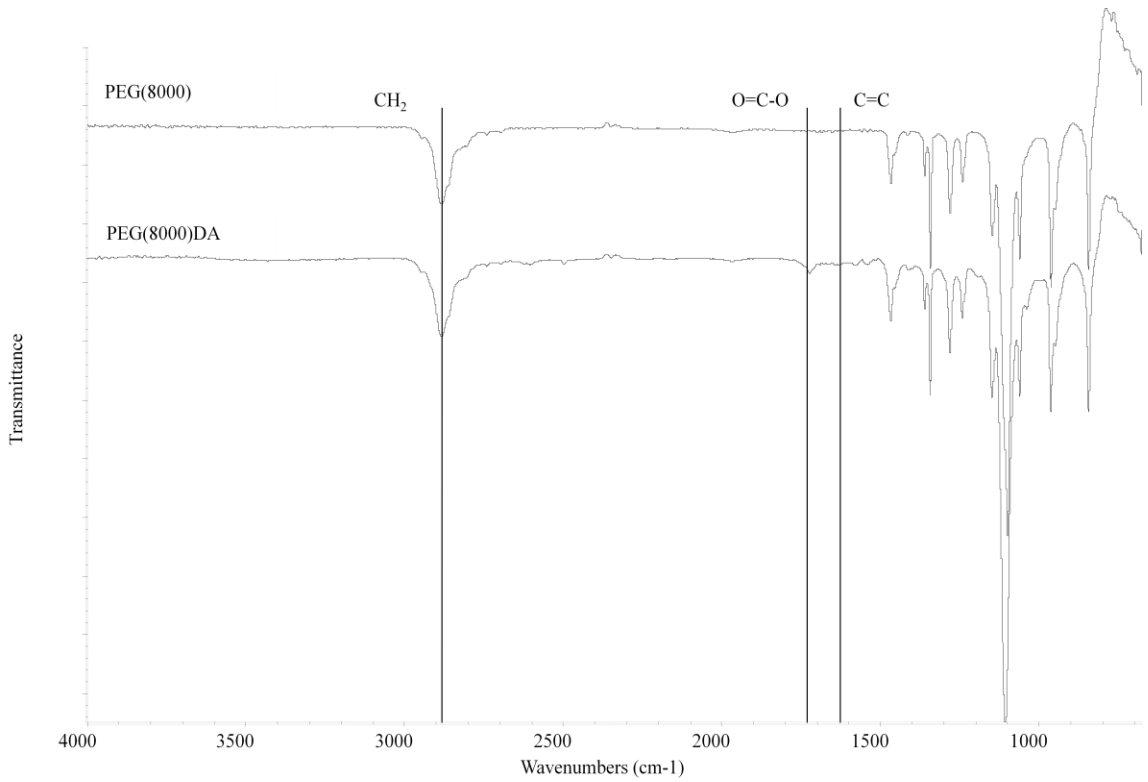

Figure 29. Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectrum of the product PEG(8000)DA and the precursor PEG(8000).

APPENDIX A (continued)

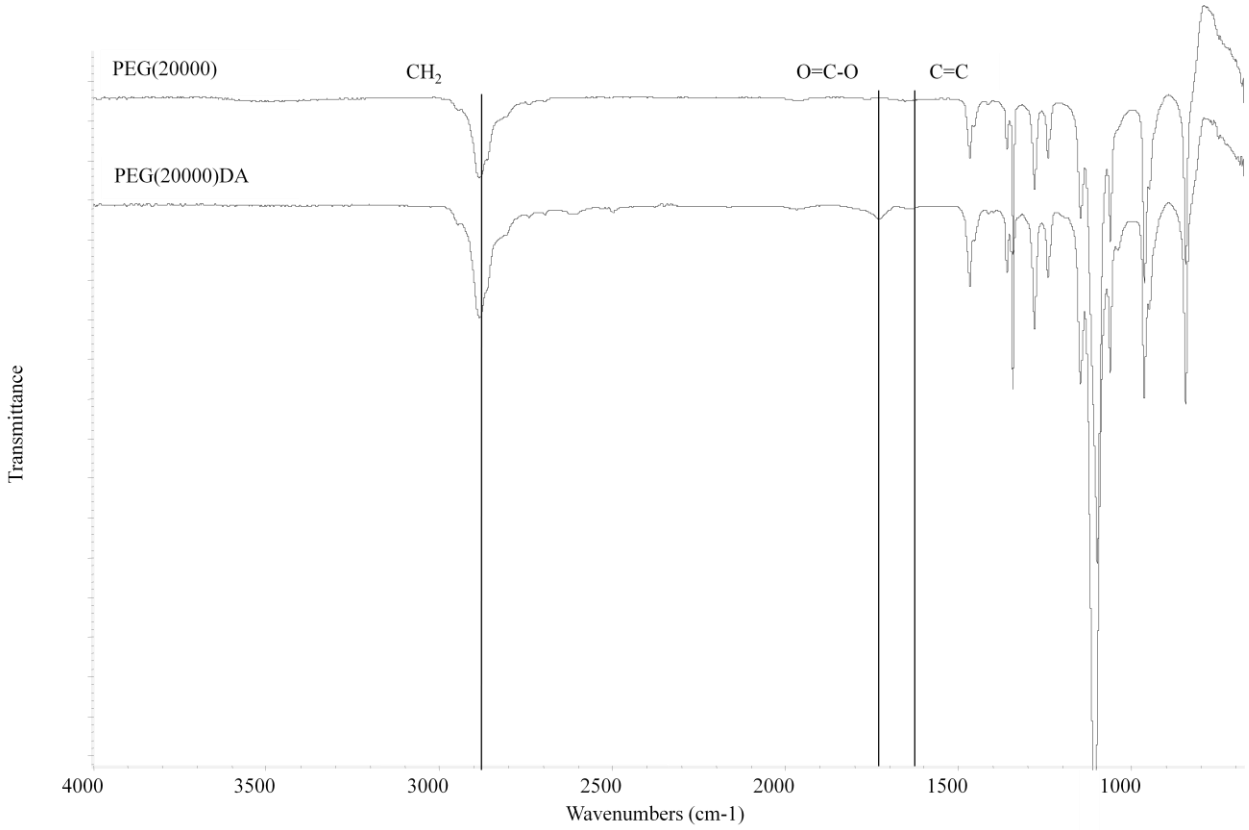

Figure 30. Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectrum of the product PEG(20000)DA and the precursor PEG(20000).

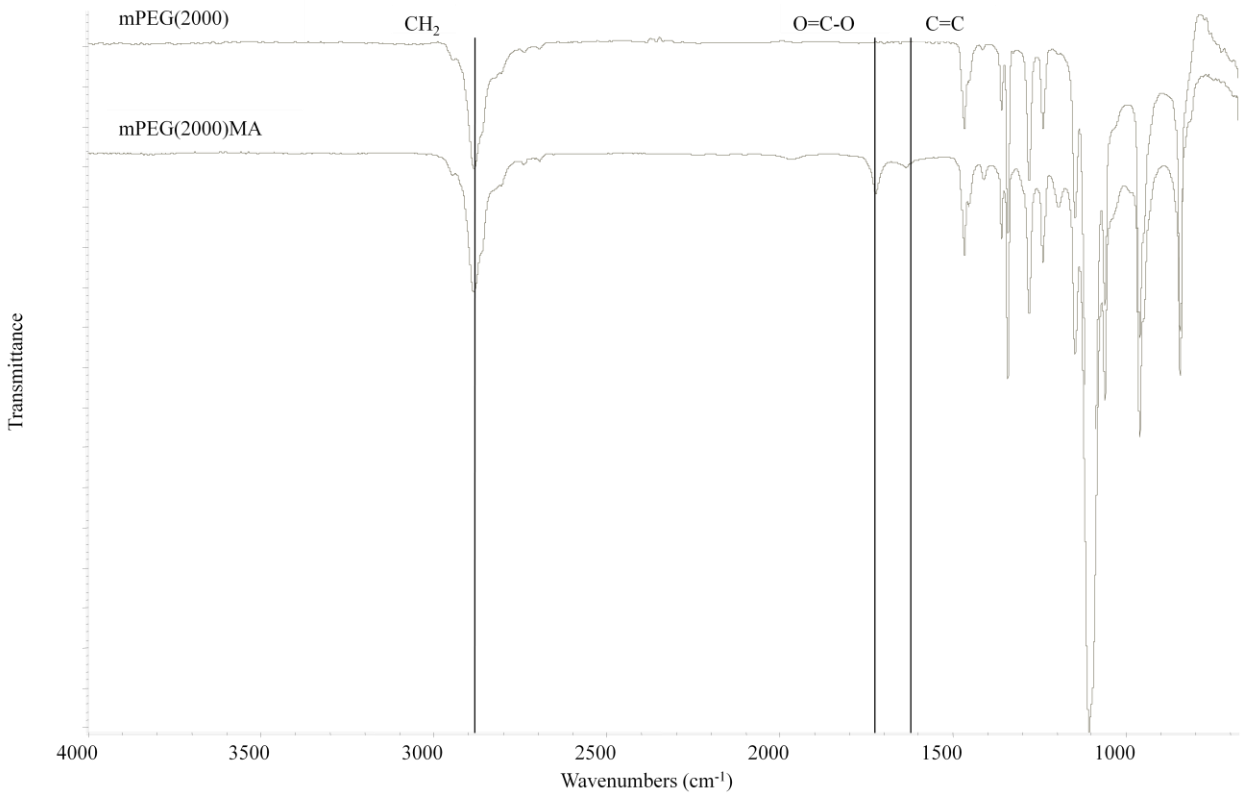

Figure 31. Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectrum of the product mPEG(2000)MA and the precursor mPEG(2000).

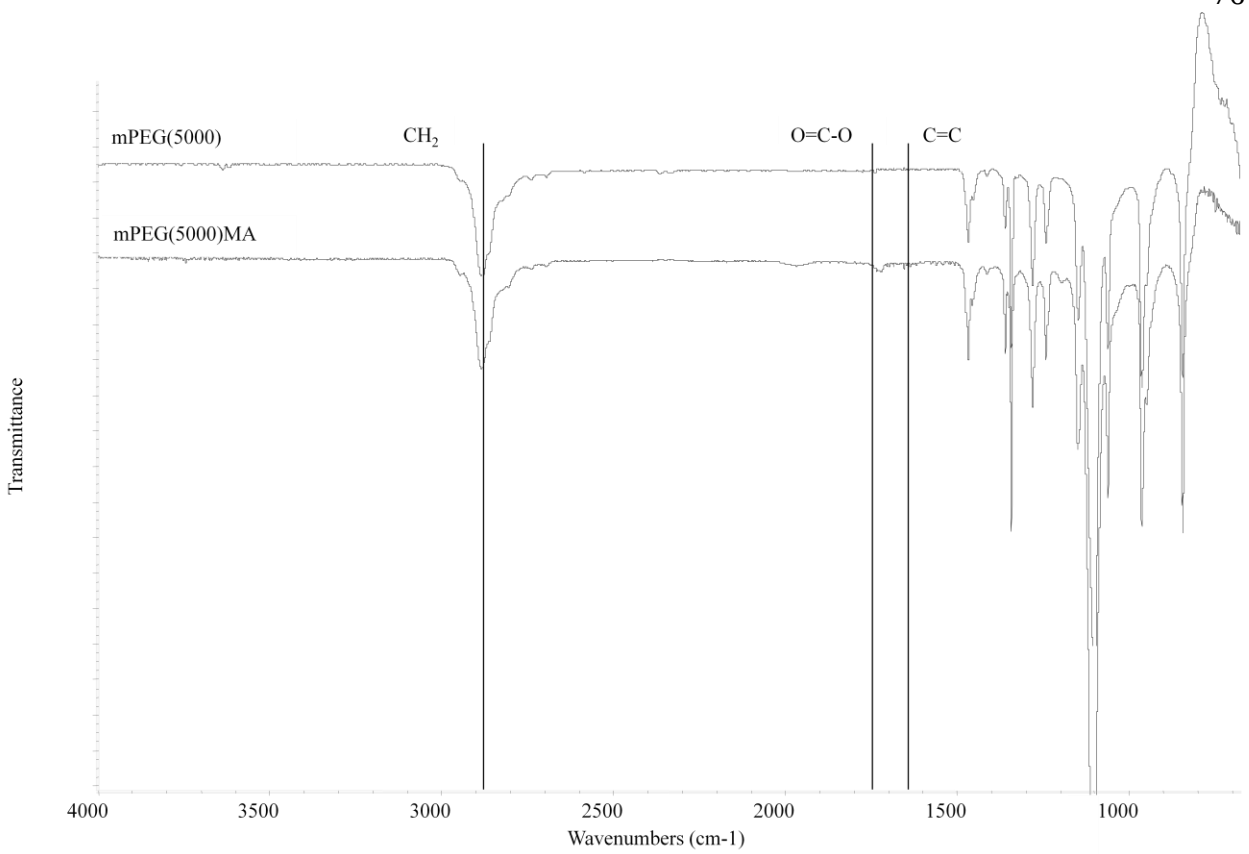

Figure 32. Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectrum of the product mPEG(5000)MA and the precursor mPEG(5000).

APPENDIX B

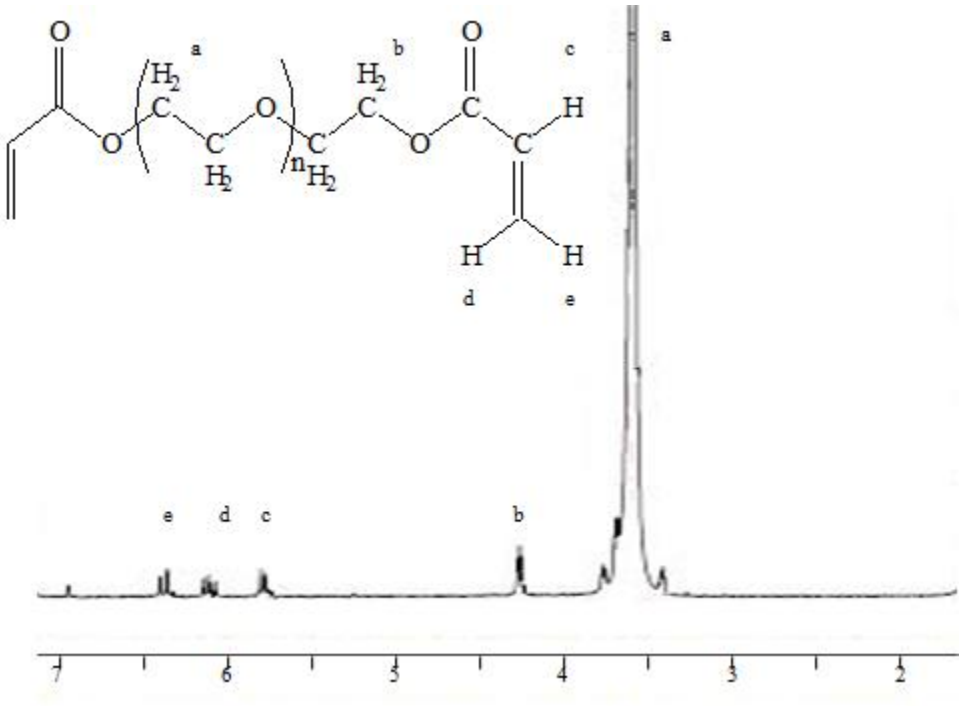

Figure 33. NMR spectrum of PEG(4000)DA. ^{1}H NMR ($CDCl_3$): a) 3.6 ppm (m, 362 H, (-OCH_2-CH_2)$_n$), b) 4.3 ppm (t, 4H, dd, -CH_2 -COO-), c) 5.8 ppm (dd, 2 H, -COOCH=CH_2), d) 6.1 (m, 2H, -COOCH=CH cis), and e) 6.4 ppm (dd, 2H, -COOCH=CH trans).

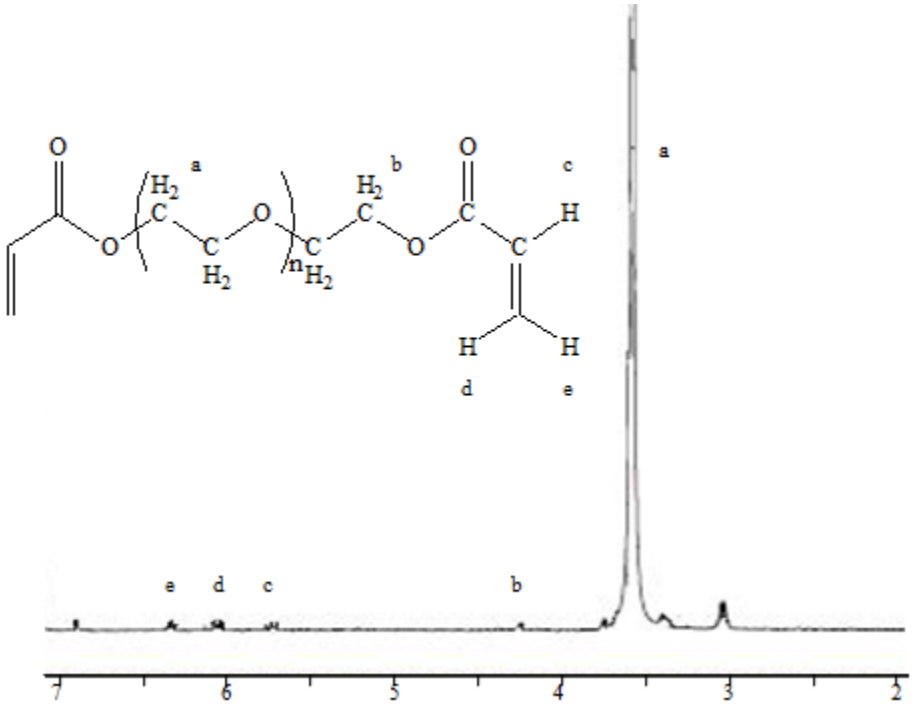

Figure 34. NMR spectrum of PEG(6000)DA. 1H NMR (CDCl3): a) 3.6 ppm (m, 544 H, (-OCH2-CH2)n), b) 4.3 ppm (t, 4H, dd, -CH2 -COO-), c) 5.8 ppm (dd, 2 H, -COOCH=CH2), d) 6.1 (m, 2H, -COOCH=CH cis), and e) 6.4 ppm (dd, 2H, -COOCH=CH trans)

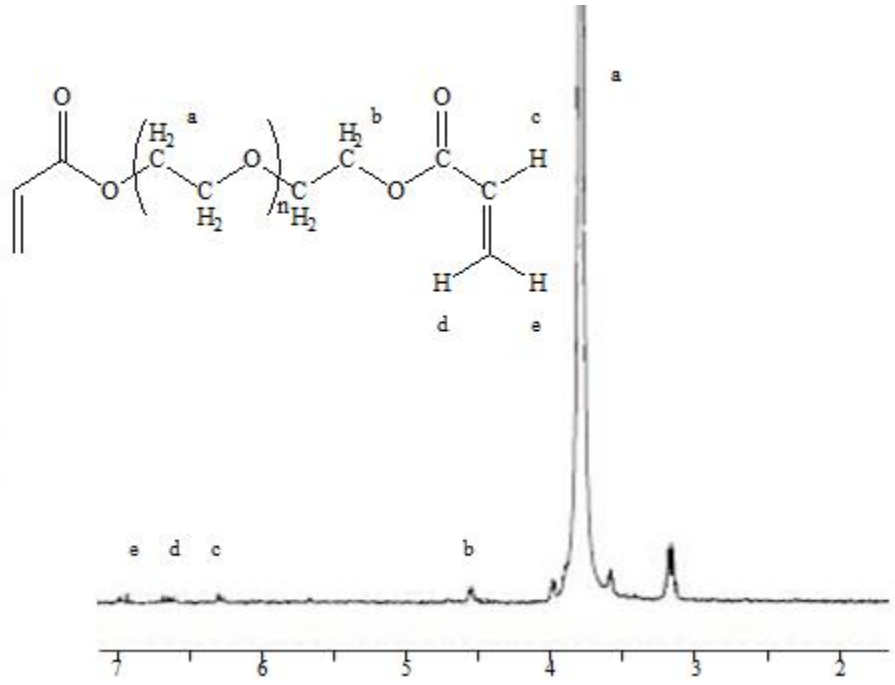

Figure 35. NMR spectrum of PEG(8000)DA. ^{1}H NMR ($CDCl_3$): a) 3.6 ppm (m, 725 H, (-OCH_2-CH_2)$_n$), b) 4.3 ppm (t, 4H, dd, -CH_2 -COO-), c) 5.8 ppm (dd, 2 H, -COOCH=CH_2), d) 6.1 (m, 2H, -COOCH=CH cis), and e) 6.4 ppm (dd, 2H, -COOCH=CH trans).

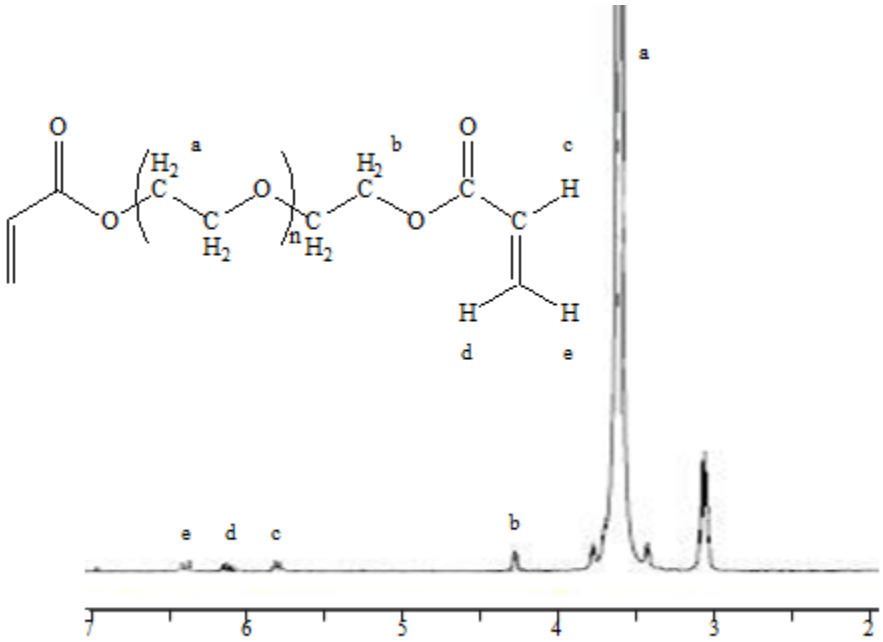

Figure 36. NMR spectrum of PEG(20000)MA. ^{1}H NMR ($CDCl_3$): a) 3.6 ppm (m, 1817 H, (-OCH_2-CH_2)$_n$), b) 4.3 ppm (t, 4H, dd, -CH_2-COO-), c) 5.8 ppm (dd, 2 H, -COOCH=CH_2), d) 6.1 (m, 2H, -COOCH=CH cis), and e) 6.4 ppm (dd, 2H, -COOCH=CH trans).

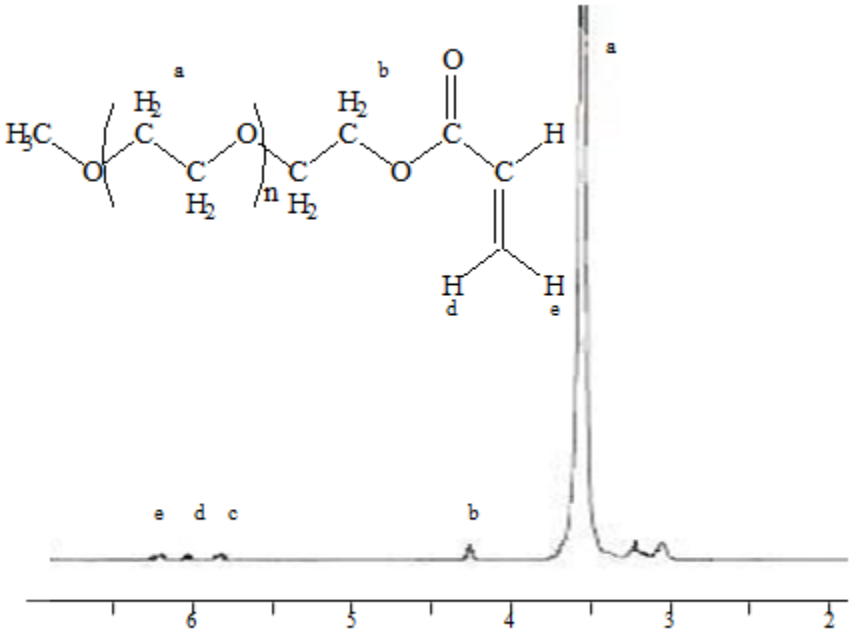

Figure 37. NMR spectrum of mPEG(2000)MA. ^{1}H NMR ($CDCl_3$): a) 3.6 ppm (m, 179 H, (-OCH_2-CH_2)$_n$), b) 4.3 ppm (t, 4H, dd, -CH_2-COO-), c) 5.8 ppm (dd, 2 H, -COOCH=CH_2), d) 6.1 (m, 2H, -COOCH=CH cis), and e) 6.4 ppm (dd, 2H, -COOCH=CH trans).

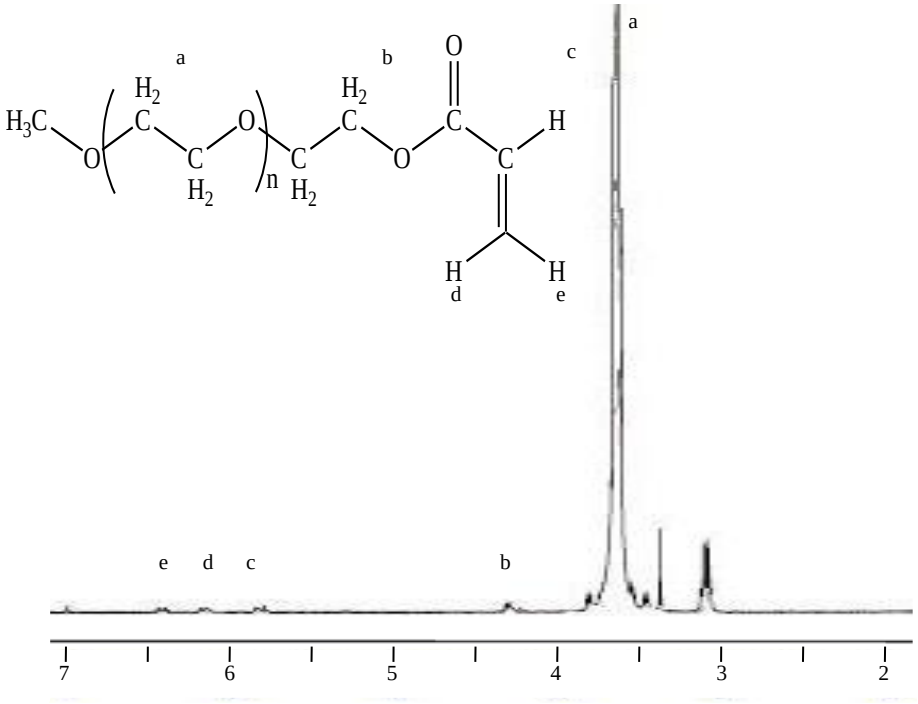

Figure 38. NMR spectrum of mPEG(5000)MA. ^{1}H NMR ($CDCl_3$): a) 3.6 ppm (m, 452 H, (-OCH_2-CH_2)$_n$), b) 4.3 ppm (t, 4H, dd, -CH_2-COO-), c) 5.8 ppm (dd, 2 H, -COOCH=CH_2), d) 6.1 (m, 2H, -COOCH=CH cis), and e) 6.4 ppm (dd, 2H, -COOCH=CH trans).

VITA

Name: Mohammad Rahman

Education:
M.S., Bioengineering, University of Illinois at Chicago, Chicago, IL, 2011

B.S., Bioengineering, University of Illinois at Chicago, Chicago, IL, 2007

Professional experience:
Laboratory Technician, Neural Engineering Laboratory,
University of Illinois at Chicago, Chicago, IL, 2002-2004

Research experience:
Research Assistant, Department of Biopharmaceutical Sciences**,**
University of Illinois at Chicago, Chicago, IL, 2007-2010

Honors and Awards:
1st Place Award in the College of Engineering Senior Design Competition Engineering EXPO for Assistive Technology and Universal Design Category**,** University of Illinois at Chicago, 2006

The Chancellor's Student Service and Leadership Award (CSSLA), University of Illinois at Chicago, 2007

Abstracts and proceedings:
Rahman M., Gemeinhart R.A.; Analysis and optimization of Parameters of Hydrogel for Diffusion for Diffusion of Biomolecules: University of Illinois at Chicago College of Pharmacy Research Day, 2010

Professional memberships:
Controlled Release Society Illinois Student Chapter