Establishing novel biomaterial applications of poly(ethylene glycol) based on its ability to bind water and control its environment

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Title: Establishing novel biomaterial applications of poly(ethylene glycol) based on its ability to bind water and control its environment

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Lay Abstract

Polymeric biomaterials have created significant advances in the field of biomedical engineering, however, very few polymeric drug delivery devices have achieved clinical and commercial success. Thus, the motivation for this thesis was to encourage long-term success of materials through expanding the fundamental understanding of polymer properties.

Poly(ethylene glycol) was specifically chosen for study due to its unique exhibition of amphiphilic character and the ability to hydrogen-bond multiple water molecules, that together suggest the possibility for PEG to control drug release and its environment.

Through strategic experimental designs, greater understanding of the abilities and limitations of PEG was established and shown to be the result of the distinct structure of PEG. Specifically, two novel drug delivery systems were developed with demonstrated understanding of the structure-function relationship between polymers and drugs, and the activity of PEG as a melanoma cell viability inhibitor was discovered and found correlated to the PEG structure. Overall the work within this thesis expanded the potential for PEG in biomedical applications to more than being used as simply a hydrophilic additive.

Abstract

Polymeric biomaterials have created significant advances in the field of biomedical engineering, however, very few polymeric drug delivery devices have achieved clinical and commercial success. Thus, the motivation for this thesis was to encourage long-term success of materials through expanding the fundamental understanding of polymer properties.

Poly(ethylene glycol) was specifically chosen for study as its polyether backbone provides it with many unique properties that are still not fully understood, and are not seen with other similar polymers. PEG has been shown to exhibit amphiphilic character, due to its high conformational freedom, and the ability to hydrogen-bond 2-3 water molecules for each ethylene oxide subunit, creating a very structured water shell and large hydrodynamic radius. Together, the properties formed the hypothesis for the possibility for PEG to control drug release and its environment, expanding its potential in biomedical applications.

This hypothesis was investigated with PEG in three states – free PEG, conjugated and blended. Free PEG was determined to inhibit melanoma cell viability by activating apoptosis via PEG effects on the osmolality of the cell medium (Chapter 3). Novel silicone hydrogels incorporating methacrylated PEG as the sole hydrophilic component showed advantageous properties for biomedical applications across a range of formulations (such as low contact angle and protein deposition), as well as altering the release of highly hydrophilic antibiotics from the materials, presumably via PEG-drug hydrogen bonding (Chapter 4). Novel siloxane-PEG blended materials were shown to have the ability to influence drug release of hydrophilic, hydrophobic and drug salts through the structure of PEG (Chapter 5).

Overall, the work within this thesis expanded understanding of the abilities and limitations of PEG based on its distinct structure, and expanded the potential for PEG in biomedical applications to more than being used as simply a hydrophilic additive.

This dissertation is dedicated to my family and dear friends. I could not have done this without you.

Thank you for your ongoing support throughout the stresses and successes.

You have always been there for me and I love you.

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List of Abbreviations and Symbols

CIP	Ciprofloxacin
DMSO	dimethyl sulfoxide
EGDMA	Ethylene glycol dimethacrylate
EWC	Equilibrium water content
HPLC	High-pressure liquid chromatography
MET	Metformin hydrochloride
Metformin	Metformin hydrochloride
MW	Molecular weight
PBS	Phosphate-buffered saline
PDMS	Poly(dimethyl siloxane)
PEG	Poly(ethylene glycol)
P-gp	P-glycoprotein
PVP	Poly(vinylpyrrolidone)
TOB	Tobramycin
TRIS	(3-methacryloyloxypropyl)tris(trimethylsiloxy)silane
UV	Ultraviolet
w/w	mass/mass

Declaration of Academic Achievement

The work described within the written thesis was conceived, conducted, analyzed and written by Ivana Postic, with support and consultation from Dr. Heather Sheardown.

1. Introduction

1.1 Motivation

The research field of drug delivery contains extensive literature showing successes in the delivery of therapeutics, including proteins, antibodies, nucleic acids and small molecules in a controlled or sustained manner [1]–[4]. Yet, the translation of academic work into the clinic has been challenging on many levels. These challenges include poor translation from the *in vitro* to *in vivo* setting, failures of safety or efficacy in clinical trials, complications in scale-up or manufacturing, and even end failure in obtaining regulatory approval [5]. The numerous attempts at developing a single new therapeutic translate to an economic cost estimated at $2.5-5 billion USD [6]. The cost to recoup this enormous expense falls into the hands of paying bodies – the end consumers / patients – and failures increase these costs.

The trend towards more sophisticated medicine such as gene therapy and biologics requires even more sophisticated and costly production methods. This brings an average daily price of a biologic in the United States to $45, compared to $2 for a small molecule drug [7]. Further compounding the price of therapeutics is the high cost associated with drugs in the rare disease space, making it clear that the drug development process must be improved, or else the risk exists that government healthcare budgets will not be able to cover or subsidize therapies, and individual citizens will be forced to find ways to pay for treatment out of pocket.

One avenue through which researchers have been working to tighten the match between the *in vitro* efficacy of a molecule and the *in vivo* or clinically desired outcomes, is through the optimization of the drug's pharmacokinetic (PK) profile. Early PK data can be used to determine which drug will be a lead candidate in the pipeline and optimized PK profiles can improve the potential of a therapeutic from both a safety and efficacy perspective [8]. Specifically, having the ability to maintain drug levels below toxic concentrations and above minimum efficacy concentrations can help to reduce downstream complications or side effects. This goal has been extensively targeted through the incorporation of pharmaceuticals within biocompatible polymer systems, or drug delivery systems.

There are a variety of polymers that can be created, assembled and explored, resulting in the possibility of producing an overwhelming number of options for the controlled or sustained delivery of a single drug. There also continues to be growing knowledge of the interaction between polymers and biology, which further supports the long-term success of polymer-based therapeutic devices. Despite the clinical potential of these systems, the

lack of sophisticated delivery devices on the market indicates an unmet need to further optimize not just the PK profiles of drug for clinical success, but to also improve the development process of the polymeric components.

1.2 Thesis Objectives

In this thesis, the overarching goal was to develop an approach to material design that supports the translation of biomedical polymers to the clinical setting. The tactics used to achieve this were to strategically define the polymers of interest, to develop streamlined manufacturing methods with minimal potential for downstream toxicity concerns, and to design studies that allow for deeper understanding of the structure-function relationship of the polymers and drug of interest. Ultimately this has the potential to reduce the time and cost spent on optimizing materials at any given stage in the process (*in vitro, in vivo* and potentially even clinical).

The specific goal of this work was expand the clinical uses of poly(ethylene glycol) through the tactics defined above. Poly(ethylene glycol) is a linear, non-ionic, hydrophilic polymer with high conformational freedom [9]. Despite its rather simple structure and properties, PEG has found use in medicinal applications as a gold standard hydrophilic polymer with inherent protein-repelling capability [10]. It is able to hydrogen bond with itself and with molecules in its environment [11]. The structure of PEG also provides it with the ability to adopt both non-polar and polar conformations, leading to amphiphilic properties. These properties presented an opportunity to expand the knowledge and clinical applications of PEG through the work in this thesis.

1.3 Hypothesis

The hypothesis of this thesis is that the unique structure of PEG and amphiphilic properties will allow it to control its environment in a variety of settings, leading to clinically relevant activity and controlled movement of small molecule therapeutics.

1.4 Design

To achieve broad understanding of the abilities and limitations of the structure-function relationship of PEG, PEG was explored in three states: conjugated, entrapped and free. In each state, the relationships between PEG concentration, PEG molecular weight, drug properties and drug concentration were examined.

In most devices developed to-date, PEG is only a component of a multi-component system, making it difficult to understand the effect of each on the resulting pharmacokinetic profile. Therefore, the experimental procedures developed herein were designed to assess PEG in simple device compositions to elucidate relationships

between the structure of PEG and its properties.

Rather than focusing solely on the physical properties of the test materials, each chapter maintains a distinct focus on expanding knowledge within a specific biomedical research area and building upon clinically relevant data by choosing to deliver drugs that pose pharmacokinetic challenges.

The specific designs and clinical applications of each chapter are as follows.

1.4.1 Free PEG influence on the cellular environment and cell activity

PEG is generally perceived as an inert polymer (exhibiting no immune response) with no inherent biological activity (not interacting with tissue). In Chapter 3, this belief is challenged with the hypothesis that the ability of PEG to strongly hydrogen bond water may disrupt the environmental conditions required for cellular activity and viability. Metastatic melanoma cells were chosen as the cell model due to an unmet need for efficacious treatment. Cellular changes such as viability, membrane transport and apoptotic markers were measured and these data were combined with observed effects on cell media (osmolality, viscosity) to determine any structure-function relationship of PEG on melanoma cells.

1.4.2 PEG influence on silicone hydrogel properties and drug delivery

Chapter 4 of this thesis explores the ability of methacrylated PEG to control its environment when conjugated as part of a silicone hydrogel. Functionalized siloxanes (such as TRIS (tris(trimethylsiloxy) silylpropylmethacrylate) used herein) are constituents of many biomedical devices, including extended-wear soft contact lenses and are therefore clinically relevant biomaterials. A short-chain PEG with two methacrylate end groups was used to crosslink the macromer chains of PEG and TRIS and engineer a novel hydrogel material. The materials were also loaded with the highly hydrophilic antibiotic tobramycin and the release rates were examined. The effect of varying PEG molecular weight and concentration were investigated as a means of controlling drug release. In addition, material properties important for potential biomaterial success, including transparency, refractive index, and contact angle were measured to evaluate these silicone hydrogels for potential contact lens application.

1.4.3 PEG influence on release rates of varying therapeutics from PDMS elastomer

In Chapter 5, highly hydrophobic and clinically approved poly(dimethyl siloxane) elastomer was used as the base material for a drug delivering device. PDMS on its own

has been widely used for the delivery of low molecular weight hydrophobic drugs, with the release mechanism being dissolution of the drug in the polymer phase and its subsequent diffusion [47]. However, the efficacy of this approach is limited by the molecular weight of the drug and its solubility in the hydrophobic PDMS phase [47]. To overcome this limitation, PEG and crystalline therapeutics were physically entrapped in the PDMS to create a novel elastomeric material with altered bulk properties. It was hypothesized that entrapped PEG may retain the ability to control its environment (via its amphiphilic character), and thus by interacting with the therapeutics, release rates may be altered / controlled. Thus, release of drugs of different solubility was examined in this study, and the data were tested against known kinetic release models, examining the potential for controlled, predictable drug release from such a device.

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2 Literature Review

2.1 Modern Drug Development

Modern research is equipped with the knowledge and discoveries of decades of fundamental research. For example, today's scientists have access to advances in high-throughput screening, *in vivo* models of disease, stem cell therapeutics, and genetic engineering. These advanced techniques continue to promise the discovery of new drugs with increased efficacy, reduced systemic side-effects and overall improved prognoses. However, *many* promising bench-top therapeutics still fail to reach the market. The reasons behind the low translation rates can vary significantly, but two major hurdles include poor translation of pre-clinical observations to clinical safety and efficacy, and significant limitations related to formulation and delivery. To overcome these challenges, researchers have expanded the scope of medical research from being focused solely on novel drug discovery. Significant preclinical research today is devoted to improving translation and drug delivery, by improving pharmacokinetic profiles through the development of better drug delivering vehicles.

2.1.1 Pharmacokinetics

Pharmacokinetics is defined through the basic stages of absorption, distribution, metabolism and elimination of a compound [1]. Absorption occurs generally via nasal, oral, pulmonary, transdermal, or parenteral routes (depending on the route of administration) [2].The site of absorption can also be described as occurring at specific tissues, such as the mouth, stomach or intestine [3]. After absorption, the distribution of the therapeutic occurs throughout the body and into tissues. This is controlled by mechanisms such as passive diffusion, active targeting and cell-uptake [2]. Metabolism of the compound can occur at varying times / locations / to varying degrees, and occurs by methods such as hydrolysis or enzymatic cleavage [2]. Finally, elimination from the body is typically via the renal or bile pathways [1].

During drug development, the appropriate delivery dosage and regimen for each drug is determined based on these pharmacokinetic parameters [1]. However, this is not a straightforward calculation, given that the action of drugs on the body and the action of the body on the drugs is unique to every compound. Numerous factors can contribute to a particular pharmacokinetic profile including [4]–[6]:

- drug size / molecular weight
- drug properties
- minimum efficacy levels
- toxicity levels
- side effects
- therapeutic range
- administration method

- the stage of the disease or disorder
- patient compliance

For example, absorption can be affected by the presence or absence of certain foods in the stomach. This can alter the concentration of drug that enters tissue to outside the therapeutic range (illustrated in Figure 2.1), resulting in toxic or ineffective concentrations at the site of action [7]. Also, metabolism and drug transport via blood plasma can expose the drug to numerous inactivating enzymes, hydrolysis, vascular barriers, and elimination sites (such as the kidney), which can significantly reduce bioavailability [8], [9].

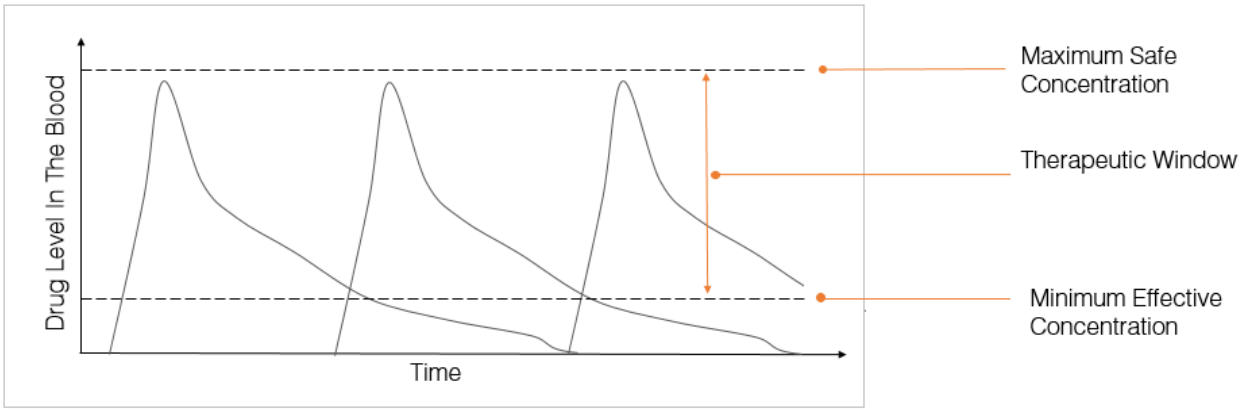

Figure 2.1. Representation of a therapeutic window for an orally administered drug. Each peak indicates a drug dose and its relevant concentration in blood plasma. Dashed lines indicate the upper and lower concentration levels between which appropriate efficacy and low toxicity are observed.

2.1.2 Polymeric materials in medicine

As far back as 2000 B.C., naturally found polymeric materials such as linen (from the flax plant), silk (from cocoons), and horsehair were used successfully in medicine [10]. The main application for many of these materials was suturing due to the strength and compatibility of the polymers in wound closure [10]. Innovations during the last century have led to the development of new synthetic polymeric materials for biomedical applications and likewise significant improvements in medical care [11]. For example, wartime surgeons were forced to make use of available materials from airplanes, clocks and automobiles, in turn, discovering the success of titanium, stainless steel, ceramics, polyurethanes, silicones, nylon, Teflon® and methacrylates as biomaterials [12]. This success prompted chemists and polymer scientists to develop novel polymeric materials, techniques for controlling and modifying reactions, and polymer combinations that could be developed into new products with clinical applications [11].

As a result, polymers are now found in almost every medical application, from ophthalmology (contact lenses, intraocular lenses), dentistry (implants), cardiology (heart valves and pump diaphragms), to widely used medical tubing (silicones, polyurethanes,

Teflon®) [12]. This success is anticipated to continue in the coming years, with the global market for implantable biomaterials estimated to grow 6.7% between 2014 and 2019, to a total of $109.5 billion USD [13]. In addition, with the variety in polymer compositions and assemblies possible, the market for *implantable* biomaterial devices accounts for only a fraction of the total biomaterials market.

2.1.3 Synthetic polymers

Natural and synthetic polymers have both been of interest to researchers for their potential use in biomedical applications. Many natural polymers have the inherent advantage of already being found in the body, thereby reducing the potential for toxicity or foreign body response [14]. Examples include hyaluronic acid (found in extracellular matrix, cartilage) and collagen (found in connective tissue, hair, basement membrane) [15]–[17]. Biocompatibility is not limited to natural polymers however, and conservatively, hundreds of synthetic polymers and scaffolds have been shown to be inert or elicit an acceptable biological response when implanted [18], [19]. For example, intraocular lenses used to replace the natural, clouded lens in cataract patients have been composed of generations of synthetic materials, from poly(methyl methacrylate), to silicone rubber to modern, foldable acrylic based lenses [20]. Thus, with expanding knowledge of host-material interactions, synthetic polymers have shown, and continue to show great progress in becoming superior materials for biological applications.

2.1.3.1 Advantages and limitations

Polymers found naturally often show significant batch to batch variation between different plant, bacterial or animal sources, and between extraction methods used for purification [21], [22]. This can result in large variation in composition, molecular weight, physical properties, rate of degradation and activity [23], [24]. In addition, chemical modifications may need frequent re-optimization in order to adjust for differences in the number of reactive groups, solubility and viscosity of natural polymers [25]. In contrast, synthetic polymers are composed of monomeric units which can be obtained in near pure form, reducing the variability in the starting materials as well as the final properties [26]. Advanced polymerization processes also contribute to synthetic polymers with more controlled properties. Further, many polymers are readily available from large-scale manufacturers, reducing both time and cost of final material production. Thus, despite the inherent success of natural polymers in biomedical applications, synthetic polymers may offer better manufacturing control. This lends support to the use of synthetic polymers in biomedical applications, as they may assist in the production of devices capable of meeting stringent regulatory standards, particularly at manufacturing stages.

2.2 Drug Delivery

2.2.1 Modeling Diffusion

Diffusion is the process of the molecules spreading/moving from high concentration to low concentration as a result of Brownian motion (random walks) [27], [29]. In order to quantify or model molecular movement mathematically, one can begin with the

movement of a particle forward or backward in one dimension. At any point, the particle is equally likely to travel forward or backward (random walk). To determine the distance travelled by the particle, simple summation of the steps would equal to zero due to the backward step being in a "negative" direction. Thus to correct for this, the distance travelled is squared, giving

$$x^2 \propto N \tag{1}$$

where x is the distance travelled and N is the number of steps. Given that the number of steps is dependent on time, this can be then understood as

$$x^2 \propto \Delta t \tag{2}$$

Plotting this, with time as this independent variable, the slope of the resulting linear curve gives the diffusivity (or diffusion coefficient, D) of that particular particle in the particular medium, giving

$$x^2 = Dt \tag{3}$$

2.2.2 Diffusivity

Diffusivity can be simply understood as the amount of motion a molecule experiences (or its mobility) within a particular medium [30]. Factors affecting diffusivity include the molecule's radius (a), the solvent viscosity (n), thermal agitation (Boltzmann's constant) (k_B) and temperature (T), which can be related through the Stokes-Einstein equation (4), specifically for hard, spherical molecules in liquid solvents:

$$D = \frac{k_B T}{6\pi a n} \tag{4}$$

In the context of drug delivery, diffusivity is influenced by the interactions between the polymeric structure of delivery device and the particular drug in question. With a higher diffusivity, it has been suggested that the dynamically fluctuating polymer structure better accommodates the random motion of the diffusing molecule [30]. Conversely, as diffusivity lowers or where the temperature of the system is close to the T_g (glass-transition temperature) of the polymer, the polymer chains will not hold as much free volume or will obstruct drug diffusion and thus, drug release will be reduced.

2.2.3 Diffusion

Diffusion occurs when there is a high molecule concentration and a surrounding medium with no (or low) molecule concentration. It is described through the flux (J): the net rate of particle movement per unit area [29]. Specifically, molecules will move towards locations with lower concentrations as the flux of molecules is proportional to the concentration gradient [29]:

$$J \propto \frac{\partial C_x}{\partial x} \tag{5}$$

Then, by including the proportional effects of diffusivity of a particle onto the system, one obtains Fick's First Law in one dimension [30], [31].

$$J_x = -D\frac{\partial C_x}{\partial x} \quad (6)$$

Here the negative sign indicates movement in the direction of the lower concentration, and gives a positive overall flux [27]. This equation can be used to determine the flux at any particular position during the random walk. However over time, the concentration gradient becomes less steep as the initial concentration is reduced (such as a depleting reservoir of drug) as depicted in Figure 2.2.

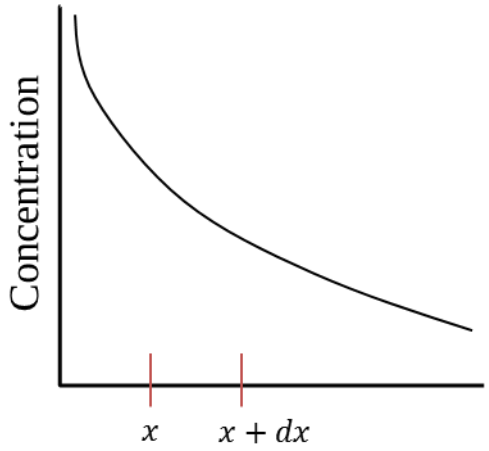

Figure 2.2. Flux of a molecule moving from a location of high concentration to low concentration.

In this case, the concentration gradient $\frac{\partial C_x}{\partial x}$ at x is steeper than at x+dx. According to Fick's first law, the flux is then higher at x than at x+dx. Therefore as molecules diffuse, the concentration also changes with time ($\frac{\partial C_x}{\partial t}$) and then to determine this rate of change, one can look at the difference between the flux in and flux out of a infinitely small volume between x and x+dx [27]. Simplified, this gives:

$$\frac{\partial C_x}{\partial t} = -\frac{\partial J_t}{\partial x} \quad (7)$$

Then, substituting Fick's first law into the equation gives Fick's second law in one dimension,

$$\frac{\partial C_x}{\partial t} = D\left(\frac{\partial^2 C_x}{\partial x^2}\right)_t \quad (8)$$

which states that the rate of change in concentration will depend on the diffusivity of the molecule in the medium and that a higher rate of change in concentration gradient produces a more rapid change in concentration [32]. In the context of drug delivery, where the delivery device is loaded with a high concentration of drug, and the surrounding medium contains little or no drug, Fick's laws can be used to understand the

fundamental theory behind the drug release and model the rate of release of drug from the delivery system.

Overall, drug release/drug delivery is a process influenced by numerous factors, including drug dissolution, drug diffusion, polymer dissolution, polymer swelling and water diffusion [30]. When examining drug release from a system, it is the slowest or rate limiting process that has the largest effect on the rate of drug release [30]. The rate-limiting step is specific to the type of delivery system and often also the polymers and molecules involved. Therefore, the particular device and drug molecule of interest must always be considered together to understand particular release rates and characteristics.

2.3 Kinetics

2.3.1 Zero order

As described earlier in Section 2.1, drug delivery for biological applications would benefit from a controlled or sustained system that maintains drug levels within the therapeutic window. Controlled release can be most simply seen in systems exhibiting zero-order release kinetics,

$$\frac{\partial C}{\partial t} = -k_o C^o \tag{9}$$

where C^o is the initial drug concentration and k_o is the rate constant (a combination of multiple components including diffusivity, and surface area) and is experimentally observed as a constant mass of drug released/time.

This type of drug release can be seen in (in principle) when a drug tablet is coated by a water-soluble polymer[1] [30], [33]. In an aqueous environment, the drug core will dissolve until a point of saturation and the polymer membrane will swell into a gel as the chains disentangle [30]. Drug release occurs through the diffusion of the drug from the reservoir, into the surrounding membrane and then into the surrounding medium. The drug in the membrane holds a saturated concentration of drug that is constantly replenished by the interior reservoir until the reservoir concentration falls below saturation [30]. Thus, a constant amount of drug is released into the medium, i.e. release is concentration independent, until the reservoir concentration is too low to maintain the initial concentration gradient/release rate [30].

While the reservoir is saturated, zero order kinetics can be seen experimentally as depicted in Figure 2.3.

[1] As noted earlier, drug release will be ultimately determined through numerous factors, including the characteristics of the drug and the polymer.

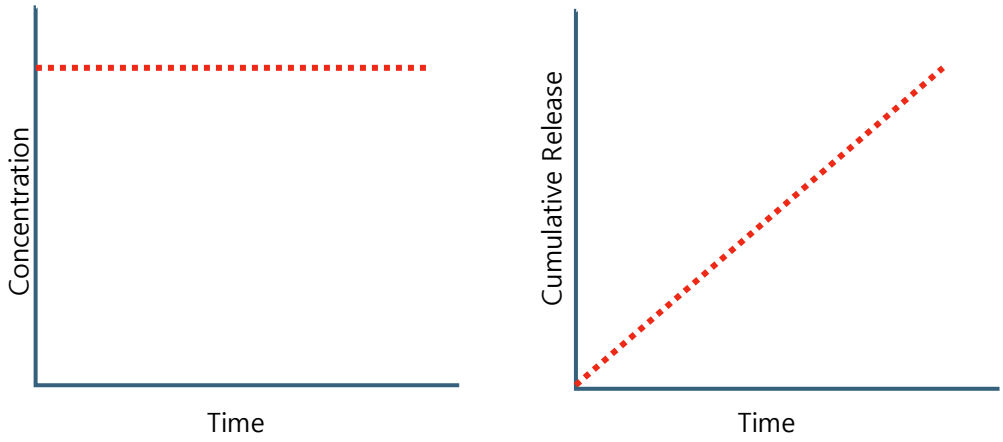

Figure 2.3. Zero-order kinetics.

Although highly desirable in drug delivery applications, zero-order kinetics are difficult to achieve in practice. In a membrane-reservoir system as described, defects such as cracks in the membrane will cause increased diffusion of water. This can increase the internal hydrostatic pressure of the membrane and lead to uncontrolled drug release through both the membrane and the cracks [30]. Alternatively, in the example of a system where drug is dispersed throughout a polymer (rather than as an interior reservoir) a burst-release of the drug will be noted. This burst is due to the rapid release of drug found closest to the surface of the polymer membrane [30]. Then, the interior drug must diffuse a further distance to be released and the release rate is affected [30].

2.3.2 First order

First-order release kinetics are typically depicted by sustained release systems where the drug release rate decreases as the concentration of drug inside the delivery device decreases [34]. For example, these kinetics are seen at the end of the zero-order reservoir release described earlier, or when the drug concentration in the reservoir is loaded below the solubility limit [30]. Thus, in this type of system, drug release is proportional to the drug concentration and can be simplified to:

$$\frac{\partial C}{\partial t} = -kC \;\; \boldsymbol{or} \;\; C = C_o e^{-kt} \;\; \boldsymbol{or} \;\; logC = logC_o - \left(\frac{kt}{2.303}\right) \qquad (10)$$

Here k is the proportionality constant, C is the released drug amount at time t and C_o is the initial drug amount. Graphically (Figure 2.4) this is depicted by a strong burst release of drug with the initial high drug concentration, followed by a proportional decrease in drug release over time.

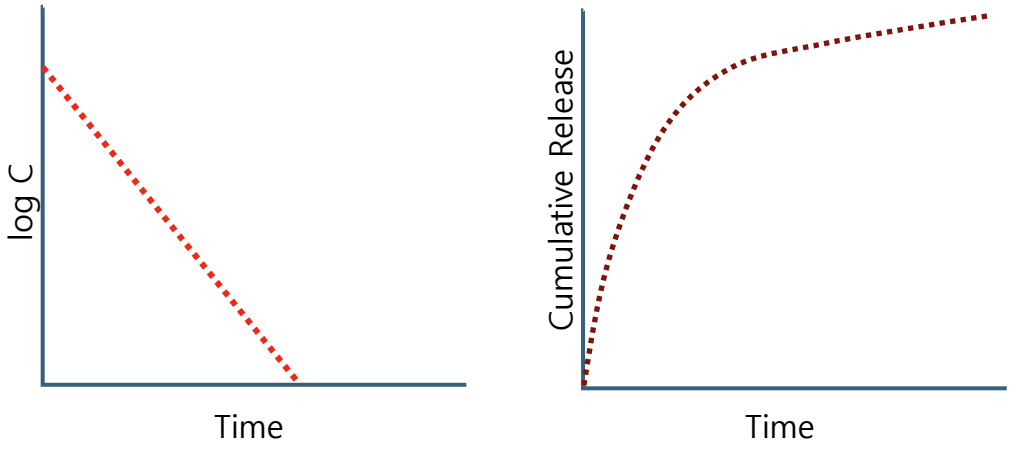

Figure 2.4. First-order kinetics.

First-order kinetics are very typical of drug tablets with no membrane coating, and are thus are not ideal given that the amount of drug released varies with time and does not provide strong support for sustained release levels within a therapeutic window.

2.3.3 Higuchi

In 1963, Takeru Higuchi developed the now famous Higuchi equation that helps to model diffusion-based drug release from drug delivery systems where the drug is dispersed evenly throughout the polymer matrix. The initial derivation was specific to ointment based drug delivery directly into the skin, but the formula has been expanded and proven applicable in numerous other systems with varying geometries. Still, in order to apply the model, multiple assumptions must be true, as follows [30], [35]:

The initial drug concentration must be much higher than the drug solubility ($C_S/C_O \leq 1$)
Perfect sink conditions must be maintained in the surrounding medium throughout the entire experiment
The drug particles loaded into the device must be much smaller in diameter than the thickness of the slab
The drug is initially evenly distributed throughout the film
The dissolution of the drug particles in the matrix is rapid compared to the diffusion of the drugs through the matrix
The (membrane) slab must be very thin so that edge effects are negligible
The slab must not swell or degrade (or changes must be negligible)
The coefficient of diffusion of the drug must be constant (does not depend on time or position within the film)

With these assumptions in place, Higuchi was able to develop a simple mathematical model to understand the release within matrix dispersed formulations:

$$C_t = k\sqrt{t} \qquad (11)$$

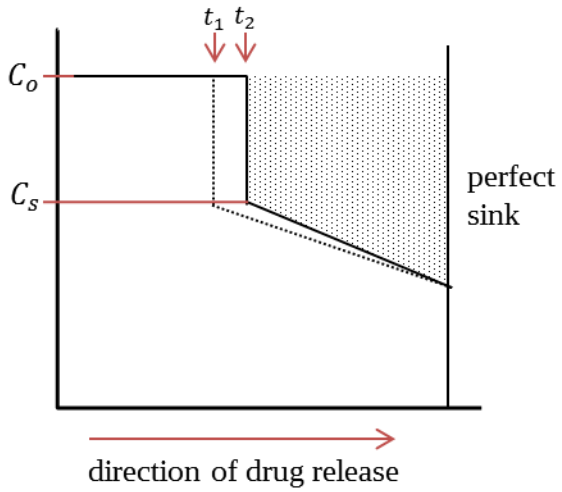

Figure 2.5. A representation of the model assumptions used to define the Higuchi equation.

In Figure 2.5, the drug molecules already dissolved in the matrix closest to the surface are able to diffuse out quickly due to the perfect sink conditions and according to Fick's laws. Since a large excess of drug is dispersed throughout the matrix, the zone of released/depleted drug near the surface is rapidly replaced with newly dissolved drug and that drug is also able to diffuse out of the matrix. This concentration gradient within the matrix forms a "moving front" [30] and an assumed pseudo-zero order (linear) concentration profile is seen throughout the matrix. This leads to diffusion based drug release from within the matrix.

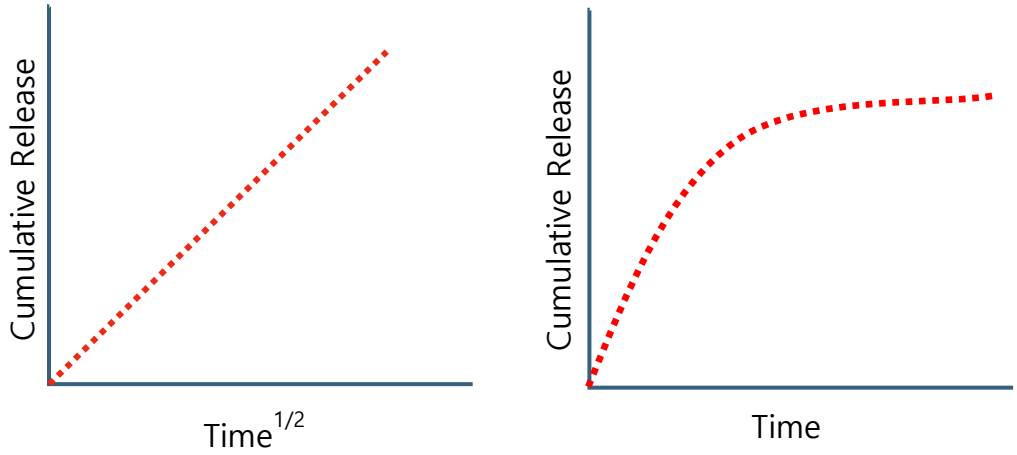

Figure 2.6. Higuchi kinetics.

Since this initial work, numerous variations and alterations to the equations have been made to better fit experimental release profiles of delivery systems. For example, it has been reported that the Higuchi equation alone is accurate in predicting release with a

maximum error of 13% [30]. Alterations to the equation have shown to be more accurate (within 0.5%) [30]. For example, where certain conditions are not satisfied (such as release from a sphere or cylinder), variations of the equation help to more accurately predict the release [30], [35]. Further, the original model assumes diffusion only through the polymer-matrix phase, however, this may not always be the only mechanism. In cases where a drug may be poorly soluble inside a polymer matrix, the drug will be able to diffuse out through the channels created by its own dissolution [36]. In this case, the drug release will depend on the drug loading concentration and the size of the drug and additional fitting is required to most accurately predict the drug release [36].

2.3.4 Korsmeyer-peppas

In cases where the previously described models do not fit experimental data well, the Korsmeyer-Peppas equation is generally used to determine the type of release involved [37]. The semi-empirical equation relates drug release and time exponentially as follows,

$$\frac{C_t}{C_\infty} = kt^n \qquad (12)$$

where k is the constant specific to the geometry and characteristics of the polymer matrix, $\frac{C_t}{C_\infty}$ is the fractional release of drug at time t and n is the diffusion coefficient. For a thin slab, differing values of n indicate the type of drug release mechanism as seen in Table 2.1.

Table 2.1. n values for thin slabs that indicate the drug release mechanism

Release exponent ($\boldsymbol{n}$)	Type of Drug Transport
n = 0.5	Fickian diffusion
0.5 < n < 1	Anomalous transport
n = 1	Polymer swelling

With a polymeric delivery system in particular, if the polymer swelling is more rapid than drug diffusion, drug diffusion will be the dominant release mechanism and thus the release is best understood by Fickian diffusion. If diffusion is much faster than polymer swelling, then n will be closest to 1 and therefore the release is controlled more by the relaxation of the polymer chains in the matrix [38]. In cases in between, a combination effect of diffusion and chain relaxation are at play [30].

The Korsmeyer-Peppas equation includes a few simple assumptions that must be valid, including that drug release occurs in one dimension and the length to thickness ratio should be at least 10. Additional variations of the equation exist for models where burst release or a lag time before release are involved [37]. Further variations are applied in the case of altered matrix geometries (e.g. cylinder, sphere) [37]. Overall, the model provides a simple initial analysis for drug release curves which may not follow simple zero-order or diffusion based release.

2.4 Silicone Materials

2.4.1 Properties

Silicones are polymers composed of repeating siloxane units, and more specifically a chain of altering Silicon and Oxygen atoms as depicted in Figure 2.7. Generally, the silicone-oxygen backbone provides silicones with a unique combination of high temperature stability and elastomeric properties at low temperatures [39]. Then by altering the chain length or side groups, properties can be altered from ultra-thin substances to greases and rubbers [39]. As a result, today there exist (conservatively) hundreds of silicone products and applications.

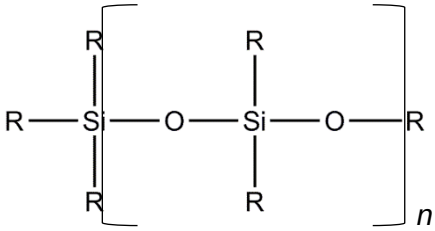

Figure 2.7. Standard molecular structure of silicones. R groups can represent varying side chains.

Silicon is found just below carbon in the periodic table, however the greater electronegativity of silicon results in more polar bonds compared to carbon, such as the Si-O bond which has high bond energy. High bond energy is correlated with a strong bond, a bond that is less likely to break apart [40]. This fundamental property contributes to the explanation for the stability of silicones in a variety of environments [41]. The side groups, typically methyl groups, then further help with stability by shielding the Si-O backbone and producing low surface energy [41]. With the methyl groups pointed to the outside, this gives very hydrophobic films [41].

The structure of a silicone system is further influenced by the high flexibility of the siloxane chain, which has few barriers to rotation and can adopt many conformations [39], [41]. As a result, silicone polymers tend to form helices with a large free volume and compressibility [39]. The high free volume (compared to hydrocarbons) produces high solubility and diffusion of gases into silicones, including oxygen, nitrogen and even water vapor (although liquid water is not capable of wetting a silicone surface) [41].

2.4.2 Production

Silicone chains are generally produced through the initial hydrolysis of certain silanes (such as dimethyldichlorosilane) resulting in a disilanol, followed by condensation polymerization in the presence of an acid catalyst (Figure 2.8).

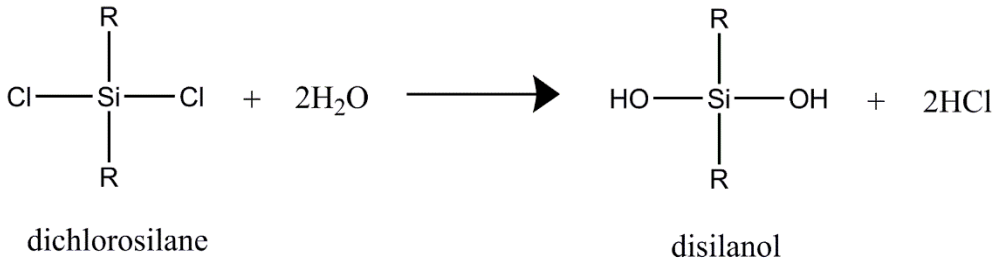

Figure 2.8. Hydrolysis of silanes to form silanols.

For silicone gels, elastomers and the sort, the siloxane chains are cross-linked (or "cured") through three main mechanisms [42]. In the first, the use of tri- or tetra-functional siloxane comonomers, allows for polymerization to occur at multiple branch points, creating crosslinks between chains (Figure 2.9).

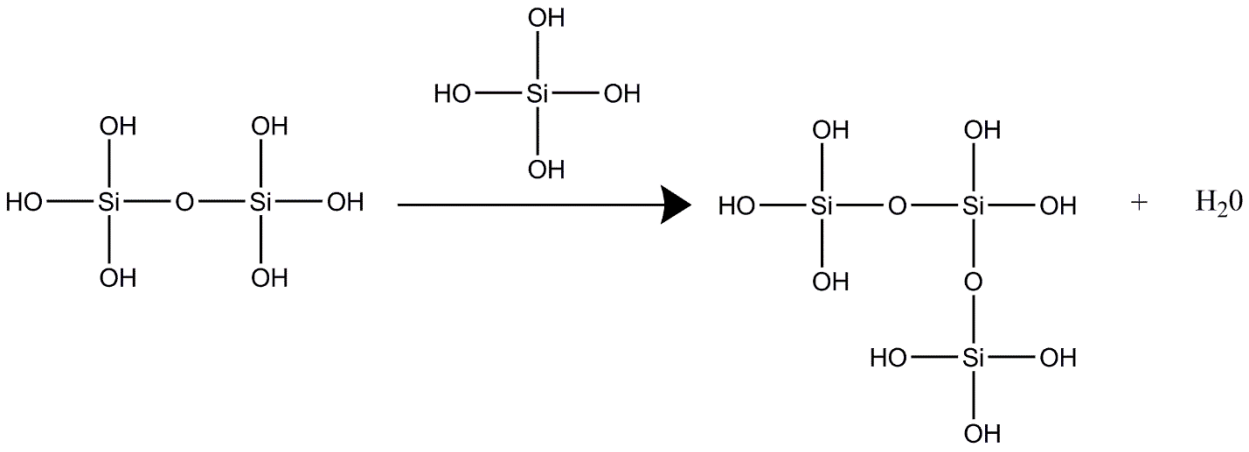

Figure 2.9. Polymerization of silicones.

A peroxide decomposition forms the radicals that initiate the reaction and by-products remain in the final product. In the second, addition reactions can be achieved through platinum catalysis of Si-H and Si-vinyl groups (Figure 2.10).

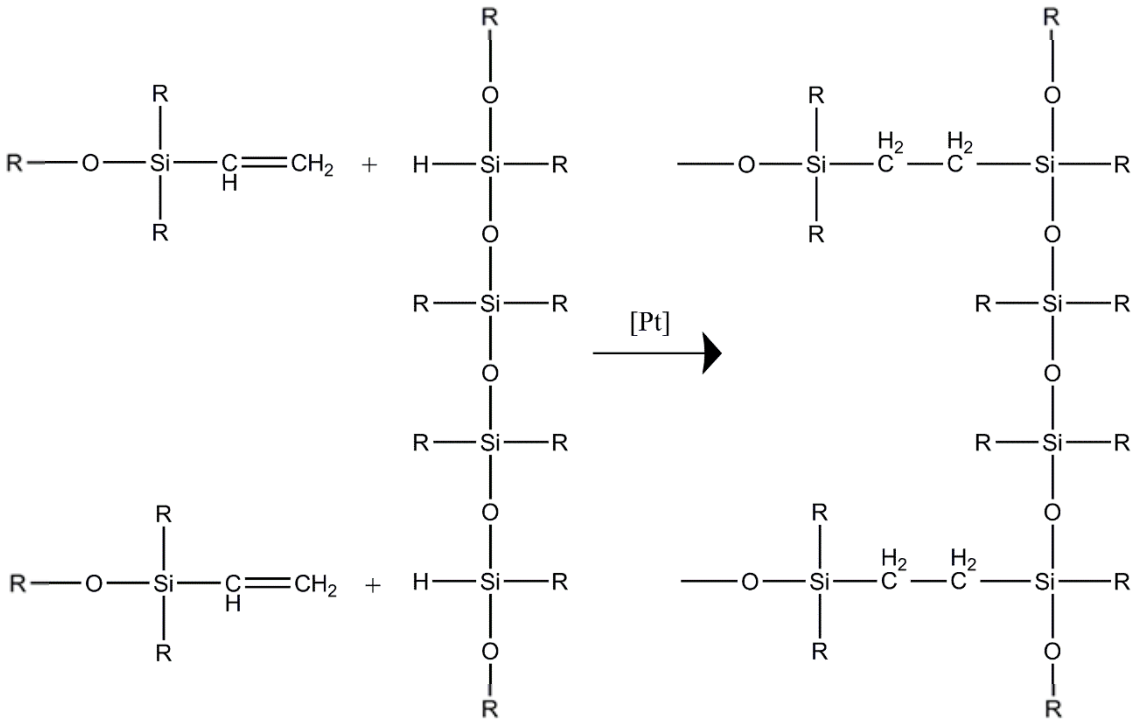

Figure 2.10. Platinum based polymerization / crosslinking of silicones.

This addition cure has no by-products [43]. In the third mechanism (Figure 2.11), a tin-cured silicone can be produced through the condensation of a silicic acid ester and a siloxane [44].

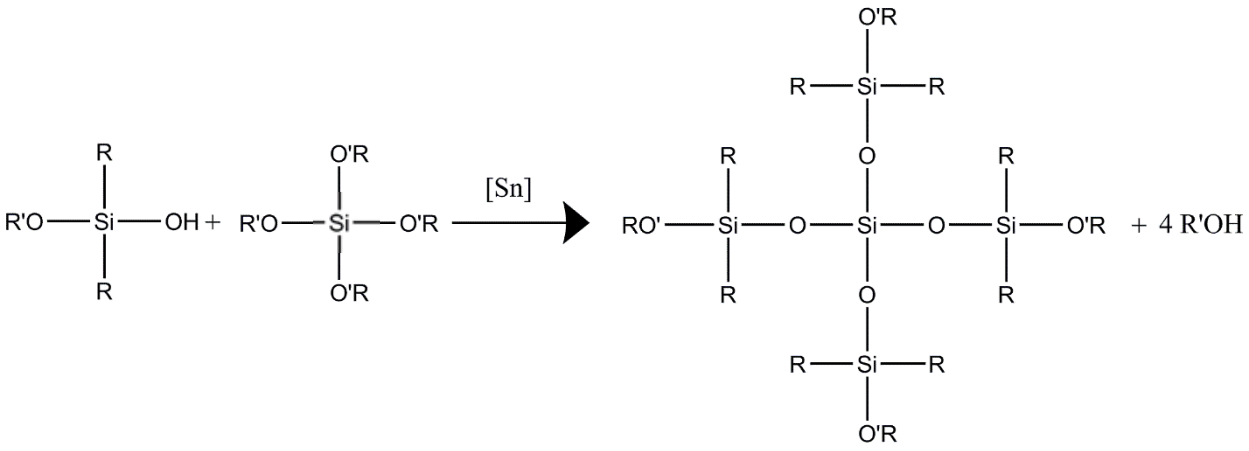

Figure 2.11. Tin-based polymerization / crosslinking mechanism of silicones.

Tin-based silicones are generally less expensive than platinum, and are not prone to inhibition through additive compounds, however in contrast to platinum products, tin is generally toxic in biological settings and cured products can become brittle within only a few years [45].

2.4.3 Biomedical applications

The properties of silicones identified earlier (low surface tension, chemical and thermal stability), led to its first tested medical use in the 1940's by Canadian researchers. Researchers discovered the ability of hydrophobic silicone coated syringes to preserve blood samples by preventing coagulation [41]. This fundamental work led to the extrapolated application of silicones to many varying biological applications and a current collective perspective of silicones as good biomaterials [39]. Today, silicones can be found as components of numerous products including pacemakers, prostheses, artificial skin, contact lenses, oxygenators, and medical adhesives [39].

In the field of drug delivery, (since the 1960's) silicones have seen similarly high uptake. As noted earlier, the structural features of silicone (e.g. high free volume), sparked early studies examining the permeation of gases in silicones and today, this fundamental work has led to the incorporation of silicones into many drug delivering devices, as a matrix, membrane and coating [39].

Silicone has especially found success as a steroid releasing device. Steroids are inherently very hydrophobic (being based on a four-ring skeleton), and thus are highly soluble in hydrophobic silicone. This translates to the ability to load large drug quantities into the matrix, and in turn, high drug loading generally allows for longer, more sustained drug release. Further, the low molecular weight of steroids allows for relatively rapid molecular diffusion through the free volume of the silicone matrix. For example, 3M's Climara® is an adhesive silicone-based film that is applied weekly for the sustained, transdermal release of estradiol. Mirena® is a PDMS based intrauterine ring which is able to release levonorgestrel for up to 5 years. The release from PDMS matrices follows that of matrix and reservoir-type devices and depends on the diffusivity and solubility of the drug in the system [46].

Further advantages to the use of silicone in biomaterials applications are seen during production. Silicone is very cost effective and can be fabricated into a remarkable variety of moulds and forms as noted earlier. It can be cured without excessive heat and is generally both chemically inert and non-toxic. By crosslinking with platinum, the biological safety is especially prominent as this method does not produce any volatile by-products and has been approved by the FDA.

As with any material, a universal biological application is difficult to achieve with a single formulation, and thus the application of silicones in the body does have its limitations. The inherent hydrophobicity which has been successfully levied in steroidal drug delivery, also poses restrictions to its long-term implanted success primarily by the buildup of proteins on its surface. One way this has been tackled is by the development of silicone hydrogels.

2.4.4 Silicone hydrogels

Hydrogels as biomaterials and delivery devices have become extremely popular due to their high water content, softness, flexibility and biocompatibility. Similar to silicone chemistry, the possible variations of hydrogels are practically limitless [47]. They have become a great foundation of numerous new research fields, including in the development of tissues engineering (e.g. tissue scaffolding, extracellular matrix replacements) and 'smart' (stimuli responsive) materials. At the clinical level, silicone hydrogels have especially achieved immense success as the base materials of contact lenses [47]. Silicone hydrogels have been altered through the addition of NVP or other hydrophilic compounds, and/or plasma treatment to improve the hydrophilic character of the material. Still, like silicones alone, silicone hydrogels can be prone to protein deposition through the exposure of the silicon group to ophthalmic proteins such as lysozyme (a fouling protein) [48]. After almost 20 years of research, a drawback of the silicone component continues to limit the use of contact lenses for some patients by contributing to the end of day dryness/irritation, and resulting in discomfort to the user and limiting wear time [49]. [50].

2.5 Poly(ethylene glycol)

2.5.1 Properties

Poly(ethylene glycol) is a synthetic, linear polymer composed of repeating ethylene glycol units $–[CH_2CH_2O]-$, and is standardly produced with one or two terminal hydroxyl groups. The polymers are identified by their chain length, which is indirectly determined through its average molecular weight (in g/mol or simplified as MW). To approximate the corresponding number of ethylene glycol units, the molecular weight can be divided by 44 (the molecular weight of ethylene oxide) [51]. PEG is historically referred to as poly(ethylene glycol) for molecular weights below 20,000 and poly(ethylene oxide) for molecular weights above 20,000, although the exact reasoning behind the nomenclature remains debated and differing definitions continue to exist [52]. Physically, PEG is available in a variety of physical states which depend on the molecular weight. Below 700 MW, PEG is a clear liquid at room temperature with an oily consistency [53]. Between 700 and 900 MW, PEG is a semi-solid and at 1000 MW, PEG is a waxy solid [53]. Above 1000 MW, PEG is available as flakes or free-flowing powder [53].

PEG is popularly perceived as a simple, non-ionic, hydrophilic polymer in the fields of science and engineering, however, the polyether (C-C-O) backbone of PEG provides it with many unique properties that are still not fully understood, and are not seen with other similar polymers [54]. The many rotations possible around each of the C-C and C-O bonds provide high conformational freedom and inherent flexibility, wide solubility, molecular weight effects and surfactant properties.

The three possible PEG conformations that have been commonly found are all-trans, alternating trans-gauche-trans, and trans-trans-gauche [55]. In its crystalline form, high-molecular weight PEG forms a 7/2 helical secondary structure (seven monomer units

form two helical turns) consisting of “ttg” sequences [56]–[59]. This conformation internalizes oxygen atoms and externalizes methylene groups [52]. When placed in an aqueous environment, the PEG molecules take on a secondary structure in the form of a broken helix, with short helical sections connected by random coils [52]. This change in structure is due to both the flexibility of the PEG backbone, as well as the ability of the PEG to exhibit hydrogen bonding, between atoms on the PEG molecule itself, and between water molecules and the oxygen atoms of PEG (this also contributes to its hydroscopic property) [61]. Notably, low molecular weight PEG chains are known to exhibit less order, but the interpretations of the chain disorder can vary in the literature [60].

2.5.2 Production

In 1859, Lourenco first described the synthesis of PEG, establishing its composition but not knowing its structure given that the concept of polymers did not exist at the time [62], [63]. From then short-chain oligomers of PEG were developed, and very slowly higher molecular weight PEGs were synthesized as well. It was not until 1958 that the first commercially synthesized PEG was made available by the Union Carbide Corporation under the trademarked name of POLYOX® [62]. At around the same time, researchers were beginning to develop more rapid and controlled polymerization methods utilizing catalysts, and today, the production of PEG is so well defined that polydispersity indexes of 1.01 are readily available at relatively low cost [64].

The production of PEG begins with the highly reactive intermediate ethylene oxide. This compound is typically used as a fumigant or sterilizing agent as it is highly diffusive and is able to enter cells reacting as an alkylating agent with cellular components which disrupt vital functions [65]. Ethylene oxide (EO) is highly reactive primarily due to the large ring strain within the structure [62]. The COC and CCO bond angles are ~60° within EO [66], whereas carbon atoms normally form single bonds (in a tetrahedral arrangement) at ~109.5° [67]. The overlap in orbitals creates repulsion between the atoms, while ring-opening relieves this stress and thus becomes the first stage of the polymerization mechanism.

Ethylene oxide first reacts with an initiator in a base catalyzed reaction also known as anionic polymerization in the following way:

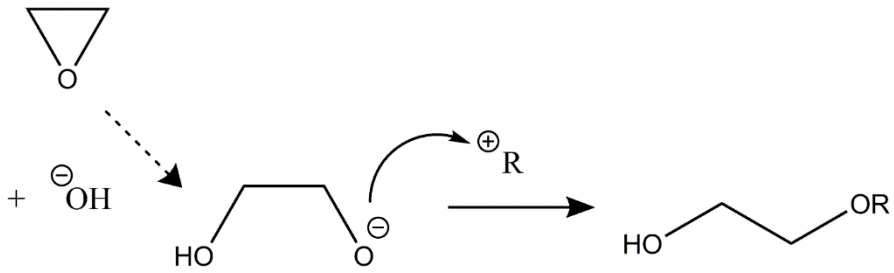

Figure 2.12. Polymerization of ethylene oxide.

Here R can represent many initiators including alcohols, water and hydrogen halides. More specifically, the gaseous EO is passed over the catalyst and initiator using a controlled process. The reaction repeats and propagates the chain extension one ethylene oxide unit at a time. The process is terminated by limiting EO or neutralizing the catalyst. By using an ethylene glycol initiator (HO-C-C-OH) or water, the common dihydroxy-terminated PEGs can be produced.

Anionic polymerization occurs rapidly and the mechanism in Figure 2.12 typically results in low molecular weights. These limitations are a result of alternative reaction pathways that can regenerate the catalyst or result in unsaturation (double bond formation) at the end terminal, effectively preventing further polymerization [62]. Yet despite these limitations, anionic polymerization has been the main method of PEG production since the 1930's [68].

To produce higher molecular weight products using this method, a higher-molecular weight PEG can be used as the initiator (e.g. PEG 600 to produce PEG 1000) or alternative initiators can be used such as alkali metal hydrides and amides [62], [68]. Acid catalysis is also possible, however, up to 20% of EO is converted into undesired side-products and thus this polymerization method is rarely used [62].

2.5.3 Interaction with water

PEG is very unique given its high water solubility at temperatures below the boiling point of water [69], [70]. This hydrophilicity is surprising when in contrast, with one less methylene (CH_2) group, poly(methylene oxide) is hydrophobic and insoluble in water [54], [71]. Likewise, with only one additional methylene group, poly(butylene oxide) is not soluble in water [54], [71].

The balance of hydrophilic and hydrophobic forces within PEG cause this high water solubility [71]. Specifically only in PEG does the oxygen-oxygen distance along the PEO helical backbone (4.7 Å) match that of water with its next nearest neighbour [71], [72]. This strong ability to hydrogen bond with water induces local order at the molecular surface. This cage of water molecules also shields the hydrophobic methylene groups, further encouraging mixing/solubility [58], [71].

Also uniquely, due to the flexibility of the PEG chain, each ethylene oxide unit is able to hydrogen bond with 2-3 water molecules, creating the very structured water shell and producing a hydrodynamic radius 5 to 10 times larger than proteins of a comparable molecular weight [64], [73]. Relaxation-time studies further show rapid motion of the polymer chain [74]. This hydrophilic property of PEG has been heavily exploited in pharmaceutical and biomaterials development as described later in this review.

PEG tends to exhibit more amphiphilic properties in water than is presumed by its popular 'hydrophilic' label [54], [73]. For example, PEG is able to form thin monolayers at an air-water interface [75]. This is surprising because both lipids and surfactants behave in this way, yet these molecules have distinct blocks of hydrophobic and hydrophilic units whereas PEG is a single repeating unit [54]. This again is due to the high conformational

freedom between atoms of the PEG chain, producing detectable conformations that can be polar or non-polar [54], [73].

The equations used to model the structure of PEG in solution are the same as those used to model random coil polymers, despite the irregular helical PEG shape [52]. Flory developed these models using first principles of molecular motion to define equations for describing the motion of polymers in varying solvents and finding that in the absence of external forces, the polymer chain will adopt an overall spherical shape [52], [76], [77]. Summarized simply in Equation 12, this work helps to describe the unique characteristics of PEG with a wide variety of solvents.

$$R_f = aN^v \tag{13}$$

The radius of the polymer coil (R_f) is proportional to the segment length (a) and the number of segments (N). v is the solvent quality or excluded volume component and R_f is strongly influenced by this component. To understand this simply using the context of earlier introduced principles, similar to the varying diffusivity of a molecule in a particular solvent, a polymer coil is also influenced by the solution in which it is found.

The radius is influenced by the balance between the van der Waals forces (and/or hydrogen bonding where applicable) between polymer segments and the entropy of mixing [78]. In a poor or bad solvent, polymer-polymer interactions dominate over the entropic [78]. More simply, there is less penetration of the solvent into the polymer coil and thus the polymer coil shrinks, resulting in a lower R_f and possibly precipitation [52], [78]. A good solvent (or high temperature) will favour entropic mixing (greater penetration of the solvent) and the polymer coil will expand producing a greater radius [78]. For example, PEG is poorly soluble in silicones, forming visible phase separation, but is highly soluble in water [79].

2.5.4 PEG interactions with biological components

The unique features of PEG described in this section have found further uses in medicinal and cellular applications. Clinically, PEG has achieved success as a front-of-the-line laxative due to its ability to retain water, softening the stool and increasing bowel movements [80]. Even at high concentrations, PEG is generally well tolerated and shows low immunogenicity [73]. PEG is also present in solutions for blood and organ storage, as it is able to reduce aggregation of cells and proteins [81]–[83]. PEG in copolymer formulations has also been shown to improve the success of cardiovascular devices such as stents by improving the host reactions to the material (reducing thrombosis) [84].

However, PEG is not only unique because it has found success in such a variety of applications, rather PEG also exhibits unique characteristics when exposed to cells, with mechanisms that are still debated and not well understood. Work in this area adds a further intriguing look at the impacts of PEG in biological settings, from influencing protein function, fusing membranes, causing localized and organ toxicity, and even preventing cancer cell growth.

2.5.4.1 Membrane fusion

The process of antibody production has remained unchanged since 1975 [85], [86]. This process relies heavily on membrane fusion, which is most commonly facilitated by the use of PEG [86], [87]. Briefly, laboratory mice are immunized with an antigen and adjuvant [86]. After a few weeks, the mouse spleen is collected and myeloma cells (cancerous white blood cells) are fused with the mouse B lymphocytes (white blood cells that produce antibodies) to produce long-term antibody-producing cells (aka hybridomas) [86]. This membrane fusing property of PEG has been more recently applied to studies focused on correcting spinal cord injuries as well [88], [89].

For many years, the mechanism by which membrane fusion occurs (in normal cells and in hybridomas) was debated and complicated by model-specific variations. For example, triggering fusion using calcium or detergents induced membranes to fuse, but also caused high leakage of membrane contents [90]. However, successful membrane fusion is defined by the mixing of entrapped cell contents with minimal leakage. This was found to be well induced by PEG, and as a consequence, there exists a significant amount of work examining the mechanisms by which PEG induces membrane fusion in model systems [90]. Ultimately, it was determined that PEG acts via a single major effect; PEG brings cell membranes very close together by causing a dehydrating effect [90].

In a typical suspension of model lipid vesicles, the polar headgroups are hydrated, creating a hydration-dependent steric barrier and repulsion between closely found membranes (preventing membranes from fusing) [91]. When PEG is present near the membranes of two cells (or model lipid vesicles), it creates a point of dehydration at the membrane surface due to its strong ability to hydrogen bond with multiple water molecules, and due to its high chain flexibility [91]. This brief dehydration produces a thermodynamic force that brings membrane lipids out of their lamellar positions and into a brief but stable fusion intermediate [91]. This also leads to asymmetry in the lipid packing pressure between the outer and inner membrane bilayer, further encouraging fusion between the two membranes [91]. A few disparities regarding the exact interaction between PEG and the lipid headgroups exist [92], however the dehydration driving force remains agreed upon.

Not every PEG can be used for membrane fusion, and typically it is a PEG between 600 and 6000 that is applied to hybridoma production [93]. Higher molecular weight PEGs greatly increase a solution's viscosity, making it more difficult to handle in the laboratory setting [93]. Lower molecular weight PEGs are more toxic to cells than higher molecular weight PEGs. The standard hybridoma protocol today lists the use of 50% PEG 1500. Still, regardless of molecular weight, in this application, PEG is known to be toxic to different cells at different concentrations so only approximately 1 minute exposure is recommended [87].

2.5.5 PEG toxicity

PEG is generally accepted as being highly biocompatible in almost any application or administration route. For this reason, it is used widely as an excipient in drug

formulations (e.g. pill binders, surface coatings, lubricants, ointments, etc), in cosmetics and fragrances (e.g. shampoos, hair gels, lipsticks, etc.) and in wound dressings. Still, there have been reports of high toxicity over the years, and more recently, an increase in reported mild to severe allergic reactions to PEGs [51]. Given that health care practitioners are generally not aware of potential toxicity of PEG, excipients are not often listed as part of formulations, and with the many different commercial names given to PEGs, it is supposed that the number of reported cases is severely underestimated [51].

A recent review by Wenande and Garvey identified 37 reports of immediate-type hypersensitivity to PEGs published between January 1977 and April 2016 [51]. Cases of hypersensitivity were reported across all PEG molecular weights commercially available (200-35,000), with the highest number of reports for PEG 3350 [51], [94]. This molecular weight is most often used for laxative purposes prior to colonoscopies and the higher reporting is likely due to the simplified cause-effect observation possible [51]. No correlation to PEG concentration could be determined in this study because concentrations of PEG and other excipients are very rarely reported on ingredient labels [51]. Still, the researchers were able to conclude that patients likely have an individual reactivity-threshold for both dose and molecular weight of PEGs [51].

PEG is regarded as safe with LD_{50}>10 g/kg and PEG up to 10,000 MW are deemed acceptable at exposures of 10 mg/kg [95]. Only a few reported cases of PEG overdose exist, and these have been clinically manifested as toxicity to renal function [95]. For example, Erickson *et al*. described an incident of a 65 year old man who exhibited acute renal failure after ingesting the liquid contents of a lava lamp [96]. The toxicity was mainly attributed to the high content of PEG 200, although additional components were also found in the patient's blood [96]. This correlation was likely drawn due to *in vivo* studies outlining the fate of PEG of different molecular weights. Specifically, PEGs under 400 g/mol are known to be readily absorbed through the gastrointestinal mucosa [51] and can be metabolized to toxic by-products [97].

Higher molecular weight PEGs are generally excreted by the GI, and although there is a correlation between less short-term toxicity with higher molecular weights, there are no data to show less chronic toxicity [97]; the work by Wenande also suggests otherwise. Further, although PEG can be highly purified to produce products with very low PDI, when a high molecular weight PEG is listed in a formulation, this may be the reported average of a mixture of molecular weights, and short PEGs may have been included in order to alter the properties of the high molecular weight PEG (e.g. to reduce viscosity) [98]. This could further complicate understanding of the safety of PEG.

2.5.6 Biomedical applications

Poly(ethylene glycol) has been the gold standard polymer for medical, cosmetic, pharmaceutical and other biological applications [68]. In a recent review article by Hutanu *et al.,* the first half of 2014 alone produced ~23 articles using PEG in drug delivery, and 12 in surface modification/nanoparticle PEGylation. This high use of PEG in biomedical applications has been ongoing (conservatively) for over 20 years – since the first approval of PEGylated products [99]. A few of these examples are described below.

2.5.6.1 PEGylation

PEGs most prominent success can be considered its simple end-terminal conjugation to molecules with less-than-favorable hydrophilicity and/or systemic circulation times. PEGylation is the term coined for the process of conjugating a PEG molecule to another small molecule (e.g. a therapeutic), antibodies, oligonucleotides, nanoparticles or siRNA [64].

PEGylation helps to increase the solubility of molecules in aqueous media, shield compounds from degrading enzymes in blood plasma, and delay their renal clearance, increasing the circulation time and reducing the number of administrations necessary [64], [100], [101]. This is possible because of the unique ability of PEG to create the large ordered cage of water molecules around it, preventing enzymes from accessing the conjugated therapeutic, and increasing the hydrodynamic size of the molecule above the renal clearance threshold (60kDa) [64], [102]. PEGylation further provides improvements to the physical and thermal stability of molecules, preventing/reducing aggregation of the drugs [64]. There are many examples of PEGylated therapeutics on the market, with a range of molecules including proteins, antibodies and small molecules [103].

2.5.7 Altering material surfaces

The ability of PEG to prevent proteins from accessing shielded molecules has further been exploited in alternative applications, a major area being the application of PEG as a surface coating onto materials which exhibit a tendency towards protein adsorption. A great example of this type work is the incorporation of PEG with PDMS (and other silicones), which inherently are very compatible in many biological applications, but due to their hydrophobic character, they are prone to protein adsorption.

Modifications using PEG can vary significantly but can be generally categorized into bulk and surface modifications. PEG can be applied to surfaces (using PDMS as an example) via adsorption, covalent attachment or graft copolymerization [104].

Coating a material with PEG by simple adsorption can be very rapid and simple to perform [105]. In these applications, PEG is typically a copolymer of PEG and an ‘interacting’ molecule (e.g. cationic poly(L-lysine), which can electrostatically interact with a PDMS surface that has been plasma treated (reactive anionic oxygen groups exposed on surface) [105]. Non-covalent coatings are generally temporary however, as the surrounding environment can weaken the attractions at the interface. Changes to conditions such as temperature, flow, osmolarity and mechanical forces can all weaken the protein resistance [105].

There exists a substantial amount of research on covalent linkage of PEG to surfaces. PEG can be grafted directly though a functionalized end group, or via another chemical linker. The chemistry used to bind PEG to PDMS can include platinum-catalyzed hydrosilation between the Si-H group on PDMS and vinyl terminated PEG (PEG-$CH=CH_2$) [105]. This chemistry is also the basis for PDMS crosslinking, which is predominantly used in biomaterials applications as it does not result in by-product

formation. Amine-terminated PEG (PEG-NH_2) has also successfully been grafted to aldehyde-silane, producing a glass surface with a dense layer of protein-resistant PEG [106].

Surface modifications are excellent choices for altering the interface properties while maintaining the desired bulk properties of the material. In cases where the bulk material could also benefit from more hydrophilic character, the introduction of PEG in the material can provide the desired material properties as well as protein resistance. This type of modification has been extensively studied in varying biomedical applications with great success.

Recently, Chen *et al* demonstrated that functionalized PEG could be integrated into the PDMS backbone to create a novel silicone elastomer [107]. The resulting materials exhibited good strength (due to the PDMS monomer), good water uptake (due to PEG), and were able to significantly reduce the adsorption of an ophthalmic protein [107]. In another study by Wang *et al.*, researchers were able to create interpenetrating networks of PEG and PDMS, also producing a silicone hydrogel with a protein resistant surface [105]. This protein repelling property is made possible as a result of the high chain mobility of PEG and its inherent predisposition to hydrogen bond with numerous water molecules (creating the steric/hydrodynamic shield). Overall, reduced protein adsorption onto materials can translate to improved clinical benefits.

This data shows that PEG can act as a highly hydrophilic molecule, but at the same time, it is able to be grafted to- and polymerized with- strongly hydrophobic silicones, without phase separation. This unique behaviour again is due to the high conformational freedom of the C-C-O bonds of PEG. At the interface with silicones, PEG adopts a more non-polar conformation [108], [109]. The C-C-O bonds rotate from gauche to trans, reducing the inherent ability to hydrogen bond with water [73]. Further from the surface, the polymer is exposed to the more aqueous environment, and gauche/polar conformations can be detected [73], [108], [109]. Thus for bulk modifications, as PEG chains are able to penetrate out of the material and away from the material surface, there are more polar conformations taken on and a strong repulsion of proteins remains possible [73].

2.5.8 PEG in drug delivery

PEG on its own does not have the inherent strength to create drug delivery systems with high tensile strength (due to its large hydrodynamic radius and chain flexibility), making it difficult to place such a device in broad biomedical applications [110]. The high water content of such hydrogels can also limit the type of drugs that can be loaded into the system and the rate of release can be quite uncontrolled and rapid [110]. The research has thus been largely focused on improving drug delivery by largely incorporating polymers of differing properties to produce devices with the desired combination of physical properties and release rates. An example of this type of work is the combination of two extensively used biomaterials with very different properties: silicones and PEG.

As noted in Section 2.4, silicone based polymers such as PDMS have found significant success in medicine due to their strong tensile strength, oxygen permeability, general biocompatibility and ability to deliver small hydrophobic compounds for long periods. The limitations of silicones are their inherent hydrophobicity that can lead to membrane fouling by proteins (and device rejection) and poor ability to release more polar compounds. PEG and other hydrophilic (or 'osmotically active') molecules/compounds have been examined as additives to silicones since the early 70's [111].

By the early 90's, fundamental work had been done that demonstrated the release of hydrophobic, hydrophilic and drug salts (e.g. melatonin, estradiol, clonidine hydrochloride) from PDMS based materials [111], [112]. Additives ranging from salts (e.g. sodium chloride), glycerols, PEGs, sodium alginates and others were tested alone and in combination with drugs in order to understand the role of the additives on drug release rates [111]. For example, Hsieh *et al* demonstrated that melatonin and estradiol showed increased release rates in the presence of glycerol, salts and PEG 400, and that the magnitude of change depended on the additive used [111]. In all cases, the drug release maintained a Higuchi type relationship, where the release rate followed a diffusion based square root time dependence [113].

Later work by Fedors *et al* showed that salt crystals specifically caused irregular pores in PDMS materials which led to drug sequestered deeper in the matrix diffusing out through the salt pore [114]. The water diffusing into the pores was also postulated to produce very high local stresses on the crosslinked PDMS network, leading to cracks in the polymer and the formation of additional channels that could release further drug [113], [114]. In the case of sulphanilamide release, it was found that glycerins and PEGs increased drug release but not to the extent of sodium chloride [113], [115]. This was postulated to be due to more rounded cavities formed by additives such as PEG, which in turn could resist swelling stresses better thus resulting in less channel formation [113], [115].

At the same time, other drugs showed the opposite results. Work by Di Colo *et al* demonstrated that prednisolone release was highest with glycerin, and lowest with sodium chloride [116]. Also, salt drugs (such as papaverine hydrochloride) were shown to be able to create their own drug release pathways due to the inherent salt presence [117]. Thus, this growing number of studies testing varying compounds and additives has resulted in a collection of work with varying results dependent on numerous variables within the system and no clear correlation between additive properties, drug properties, swelling, compound concentrations and drug release rates.

In 2008, work by Brook *et al*. began to examine more of the correlations between individual additive properties and drug properties from silicone based materials [118]. They showed that nicotine release could be increased by increasing PEG concentration, and that burst release could be controlled by the addition of another additive (linoleic acid) which increased the solubility of nicotine in the base polymer [118]. Panou *et al.* then further expanded fundamental understanding of these results by more clearly showing the effects of PEG concentration on silicone materials [119]. They were able to

show that increasing PEG 3000 content within a PDMS network interfered with the crosslinking between PDMS chains, creating a looser PDMS network, lower mechanical PDMS strength and greater bulk hydrophilicity, where the movement of water inside the bulk material was osmotically induced [119]. This correlated greatly with previous studies having found consistent diffusion based (square root time) release of drugs from silicone-PEG matrices [119].

Most recently, work by Gehrke *et al* began attempts to model the drug release using diffusion based equations (e.g. fitting Fick's second law to drug release curves) [120]. The model systems examined included various medical grade curable silicones with and without PEG (of varying molecular weights) and dexamethasone, a steroidal, hydrophobic drug [120]. They demonstrated that Fick's second law fit the drug release curves, both with and without PEG [120]. However, upon testing the models by creating material formulations that should produce predicted release curves, they were unable to model the data, with the exception of PEG-exempt formulations [120]. They believed that the poor predictability of the release was the result of non-diffusion kinetics influencing drug release rates when PEG was present in the materials [120].

Clearly the literature does not provide a clear story of the kinetics of PDMS-PEG drug release, the inter-compound relationships between silicone, PEG and drug properties, and the influence of concentrations although there is clear evidence that the addition of hydrophilic compounds such as PEG to hydrophobic matrices such as PDMS can be useful for enhancing drug release kinetics. The sheer variability in each silicone-PEG model system (from silicone type, PEG molecular weights, concentrations, drug types, curing agents, solvents, solubility enhancers, etc.), complicates the extrapolation of the results to any novel system, as any of the individual variables likely influence the drug release rate and thus the fundamental understanding of the release system kinetics and molecular interactions. Thus, there is a need to better understand polymer blend drug delivery systems.

2.5.9 PEG in novel applications

As is seen throughout this review, PEG is quite flexible, and where PEGylation or surface immobilization will repel proteins, there is additional literature work indicating a strong influence on/interaction between proteins and PEG (and similar surfactants). This is especially relevant for more recent drug delivery applications where polymeric micelles may be the drug delivering carriers, and polymer components could disassociate *in vivo*.

For example, it has been shown by a number of groups that PEG and other non-ionic surfactants (e.g. Tweens, Pluronics, etc.) can inhibit membrane efflux proteins. Efflux proteins such as P-glycoprotein are evolutionarily conserved due to their role in removing non-specific toxic molecules which can enter cells via passive membrane diffusion. They have also been found to be over-expressed in numerous multi-drug resistant (MDR) cancers, where they also play a role in protecting the cancer cell from chemotherapeutics. There exist no successful clinical P-gp inhibitors, however more recent research has demonstrated that polymers may hold potential in this application.

Given that P-gp is found at high density on intestinal cells and also throughout the entire body, potential polymeric inhibitors were tested in the early 2000s for their ability to inhibit P-gp function and increase bioavailability [121]. Prior to this, PEG and other similar surfactants were chosen as test compounds and investigated in similar experiments with the metabolic enzyme cytochrome P450 and Caco-2 model intestinal cells due to the widespread use of these additives in drug formulations [122], [123].

Work by Johnson *et al* found that 0.1-20% of PEG 400 improved verapamil uptake in rat intestinal tissue by possible alterations to P-gp or CYP-3a [121]. The mechanism of inhibition was not determined at the time but was postulated to be due to imbalances in osmotic pressure of the buffer, given that 5% w/v PEG 400 increases the pressure by 125 mOsM [121]. P-gp has twelve membrane spanning domains and requires a certain membrane fluidity to change conformation and exert its activity, thus logically changes in osmotic pressure could indirectly also impact P-gp function [121]. Pluronic P85 (a triblock copolymer of PEG-polypropylene oxide-PEG) showed greater inhibition at lower doses, however this mechanism of inhibition may be attributed to the more hydrophobic PPO component [121].

Hugger *et al* found that up to 20% PEG 300 improved compound transport across Caco-2 cells (without impacting monolayer integrity), and fluorescent membrane probe tests (using DPH and TMA-DPA) determined that PEG reduced the fluidity of lipid headgroups, but not the side chains, suggesting that the mechanism of action was through an alteration in membrane rigidity which impacted conformational protein activity [92].

Additional work by Roy *et al* found concentration-dependent induction of apoptosis in HT-29 colon cancer cells in the presence of PEG [124]. They also determined that 50 mM PEG induced the expression of the pro-apoptotic protein PAR-4 17-fold [124]. In this case, no osmotic effect was found to be relevant when tested with sodium chloride and sorbitol controls up to a concentration of 100 mM and thus the apoptotic effect was due to PEG specifically [124]. Follow-up work determined PEG 3350 mediated apoptosis was due to the suppression of the membrane protein epidermal growth factor receptor (overexpressed in ~80% of human colorectal cancers) through increased lysosomal degradation of the protein [125].

Thus these varying results do not help to establish a strong picture of the mechanism of action of polymers on membrane proteins but do provide evidence for an effect. Further, in some studies the polar head groups were impacted, in others they were not and it seems that the mechanism of action varies both by cell type and excipient/polymer type. There also exists the possibility that the *in vitro* models are molecularly different from their natural tissue counterparts, further complicating the elucidation of the inhibition mechanism.

Significant ongoing work into the safety and cellular interaction of excipients with cells and tissues is imperative, and will help to fully understand the potential and limitations of polymers such as PEG. More specifically, although numerous reference books and protocols note the toxicity of PEG to cells during hybridoma formation, there is extremely

limited information regarding the cell death process [126], [127] and PEG is still generally regarded as a highly biocompatible polymer in all applications and variations.

2.6 References

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3 Poly(ethylene glycol) induces cell toxicity in melanoma cells by producing a hyperosmotic extracellular medium.

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3 Poly(ethylene glycol) induces cell toxicity in melanoma cells by producing a hyperosmotic extracellular medium.

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3.1 Abstract

Poly (ethylene glycol) (PEG) is a polymer that is widely used as a biomaterial and has been approved in a host of applications. While generally viewed as inert, recent studies with PEG suggest that it may have some effects on cells and tissues, making it potentially attractive as a therapeutic agent. In this study, the effect of PEG on the cell viability, membrane transport and apoptotic markers of metastatic melanoma cells was examined. The data were combined with observed effects of the polymer on the cell media, including osmolality and viscosity in order to elucidate any structure-function relationship between the polymer and cells. It was observed that PEG reduced the cellular viability of A375 cells, and that the effect was dependent on PEG molecular weight and concentration. The mechanism was highly correlated with changes in the osmolality of the cell medium, which is determined by the inherent structure of PEG, and in particular the ethylene oxide units. This mechanism was specific to PEG and was not observed with the similar linear, hydrophilic polymer poly(vinyl pyrrolidone). Overall the data suggest that PEG and PEG-like compounds have a distinct effect on cellular activity, presumably mediated in part by their osmotic effects, supporting the further investigation of these polymers as pharmaceutically active compounds.

3.2 Introduction

Polymeric biomaterials, long used as implants and in other applications, are emerging as novel therapies in a variety of applications [1], [2]. In fact, over the past decade, there have been a number of polymeric compounds that have been approved for clinical use due to their therapeutic effects. The main application for these materials has been in the sequestering and/or removal of excess ions and small molecules from the gastrointestinal system. For example, polymers have been indicated as a treatment for high levels of phosphate (hyperphosphatemia) [3], potassium (hyperkalemia) [4] and cholesterol (hyperlipidemia) [5]. There is also some preclinical evidence that polymers have efficacy in the inactivation of bacteria and toxins such as *Clostridium difficile* and anthrax [6], [7], in entrapping viruses such as influenza [8], producing sensitised multi-drug resistant cells [9], and as a potential treatment for autoimmune diseases [10]. However, there have been no polymers approved as therapeutics in any of these applications to-date.

Poly(ethylene glycol) (PEG) is a polymer that is widely used in biomedical applications, although it is generally regarded as inert [11]–[14]. There is recent evidence to suggest that PEG and PEG like polymers, specifically poloxamer and Triton X-100 may have biological activity in certain applications. Poloxamers, composed of blocks of poly(ethylene glycol) and poly(propylene oxide), are widely used in medical applications, largely as drug carriers [15]. However, they have also been shown to enhance the transport of small molecules across cell membranes, including the membranes of multi-drug resistant cells, and the blood-brain-barrier [15]. Possible mechanisms for this transport include microviscosity modification of the cell membrane, inhibition of drug efflux transporters such as P-glycoprotein and enhancement of pro-apoptotic signalling pathways. Triton X-100 is a non-ionic surfactant composed of a poly(ethylene glycol) chain and an aromatic, lipophilic group. Due to its polar head group, it is widely used in biology to disrupt cell membranes, allowing for permeabilization or protein extraction [16]. However, surprisingly, it has also been shown to produce the hallmarks of apoptosis in human carcinoma cell lines within 60 minutes of exposure [17]. Poly(ethylene glycol) alone has been shown to exhibit these properties as well, specifically inhibiting P-glycoprotein in intestinal cells, and causing apoptosis of human colon cancer cells [18]–[24].

The goal of this study was to examine the effects of PEG on a human melanoma cell line. While an extremely simple polymer from a structural perspective, in solution PEG is able to produce strong changes in its environment. Specifically, it is able to hydrogen bond 2-3 water molecules per ethylene oxide unit, effectively creating a surrounding ‘cage’ of water [25]–[27]. This property has been exploited in PEG modified surfaces or PEG-drug conjugates with enhanced solubility or increased residence time [28]. PEG has also been shown to exhibit amphiphilic character, secondary structures and molecular-weight-dependent properties [29], [30]. The basis for the hypothesis that PEG in particular may have biological activity thus stems from these unique PEG properties and the knowledge that cells are highly sensitive to their extracellular environment, which is composed mainly of water [31].

Despite this, there remains a major gap in the current literature in terms of understanding the relationship between the structure of PEG and any observed biological effects. Therefore this study was performed to better understand the interactions between PEG and cells and how these interactions result in cellular changes by specifically examining the changes in cellular activity, morphology, membrane integrity and transport as well as apoptosis markers, that are affected by the presence of PEG and the changes to the cell culture medium that are the result of PEG.

Since it has been suggested that PEG exerts effects on important membrane proteins such as P-glycoprotein and may induce apoptotic effects, studies were performed on an *in vitro* culture of *A375* metastatic melanoma cells which are known to express P-glycoprotein [32]. There is significant clinical need for improved treatments for metastatic melanoma; cutaneous malignant melanoma represents only 2% of skin cancers, but the survival rate of patients is 10-15% over 10 years [33]. PEG specific-effects on A375 cells were compared with two other cell types – a 3T3 murine fibroblast cell line and a human corneal epithelial cell line to determine whether any effects observed were cell specific. Furthermore, the specificity of the effects of PEG was investigated by comparing with poly(vinyl pyrrolidone) (PVP), a non-ionic, linear, hydrophilic, perceived as 'inert', medically approved polymer. However, in contrast with PEG, the structure of PVP contains a bulky, 5-membered lactam ring. The results of this work provide insight into the nature of the interactions between hydrophilic synthetic polymers and cells, and the potential of synthetic polymers as pharmacologically active compounds.

3.3 Materials

A375 (ATCC®CRL-1619™) metastatic melanoma cells and 3T3 (ATCC®CRL-1658™) mouse fibroblast cell line were obtained from ATCC via CedarLane (Burlington, ON). Human corneal epithelial cells were the generous donation of Dr. May Griffith. Keratinocyte serum-free media with growth factors, Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), phosphate buffered saline (PBS) (pH 7.4), TrypLE, PrestoBlue, Click-iT® TUNEL Alexa Fluor® 594 Imaging Assay and CellEvent Caspase-3/7 Green Detection Reagent were obtained from ThermoFisher Scientific (Burlington, ON). T-75 culture flasks and black, clear bottom, 96-well plates were obtained from Corning (Corning, NY). Poly(ethylene glycol) with molecular weights of 200 (M_r 190-200), 2000 (M_r 1900-2200), 8000 (M_r 7000-9000), and 20,000 (M_r 16000-24000), were obtained from Sigma-Aldrich as BioUltra grade (St. Louis, MO). Poly(vinyl pyrrolidone) with a molecular weight of 8000 was obtained from Alfa Aesar (Ward Hill, MA). Daunorubicin hydrochloride and sodium dodecyl sulfate were obtained from Sigma-Aldrich (St. Louis, MO). Apo-ONE® Homogenous Caspase 3/7 Assay was obtained from Promega (Fitchburg, WI). Annexin V apoptosis kit was obtained from Sigma-Aldrich (St. Louis, MO). 0.22 µM syringe filters were obtained from Millipore (Etobicoke, ON).

3.4 Methods

3.4.1 Polymers

PEG and PVP were weighed and dissolved at room temperature (~22°C) in DMEM+FBS to a final stock concentration of 20% w/v. For cell culture studies, solutions were filtered using a 0.22 µM filter. Daunorubicin hydrochloride and sodium chloride were solubilized by the addition of sterile DMEM+FBS. All solutions were warmed to 37°C prior to use.

3.4.2 Viability Assay

A375 metastatic melanoma cells (A375 cells) were cultured in DMEM containing 10% FBS in T-75 filter-top flasks for at least 2 weeks and were subcultured at least 3 times after removal from cryogenic storage. After this point the cells were microscopically seen to grow at a consistent rate. Cells were kept in an incubator at 37°C with 5% CO_2. A375 cells were seeded at a density of 1000 cells/well into black, clear-bottom 96-well plates with 200 µL of DMEM+FBS. Because A375 cells were found to divide very rapidly, this density was used to ensure subconfluence at the measurement time of 96 hours after seeding. To ensure a constant temperature, cells were seeded only in the interior wells and only DMEM+FBS was placed in the exterior wells.

Seeded 96-well plates were incubated for 24 hours to allow cells to adhere and begin normal proliferation after trypsinization. Test compounds were then added and plates were incubated for 72 hours. Each condition was plated in triplicate on a single 96-well plate and each test was repeated in three independent experiments. After incubation, cells were washed three times with PBS and a PrestoBlue® assay was performed according to the manufacturer's protocol. Briefly, 90 µL of pre-warmed (37°C) cell media and 10 µL of PrestoBlue® cell viability reagent were added to each well, and plates were incubated at 37°C for 10 minutes. PrestoBlue, a non-toxic resazurin-based reagent, is modified by the reducing environment in living cells, which alters its fluorescence, allowing for quantification of cellular viability in comparison to control. PrestoBlue® fluorescence was measured at excitation/emission wavelengths set to 560/590nm.

3T3 cells were grown and assayed under the same conditions. HCEC cells utilized Keratinocyte serum free media and were plated at 2500 cells/well, as lower seeding density provided optimal timing of log growth.

3.4.3 Controls

Cells incubated in cell media only were used as blank and negative controls, indicating the optimal cell viability (100%) at the endpoint. Sodium dodecyl sulfate , a surfactant, was used as a positive control for cell toxicity. PVP was chosen as the polymer-specific control. While saline is traditionally used as a control in many preclinical and clinical trials, the use of another polymer (PVP) as a control was chosen in these *in vitro* assays as it represents a better direct comparator of polymer-specific activity. Daunorubicin (DNR) was included as a comparator for cell viability, as it acts by intercalation, inhibiting macromolecule biosynthesis and leading to cell death by caspase mediated apoptosis [34]. It passively permeates through the cellular membrane and is also a P-glycoprotein efflux substrate [35], allowing for observations of membrane transport changes and

observations of possible membrane efflux protein inhibition. DNR is not commonly used for treating cutaneous melanoma clinically, as more targeted therapeutics with less systemic side effects are the standard of care [36]. However, its availability, mode of action and drug properties allowed for design of experiments that can provide a broad variety of information on possible interactions between PEG and cellular membranes. Doxorubicin is of the same class and properties as DNR, and was used for the TUNEL assay based on availability.

3.4.4 Viscosity

Polymer solutions in DMEM + 10% FBS were warmed to 37°C in a water bath for 30 minutes prior to measuring viscosity. Viscosity was measured using a Malvern SV-10 viscometer (Malvern, UK). The mean recording temperature was approximately 34°C, due to non-insulated instrumentation. Two or three concentrations of each test compound were measured; these PEG concentrations represented a range of cell toxicity values.

3.4.5 Osmolality

Polymer solutions were prepared as described by dissolution into DMEM + 10% FBS. The osmolality of the solutions was measured using a 3320 MicroOsmometer (Advanced Instruments, Inc. - Norwood, MA), which measures the freezing point to determine solute concentration.

3.4.6 Membrane Transport

The foundation of this assay is that daunorubicin hydrochloride (DNR) is able to enter cells via diffusion through the cell membrane and into the nucleus, and it can be effluxed by non-specific membrane proteins such as P-glycoprotein [35]. By monitoring DNR transport, changes to the membrane permeability or membrane protein activity can be detected. DNR transport was tracked via its inherent fluorescence (emission/excitation wavelength of 490/595 nm). Specifically, daunorubicin hydrochloride is fluorescent in solution, but loses fluorescence upon binding to DNA. The fluorescence of DNR was monitored every 5 minutes for 3 hours at 37°C, as adapted from an assay developed by Regev *et al* [37].

3.4.7 Caspase 3/7 Apoptosis Assay

Apoptosis was measured by detecting the activity of apoptosis executioner caspases 3 and 7 at various time points using a commercially available kit (PROMEGA). Cells were cultured as described in the viability assay, but measurements were taken at 2, 4, or 6 hours because caspases are early apoptotic markers, and the initiation of apoptosis is unique to each cell type and compound combination. The complete caspase assay medium was prepared according to the manufacturer's protocol. The complete assay medium was then added in a 1:1 ratio to wells and cells were incubated at room temperature for 16 hours. Fluorescence was measured at an excitation/emission wavelength of 499/521 nm.

Caspase 3/7 activity was also measured using the ThermoFisher detection agent at 24 hours (due to availability), according to manufacturer's directions. Briefly, cells were

cultured as described for the viability assay, then washed with PBS before the addition of the caspase detecting agent for 30 minutes. Fluorescence was measured at 503/530 nm.

3.5.8 DNA Fragmentation Assay

The Click-iT® TUNEL Alexa Fluor® 594 Imaging Assay was obtained from ThermoFisher Scientific and used according to manufacturer's instructions. Briefly, cells were fixed using 4% paraformaldehyde in PBS and permeabilized using 0.25% Triton® X-100. Terminal deoxyonucletidyl transferase (Tdt) was then applied with modified deoxyuridine triphosphates (dUTPs). The Click-iT® reaction cocktail containing the Alexa Fluor® 594 antibody was then applied to cells. Imaging was performed with an inverted confocal microscope using a Texas Red filter.

3.5 Results

CELL-SPECIFIC ASSAYS

3.5.1 Poly(ethylene glycol) exposure induced morphological changes to A375 cells

The morphology of A375 cells was examined after 72 hours of exposure to PEG (Figure 3.1A). Control cells, with no polymer exhibited a morphology that is typical for A375 epithelial cells in culture [38], as did cells exposed to 1% w/v of PEG_{200}, PEG_{2000} or $PEG_{20,000}$. Cells exposed to 10% w/v of PEG of all molecular weights were significantly fewer in number and smaller in size. These cells adopted a spherical shape and appeared condensed. The morphological changes were detected visibly as early as 24 hours after PEG exposure (data not shown).

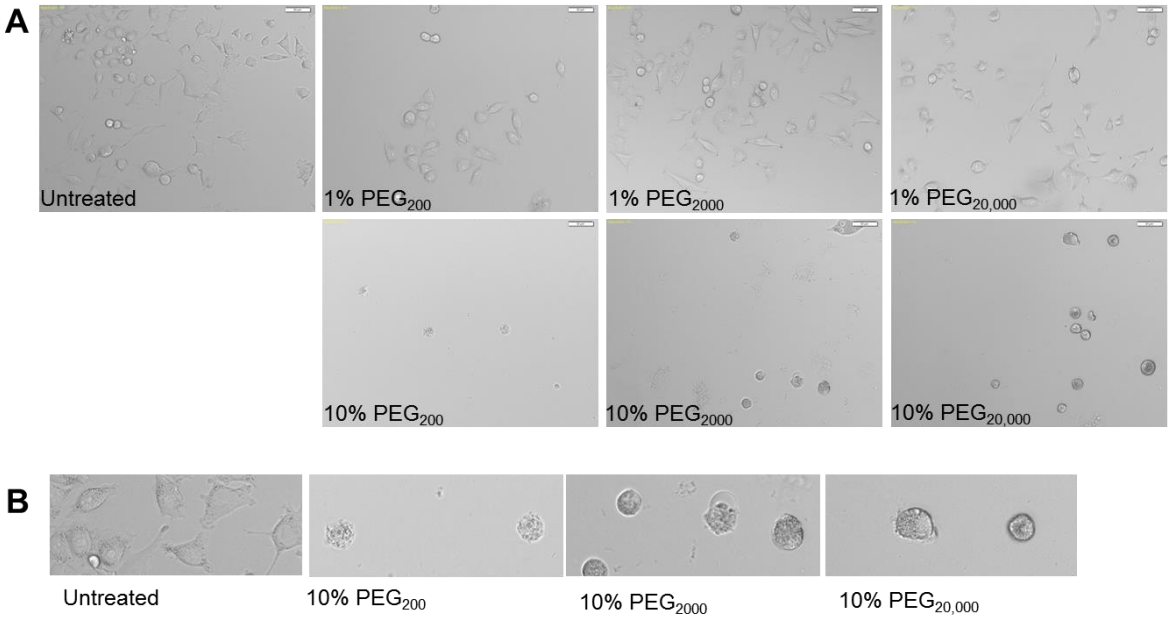

Figure 3.1. Comparative morphology of untreated and PEG treated A375 metastatic melanoma cells show morphological changes indicative of low cell viability at higher PEG concentration. 1A: Bright-field images at 20x magnification. Scale bars represent 50 µM. 1B: Unscaled close-up of cells from 1A images.

Supplementary Figure 3.1 demonstrates the morphology of cells exposed to the surfactant sodium lauryl sulfate for comparison with cells exposed to PEG. SLS-exposed cells appear elongated at lower concentrations and highly fragmented at high concentrations. In contrast, PEG-exposed cells appear shrunken but not elongated or fragmented (Figure 3.1).

In order to determine whether the effects noted were PEG specific, cells were also exposed to PVP, another hydrophilic polymer that does not have the same water-binding and amphiphilic properties. A molecular weight of 8000 was selected for comparing PEG and PVP. At high concentrations of PEG_{8000} (Supplementary Figure 3.2), the morphology of A375 cells appeared more spherical and condensed / shrunken, which is similar to the

morphology of cells exposed to PEG compounds, however they appear to be even smaller in size than cells exposed to PEG.

3.5.2 PEG caused a decrease in cell viability as measured using a resazurin reduction assay

The reduction of the resazurin based compound PrestoBlue® was used to measure the viability of A375 cells after 72 hours of PEG exposure. The effects of PEG concentration were observed by plotting cell viability with increasing ethylene oxide content (Figure 3.2). Supplementary Table 3.1 lists the estimated number of moles of each PEG compound at ~100% and ~40% viability, and the corresponding number of EO units for each compound.

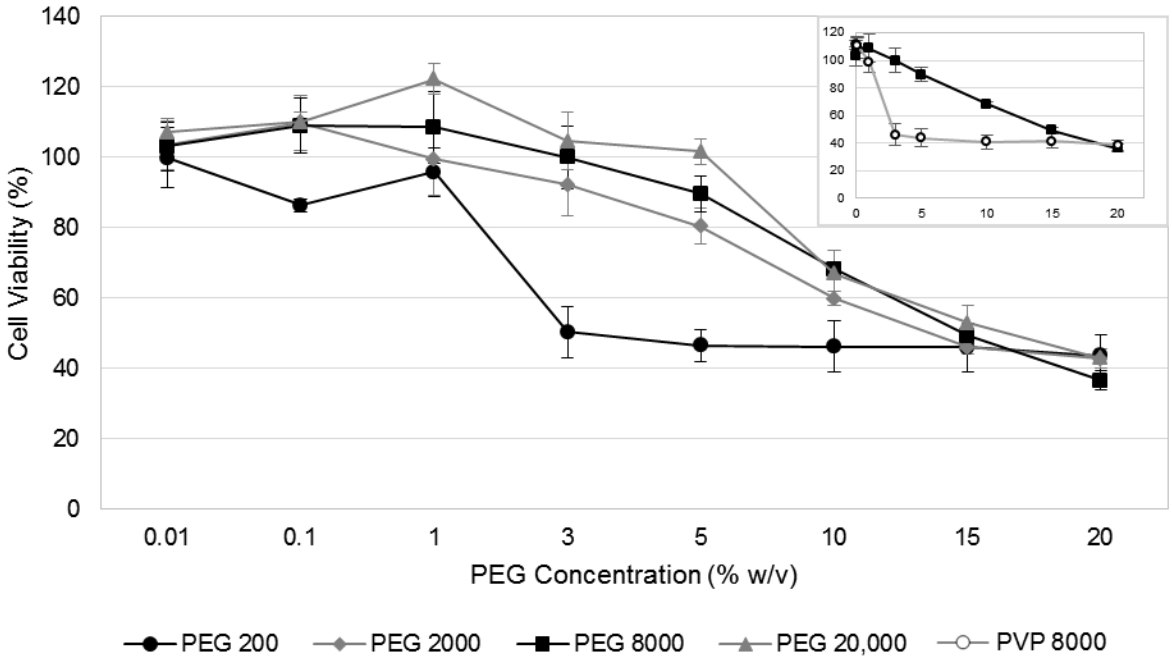

Figure 3.2. Cell viability of A375 melanoma cells is influenced by the presence of PEG. A: An inverse relationship is observed between PEG concentration (ethylene oxide content) and cell viability. The inset shows cell viability in the presence of PVP_{8000} and PEG_{8000}. Error bars denote standard error (n=3, triplicate samples, repeated three times).

In Figure 3.2, it can be seen that across all PEG compounds tested, a PEG concentration between 0.01 and 1% w/v produced no discernible difference in cell viability from cells not exposed to PEG. Changes to cell viability were first observed with PEG 200 (~50% reduction at 3% w/v), followed by a more gradual reduction in viability

with higher molecular weight PEGs. At 15% w/v PEG, cell viability was approximately equal (45%) across all PEG compounds. These data indicate an inverse correlation between PEG concentration and cell viability. Specifically, as the number of ethylene oxide units increases, cell viability decreases.

A very different trend was observed with PVP (Figure 3.2 inset), with a sharp decrease in the cell viability being observed at concentrations greater than approximately 1%, beyond which the level of cell viability were less than 50%. Therefore it appears that the mechanism by which these polymers induce cellular changes is very different.

Overall, Figure 3.2 demonstrates an inverse correlation between PEG concentration and cell viability for each PEG that was tested. The data also suggest PEG chain length influences cell viability, as observed by the distinct variation in the class-trend in cells exposed to PEG_{200}.

3.5.3 Low PEG concentration did not alter rates of daunorubicin hydrochloride membrane transport

PEG has a linear structure with a very high degree of conformational freedom, making both hydrophobic and hydrophilic conformations, as well as surfactant-like properties possible [39], [40]. Thus, given the potential for PEG to have surfactant-like properties [29], membrane permeabilization and changes in membrane transport were examined using the small, hydrophobic intercalating agent daunorubicin hydrochloride (DNR) [34]. DNR toxicity over a large concentration range is shown in Supplementary Figure 1, confirming that DNR was transported into the nucleus of A375 metastatic melanoma cells, inducing cell death.

Note, that surfactants can permeabilize cells at low concentrations without inducing toxicity [16]. Specifically, they are able to break hydrogen-bonds between phospholipid head groups, which changes the membrane integrity [16]. This experiment was designed to examine whether a similar effect would be observed with PEG. In this case, cells were exposed to DNR alone or 1% w/v PEG with DNR. A non-toxic concentration of PEG was chosen to prevent artifacts due to cell death or membrane disintegration, but still allow for the potential observation of early changes to the membrane due to PEG. Two DNR concentrations were chosen for tracking as shown in Figure 3.3, with 0.055 μM DNR on the left vertical axis and 0.01 μM on the right vertical axis. Daunorubicin transport was measured for approximately 3 hours. The transport was measured in the presence and absence of PEG of three molecular weights (200, 2000, and 20,000).

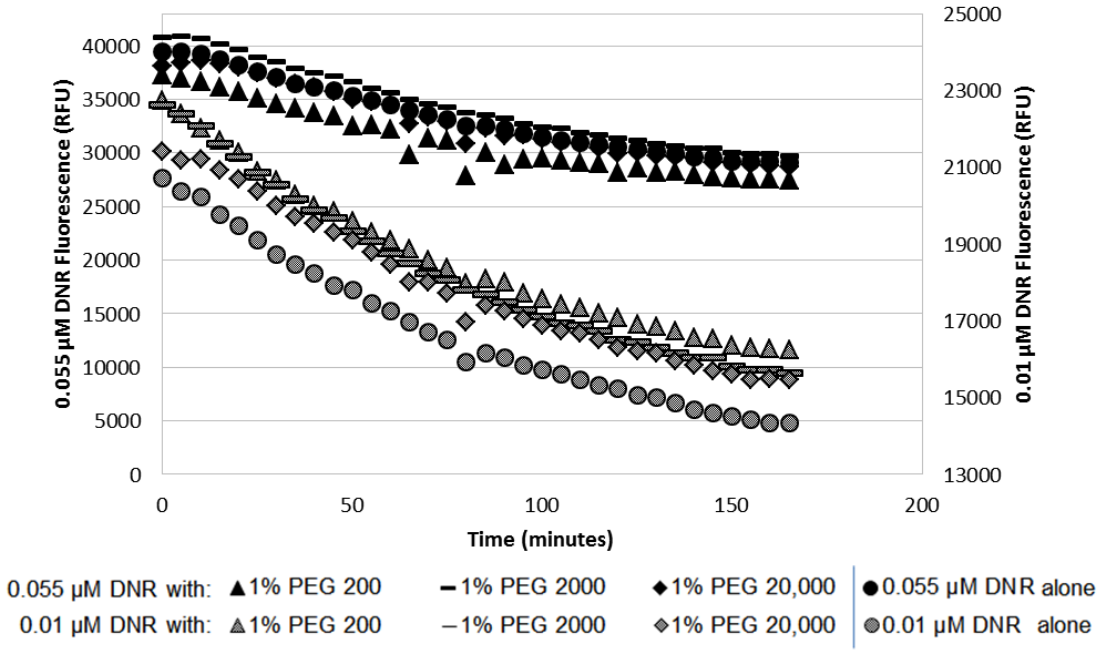

Figure 3.3. Daunorubicin hydrochloride (DNR) transport into cells, as measured by fluorescence quenching upon nucleic acid binding. Error bars (n=3) were omitted from the figure for better visualization of the data points.

As expected, the higher DNR concentration produced greater fluorescence values, while the lower DNR concentration produced lower values. There were no statistically significant changes in DNR fluorescence at each timepoint for DNR alone versus DNR in the presence of PEG of any molecular weight.

Table 3.1 The slope of daunorubicin hydrochloride (DNR) uptake and quenching in the absence and presence of poly(ethylene glyol).

		Daunorubicin hydrochloride +		
	DNR alone	1% PEG 200	1% PEG 2000	1% PEG 20,000
0.055 µM DNR				
Slope	-70.39	-63.92	-75.82	-69.46
R^2	0.993	0.968	0.999	0.955
0.01 µM DNR				
Slope	-41.50	-40.93	-45.19	-41.24
R^2	0.995	0.9717	0.994	0.993

Given the apparently linear decrease in DNR fluorescence observed in Figure 3.3, the slope was determined using linear regression of the data. Table 3.1 depicts the change in DNR concentration over time. A more gradual slope in the presence of PEG would indicate less fluorescence quenching – i.e. a reduction in the transport / binding of DNR to nucleic acids. A steeper slope would indicate more fluorescence quenching – i.e. an increase in the transport / binding of DNR to nucleic acids, presumably as a result of the

presence of the PEG. However, results indicate that there was no statistically significant variation/difference between the slopes and thus no statistically significant changes in the transport of DNR as observed using this assay.

3.5.4 PEG increases the presence of apoptosis markers: Caspases 3 and 7

Caspase activity was measured at multiple timepoints (after 2, 4 and 6 hours of exposure to polymer), because the initiation of apoptosis is not predictable [41]. The caspase 3/7 substrate fluorescence was normalized to the baseline values (cells with no test compound added), thus, the value ‘1’ on the vertical axis represents the baseline, and increases above this value indicate increases in caspase 3/7 activity.

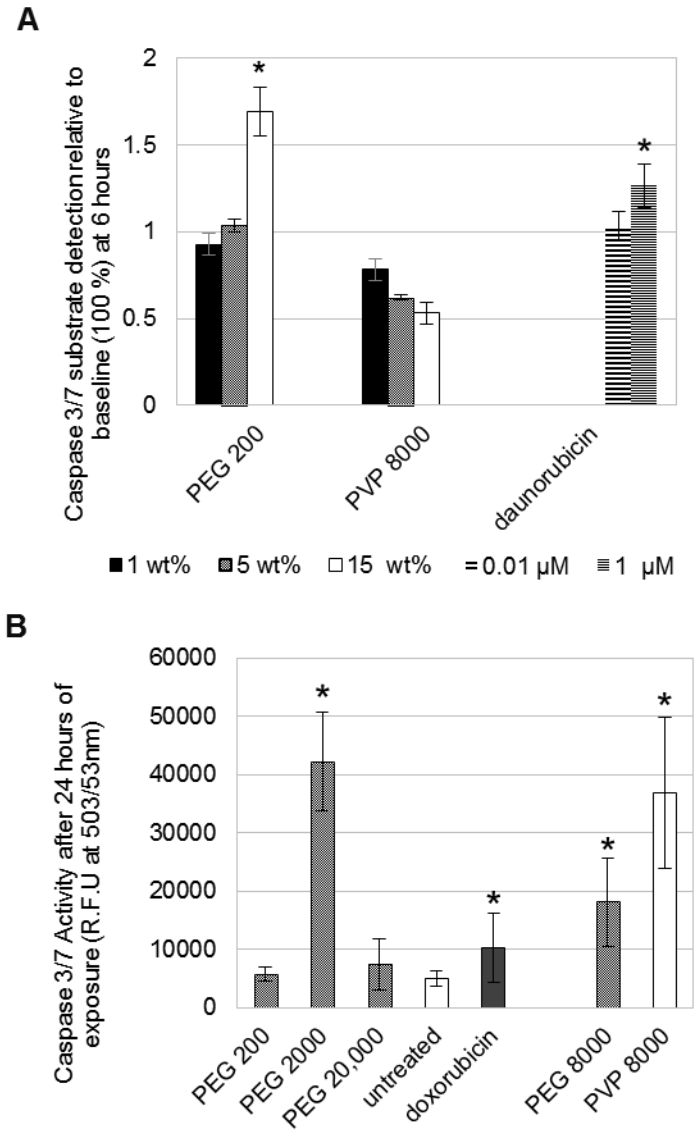

Figure 3.4. Caspase 3/7 levels after exposure to polymers or controls. Error bars represent standard deviation (n=3). Asterisk represents statistically significant (p<0.05) change from baseline. A: Caspase 3/7 activation relative to baseline (1) after 6 hours of exposure to PEG or daunorubicin-HCl. B: Caspase 3/7 activation after 24 hours of exposure to PEG or control.

The results after 6 hours are shown in Figure 3.4a. A statistically significant ($p<0.05$) increase in caspase 3/7 activity was detected with 15% w/v PEG_{200}. PEG_{2000} and $PEG_{20,000}$ did not demonstrate statistically significant increases in caspase 3/7 activation at 6 hours. 1 µM DNR showed a statistically significant increase in caspase 3/7 activation after 6 hours of exposure. At 2 and 4 hours, 15% w/v PEG_{200} also demonstrated statistically significant ($p<0.05$) increase in caspase 3/7 activity, while PEG_{2000}, $PEG_{20,000}$ and DNR did not (data not shown).

Caspase 3/7 activation was further tested after 24 hours of exposure to PEG or control (Figure 3.4b). At this timepoint, exposure to PEG_{2000} showed an over 8x increase in caspase activity versus control, which was statistically significantly different. Doxorubicin control also demonstrated a statistically significant increase (~2x) in caspase activity at this timepoint. These data further support the case for induction of apoptosis by PEGs through the detection of early apoptotic activation of caspases 3 and 7. Of note, PEG_{200} cells may still have caspase activity at 24 hours, however due to the lower number of cells (as observed microscopically) the fluorescent signal was not higher than that observed with the untreated cells.

3.5.5 PEG increases the presence of apoptosis markers: TUNEL signal

TUNEL staining was used to determine whether DNA fragmentation (a late apoptosis marker) can be detected in cells exposed to PEG for 24 hours (Figure 3.5).

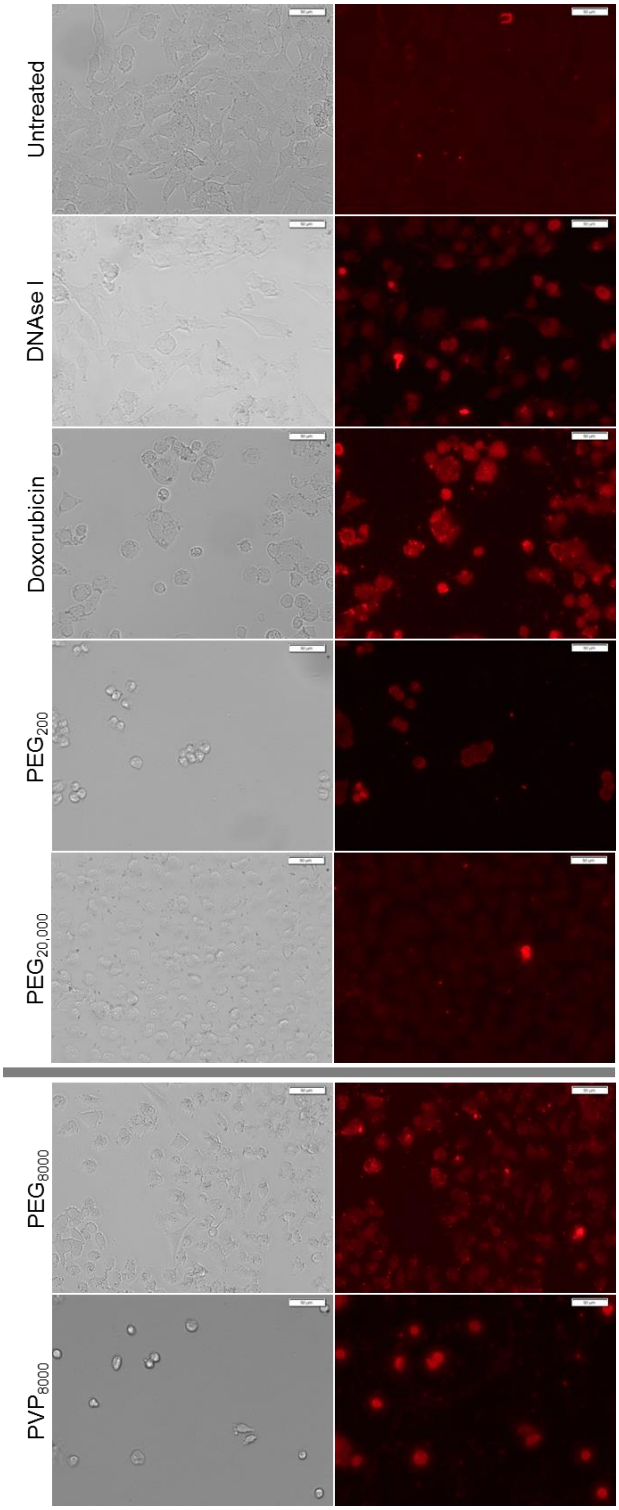

Figure 3.5. TUNEL staining of A375 cells after 24 hours of exposure to PEG, PVP or controls.

The DNAse I control showed both strong staining of the nucleus and some visible staining of DNA fragments. Doxorubicin-HCl exposed cells show more staining of DNA fragments within the A375 cells versus DNAse exposed cells. This difference in staining across controls is expected, given the short time the cells were exposed to DNAse versus the longer exposure to doxorubicin. Cells exposed to PEG_{200} were visibly fewer in number, with some staining of fragmented DNA. Cells exposed to $PEG_{20,000}$ showed visible condensation in the brightfield image, and minimal DNA fragmentation, indicating minimal detection of late apoptosis. Cells exposed to PEG_{8000} showed DNA fragmentation across all cells visualized. Thus, 24 hours after polymer exposure, the staining of this late apoptosis marker complements the data seen with the activation of caspases 3/7 (early apoptosis makers) in Figure 3.4, as well as the toxicity trends seen in Figure 3.2. Further, in contrast to PEG_{8000}, PVP_{8000} exposed cells showed strong nuclear staining, but with residual cell fragments seen in the background of the fluorescent image, indicating an alternative timing of apoptotic events.

Taken together, the apoptosis markers (caspases and TUNEL staining) and morphological analysis demonstrate apoptosis induction by PEGs. The data show apoptosis occurring at different timepoints depending on the type of PEG to which the cells were exposed.

3.5.6 Comparison of PEG effects with other cell types

The impacts on cell viability were examined for cell-specificity by performing the PrestoBlue® viability assay with a mouse cell line and with non-cancerous human cells (Figure 6).

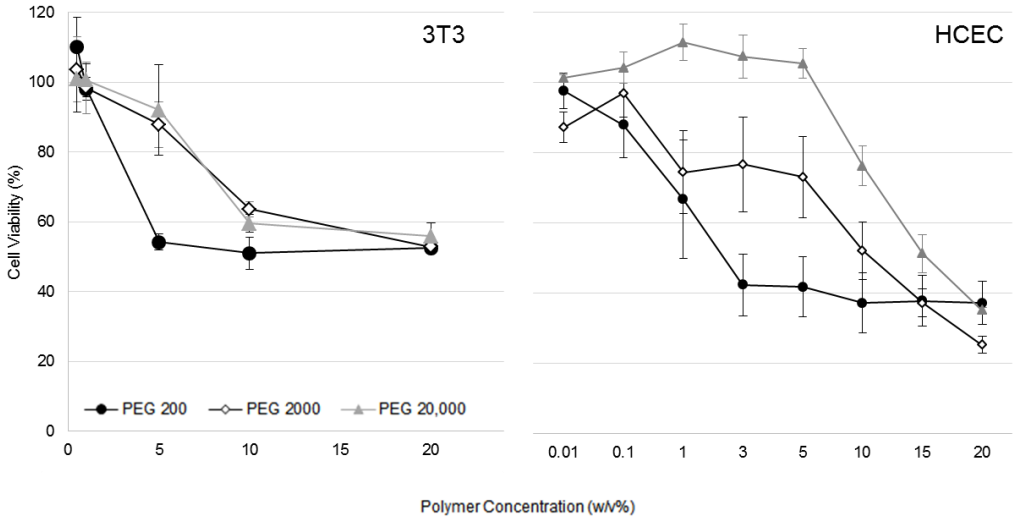

Figure 3.6. Influences of PEG_{200}, PEG_{2000}, and $PEG_{20,000}$ on murine 3T3 fibroblast cells (A) and human corneal epithelial cells (B). Error bars denote standard deviation (n=3).

In Figure 3.6a, a 3T3 mouse fibroblast cell line was exposed to $PEG_{200,}$ PEG_{2000} or $PEG_{20,000}$ for 72 hours. At 0.5 and 1% w/v, there was no discernible reduction in cell viability for all PEG molecular weights. However, b y 10 % w/v, PEG_{200} and $PEG_{20,000}$ reduced cell viability to ~60%, and at 20% w/v, viability was approximately 50%. In Figure 3.6b, human corneal epithelial cells were exposed to $PEG_{200,}$ PEG_{2000}, or $PEG_{20,000}$ for 72 hours. From 0.01-0.1 % w/v, there was no discernible reduction in cell viability for all PEG molecular weights. At 1% w/v, PEG 200 demonstrated a reduction in cell viability to ~70%, which further decreased to ~40% at 3 % w/v. Thus, the cellular effects of PEG on A375 cells appear to be maintained across species and cell types.

ASSAYS OF THE CELLULAR ENVIRONMENT

3.5.7 PEG effects on media viscosity

To assess whether the impact of PEG is not necessarily biological but rather the result of changes to media surrounding the cells, the physical properties of the PEG containing media were examined . The viscosity of PEG containing medium at various PEG molecular weights and concentrations is shown in Figure 3.7. The viscosity increased with increasing PEG concentration for all PEG molecular weights, although the magnitude of increase in viscosity differed. For example, a solution of 15% PEG_{2000} was approximately 2.6x more viscous than 5% PEG_{2000}. At the same time, the viscosity of 15% $PEG_{20,000}$ was approximately 6.2x higher than 5% $PEG_{20,000}$.

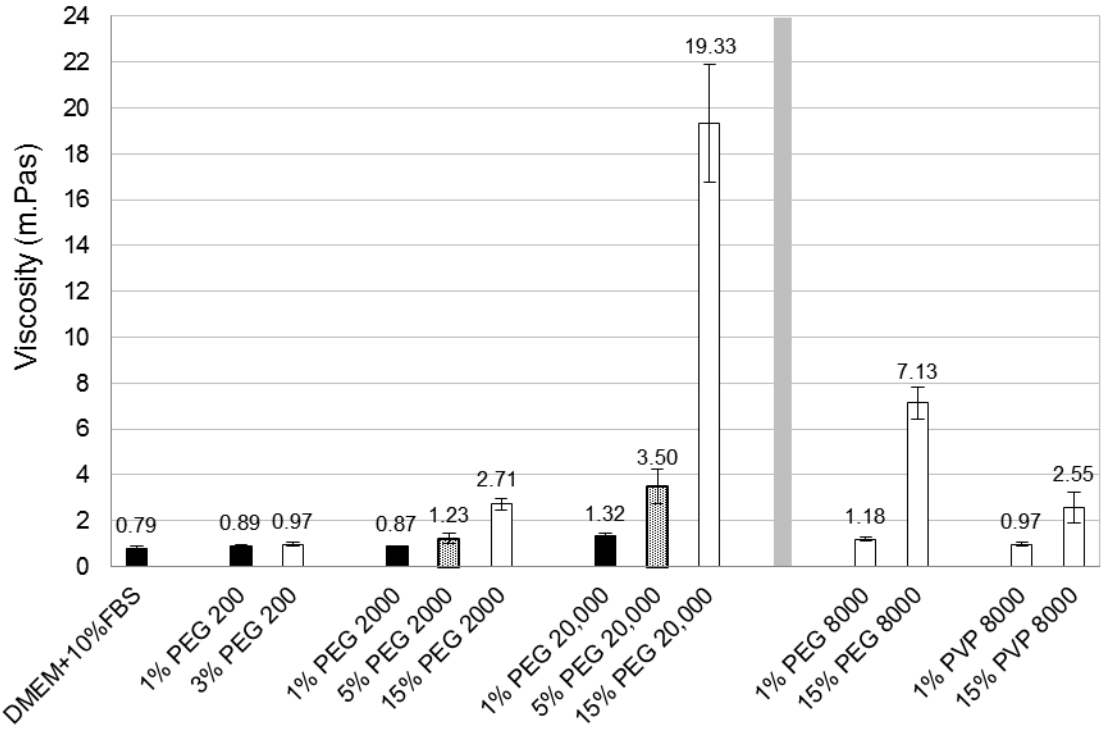

Figure 3.7. Viscosity of cell media with varying polymer molecular weights and concentrations. Precise average viscosity values are stated above each bar for ease of comparison. Error bars denote standard deviation (n=3).

The magnitude of change in viscosity did not correlate directly with the magnitude of change seen in cell viability although (Figure 3.2), although there was a general trend toward decreased cell viability with increased medium viscosity. For example, ~50% cell viability was seen with a solution viscosity of 1.09 mPas with PEG_{200}. At approximately the same viscosity, cells exposed to media with PEG_{2000} were 75% viable and cells exposed to $PEG_{20,000}$ were 100% viable. PEG 200 was an exception, as there was no real difference in viscosity (1% vs. 3%) but yet there was a large drop in viability. Therefore it can likely be concluded that the changes to the viability of the cells are not the direct result of alterations to the viscosity of the medium.

3.5.8 PEG effects on media osmolality

Cells require a plasma osmolality of approximately 280-295 mOsm/kg for adequate cellular activity [42]. Hypertonic solutions are well known to cause biological changes [31], [43]–[45]. Given that the dissolution of PEG in cell media alters the osmolarity, it was investigated whether PEG may exert toxicity towards A375 cells through the alteration of the media osmolality, specifically by producing hyperosmotic cell media (Figure 3.8). Note, the viability values in this Figure represent viability after 72 hours of exposure to test compounds.

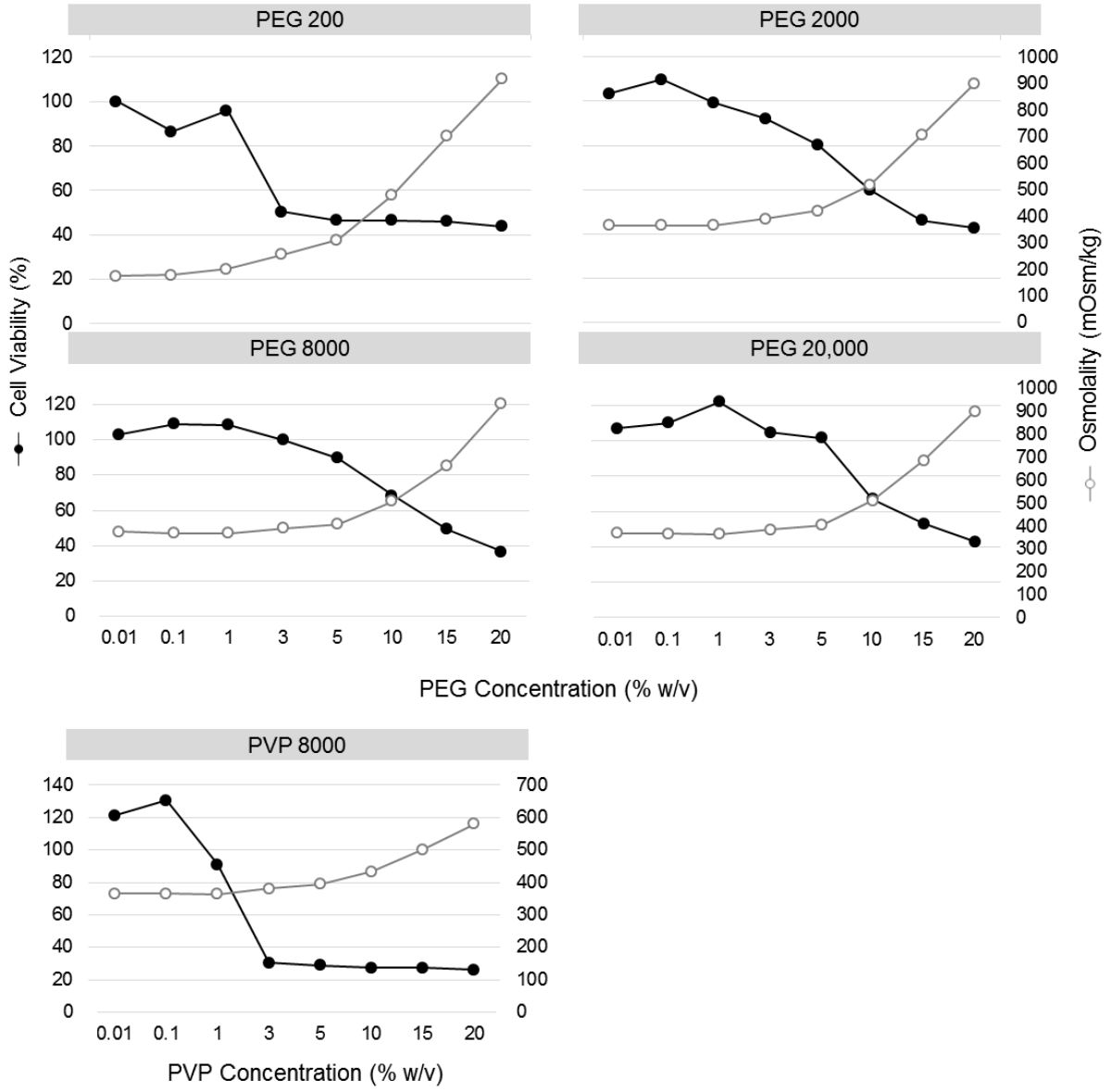

Figure 3.8. The effects of PEG and PVP on media osmolality and cellular viability.

Cell viability was observed to decrease with increasing osmolality of the cell media consistently across all PEG molecular weights. Reduction in cell viability began at a media osmolality of ~400 mOsm/kg (DMEM + FBS alone was ~360 mOsm/kg). This negative correlation continued to ~40% viability in the presence of media with an osmolality of~900 mOsm/kg. The correlation was independent of PEG molecular weight. Cells exposed to PEG_{200} experienced stronger reductions in cell viability at lower media osmolality than when exposed to PEG_{200}, PEG_{8000} or $PEG_{20,000}$ (seen at ~3% w/v PEG_{200}). Looking specifically at PVP_{8000} alone, there does not appear to be an inverse correlation between osmotic effects and cell viability. This is observed in particular

between PVP_{8000} concentrations of 1 and 3 % w/v, where cell viability rapidly decreased, while osmolality increased only slightly. Thus, the data indicate that there is a direct correlation between cellular viability and media osmolality in the presence of PEG.

3.6 Discussion

3.6.1 PEG effects on A375 cells

Poly(ethylene glycol) of three different molecular weights (200, 2000 and 20,000) was investigated for its biological activity using A375 metastatic melanoma cells, based on literature that indicates PEG may have some inherent biological activity. Both changes to the cells and the effect of PEG on the cell medium were examined to determine the cause of any biological effects. The most significant finding was that all the PEG compounds tested demonstrated toxicity towards A375 cells. This toxicity was dependent on PEG concentration / structure as seen in Figure 3.2.

Despite literature reports which suggest that PEG may have an effect on P-glycoprotein, the results of this work suggest that the mechanism of toxicity is more physical than biological. In fact, as shown in Figure 3.8, the effect of PEG is thought to be primarily related to the change in the osmolality of the medium and there was a direct inverse correlation between osmolality of the PEG solution and the viability of the cells. While for most compounds osmolality is only dependent on the concentration of the solute, PEG does not act as an ideal solute [46], and therefore, the measured osmolality represents an interaction between the concentration of PEG in a solution and its molecular weight (i.e. the number of EO units present). Taken together, these data suggest that the ethylene oxide subunits alter the osmolality of the cell media, and the ensuing hyperosmolality then induces cell death.

It is important to note that there exists a good understanding of the relationship between media osmolarity and cell toxicity. Upon the addition of solute into the extracellular environment, a hyperosmotic condition is created where the extracellular concentration of solute is greater than the intracellular concentration of solute. The spontaneous, natural phenomena of osmosis then occurs, which results in the passive diffusion of water from inside the cell to the outside. This movement results in a net water loss for the cell, and leads to cell shrinkage [47]. Cell shrinkage (or “apoptotic volume decrease” (AVD)) contributes to the signaling of apoptosis, and is an early marker of apoptosis, sometimes occurring even prior to the activation of executioner caspase 3 [48]. In some cell types, cell shrinkage has also been shown to be essential for apoptosis to occur [49].

Based on this knowledge, it was expected and indeed observed that the morphology of A375 cells is ‘shrunken’ after being exposed to PEG. The cells exposed to higher concentrations of PEG (correlating to low cell viability and high osmolality) were spherical in shape and appeared highly condensed (based on the darker pigmentation inside the cells). In contrast, cells exposed to lower concentrations of PEG (correlating to high viability and osmolality similar to control) appeared similar in morphology to control cells.

The activity of apoptotic executioner caspases 3 and 7 were also measured. PEG_{200} demonstrated statistically significant increases in caspase 3/7 activation as early as 2 hours after PEG exposure and PEG_{2000} and $PEG_{20,000}$ generally demonstrated increases in mean caspase 3/7 activation with increasing PEG concentration although later (Figure 3.4). The initiation of apoptosis is known to vary between different compounds and thus additional later timepoints should be tested to obtain a full spectrum of induction times.

The resazurin viability results, osmolality measurements, cell morphology, viability threshold of ethylene oxide units, caspase 3/7 activation and TUNEL staining results strongly support the hypothesis that PEG has biological activity; specifically, the activity is reduced cellular viability due to altered osmolality of the cell medium, which is related to the PEG structure (hydrogen bonding via its ethylene oxide subunits).

3.6.2 Specificity of Results

The observations of PEG toxicity towards A375 cells were unique when compared with another linear, non-ionic, hydrophilic polymer: poly(vinyl pyrrolidone). Overall, the data demonstrated that PVP reduced cellular viability. However, there is likely an alternative mechanism of action for PVP, because all PVP data showed a distinctly different pattern than was observed with the PEG exposed samples of the same molecular weight. For example, PVP was shown to activate caspase 3/7 dependent apoptosis after 24 hours of exposure, but it also significantly reduced caspase activity after up to 6 hours of exposure. This suggests that early triggering of cell death may be via a different pathway than PEG.[2] Further investigation of viscosity effects may be warranted to better understand the PVP mechanism of toxicity.

The cell-specificity of the PEG effects was consistent across multiple cell-types and they were not shown to be exclusive to A375 metastatic melanoma cells. PEG produced very similar reductions in cell-viability with a murine 3T3 fibroblast cell line and human corneal epithelial cells (Figure 3.6). In both cases, the viability was also concentration and molecular-weight dependent. These data support the osmotic mechanism of action of PEG, as hyperosmotic conditions are expected to produce similar effects in all cell types.

An early hypothesis for the viability effects of PEG on A375 cells was that PEG could act as a surfactant on the cellular membrane. This was hypothesized to be possible due to the amphiphilic character of PEG that has been reported in the literature [29]. Morphology data (Figure 3.1) suggested that this was not the case, as cells exposed to the surfactant were not shrunken, but were rather visibly elongated (at lower surfactant concentration) or fragmented (at higher surfactant concentration) (Supplementary Figure 3.1) in contrast to the PEG exposed cells. In addition, the transport of DNR was not affected. These data suggest PEG does not act as a surfactant on A375 cell membranes.

3.6.3 Additional Insights and Potential Future Studies

While viscosity may be a factor with lower molecular weight PEG, somewhat surprisingly, similar viscosity related effects were not observed with higher PEG molecular weights of

[2] In addition to the strong hydrogen bonding ability of PEG, this difference may also be due to the ability of PEG to hold a larger volume of water due to its large conformational freedom in comparison to PVP.

2000 and 20,000. Thus it is likely that viscosity is not directly correlated with cell viability changes, however it is theoretically possible it may have some influence at the highest measured value seen with $PEG_{20,000}$. At high viscosity, a hydrated gel may form due to the ability of PEG to form hydrogen bonds with water, thereby limiting diffusion in these gels. Viscosity-dependent cell effects have been previously observed with alginate solutions [50]. The unique effects of PEG_{200} may alternatively be due to the possibility that PEG_{200} is more readily broken down into the toxic ethylene oxide than the higher molecular weight PEGs.

Taken together, the data strongly suggest a structure-function relationship mediated by osmotic effects. It may also be possible that PEG also acts on the cell membrane, a protein, a cellular cascade or in the production of toxic metabolites. The current membrane transport data (Figure 3.4) suggests that there are no changes to the membrane with PEG exposure. However, some studies using intestinal cells have shown that PEG increases the rigidity of the phospholipid head groups [20], which could prevent the function of membrane proteins, or lead to initiation of the apoptotic pathway. Further studies on membrane and protein effects are needed to better understand the downstream effects of PEG on cells and it is important that future experiments elucidate the process in more detail, as cancer cells can become drug resistant over time by modulating ion channels and transporters to prevent cell apoptosis [51].

3.6.4 Potential Applications of PEG

Given the mechanism of action by osmotic effects, it is proposed that any potential application will require targeted administration of PEG in order to produce and maintain osmotic effects. Potential applications could include PEG as a topical cream for dermal cancers, or gastrointestinal cancers (given that PEG > 400 MW is generally not absorbed and is passed through the GI tract). In fact, the work by Roy *et al* has already demonstrated that PEG_{3350} (a strong laxative administered prior to colonoscopy) produces apoptosis in HT-29 colon cancer cells [18]. A limitation of this work was that upstream events leading to cell death were unclear and this current study may provide a possible explanation for their observed results.

Another mechanism by which localized, targeted PEG effects may be achieved is by exploring the therapeutic potential of PEG when conjugated to a targeted therapeutic or as a drug delivery vehicle. PEG is the first-line polymer for improving drug solubility and increasing residence time by being directly conjugated to the therapeutic [52]. In this case, this established system may provide a unique and simple mechanism for exploring the chemotherapeutic potential of PEG. PEG is also frequently investigated as a drug delivery vehicle or part of a drug delivery vehicle, and with this knowledge it may be possible to improve the delivery system by capitalizing on the biological activity of PEG against cancer cells. PEG can also be extensively modified from a structural perspective, and with appropriate modification, there is the potential for targeted, localized delivery of PEG alone to disease sites as part of this approach as well.

3.7 Conclusion

Overall, this study indicates for the first time that PEG has inherent biological activity in human and non-human cells that is linked to its structure and properties. The results specifically indicate that the mechanism of toxicity is due to changes in extracellular osmolality, which can be correlated with the number of ethylene oxide units present in the extra-cellular space. It is important to note that these data do not suggest new clinical concern for the safety of PEG in pharmaceutical, cosmetic or food applications, especially given the long-term evidence of PEG safety in these applications. Rather, this study highlights the untapped potential of PEG, and possibly other polymers, as therapeutic agents. Given that the structure and properties of polymers, particularly synthetic polymers, can be very finely tuned, there is limitless potential for polymers (and PEG specifically) to become part of modern medical treatments, rather than being solely used as a pharmaceutical filler or as therapeutic carriers.

3.8 References

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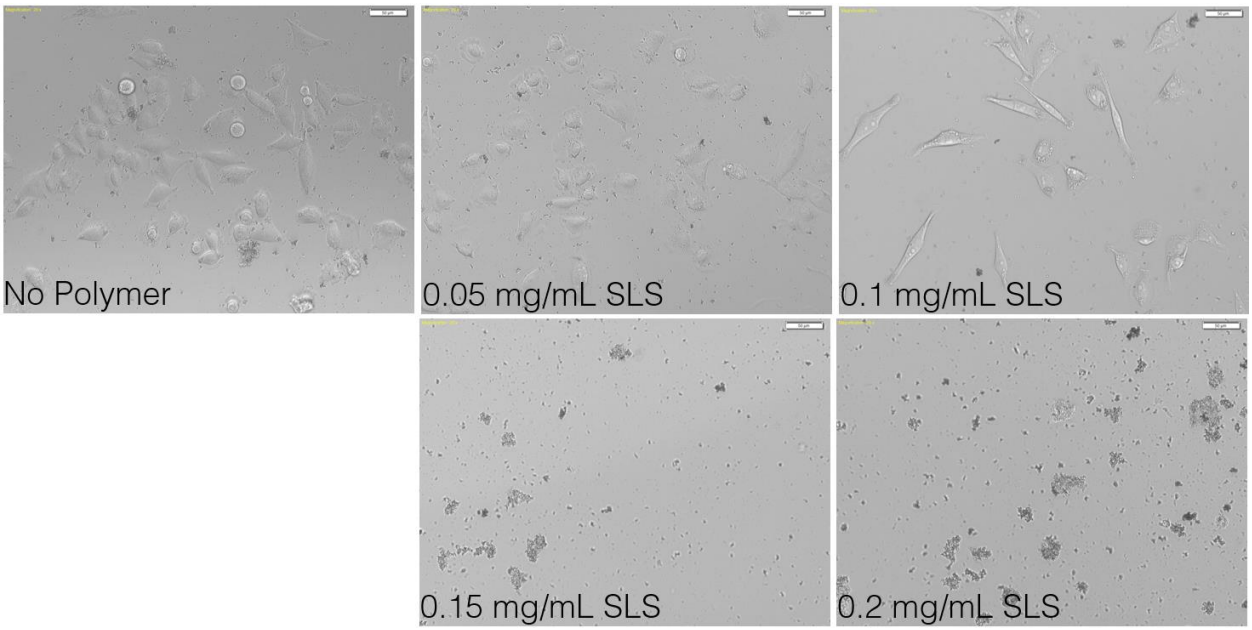
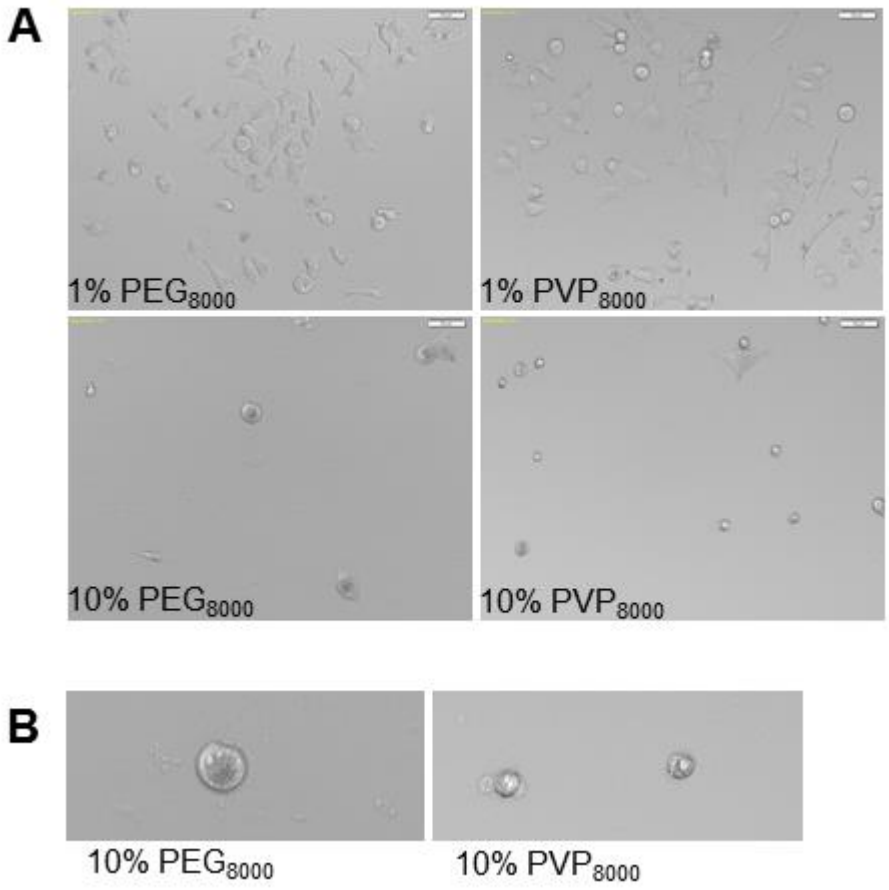
3.9 Supplementary Data

Supplementary Table 3.1. Comparison of the approximate number of PEG moles at ~100% or 40% cell viability, and the corresponding number of ethylene oxide units. Ethylene oxide (EO) content was calculated based on the 1% w/v of PEG data point seen in Figure 3.2.

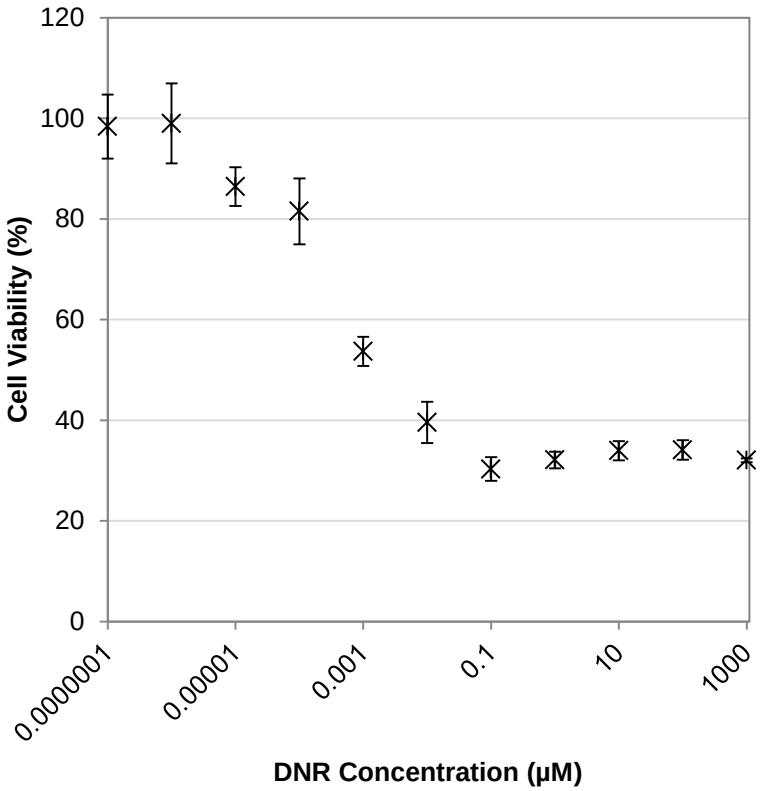
	PEG_{200}	PEG_{2000}	PEG_{8000}	$PEG_{20,000}$
Viability of ~100%				
# of moles	50	5	3.75	0.5
# of EO units	161	161	483	161
Viability of ~40%				
# of moles	1000	100	25	10
# of EO units	3222	3222	3222	3222



Supplementary Figure 3.1. Morphology of A375 cells exposed to sodium lauryl sulfate for 72 hours



Supplementary Figure 3.2. Morphology of A375 cells exposed to PEG8000 or PVP8000 for 72 hours.



Supplementary Figure 3.3. Daunorubicin hydrochloride reduces cellular viability of A375 metastatic cancer in a concentration-dependent matter, after 72 hours of exposure. Error bars denote standard error.

4 Controlling the release of tobramycin by incorporating poly(ethylene glycol) into silicone hydrogel contact lens materials

Authors

Ivana Postic and Dr. Heather Sheardown

Author contributions

The work described in this chapter was conceived, conducted and analyzed by Ivana Postic with support, consultation and editing from Dr. Heather Sheardown.

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The Literature Appendix and Figures 1 and 3 were omitted from the submitted manuscript.

4 Controlling the release of tobramycin by incorporating poly(ethylene glycol) into silicone hydrogel contact lens materials

4.1 Abstract

The controlled delivery of hydrophilic drugs can be difficult to achieve due to the burst release of drug that is associated with materials of high water content, such as hydrogels. Silicone hydrogels have significant potential for drug delivery due to their increased hydrophobicity and the tortuous nature of the pores, overcoming some of the limitations associated with conventional hydrogel materials. The aim of this study was to examine the potential of poly(ethylene glycol) (PEG) containing silicone hydrogels for delivery of the hydrophilic aminoglycoside antibiotics. It was hypothesized that PEG, a polymer that has seen extensive use in biomedical applications, will provide, in addition to hydrophilicity and protein repulsion, a mechanism for controlling the delivery of this hydrophilic drug. PEG was combined with the macromer TRIS to create the model silicone hydrogel materials. The optical and physical properties of the novel TRIS-*co*-PEG silicone hydrogels showed materials with excellent transparency, low refraction and high transmittance indicating minimal phase separation. Desirable properties such as wettability and protein repulsion were maintained across a wide range of formulations. The water content was found to be highly correlated with the ethylene oxide content. Drug release could be influenced through PEG content and was found to fit Higuchi-like kinetics. Overall, the study demonstrates that incorporation of PEG into a model silicone hydrogel could be used to establish more gradual release of a hydrophilic compound. Data suggests this is related to the unique structure and properties of PEG which alter the types of water found in each formulation and the water content.

4.2 Introduction

In order to provide better therapeutic efficacy and safety, drug delivery systems have been widely applied in pharmaceutical formulations. Hydrogels in particular have received a great deal of attention as potential candidate materials for controlling the release of drugs [1], [2]. These water swollen hydrophilic network polymers impart their controlled release by acting as a partition through which the drug compound of interest must diffuse [3]. True controlled release of hydrophilic compounds, however, can be very difficult to attain. This is primarily due to burst release associated with a hydrophilic drug that occurs almost immediately upon placing the drug-loaded device in contact with an aqueous solution [3]–[6]. In addition, protein deposition can lead to fouling, which can alter drug release rates and result in inflammation [7].

Despite these limitations, hydrogels remain one of the most widely used type of biomaterials due to their biocompatibility [8], versatility [9], and low material cost [10], [11]. While hydrogels remain the standard for drug delivery in numerous clinical applications [12], [13], substantial research has been devoted to the development of strategies to overcome the limitations of these materials. For example, increasing the crosslinking density within a hydrogel can reduce the rate of hydrophilic drug release [9], [14]. Covalent tethering of a drug via hydrolysable or biodegradable linkers is another strategy for controlling the release of the drug [15], [16]. These approaches may not, however, be optimal for clinical translation due to the changes in the desired physical properties of the material (e.g. transparency, contact angle) that may be the result of these modifications [17], [18].

An alternate strategy is to use physical interactions between the polymer and the drug to tune drug release. This has been seen frequently seen in molecular imprinting, where physical interactions (such as ionic and hydrogen-bonds) between the polymer and drug are used to first assemble the polymer in a way that creates ‘pockets’ of drug. These ‘pockets’/ drug templates can then increase drug uptake and slow release of the polymer [19]–[23]. These interactions are often ionic and require the incorporation of ionisable polymers. However, increased surface charge on a material can lead to increased protein deposition and other deleterious effects [24], [25]. Protein deposition can subsequently lead to undesirable activation of inflammatory processes and ultimately in the rejection of the material by the body [3][26], [27]. Although ionic polymers tend to be non-toxic and excellent for oral drug delivery, their widespread application as hydrogel biomaterials may not be optimal, especially where protein deposition may increase the risk of complications such as in blood contacting devices and contact lenses[4].

Outside of molecular imprinting, a physical interaction that has not been widely explored for drug delivery alone is hydrogen-bonding between the polymer and drug, likely due to this bond being weaker than ionic bonds. There are only a few examples where this bonding has been used alone for drug delivery. For instance, Papageorgiou *et al*. were able to demonstrate hydrogen bonding between solid dispersions of hydrophilic drug and

[3] Refer to Literature Appendix I for details
[4] Refer to Literature Appendix II for details

chitosan matrices (28). This physical interaction led to a slowed drug release and hydrogel formulations with minimal burst release (28). The release data suggest that the very small amount of drug available at the surface releases more easily than the deeply embedded drug, leading to a smaller burst release (28). Ozeki *et al* were similarly able to show that hydrogen bonding between solid dispersions of a drug in poly(ethylene glycol)-containing matrices altered drug release rates (29). Despite these studies indicating that hydrogen-bonding can control drug release, a limitation of these formulations is the loading of drugs as solid dispersions within the matrices which may lead to changes in the physical properties of the hydrogels as the drug is released (such as changes in crystallinity leading to changes in elasticity or mechanical strength) (30–33).

In this work, it was hypothesized that by incorporating the hydrogen bonding potential of poly(ethylene glycol) (PEG) as the hydrophilic component of silicone hydrogel materials, burst release of hydrophilic drugs loaded by soaking can be reduced, and more gradual release can be attained. Specifically, similar to PEG binding with water, which can have three states (tightly bound, loosely bound and free (34)[5]), it was hypothesized that hydrogen binding between PEG and a hydrophilic drug will occur and that this will lead to more gradual release of the drug. A silicone hydrogel system was chosen for this study, as they are currently the most commonly prescribed contact lens materials (35), and they have numerous properties that make them excellent biomaterials including high oxygen permeability and, with appropriate surface modification, minimal protein deposition. Further, the siloxane component of these materials could provide a physical barrier to drug transport, slowing drug release compared to more conventional hydrogel materials. While there is interest in using these materials for drug release, they have, somewhat surprisingly, not been widely successful in their commercialization.

In addition to potential drug influencing properties, PEG is of particular interest in this application as it has the unique property of being able to hydrogen bond 2-3 water molecules for every ethylene oxide unit (36,37). Its high chain flexibility adds to the ability of PEG to create a large cage of hydrogen-bonded water around it (36,37). Steric hindrance then produces the protein repelling properties that are desirable in biomedical applications (38). Notably, PEG is known to maintain many of its free-chain characteristics when incorporated into a hydrogel. In these applications, the strong hydrogen-bonding ability of PEG is able to mask hydrophobic surfaces that can be prone to protein adsorption (39,40), allowing for the creation of surfaces with high surface energy and low protein adsorption.

Thus, in order to test the hypothesis that PEG containing silicone hydrogels could be used to control drug release via hydrogen-bonding, the release of the highly hydrophilic drugs (tobramycin and amikacin) was tested using novel model silicone hydrogels composed of co-polymerized macromers methacrylated -PEG and TRIS. Tobramycin is specifically used in ocular applications as an antibiotic eye drop (41) for the treatment of a host of ocular infections. Due to the fast washout times, it must be applied multiple times each day for efficacy and therefore would benefit from controlled release to

[5] Refer to Literature Appendix III for details

maximize patient compliance (42) and improve drug bioavailability (43). In addition to drug release, polymer properties including surface and bulk characteristics were examined.

4.3 Materials

(3-methacryloyloxypropyl)tris(trimethylsiloxy) silane (TRIS), poly(ethylene glycol) methyl ether methacrylate (PEG, with M_n of 500 or 300), ethylene glycol dimethacrylate (EDGMA), isopropyl alcohol, inhibitor remover beads, tobramycin (≥98%), fluorescamine (≥98%), amikacin (European pharmacopeia standard), lysozyme from chicken egg white, bovine serum albumin, and sodium dodecyl sulfate were purchased from Sigma-Aldrich (Oakville, ON). The photoinitator 1-hydroxy-cyclohexyl-phenyl-ketone (Irgacure®184) was generously donated by BASF Chemical Company (Vandalia, IL). 10x phosphate-buffered saline was obtained from Bioshop Canada Inc. (Burlington ON) and diluted to 1x for experiments. The UV-permeable acrylic mold (Plexiglass® G-UVT) was generously donated by Altuglass International (Bristol, PA). A Cure Zone 2 CON-TROL-CURE (Chicago, IL) chamber with a 400 W UV lamp and 365 nm wavelength light was used for polymer preparation. A Tecan Infinite® M1000 PRO plate reader spectrophotometer was used for all spectrophotometry. A Hyperion 3000 microscope (Bruker Corporation - Billerica, MA) was used for FTIR measurements. SEM was performed using a FEI-Magellan 400, XHR FE-SEM. Surface wettability of each material was determined using contact angle measurements made on a Dataphysics OCA20 goniometer (Dataphysics Instruments GmbH - Filderstadt, Germany). An Atago Pal-1 pocket refractometer (Atago Co LTD., Japan) was used to measure the refractive index of the materials. Proton NMR was performed on a Bruker AV 600 spectrometer at 600 MHz. CryoTEM was performed on a JOEL 1200EX TEMCAN. Mechanical testing was performed on an Instron 4411 Universal Tester.

4.4 Methods

4.4.1 Silicone hydrogel synthesis

Macromers and crosslinkers were passed through a syringe column containing inhibitor remover prior to polymerization. The compositions of the polymers prepared are summarized in Tables 4.1 and 4.2. Note that the molar ratios presented in Table 4.1 are based on the full chain length of the PEG rather than the repeat units of ethylene oxide.

Table 4.1. Composition of silicone hydrogel formulations (mol%).

Formulation	**Ratio**	**Macromer**			**Crosslinker**	Inclusion in study
		TRIS	PEG_{500}	PEG_{300}	EGDMA	
TRIS-*co*-PEG_{500}	1 : 1	48.5	48.5	–	3	+
TRIS-*co*-PEG_{300}	1 : 1	48.5	–	48.5	3	+
TRIS-*co*-PEG_{500}	7 : 3	67.9	29.1	–	3	+
TRIS-*co*-PEG_{300}	7 : 3	67.9	–	29.1	3	+
TRIS-*co*-PEG_{500}	3 : 7	29.1	67.9	–	3	–
TRIS-*co*-PEG_{300}	3 : 7	29.1	–	67.9	3	–

Table 4.2. Composition of silicone hydrogel formulations (wt%). The crosslinker was not included in these calculations for simpler ratio visualization.

Formulation	Ratio	Macromer		
		TRIS	PEG_{500}	PEG_{300}
Tris-*co*-PEG_{500}	0.85 : 1	45.8	54.2	
Tris-*co*-PEG_{300}	1.41 : 1	58.5		41.5
Tris-*co*-PEG_{500}	1.98 : 1	66.4	33.6	
Tris-*co*-PEG_{300}	3.29 : 1	76.7		23.3

200 µL (2.61 mol) of isopropyl alcohol was added to the macromer solution to facilitate mixing between PEG and TRIS as it was found that macromer solutions prepared without IPA had visible phase separation, and incomplete polymerization. In addition, the length of PEG macromers were selected to be as small as possible as it was hypothesized that increased phase separation would result from the use of longer chain PEGs. Following mixing, 7.35 mg of photoinitiator (Irgacure® 184) was added. The resulting solution was thoroughly mixed by vortexing and subsequently transferred using a 20 gauge needle to a custom hydrogel-mold (polystyrene sheets separated by a Teflon spacer) (Figure 4.1). The mold was then placed in the UV chamber and activated with light at 365 nm for a period of 15 minutes.

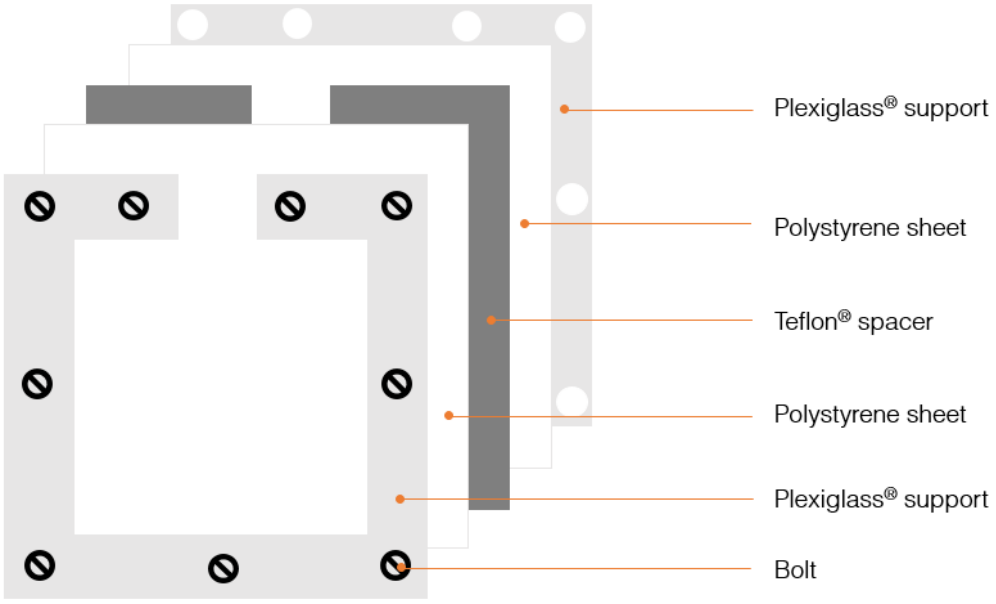

Figure 4.1. The hydrogel mold consisted of a 0.5mm Teflon® spacer placed between two polystyrene anti-stick sheets. Thick Plexiglass® surrounded either side of the polystyrene sheets for physical support.

The cross-linked hydrogel materials were then soaked in 20 mL of IPA for 4 x 30min then in 20 mL water for 2 x 30 minutes to remove residual unreacted components and IPA. Hydrogels were placed in fresh distilled water for long-term storage. As noted in Table 2, crosslinked formulations containing TRIS-*co*-PEG (3:7) were not included in this study,

as they were determined to be too fragile to be handled. Figure 4.2 shows the reaction process.

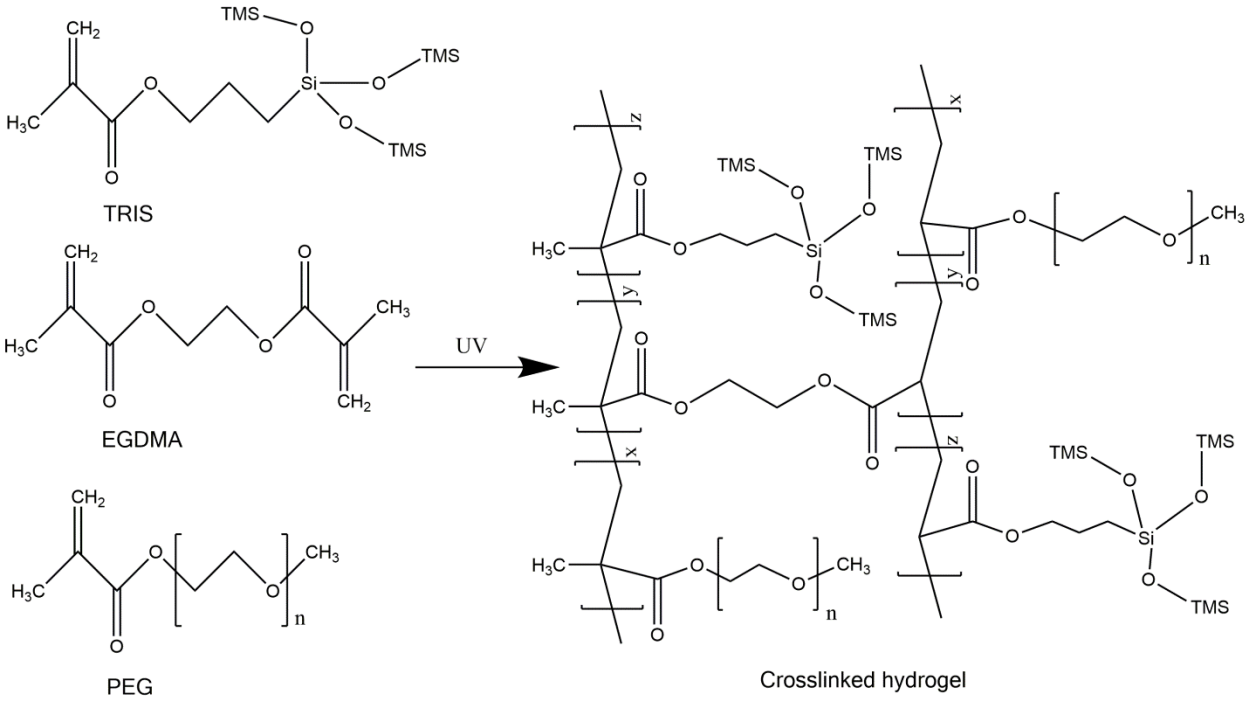

Figure 4.2. Schematic depicting the reaction between macromers and crosslinker to form the novel hydrogel materials. n: repeating ethylene oxide units corresponding to 300 or 500 molecular weight PEGs. x,y,z: varying macromer ratios that exist within the hydrogels. TMS: trimethylsiloxane

4.4.2 ATR-FTIR

Attenuated-total reflection - Fourier transform infrared spectroscopy (ATR-FTIR) was used to evaluate the UV polymerization procedure by characterizing the materials for the presence of specific chemical groups present in the TRIS and PEG macromers.

4.4.3 NMR

Extracted hydrogels were dried overnight at 37°C. Macromers (with inhibitors removed) and dried hydrogels were solvated in $CDCl_3$ and ^1H NMR was performed at room temperature.

4.4.4 SEM

Scanning-electron microscopy (SEM) of the materials was performed to examine the surface composition of the hydrogels and surface roughness. All materials were examined on glass-polished steel supports to avoid the formation of dehydration artifacts. Two states of materials were compared: (1) Dry materials formed immediately after polymerization, and (2) hydrated materials dehydrated on the glass-polished supports. Dehydration was performed at 37°C.

4.4.5 Contact Angle

Surface wettability of the hydrogels was measured using the captive bubble method (Figure 1.3) to ensure hydrogels remained hydrated during measurement, and that artifacts from material dehydration were avoided. Captive bubble measurements have also been determined to be more clinically relevant than sessile drop measurements (44). However, given that sessile drop angles are more commonly reported and understood, the captive bubble contact angles are reported similarly to sessile drop angles (180-θ) for easier comprehension of the material hydrophilicity.

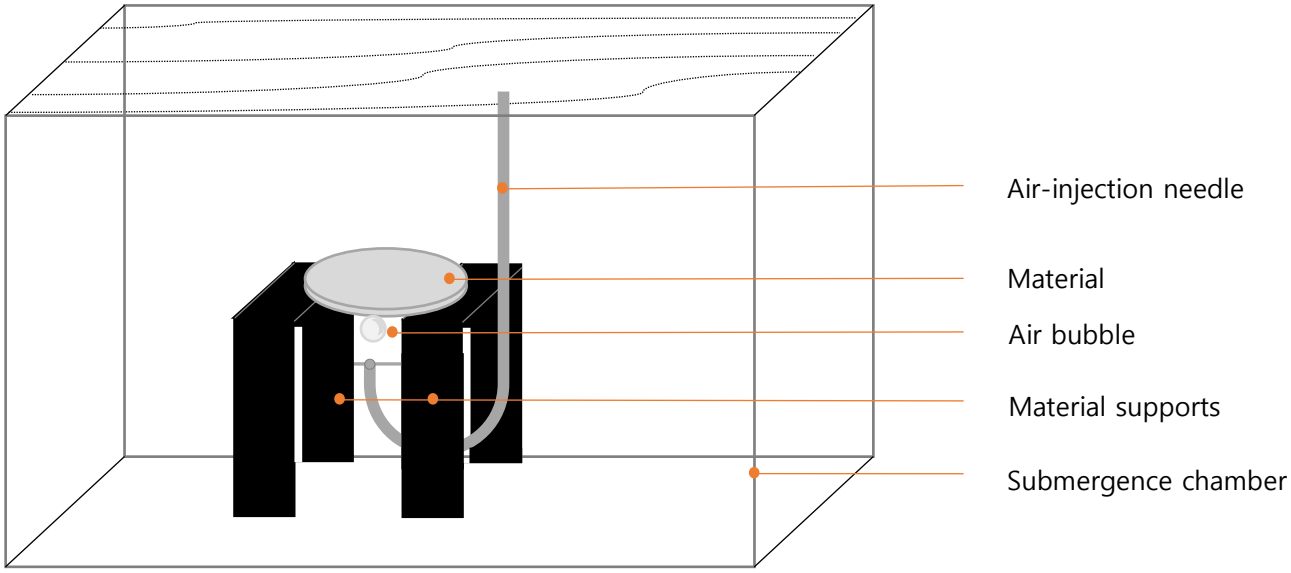

Figure 1.3. Schematic of the captive bubble set-up. Support blocks were placed at the bottom of a small, water-filled glass box and the hydrogel was placed gently on top of the supports. A hollow needle was placed underneath the centre of the hydrogel and connected to an air-pump. A 1 µL bubble of air was released, which travelled upwards to sit against the hydrogel. The instrument camera was then used to magnify the point-of-contact between the air bubble and hydrogel and determine the contact angle.

4.4.6 Protein Adsorption

Protein deposition studies were performed to assess the non-fouling effect of PEG incorporation in these model contact lens materials. Lysozyme and albumin were chosen as model proteins for study. Lysozyme is the most abundant protein in the tear film; albumin has been touted as having a passivation effect and is also abundant in the tear film, particularly during times of stress such as would be the case in an inflamed or infected eye.

Four samples of each material (1/4") were placed in 0.2 mL of 1 mg/ml protein solution (lysozyme or albumin) radiolabeled with 10% Iodine^{125}. Samples were incubated in protein solution for 3 hours at room temperature. The hydrogels were then rinsed three times for five minutes each in 0.2 mL of PBS in order to remove any loosely bound protein. Samples were then carefully dabbed with a KimWipe to remove excess surface droplets. Adsorbed protein on the samples was measured by reading samples for 5 minutes using a gamma counter (1470 Wallac Wizard; PerkinElmer, Woodbridge, ON).

4.4.7 Mechanical Testing

The strength and elasticity of the materials was measured on materials formed into a barbell mold (9 mm width, 35 mm grip distance). A 50N load cell was used to collect measurements and speed was 10 mm/min.

4.4.8 Light Transmittance

Materials were tested for transparency by measuring the light transmittance over the UV and visible spectrum (200-400, 400-700 nm, respectively). PBS-hydrated discs were placed on the bottom of a 96-well plate with an overlay of 100 µL of PBS and measured using a spectrophotometer.

4.4.9 Refractive Index

The refractive index of the materials swollen in 300 µL of PBS was measured at ambient temperature using a pocket refractometer.

4.4.10 TEM

Transmission electron microscopy was performed on materials hydrated in water then cut with a microtome while under cryogenic conditions. TEM was performed in order to better visualize the potential phase separation between TRIS and PEG within each material.

4.4.11 Water Content

Samples a quarter-inch in diameter were swollen in distilled water for a minimum of 24 hours at room temperature, subsequently removed and excess water gently removed using a KimWipe. The sample was then weighed and placed in a 37°C oven until completely dry. The dry samples were weighed and the equilibrium water content was calculated using Equation 1.

$$EWC\ (\%) = \frac{mass\ of\ hydrated\ gel\ (m_h) - mass\ of\ dry\ gel\ (m_d)}{mass\ of\ hydrated\ gel\ (m_h)} \cdot 100\% \quad (1)$$

Swelling ratio was calculated using Equation 2.

$$Swelling\ (\%) = \frac{mass\ of\ hydrated\ gel\ (m_h) - mass\ of\ dry\ gel\ (m_d)}{mass\ of\ dry\ gel\ (m_d)} \cdot 100\% \quad (2)$$

4.4.12 Differential Scanning Calorimetry

Hydrogels swollen in distilled water were placed into aluminum pans. Lids were placed immediately and the pans were sealed to prevent evaporation. DSC was performed using a DSC200 (TA Instruments, Newcastle, DE) by ramping temperature from -40°C to 15°C at 5°C/min. The free/bulk/freezable water, loosely bound/intermediate water and tightly bound/non-freezable water were calculated according to Ping et al (45). Water was chosen as the solvent rather than PBS as it is the standardized method for characterizing hydrogels in the literature, but also to achieve a more direct understanding of any relationship between PEG and water molecules and for relating resulting data to

the equilibrium water content of each hydrogel. DSC thermograms were normalized to the mass of water in each gel (product of EWC and mass of hydrated material).

4.4.13 Drug Loading and Release

Phosphate-buffered saline, pH 7.4, was sterile-filtered prior to use by passing it through a 0.2 μM filter. Four 1/4” samples of each material were equilibrated in PBS for at least 24 hours. The samples were then loaded with drug solution by placing them in 1 mL of 5 mg/mL tobramycin or amikacin (solvated in PBS) for 24 hours. Samples were then carefully wiped using a KimWipe to ensure all surface drops were removed. Drug release was performed by placing samples individually in 0.5 mL of PBS in a VWR shaking incubator (37°C, 100 rpm). PBS was replaced at predetermined timepoints over 6 hours and at 24 hours.

4.4.14 Drug Quantification

For quantification of the released drug, an adapted literature procedure involving conjugation of fluorescamine through the free amine and subsequent fluorimetry was performed (46–48). Within each well, 150 μL of release solution from the study was incubated with 50 μL of 5 mg/mL fluorescamine dissolved in DMSO. The fluorescent compound was measured at excitation/emission wavelengths set to 380/480 nm. The assay was performed in black 96-well plates in order to reduce background fluorescence and prevent cross-talk between wells.

4.4.15 Statistical Analysis

A one-factor analysis of variance was used to analyze the equilibrium water content and contact angle. A Tukey test was performed post-hoc when significant differences were identified ($p<0.05$). All error bars represent standard deviation.

4.5 Results

4.5.1 Confirmation of synthesis

4.5.1.1 ATR-FTIR

In order to confirm the conjugation of TRIS and PEG, ATR-FTIR was performed on each of the samples. The FTIR spectra are displayed in Figure 4.4.

There were 6 regions of peaks identified in the spectra (labelled A-F). C and D are peaks that correspond to the bonds of Si-O-R and Si-CH_3, respectively. The ether bonds of PEG are also found within this region (1000-1300) and are not distinguishable as they likely directly overlap with silicone peaks. Regions A and F indicate the presence of PEG. Region F corresponds to the O-H bond of water. Region A (the “fingerprint region”) shows the presence of PEG, as this region is altered in the presence of PEGs with different molecular weights. Specifically, PEG_{500} has a unique fingerprint in comparison to PEG_{300}. Peaks in the B region correspond to the benzene groups of residual photoinitiator.

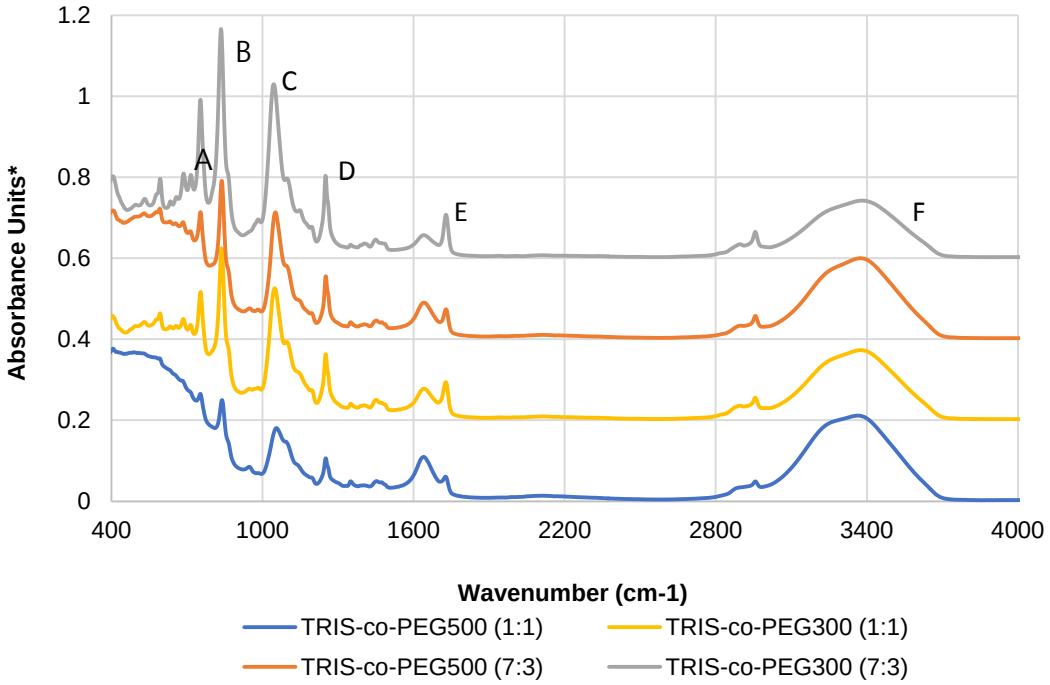

*Figure 4.4. FTIR spectra of TRIS-co-PEG materials. *Note, baseline for blue is 0 A.U, yellow is 0.2 A.U, orange is 0.4 A.U, grey is 0.6 A.U.*

4.5.1.2 NMR

The ^{1}H NMR results of the macromers and hydrogels are reported in Table 4.3. Full spectra are available in Supplementary Information. Macromer spectra were as expected, and crosslinked hydrogels showed some peak broadening as was expected.

Table 4.3. 1H NMR shifts of macromers and hydrogels in CDCl3. Hydrogel shifts are reported only for the EO chain and TMS for ease of reading.

Macromer	^{1}H NMR (CDCl3, 600 MHz) δ	Hydrogel	^{1}H NMR (CDCl3, 600 MHz) δ (for EO chain, TMS)
TRIS	7.16 (s, solvent peak); 6.00 (q, 1H, CH_2=C); 5.44 (t, 1H, CH_2=C); 3.98-4.01 (m, 2H, OCH_2); 1.85 (t, 3H, CH_3); 1.59 (q, 2H, CH_2); 0.4 (m, 2H, CH_2Si); 0 (s, 27H, TMS)	TRIS-*co*-PEG_{500} (1:1)	2.4-4.41 (m, 33H, $(CH_2CH_2O)_n$); 0.53-0.86 (s, 27H, TMS)
PEG_{500}	7.29 (s, solvent peak); 6.10 (s, 1H, CH_2=C); 5.55 (d, 1H, CH_2=C); 4.26-4.29 (m, 2H, OCH_2); 3.51-3.75 (m, 33H, $(CH_2CH_2O)_n$); 3.35 (s, 3H, PEG-CH_3); 1.92 (s, 3H, CH_3)	TRIS-*co*-PEG_{300} (1:1)	2.31-4.88 (m, 18H, $(CH_2CH_2O)_n$); 0.52-0.59 (s, 28H, TMS)
PEG_{300}	7.29 (s, solvent peak); 6.12 (s, 1H, CH_2=C); 5.57 (d, 1H, CH_2=C); 4.27-4.31 (m, 2H, OCH_2); 3.52-3.80 (m, 16.16H, $(CH_2CH_2O)_n$); 3.37 (s, 3H, PEG-CH_3); 1.94 (s, 3H, CH_3)	TRIS-*co*-PEG_{500} (7:3)	2.51-4.59 (m, 33H, $(CH_2CH_2O)_n$); -0.71-0.49 (s, 59H, TMS)
EGDMA	7.29 (s, solvent peak); 6.12 (s, 1H, CH_2=C); 5.57 (d, 1H, CH_2=C); 4.27-4.31 (m, 2H, OCH_2); 3.52-3.80 (m, 16.16H, $(CH_2CH_2O)_n$); 3.37 (s, 3H, PEG-CH_3); 1.94 (s, 3H, CH_3)	TRIS-*co*-PEG_{300} (7:3)	2.76-4.6 (m, 16H, $(CH_2CH_2O)_n$); -0.93-0.60 (s, 66H, TMS)

Due to the varying molecular weights of PEG and macromer molar ratios, the expected number of protons (based on the macromere NMR peak integration performed in spectra from Table 4.3), was compared with the measured number of protons (based on the hydrogel NMR spectra, and using the PEG peak as the reference value). These results seen in Table 4.4 confirm that the hydrogels were successfully polymerized at the input molar ratios. Some difference between expected and measured proton numbers was expected and is seen (due to the broadened peaks in the crosslinked, polymerized hydrogels).

Table 4.4. The structure of each hydrogel formulation is confirmed using ^{1}H NMR data.

	Expected # of Protons		Measured # of Protons	
	$(CH_2CH_2)_n$	TMS	$(CH_2CH_2)_n$	TMS
TRIS-*co*-PEG_{500} (1:1)	33	27	33	27
TRIS-*co*-PEG_{300} (1:1)	16	27	16	25
TRIS-*co*-PEG_{500} (7:3)	33	63	33	59
TRIS-*co*-PEG_{300} (7:3)	16	63	16	66

4.5.2 Surface Properties

4.5.2.1 Surface morphology

The surface morphology of silicone hydrogels can demonstrate the presence of phase separation and provide an indication of surface roughness. Both factors are important in producing materials that are more biologically compatible, as exposure of the hydrophobic TRIS component on the surface can lead to protein denaturation, irritation, and inflammation.

The surface morphology was measured using SEM under two conditions: materials were either dry (tested after polymerization) or dehydrated (swelled in water then dehydrated) on the SEM stand. These conditions were tested in order to observe for the possibility of dehydration artifacts. Dehydrated samples are likely more accurate representations of the material surface in biological settings, as the presence of water is very likely to alter the position of macromer chains.

In Figure 4.5, SEM images at 25,000x magnification are shown. The representative images were chosen because they captured defects in the material, indicating that the surfaces are in focus. With the exception of TRIS-*co*-PEG_{500} (1:1), the materials were highly consistent and very smooth. TRIS-*co*-PEG_{500} (1:1) was seen to have more channel like formations under dry conditions, with some faint waves after dehydration. There is no indication of artifacts from the dehydration process for hydrated materials.

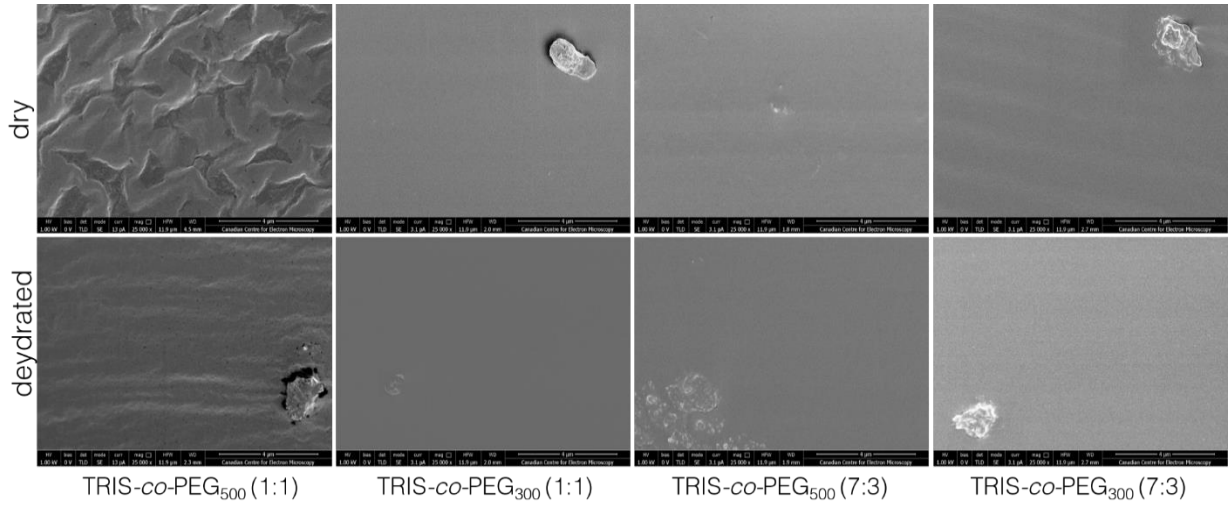

Figure 4.5. Surface morphologies of TRIS-co-PEG materials as observed using SEM. Representative images shown are taken at 25,000x magnification.

4.5.2.2 Contact Angle

The surface hydrophilicity of a material can provide significant information on its potential applications. Highly hydrophobic materials may not be suitable for biomaterial applications, due to the potential for protein deposition and denaturation. The surface hydrophilicity was assessed using contact angle measurements and a protein deposition assay.

All materials were very hydrophilic at the surface (Table 4.5). The differences in contact angle across the materials was not significant ($p>0.05$). This indicates that PEG is able to produce similar interactions with water on the material surface, regardless of the PEG concentration or molecular weight tested.

Table 4.5. Contact angles of TRIS-co-PEG materials (n=3).

Material (mol ratio)	Contact Angle (°)
TRIS-*co*-PEG_{500} (1:1)	35.02 ± 4.86
TRIS-*co*-PEG_{300} (1:1)	40.66 ± 5.19
TRIS-*co*-PEG_{500} (7:3)	46.11 ± 6.08
TRIS-*co*-PEG_{300} (7:3)	40.46 ± 0.87
TRIS-only	55.09 ± 9.75

Surprisingly, TRIS-only materials showed to be hydrophilic as well. It is known that TRIS macromers are hydrophobic, however, when polymerized it is possible that the conformation of the side chains impacts the surface hydrophilicity. Specifically, the contact angles may show a hydrophilic surface due to the hydrophobic trimethylsiloxane chain ends being entropically more favoured to be tucked inside of the material. At the same time, the carbonyl of the polymerized methacrylate backbone is preferentially

exposed to the surface, where the oxygen can hydrogen-bond with two water molecules and form the hydrophilic surface that was observed.

4.5.2.3 Protein Adsorption

Protein adsorption measurements further confirm the hydrophilicity of the material surface. After incubation in 1 mg/mL of protein (hen egg lysozyme or bovine serum albumin), there was less than 1 µg protein/cm^2 adsorbed (Figure 4.6).

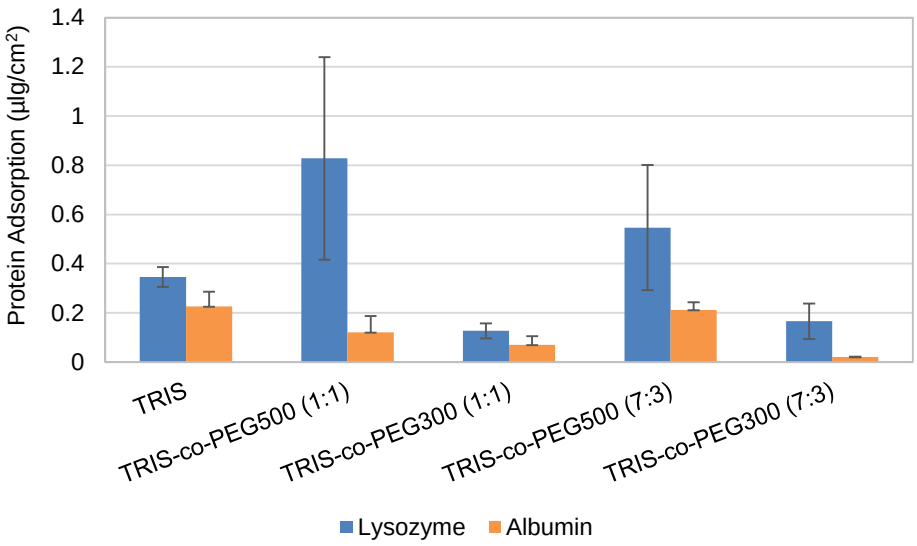

Figure 4.6. Adsorbance of lysozyme or bovine serum albumin onto the hydrogels (stdev, n=4).

Notably, lysozyme (14.3 kDA) is the smaller of the two proteins and seen to be more greatly sorbed within the materials, likely because it can diffuse into the material and become sorbed there, instead of being adsorbed only on the surface. The highest lysozyme sorption is seen with the materials with highest EWC (see Table 4.7). TRIS-only material was included for comparison, and results show that the materials with PEG_{300} (lowest EWC) show lower protein adsorption than TRIS-only material, indicating the protein repelling property of PEG. The data also suggests that TRIS-only materials swell/retain water (due to the increased lysozyme sorption) and this data was confirmed in EWC/swelling studies shown later (Table 7).

Albumin-adsorbed materials show fairly consistent and low protein adsorption (<0.1 µg/cm^2). The lower adsorption (in comparison to lysozyme) is attributed to the lower molecular packing due to the larger size of albumin, and lowered ability to diffuse into the material.

Overall, this data indicates that TRIS-*co*-PEG materials show good surface wettability, based on its low overall protein sorption and surface contact angle.

4.5.3 Bulk Properties

In order to fully characterize the influence of PEG structure on the hydrogel, the bulk properties of the material were assessed through measurements of tensile properties, optical qualities and water content.

4.5.3.1 Tensile Testing

The tensile strength and elasticity of a biomaterial is important for both the handling of the material and its successful application at the site of use. For example, for contact lenses, the materials must be strong enough to be handled without damage by a patient, and not too soft that they bend when blinking forces are applied (49,50). Figure 4.7 shows the strength and elastic modulus of the TRIS-*co*-PEG materials. The PEG_{500} (7:3) formulation shows both the greatest modulus and strength. The PEG_{300} (7:3) formulation shows the lowest modulus. PEG_{300} (7:3) and PEG_{500} (1:1) show the lowest strengths. This data is somewhat surprising given the knowledge that siloxanes provide strength due to the Si-O bonds, thus this unique data is later further explored in the context of the hydrated material and its relationship with water. Still, the modulus data is within range of reported values for conventional hydrogels in the literature (51,52).

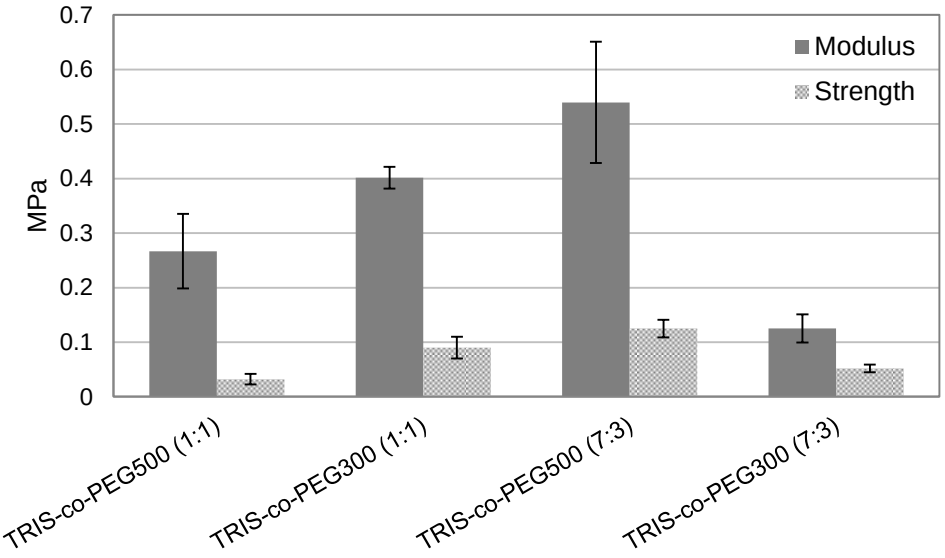

Figure 4.7. Mechanical properties of materials (stdev, n=4).

4.5.3.2 Optical Qualities

Material transparency is important to evaluate because it can provide information on the polymerization efficiency and optical qualities which may be important in certain biomedical applications such as ophthalmology. It was hypothesized that the materials may be translucent or opaque, due to possibility for phase separation between the hydrophobic TRIS and hydrophilic PEG. However, all the materials were highly transparent when hydrated in PBS as seen in Figure 4.8. PEG_{300} (7:3) materials showed opacity when hydrated only in water, indicating that the PBS salts help to create more favorable PEG conformations for less phase separation between the short-chain PEG and the high TRIS content.

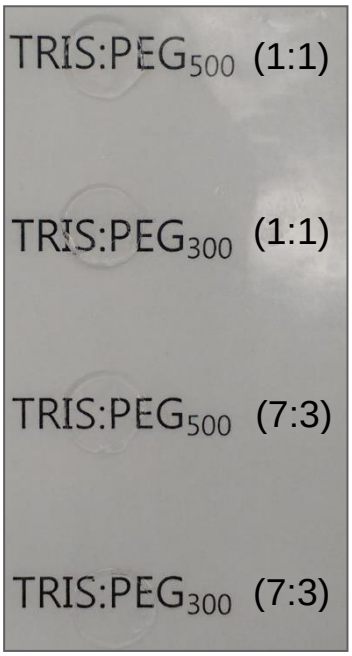

Figure 4.8. Photograph image of novel TRIS-co-PEG hydrogels swollen in PBS.

The optical qualities were further examined by measuring the refractive index of the materials. The refractive indexes of the materials were all within 0.02% of the refractive index of PBS alone, and within measurement error of the device (±0.00005), as described in Table 4.6. This data is lower than that of commercial corrective lenses (53), however it does not impeded further study of the materials as model silicone hydrogels for understanding the structure-function properties of PEG.

Table 4.6. Refractive indexes of TRIS-co-PEG materials.

	Refractive Index
TRIS-*co*-PEG_{500} (1:1)	1.3326 ± 0.0002
TRIS-*co*-PEG_{300} (1:1)	1.3329 ± 0.0001
TRIS-*co*-PEG_{500} (7:3)	1.3328 ± 0.0001
TRIS-*co*-PEG_{300} (7:3)	1.3329 ± 0.0001

The light transmittance of the materials (Figure 4.9) was measured across UV and visible wavelengths. All materials transmitted about 100% of light across the visible spectrum. TRIS-co-PEG_{500} (7:3) materials showed slightly lower (~95%) transmittance across the visible spectrum in comparison to the other materials. All materials similarly transmitted UV light across the 200-400 nm wavelengths, with a sharp rise in transmittance with increasing wavelength.

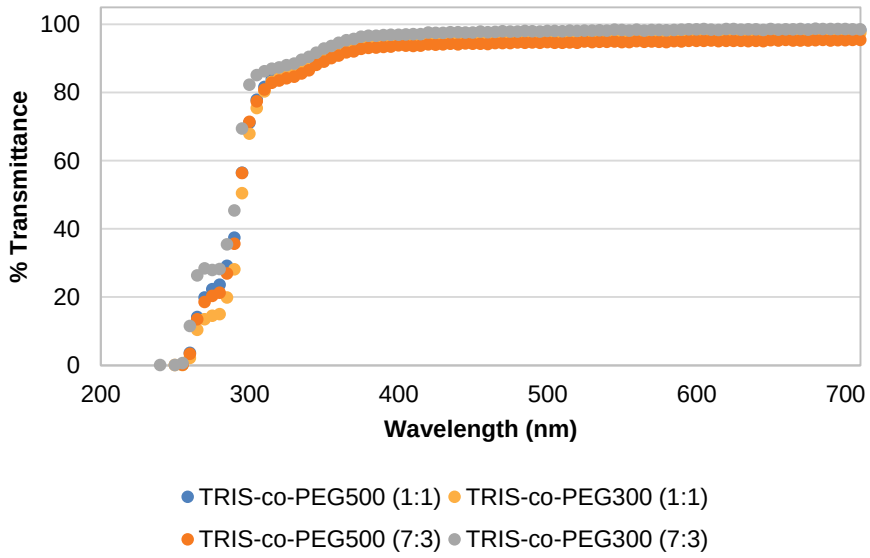

Figure 4.9. The light transmittance of each material across the UV and visible spectrums (n=3).

Overall, despite the opposing solubilities of the macromers, all materials were highly transparent in terms of visual clarity, refractive index and transmittance across the visible spectrum when hydrated in PBS.

4.5.3.3 TEM

The internal structure of the hydrogels is seen through TEM imaging in Figure 4.10. Even at high magnification (30,000x), large phase separation is not seen, rather, there is some visible nanometer-sized phase separation. The size of this phase separation is not dissimilar from that seen in TRIS-only materials containing EGDMA crosslinker. Overall, this data provides support for the optical material properties, as the minimal phase separation that is seen does not impede the transport of light through the material.

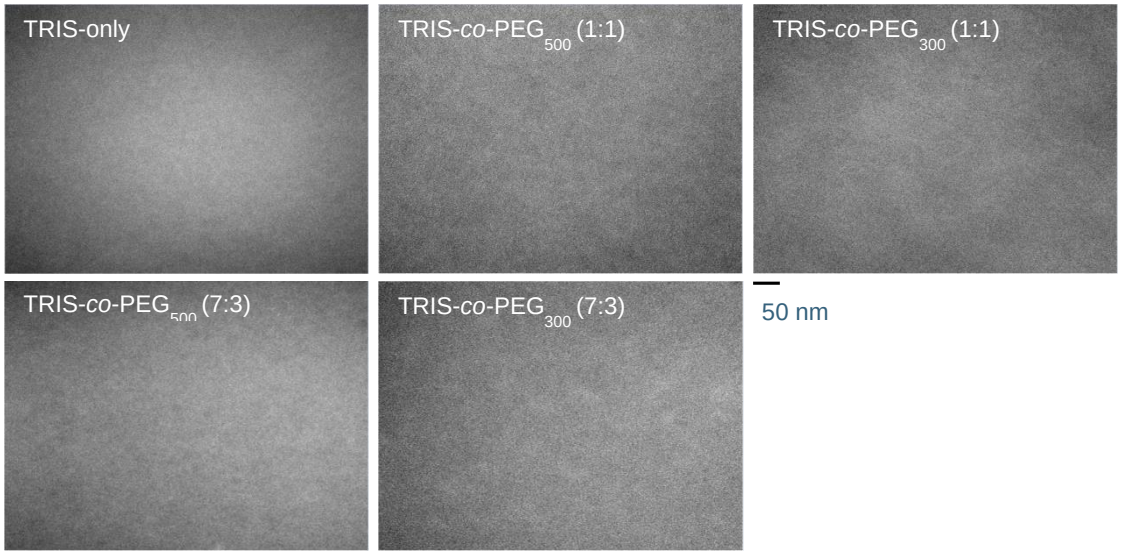

Figure 4.10. cryoTEM images of materials at 30,000x magnification.

4.5.3.4 Equilibrium water content and swelling

The potential to hold large amounts of water make hydrogels attractive in a variety of medical applications, allowing for permeation of nutrients and other small molecules. In Table 4.7, the equilibrium water content and swelling can be seen to vary significantly across the material formulations.

Table 4.7. The EWC and swelling of each formulation was statistically ($p<0.05$) significantly different from the other ($n=4$).

	Equilibrium Water Content (%)	**Swelling Ratio (%)**
TRIS-*co*-PEG_{500} (1:1)	57.88 ± 1.37	137.6 ± 7.69
TRIS-*co*-PEG_{300} (1:1)	18.75 ± 2.09	23.14 ± 3.12
TRIS-*co*-PEG_{500} (7:3)	28.62 ± 2.94	40.27 ± 5.88
TRIS-*co*-PEG_{300} (7:3)	5.72 ± 1.52	6.09 ± 1.71
TRIS-only	6.30 ± 1.17	6.74 ± 1.34

As expected, the highest water content/swelling was seen with the longest PEG chain (PEG_{500}) at the highest concentration (1:1 TRIS) while the lowest water content/swelling was seen with the shortest PEG chain (PEG_{300}) at the lowest PEG concentration (3:7 TRIS). Other formulations had intermediate water contents/swelling.

4.5.4 Relationship with Water

4.5.4.1 The relationship between EWC/swelling and PEG

Given the understanding that PEG is responsible for the hydrophilicity of the material, and that each ethylene oxide subunit of PEG can hydrogen-bond 2-3 water molecules, the relationship between EWC/swelling and the number of EO moles per material was investigated (Figure 4.11). The number of EO moles per material is the product of the moles of PEG per material and the average number of EO units per PEG chain (9 for PEG_{500} and 4.5 for PEG_{300}). There is a high, linear correlation (R^2 = 0.99) with the EWC and a similarly linear correlation (R^2 = 0.95) with swelling. This data supports the molecular relationship of PEG with water, and indicates an ability to control the EWC and swelling by altering the number of EO moles per material.

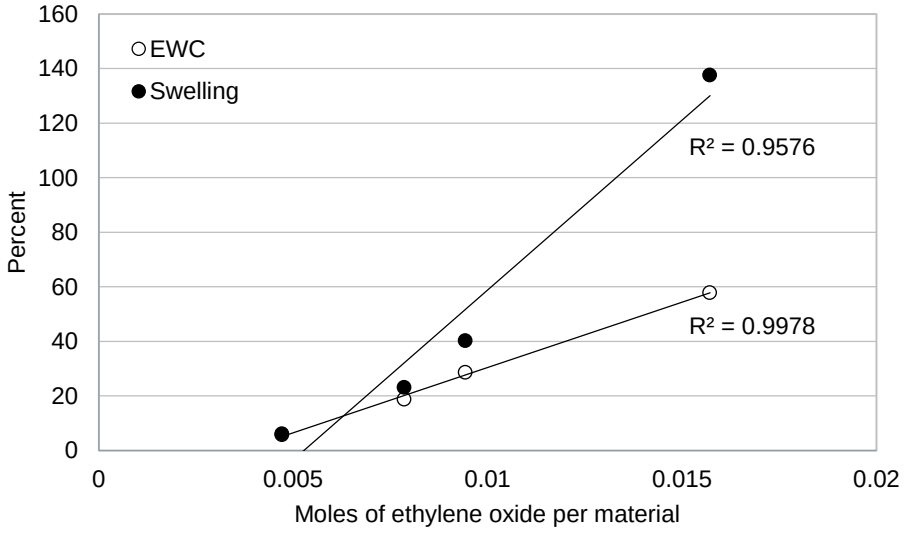

Figure 4.11. Equilibrium water content and swelling ratio of each formulation (n=4).

4.5.4.2 DSC analysis of the types of water in each material

The type of water in each hydrogel can further provide information on the influence of PEG on material properties and the potential of the hydrogels for biomedical applications. Differential scanning calorimetry was performed on each of the formulations to determine the amount of free, intermediate/loosely-bound and non-freezable/bound water in each hydrogel.

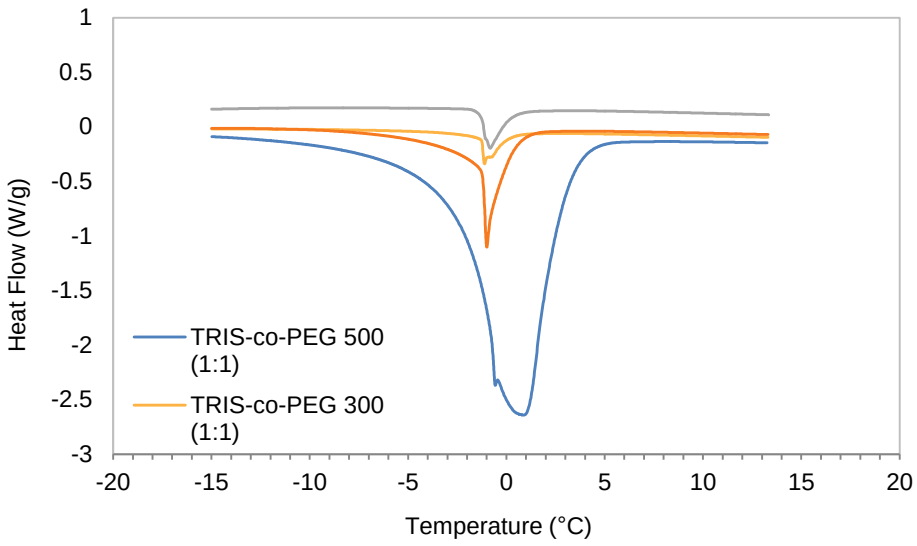

Figure 4.12. Representative DSC thermograms of materials (based on polymer and water weight).

The DSC data (Figure 4.12) shows unique spectra for each hydrogel. Noteably, intermediate water (melting at temperature lower than 0°C), is seen to some degree across all materials. Because this area of the curve for intermediate water is not distinct from that of the free water peak at 0°C, it is not possible to integrate it separately. However, visually, it can be observed that the amount of loose water is greatest in the PEG_{500} (1:1) material, and lowest in the PEG_{300} (7:3) material. Because the two peaks are not distinguishable, these integrated DSC peaks will thus be referred to as the 'freezable' water peak.

4.5.4.3 Relationship between the types of water and PEG

The freezable and bound water were calculated according to Ping et al (45) and placed in relationship to the amount of water in each hydrogel (EWC) as seen in Figure 4.13. The data show that the higher the EWC, the larger the amount of tightly bound water. In terms of the relationship to PEG molecular weight, formulations containing PEG_{300} also showed the lowest amount of free water and formulations containing PEG_{500} showed the greatest amount of tightly bound water. Therefore together the DSC and EWC data demonstrate an influence of the structure of PEG on material properties.

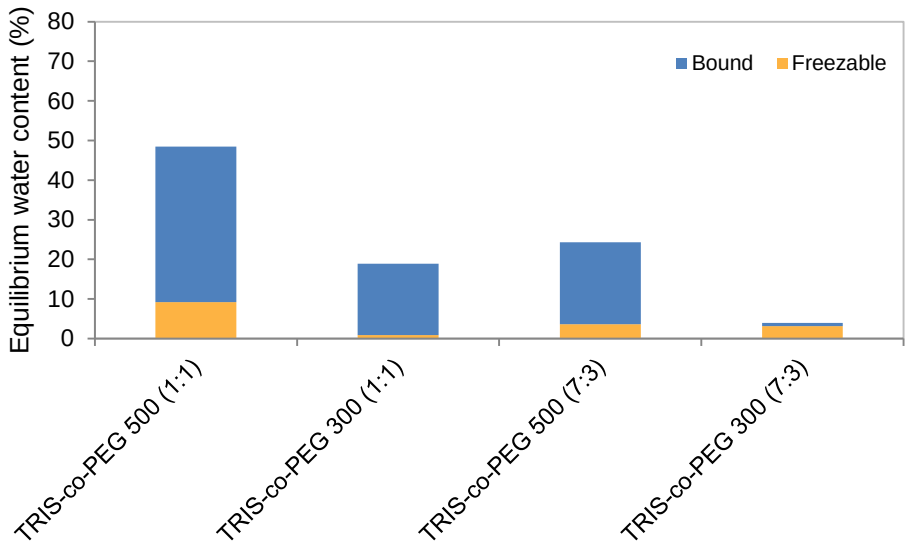

Figure 4.13. Correlation between the number of moles of ethylene oxide in each material and the corresponding equilibrium water content.

Given the linear relationship between PEG and EWC (Figure 4.11), and correlation between PEG content and the type of water, it was postulated that there may be a linear relationship between PEG and the bound water. This was confirmed in Figure 4.14 where a strongly linear (R^2 = 0.99) relationship is seen. Overall, this data indicates the strong influence of PEG on the amount of water and the types of water that are present in each hydrogel.

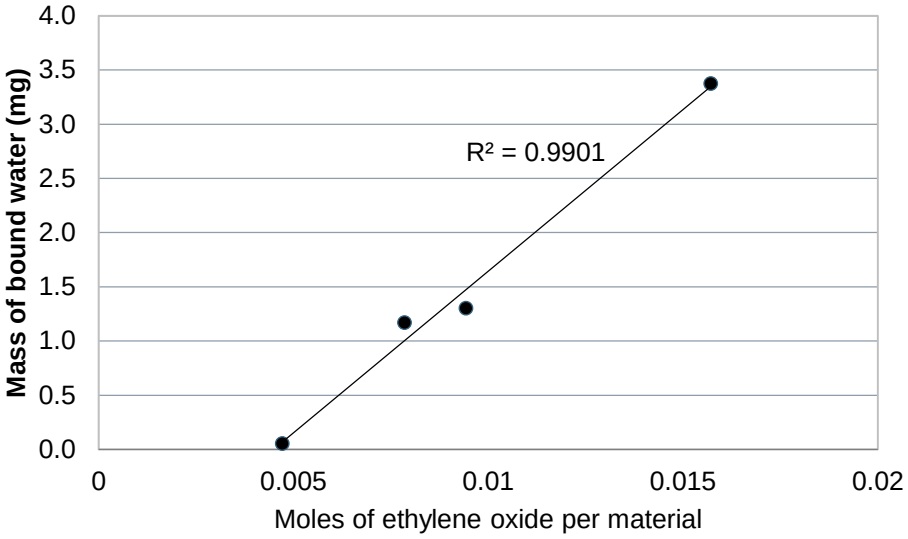

Figure 4.14. Relationship between the number of moles of ethylene oxide units and the amount of bound water.

4.5.4.4 Relationship between mechanical properties and water

Mechanical testing showed interesting results that did not directly correlate to the amount of TRIS in the hydrogels (Figure 4.7). It was postulated that the amount of each type of water may be influencing the strength and modulus. Specifically, the amount of free water in each material could reduce mechanical properties due to its weak association only with itself, leading to areas of the hydrogel which are less strong and elastic. In Figure 4.15, the material strength and elasticity was plotted against the amount of free water to examine this relationship. The correlation with elastic modulus is quite linear (R^2 = 0.92), while the relationship with material strength is less linear (R^2 = 0.84). Thus the data suggests the amount of free water in each material may be an influencing factor for the tensile strength and elasticity of the material, in addition to the TRIS content.

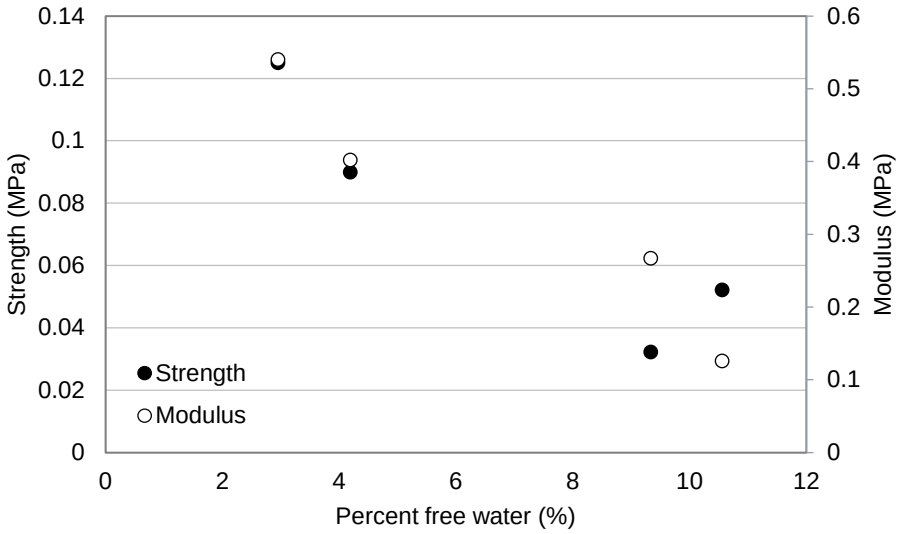

Figure 4.15. Relationship between the mechanical properties and amount of free water (% of EWC) in each hydrogel.

4.5.5 Drug Release

The release of hydrophilic drugs was examined to understand the influence of PEG properties on drug release. Specifically, the release of two structurally similar, very hydrophilic aminoglycoside antibiotics was examined from each material type. Overall, the total 24 hour release amount for each drug was found to be similar for each formulation.

The release of tobramycin (Figure 4.16) showed the largest burst from material with the longest PEG chain (PEG_{500}) and the highest PEG concentration (50%); the lowest burst of drug was seen from materials containing PEG_{300}. Altered, more gradual drug release was seen for up to 6 hours with TRIS-*co*-PEG_{500} (7:3). This gradual release data showed poor fit when examined against first order release kinetics (data not shown), however it showed good fit against the Higuchi diffusion model for the first 6 hours (Table 4.8).

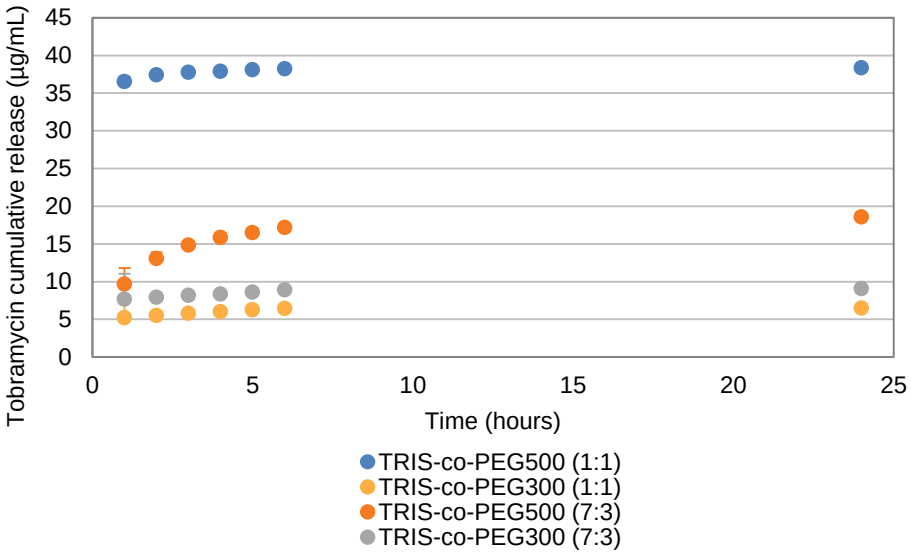

Figure 4.16. Release of tobramycin from silicone hydrogels, measured over 24 hours (stdev, n=4).

The experiment was repeated with amikacin (a structurally similar aminoglycoside) to determine whether the results would be maintained with another highly hydrophilic and hydrogen-bonding-capable small molecule. As seen in Figure 4.17, the same release patterns were noted as with tobramycin release.

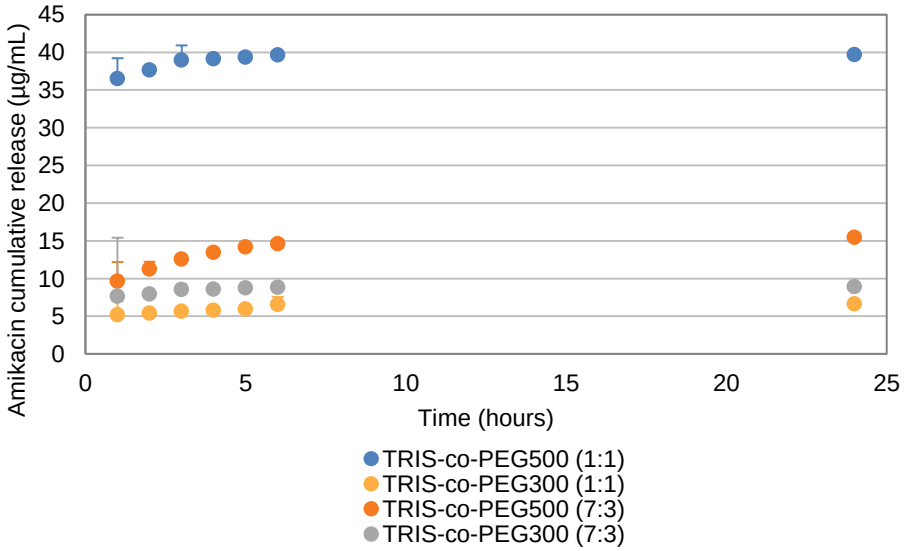

Figure 4.17. Release of amikacin from silicone hydrogels, measured over 24 hours (stdev, n=4).

Overall the data indicates that the PEG structure (concentration and molecular-weight) is an influencing factor in hydrophilic drug release from the material.

Table 4.8. The fit (R^2) to the Higuhchi model ($t^{1/2}$) of TRIS-co-PEG_{500} (7:3) drug release over the first six hours.

	Tobramycin Release (R^2)	Amikacin Release (R^2)
TRIS-*co*-PEG_{500} (7:3)	0.97	0.99

Further plotting of the data on a Higuchi plot (Table 4.8), demonstrated a good fit over the first 6 hours.

Overall the data indicates that the PEG structure (concentration and molecular-weight) is an influencing factor in hydrophilic drug release from the material.

4.6 Discussion

While contact lenses have the potential to increase the on eye residence time of drugs for treating a host of different conditions, they have not reached their potential in terms of application. Silicone hydrogels in particular have the potential to better control the release of drugs compared to conventional materials and thus TRIS was chosen as the hydrophobic component of a novel silicone hydrogel material. Then, the specific objective of this work was to investigate the unique structure-function relationship between PEG and water, when PEG is chemically incorporated as part of a silicone hydrogel material and to determine whether the incorporation of PEG could be used to control the release of hydrophilic drugs, presumably through hydrogen bonding. Together, then a novel silicone hydrogel was created based on the methacrylated macromers TRIS and PEG, and the properties of the materials were assessed through chemical characterization, surface characterization and bulk characterization.

Successful synthesis of TRIS and PEG-based hydrogels

It was found that TRIS and PEG macromers can be co-polymerized to produce a highly wettable material with no macroscopic phase separation. While it can be difficult to directly incorporate polymers of opposing solubility, in this case, the use of low molecular weight PEG and the addition of a small amount of IPA as a solvent (which can be easily removed), resulted in materials with appropriate optical clarity, refractive index, and high transmittance. Due to the relatively short PEG chains chosen, it is likely that PEG is able to adopt non-polar conformations (54,55), resulting in more compatibility with adjacent TRIS molecules. Of note, the oxygen permeability of these materials was not measured and it may be necessary to incorporate an additional siloxane macromer to generate materials with better potential on-eye properties.

Influence of PEG structure on water content

The magnitude of the effect of PEG molecular weight on the equilibrium water content (EWC) was unexpected. At the same molarity, there was more than a 30% increase in EWC in PEG_{500} formulations versus PEG_{300} formulations. This was despite only an

approximately 4.5 ethylene oxide (EO) unit difference between PEG_{500} and PEG_{300} chains. However, developing materials based on molar concentration in this conjugated macromer system results in differing numbers of EO units per material. Therefore, the variables of molecular weight and concentration couldbe independently studied. In order to fully understand the effects of PEG on the material properties then, a structurally deeper perspective had to be taken – looking at the effect of the overall number of EO subunits on material properties. With the knowledge that each EO subunit of PEG can hydrogen-bond 2-3 water molecules, the relationship between EO units and the EWC/swelling was explored. In Figure 4.11, a highly correlative, linear relationship was seen between the number of EO units per material, and the EWC (R^2=0.99) and swelling (R^2=0.96). This data indicate that EWC and swelling are directly dependent on the number of EO subunits in the material introduced through the incorporation of PEG. Future work in modelling the equilibrium water content and the affine deformation of network chains (based on Flory-Rehner theory), will provide deeper understanding of the interactions between polymer and water, and the parallel effects on elasticity/tensile properties [56]. Taken together, these results indicate that the water content of TRIS-co-PEG hydrogels may be finely tunable, simply by altering the number of EO units. Future work should continue to explore this relationship, starting with the manufacture of a TRIS-*co*-PEG material based on the calculated number of EO units required for a desired EWC. Given that EO units can be introduced by altering either the PEG concentration or the PEG molecular weight, the effect of one approach over another could then be explored. Further, the influence of TRIS content on EWC can likewise be explored, as there may be added opportunity for tuning the material through adjusting this variable as well.

The relationship with water showed some interesting results when related to the presence of PEG (Figure 4.13). With increasing EO content the amount of bound water increased – this was expected given that increased EO content provides increased binding sites. However surprisingly, each water profile was unique. For example, the PEG_{300} (7:3) formulation showed negligible bound water, while the PEG_{500} (7:3) formulation showed the least amount of freezable water. Thus the water profiles are not simply related to either the EWC or the EO content, rather the data suggests an influence of the PEG hydrogen-bonding ability and EWC/swelling together. In the case of the PEG_{300} (7:3) formulation, it has the inherent ability to bind water (as a result of the presence of PEG), but it also has a very small EWC/swelling (~5 / 6% respectively). This low EWC/swelling suggests that water does not penetrating deeply into the material, leading to it being mostly associated at the surface layers, rather than throughout the material (and bound to PEG). Then, the amount of bound water is negligible and the majority of water is free (or loosely bound) at the surface (and this is indeed observed in the thermogram). Using this same approach to understand the PEG_{500} (7:3) data, we see the EWC/swelling is larger (~28 / 40% respectively), suggesting that a larger amount of bound water is present in the material and available for binding with PEG – and this larger bound mass of water is indeed observed according to the thermogram. At the same time, because of the longer chain length of PEG_{500}, water found at the surface

layers may be more likely to be associated with the PEG, rather than freely or loosely bound to it, leading to less freezable water being detected in the system.

Hydrophilic drug release is influenced by PEG structure, and the unique types of water in each material

The controlled release of highly hydrophilic molecules from a hydrogel remains a challenge. It was hypothesized that the structured interactions between PEG and drug/water can be used to control movement through the material and influence drug release. Specifically the investigated hydrophilic drugs contain multiple amine groups and hydroxyl groups capable of hydrogen bonding, and thus it is reasonable to hypothesize that once drug is in the material, it is able to hydrogen bond with the EO groups on PEG, and have release altered. Thus, the release of the hydrophilic ophthalmic drug tobramycin (and structurally similar amikacin) from TRIS-*co*-PEG hydrogels was investigated.

The release trends were similar across both drugs tested. For most formulations, an early release was seen, followed by a small, residual release of remaining drug. This is a commonly observed release curve for hydrophilic compounds in hydrogels. However, with TRIS-*co*-PEG_{500} (7:3), a more gradual release was seen over 6 hours, with some additional release over 24 hours. Somewhat unexpectedly, the drug release over the first 6 hours release fits the Higuchi drug release model for all formulations. Higuchi kinetics are based on a model where a solid drug is incorporated during fabrication and is dispersed throughout a polymer matrix. The release first begins with drug located closest to the surface. Then, as water enters the matrix, deeper drug is able to be dissolved and released. Given that in the investigated materials the drug was loaded by *soaking* the silicone hydrogels in drug solution, Higuchi release kinetics would indicate that the hydrogen bonding ability of PEG can control the movement of water and drug resulting in the release observed.

If the state of water in the hydrogels is taken into account, this provides us with the ability to view the material release kinetics from a perspective that aligns with and helps to explain the Higuchi fit seen. Similar to the equilibriums that occur in a Higuchi model – as free water enters the system, free drug not hydrogen-bonded to PEG, is first released. This can be conceptually related to the initial equilibrium that occurs in the Higuchi model at the surface of the material. Then, as free water is exchanged with loosely bound water and drug, the associated drug then becomes free drug and is able to be released from the system. This is similar to the second equilibrium of a Higuchi release model, where more deeply embedded drug is dissolved and then able to be released. Finally, as free water continues to penetrate throughout the material, it is also exchanged with the tightly hydrogen bonded water and drug found at the PEG chains. This is related to the third equilibrium stage of Higuchi release where most deeply embedded drug is dissolved, and must navigate through the material and is then released.

This system provides a good understanding of the equilibriums that are suggested to occur based on the Higuchi fit that is seen. However the water profile (free, intermediate, bound) of each formulation is dependent on both the presence of PEG, but also the

EWC/swelling of each material (as described earlier). By taking both into account, we can then comprehend the data more fully. For example, PEG_{500} (1:1) shows the most amount of bound water (Figure 4.13), but the release data shows negligible release after the first 3 hours. It would be expected that this formulation would should the longest controlled release. However, the high EWC of the material (~60%) provides a large area for water exchange, leading to a more rapid exchange of free water with the bound drug at the polymer, and thus a more rapid depletion of the stored / bound drug within the material. In contrast, the PEG_{500} (7:3) formulation has less bound water but more gradual release, and as the data indicates, this is likely due to the lowered EWC of the material, reducing the area available for water exchange within the polymer and reducing the rate at which the bound water and loosely bound water are released.

This analysis approach also provides understanding of the release from the PEG_{300} formulations. PEG_{300} (1:1) has more loosely and tightly bound water than the PEG_{300} (7:3) formulation, forming the expectation for greater overall drug release from the (1:1) formulation. However, drug release amounts are observed to be fairly similar across the two formulations. Considering that the ~19% EWC of the (1:1) formulation provides low area available for water exchange (than PEG_{500} formulations), the amount of loosely and tightly bound water would be very slow to equilibrate with free water, creating the small initial burst and very gradual release over time. Taken together, the data demonstrates that the presence of PEG in the TRIS-*co*-PEG hydrogels provides ability to control the release of very hydrophilic small molecules through its ability to hydrogen-bond with water and hydrophilic small molecules – where the effects of hydrogen-bonding can be understood through the unique water profiles and EWCs of each formulation.

It is important to note that additional features likely contribute to the effects seen, and could also be explored to understand how to alter hydrophilic drug release from these materials. For example, in materials containing less EO (and therefore higher TRIS), there will be a lowered driving force of hydrophilic drug into the material, and an increased driving force out. The altered loading values contribute to the overall amounts released. It may also be possible that the gradual release is influenced by the physical barrier presented by the TRIS component in the presence of PEG-created water channels. Specifically, the hydrogels contain a significant fraction of hydrophobic TRIS which may obstruct the formation of long, direct channels of hydrated PEG throughout the material. As a result, hydrophilic drug solution that is loaded into the gel must diffuse out by navigating through channels that may be open or obstructed based on the movement of both TRIS and PEG chains. This obstructed pathway out of the material may contribute to the more sustained, controlled release of drug that is seen in formulations showing more gradual release, such as the PEG_{500} (7:3) formulation. Likewise with a lower TRIS content, larger burst release can be explained because the equilibrium water content is significantly greater, so any channels are much larger (and less obstructed) and drug release is no longer controlled. Therefore the addition of a siloxane component into the gels may also further prolong the release by creating a more tortuous barrier to drug diffusion.

4.7 Conclusion

In this work, a novel hydrogel was developed. It was demonstrated that macromers of opposing solubility can be directly co-polymerized to produce optically transparent materials with no phase separation when hydrated in PBS. In addition, the resulting materials were found to be highly hydrophilic, and yet could be reformulated to maintain desired properties, with a water content that was tunable between ~5 to 60% while maintaining a highly hydrophilic contact angle of approximately 40°. This tuning is thought to be possible based on the structure of PEG, and specifically the number of EO units introduced by PEG. Finally, the controlled release of hydrophilic antibiotics from TRIS-*co*-PEG hydrogels was demonstrated, with Higuchi-like kinetics providing a conceptual understanding of the molecular-level equilibriums occurring between the hydrogen-bonded drugs and free drugs. Further incorporation of EWC/swelling data provides a basis for understanding the unique drug release profiles of each formulation. Overall, the development and investigation of TRIS-*co*-PEG hydrogels in this work provides a novel platform for expanding the development and understanding of PEG as a biomaterial with the ability to influence drug release kinetics upon incorporation into materials.

4.8 Supplementary Information

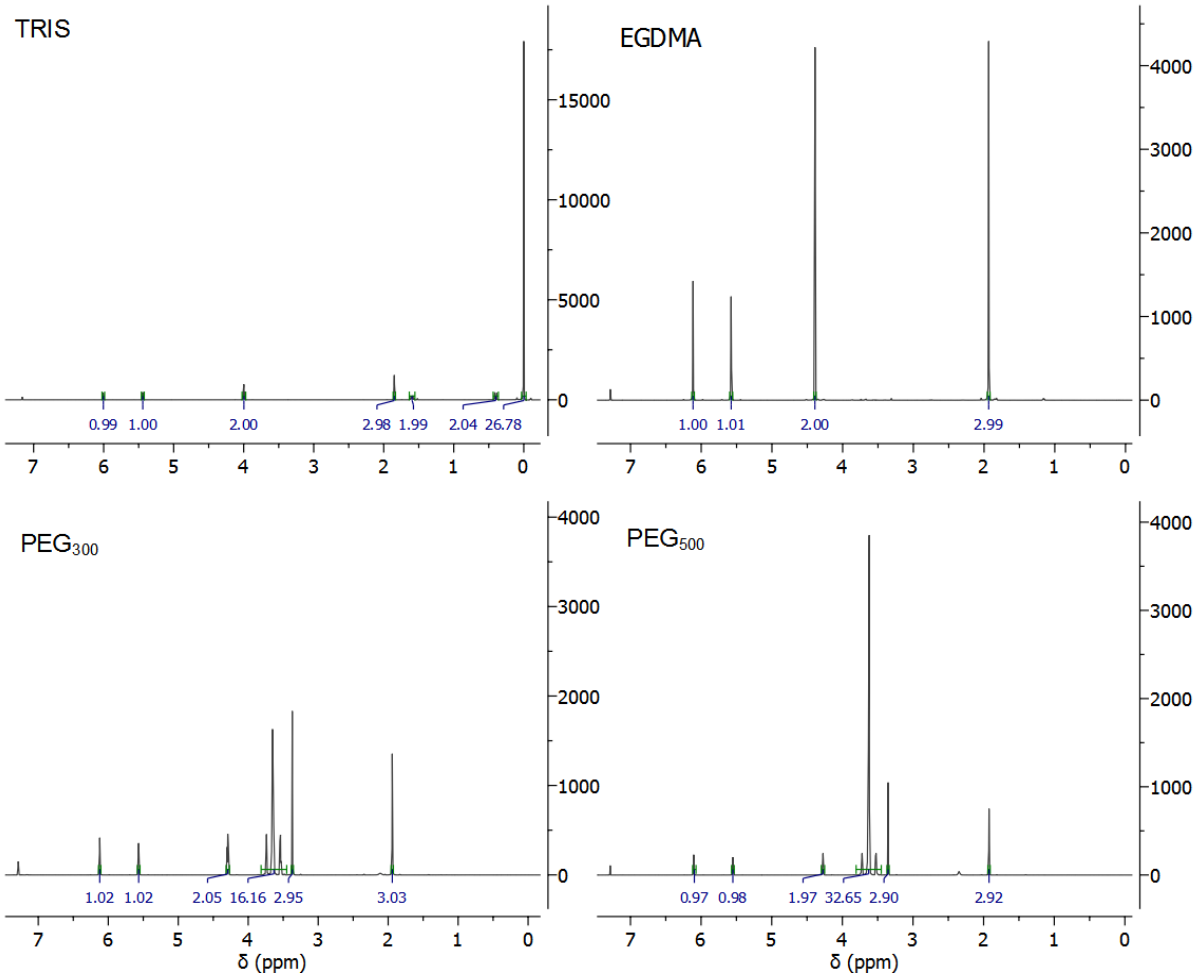

Figure SI4.1. Macromer 1H NMR spectrums. Macromers were solvated in CDL3. Refer to Results for analysis and peak identification.

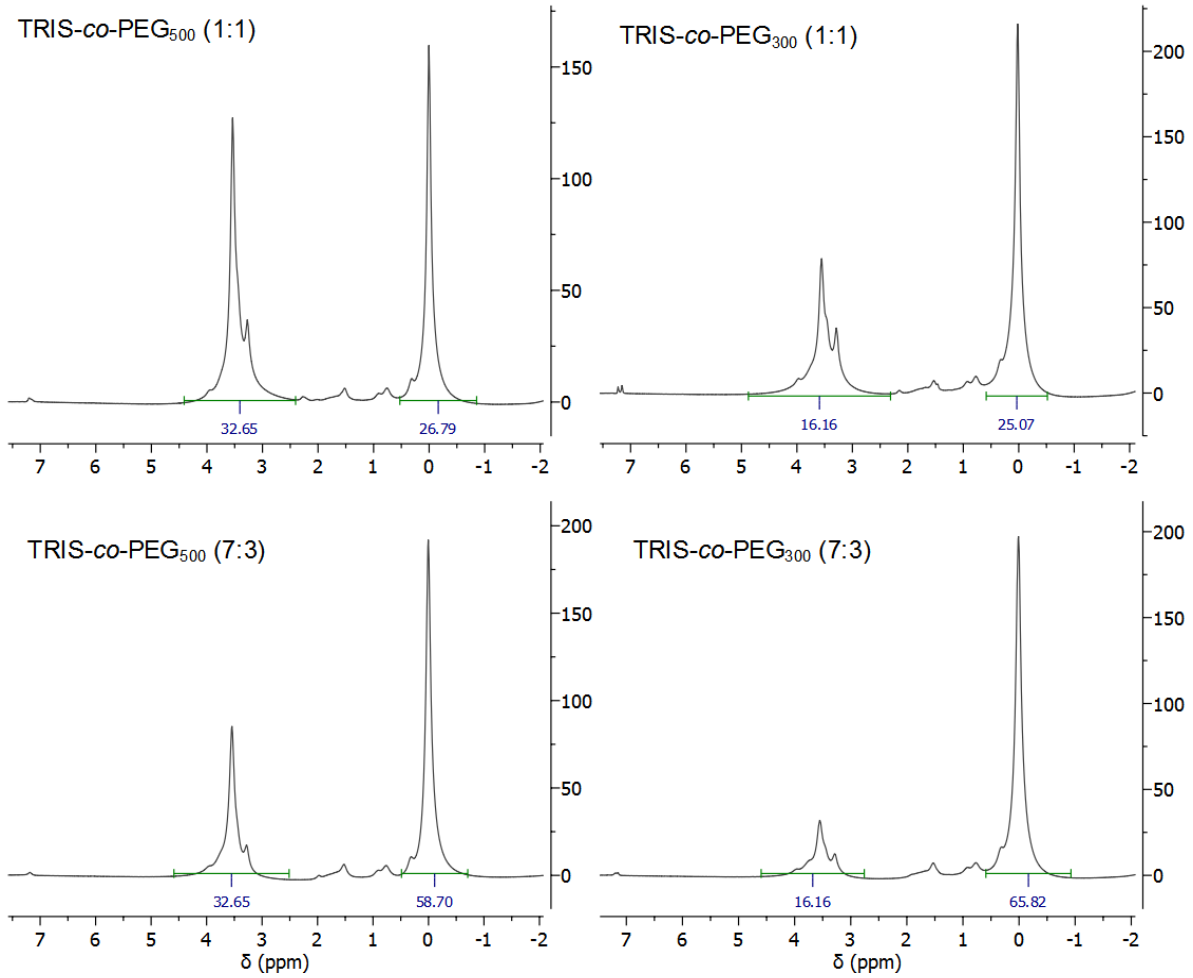

Figure SI4.2. Hydrogel 1H NMR spectrums. Hydrogels were solvated in CDL3. Identifying peaks were integrated only. Refer to Results for analysis.

4.9 References

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Literature Appendix

I. Physical and chemical properties influencing adsorption

Given that proteins are present in all bodily fluids and are vital for all cellular functions [51], it is important to understand the implications of protein adsorption onto materials with proposed biological applications.

The properties of proteins (such as hydrophobicity, solubility, electric charge and charge density) can vary significantly depending on their amino acid make-up. Also, the conformation of the protein (a combination of the 2° and/or 3° structure) will influence which amino acids are exposed to the exterior surroundings and can potentially interact with the biomaterial. From the material perspective, the chemical makeup of a biomaterial – and specifically the surface composition – will influence the degree to which a surface will interact with a given protein.

Proteins adsorb to a surface in a non-specific way, whether via hydrophobic attraction, hydrogen bonding or ionic interactions [52]. Because the adsorption of proteins occurs almost instantaneously, interactions between cells and biomaterials is due to the cellular interaction with the adsorbed proteins rather than the material surface itself [53], [11] This spontaneous protein adherence is entropically (ΔS) favoured; the attraction of hydrophobic protein segments to a hydrophobic surface leads to less translational entropy loss (ΔS_{mix}) versus having the presence of hydrophilic water on the hydrophobic material surface [54]. The denaturation of proteins is also entropically favoured, where a gain in entropy occurs when water at the material surface is displaced as the protein unfolds, and the protein gains more chain mobility with a loosening of its intramolecular forces [54]. Overall, the increases in entropy lead to a more negative Gibb's free energy value.

Protein deposition can activate the inflammatory process, which is a natural host reaction to an implanted biomaterial [55]. The deposited protein attracts first-responder cells (neutrophils – white blood cells) to the site of injury [56]. Neutrophils are triggered by the adsorbed (and generally denatured) proteins on the material surface to release signalling molecules (cytokines) for the recruitment of more specialized immune cells (macrophages) [55], [56]. Macrophages can then begin to attempt to engulf and destroy the biomaterial, and can later fuse to form foreign body giant cells [56]. These cells further release digestive lysosomal enzymes until the entire implanted material is isolated from the natural tissue and likely no longer medicinally operational [54], [56]. Fibrous encapsulation of the biomaterial can also occur as an end-stage event of a failed implantation, as a result of fibrous connective tissue (normally present during the tissue regeneration process) forming in excess and preventing the material from integrating appropriately [54], [56].

Thus it is extremely important to limit protein deposition and denaturation on biomaterials in order to produce therapeutic effects both in the short and long-term. In polymer research, this has been pursued through the modification of either the surface or bulk material. Examples of surface modifications include plasma treatment to introduce polar

oxygen species at the surface (and thus a more polar, hydrophilic surface) and the application of hydrophilic polymers in numerous forms, including as chemically-linked brushes or surface adsorbed. Bulk modifications can be performed through blending or copolymerizing a more hydrophilic compound, or through the formation of interpenetrating hydrophilic networks.

II. Contact Lenses

The first report of a potential 'soft' contact lens material came in 1960, when poly-2-hydroxyethylmethacrylate (pHEMA), a synthetic polymer, was found to be compatible for ocular applications. Prior to pHEMA, contact lenses were generally made of a 'hard' material such as glass, designed to cover the entire eye. With this discovery, significant innovation in the field began to optimize the clinical outcomes of soft contact lens wear and especially to reduce hypoxic (low oxygen) response from the ocular tissue to extended contact lens wear. Numerous additives (such as hydrophilic N-vinyl-2-pyrrolidone) and chemistries were developed to improve the oxygen permeability of pHEMA, however in 1999, a novel generation of contact lens material came to market. Given the ability of silicones to permit strong gas permeability, a combination of this property with the high wettability of hydrogel systems was proposed and shown to be extremely successful by Lai and Quinn [8]. In general, to create a silicone hydrogel, the alternating silicon-oxygen backbone is copolymerized or crosslinked with a more hydrophilic side group, comonomer or macromere.

After almost 20 years of research, a drawback of the silicone component continues to limit the use of contact lenses in all patients. Silicone hydrogels, like silicones alone, can be prone to protein deposition through the exposure of the silicon group to proteins such as lysozyme. The protein fouling results in discomfort to the user, limiting wear time and compliance. Similar to pHEMA optimization, silicone hydrogels have been altered through the addition of NVP or other hydrophilic compounds, and/or plasma treatment to improve the hydrophilic character of the material, however continued clinical issues due to surface fouling have resulted in ongoing research and development in contact lens research today.

III. Drug Delivery To The Eye

In addition to the use of contact lenses as biomaterials for correcting vision, they have also been proposed for drug delivery applications to the eye.

The human eye is innately protected from the environment by static and dynamic barriers, including specialized tissue structures and physiological mechanisms [57]. At the anterior surface alone, a number of obstacles exist to shield the eye from foreign substances and potentially toxic molecule exposure [58]. Tear film properties such as rapid turnover, dilution, blinking reflexes, the presence of protein-inactivating enzymes and mucin, significantly reduce the penetration of both hydrophilic and hydrophobic molecules [58]–[61]. The conjunctiva, sclera, and cornea provide additional physical barriers to drug penetration, such as the tight junctions between epithelial cells that reduce paracellular drug permeation [58], [62]–[64]. Thus, in order to treat ocular conditions, the properties of the therapeutic as well as administration method must be optimized to overcome ocular drug delivery obstacles.

Systemic administration of therapeutics is not commonly indicated, given the tight blood-retinal barrier and reduction of active therapeutic concentrations through hepatic first-pass metabolism [65], [66]. Rather, topical administration is the most convenient, non-invasive and effective treatment method at this time, with 90% of ophthalmic treatments formulated as eye drops, gels or similar [59], [66]. However, current topical treatment methods are not optimized to overcome the ocular barriers mentioned, and there continues to be a need to enhance bioavailability to ocular tissues [67]. The reality today is that most eye drops have only 1-7% of each drop of medication reach the drug target site.

This topical administration method also suffers from low compliance [67] with as many as 50% of patients either failing to adhere to the prescribed regimen or failing to instill the drops altogether [68]. Even with complete compliance, the low bioavailability of the active agent for topical therapies necessitates frequent administration and high doses with the concomitant cost and potential for systemic side effects [68], [69]. For example, in the case of the glaucoma drug timolol, within 5 minutes, 70% of the dosage can be found present in the systemic circulation [70]. This rapid systemic absorption can lead to further complications. For timolol, which acts as a non-selective beta blocker, ophthalmic drops accumulate at systemic concentrations similar to intravenous injection of timolol, leading to hazardous drug levels for patients already experiencing cardiovascular / pulmonary disease [71]–[73].

Numerous creative approaches have been investigated to achieve improved bioavailability in this challenging application. Pro-drug derivatives of therapeutic molecules such as timolol have helped enhance corneal penetration and reduced systemic side effects [74]. Also, many penetration enhancing molecules or corneal residence enhancers such as Pluronic®'s (block co-polymers based on ethylene oxide and propylene oxide), lipid-based nanocarriers, cyclodextrins, dendrimers, and mucoadhesive polymers have seen both *in vitro* and clinical success in improving bioavailability [74]–[77].

To improve long-term anterior drug delivery, altering the administration method to include

the use of contact lenses has also been investigated [78]. A great advantage to contact lenses over novel formulation developments is the deep understanding of their manufacture and biocompatibility, as well as their incomparable clinical success (expecting to reach a demand of 1.13 billion units / year by 2020) [79]. Their once-daily application and long-term residence time at the cornea can improve patient compliance and reduce post-lens tear film turn-over, improving drug penetration [80]–[83]. There exist no DDCLs that have been approved by regulatory bodies to-date, but recent advances have developed a DDCL that shows zero order release values and thus the clinical potential remains strong [63], [84], [85].

IV. Hydrogen bonding in relation to water content

The fundamental principle of hydrogen bonding controlling water could be extended to control the release of hydrophilic compounds. This is because hydrophilic compounds can often participate in hydrogen bonding and thus interact with PEG, possibly leading to controlled release.

The basis for this hypothesis is that it has been well established that polar hydrogels can influence the state of water that is associated with it [36]. Figure A1 illustrates these states of water as they may be associated with poly(ethylene glycol) and in relation to equilibrium water content. Polar polymer chains in a hydrogel can create three states of water: tightly bound, loosely bound and free water [36]. These types/states of water can also be interchangeably referred to as non-freezable/immobile, intermediate/freezable, and bulk/freezable/mobile water.

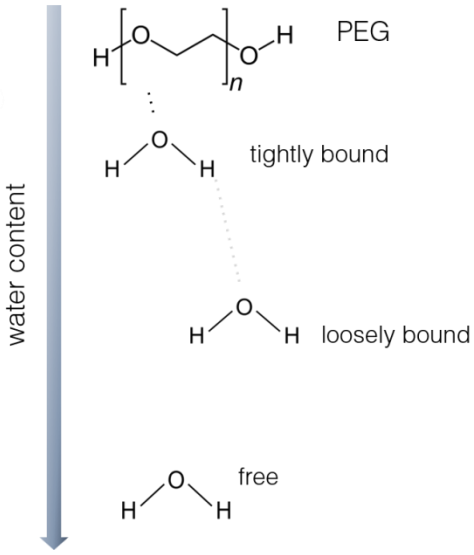

Figure A1. States of water in the presence of poly(ethylene glycol), with increasing water content. Image is adapted from Tranoudis et al [36].

Tightly bound water is found closest to the polymer surface, and is directly hydrogen bonded with the polymer. Loosely bound water is more vaguely defined as it refers to water that is weakly bound to the polymer. Free water is found farthest from the polymer

surface and experiences only water-water hydrogen bonding, and no polymer-interactions [48]. Because a hydrophilic drug could also interact with the polymer in these ways, this could lead to controlled release and diminished burst release.

5 Release of hydrophilic, hydrophobic and pharmaceutical salts from siloxane elastomer can be tuned using poly(ethylene glycol)

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Author contributions

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5 Release of hydrophilic, hydrophobic and pharmaceutical salts from siloxane elastomer can be tuned using poly(ethylene glycol)

5.1 Abstract

Siloxane-based drug delivery has achieved proven application in the clinical setting. However, its potential has not been fully realized due to numerous factors, including the inherent biological limitations of siloxanes and limited knowledge of the structure-function relationship between polymeric materials and therapeutics. Thus, the aim of this study was to develop a novel model siloxane-based material for controlled drug delivery, which will provide the foundation for optimized and predictable drug delivering devices. Materials were based on regulatory-approved polymers poly(ethylene glycol) and poly(dimethyl siloxane) and developed using a simple blending and crosslinking procedure for ease of manufacturing and translation. Small-molecule therapeutics were directly loaded prior to polymerization, eliminating the use of potentially toxic solvents, and providing high loading capacity regardless of drug solubility. Based on the hypothesis that PEG as an amphiphilic and hydrophilic polymer could be used to control the release of drugs regardless of solubility, its influence on hydrophilic, hydrophobic and pharmaceutical salt therapeutics was examined. The data demonstrates the ability of PEG to control drug release. Briefly, PEG_{400} increased hydrophobic drug release, PEG_{400} and $PEG_{20,000}$ decreased hydrophilic drug release, and $PEG_{20,000}$ provided gradual early release with no burst with a pharmaceutical salt. Further, the release rates could be correlated/fitted to kinetic models such as Higuchi and the relationship between the structure of PEG and drug properties/release could be inferred due to the strategic design of the material and study. Overall, the work describes the simple development of a novel siloxane-PEG based system with the ability to control drug release over the long-term by finely tuning with PEG.

5.2 Introduction

To be effective, drug delivery must result in sustained and therapeutic levels of the active compound at the target site. However, standard drug administration methods such as oral delivery often do not maintain therapeutic levels at the target tissue, which can result in periods of under-dosing and the potential for over-dosing [1]. This decreases drug efficacy, increases the risk for systemic toxicity, and can overall impact the health of the patient [2]. Many techniques have been investigated to improve drug delivery, such as coating drug pellets to increase absorption [3]–[6] or altering the administration method (e.g. reformulating to a dermal patch or cream) [7]. However, the method that shows the most promise for sustained delivery involves the incorporation of the drug into an appropriate polymeric vehicle that can be formulaically tuned to provide controlled and sustained release [8].

Siloxane-based materials have been widely used in drug delivery applications due to their favourable drug release kinetics and excellent biomaterial properties [9], [10]. For instance, the implantable siloxane-based device Norplant® acts as a contraceptive reservoir, releasing appropriate levels of levonorgestrel for up to 5 years [11]. Silicone based polymers (and in particular siloxanes) have been used in the medical device industry for decades due to such properties as ocular transparency, high gas permeability, stability, low toxicity, and acceptable blood compatibility [12]. However, the hydrophobicity of these materials has limited their potential in biological applications since it has been shown that hydrophobic surfaces are prone to the irreversible adsorptions of biopolymers including proteins, small hydrophobic molecules and cells [12]. Clinically, this can potentially lead to irritation, inflammation, as well as coagulation and complement activation in blood-contacting devices [13]. Further, much of PDMS-based drug delivery is limited by poor control of the initial burst release, and the inability to control drug release rates at the same time as drug dosage (e.g. low drug loading results in low release rate and vice versa) [17].

A strategy undertaken to overcome the limitations of PDMS as a drug delivering device involves adjusting the hydrophilicity of the material. Varying approaches to this include surface modification (e.g. grafting of hydrophilic monomers), bulk modification, physical modification (UV), and copolymerization with hydrophilic monomers [13]–[16]. For example, osmotically active excipients have been reported to alter the physical properties of PDMS devices by swelling the hydrophobic matrix, increasing the flow of water molecules inside and ultimately releasing more deeply embedded drug [17]–[19]. Salt, glycerin, ethylene glycol, or low molecular weight PEG are examples of excipients that have been shown to influence drug release rates using this mechanism [17].

However, despite the numerous studies that have been performed to date in the area of elastomer modifications, there is a consistent gap in (1) the understanding of the structure-function relationship of polymers and drug release rates and (2) using this to optimize the development of drug delivering devices. Specifically, despite reporting of drug release curves fitted against kinetic models, there is very minimal exploration of the

relationship between the polymer structure and device performance. Instead, the focus in most cases is the development of an appropriate drug delivery system with material or drug release properties that are sufficient for a desired application. As a result, across the literature, this approach to material development has led to the development of many devices that use similar drugs or polymers, with inconsistent conclusions on the influence of the polymer / formulation in any particular device. For example, PDMS modified with osmotically active sodium chloride has shown increased drug release in some studies, while minimal release has been reported in others [17], [20], [21]. Furthermore, pharmaceutical salts such as papaverine hydrochloride were shown to be able to create their own drug release pathways [20]. These varied results can cloud the understanding of the action of specific additives in drug delivering systems. This can lead to lost time performing laborious drug release studies and costly reformulations to understand how to optimize the delivery of any new therapeutic. Thus, the goal of this study is to develop a generic drug delivering platform that will serve as the foundation for understanding the relationship between the nature of the siloxane, an additive (poly(ethylene glycol), small-molecule therapeutics, and the resulting drug release characteristics.

Poly(dimethyl siloxane) from a commercially available source was chosen as the primary release material. This allows for simple reproducibility, in contrast to the variability in PDMS chain lengths, crosslinkers and solvents that have been used in the literature. To overcome the limitations of PDMS alone as a drug delivering device, the hydrophobic properties of PDMS were contrasted with the incorporation of hydrophilic polymer. Hydrophilic poly(ethylene glycol) (PEG) is FDA approved for use in numerous biomedical applications, it has the unique characteristic of being able to effectively bind 2-3 water molecules per ethylene glycol unit, and it has been shown to exhibit amphiphilic properties due to its linear chain structure and conformational bond freedom [22], [23]. Together with this amphiphilic property, it was hypothesized that this would lead to the creation of a PEG-PDMS material that would be able to control the release of therapeutics of varying solubility. Three general types of small molecules – hydrophobic, hydrophilic and drug salts – were investigated as model drugs for release from PEG-PDMS materials. A straightforward manufacturing method was used to maintain consistency of the systems prepared. The bulk modification chosen for this study using physical entrapment of PEG into PDMS allows for consistent manufacturing [24].

The pharmaceutical drugs ciprofloxacin, tobramycin and metformin hydrochloride were selected for comparison in this work (see Literature Appendix 1 for details on the therapeutics). When developing PEG-PDMS materials, drugs were directly incorporated in their solid form during polymerization. This resulted in a material with high drug loading capability, independent of limits posed by the variations in drug solubility. Given that each drug has a unique solubility within a material (PDMS or PEG component), as well as a unique solubility in the extracellular solution (phosphate buffered saline), the relationship between the drug, polymer and release rate could be elucidated in the study.

5.3 Materials

Poly(dimethylsiloxane) (PDMS) and platinum curing agent were obtained as the two-part Sylgard® 184 Silicone Elastomer kit from Dow Corning (Midland MI). Metformin hydrochloride, ciprofloxacin and tobramycin were obtained from Sigma-Aldrich (Oakville ON). Hydroxylated poly(ethylene glycol) (PEG) of 400 and 20,000 molecular weight (M_n), was obtained from Sigma-Aldrich (Oakville ON). A vacuum oven was used to maintain polymerizations at constant temperature and for the removal of air from the material. BIO-RAD phosphate buffered saline pH 7.4 was used as the external medium for the drug release studies. A TECAN Infinite® M1000 PRO plate reader was used for all spectrophotometric measurements.

5.4 Methods

5.4.1 Preparation of novel PEG-PDMS blended drug delivering materials

PEG (400 MW (~8 ethylene glycol units), 20,000 MW (~450 ethylene glycol units)) was weighed out at 1, 5, or 10% w/w, relative to the weight of PDMS. PEG and PDMS were placed into 35mm x 10mm polystyrene dishes and into a 70°C oven until all of the PEG had visibly melted, changing from a white solid to transparent liquid. 5% or 10% w/w of drug (metformin-HCl, ciprofloxacin or tobramycin) and 10% w/w of curing agent were then added to the mixture. The solution was thoroughly mixed using a glass stirrer and then returned to the 70°C oven. Mixing was completed within ~1 minute and before the PEG had begun to solidify from being exposed to room temperature. A vacuum of 30 mmHg was applied until all air was completely removed (~3 minutes). After 45 minutes at 70°C, the solution was fully cured (confirmed by solidification for the polymer mixture). Control discs with no PEG were manufactured using the same method. Table 5.1 describes the composition of each of the materials prepared in this study.

Table 5.1. Composition of materials used in the study (including controls). Three sets of 15 materials were manufactured containing a different drug type in each set (tobramycin, ciprofloxacin or metformin-HCl).

Material	PEG concentration (% w/w)	PEG molecular weight (M_n)	Drug concentration (% w/w)
1	0	0	0
2	0	0	5
3	0	0	10
4	1	400	5
5	5	400	10
6	10	400	5
7	1	400	10
8	5	400	5
9	10	400	10
10	1	20,000	5
11	5	20,000	10
12	10	20,000	5
13	1	20,000	10
14	5	20,000	5
15	10	20,000	10

5.4.2 Drug release study design

Quarter-inch discs were cut using a cork borer, placed into the wells of a 48-well plate and immersed in 1 mL of phosphate-buffered saline (PBS) pH 7.4. The plates were sealed with an adhesive plate seal to prevent evaporation of PBS, and placed in a VWR shaking incubator at 37°C and 100 rpm. At predetermined time points, the discs were transferred to a new solution of 1 mL PBS, the plate was resealed and the drug release was continued in the incubator. For longer incubations (more than 24hrs) the discs were transferred to 1.5 mL microtube vials to ensure evaporation did not occur, as the plate seal adhesive was not consistently effective after 2 days at 37°C.

5.4.3 Quantification of drug release of PEG-PDMS materials over time

Metformin hydrochloride is freely soluble in water and can be readily detected using spectrophotometric methods at a wavelength of around 232 nm [25]. Direct spectrophotometric detection was used in this study, as release data using control discs indicated no interference to metformin absorption at 233 nm, and preliminary drug release studies indicated the amounts of metformin release from discs could be readily detected.

Ciprofloxacin is very poorly water soluble. However, testing indicated the amounts of ciprofloxacin released from discs could be readily detected using direct spectrophotometry (277 nm) in 96-well-plates.

Tobramycin does not contain any UV absorbing groups and therefore chemical derivatization with fluorescamine was required (50:1 tobramycin releasate : 3 mg/ml fluorescamine in DMSO). This method adapted from Dash *et al*. allows for rapid high-

throughput spectrophotometric detection of tobramycin [26]. The fluorescent compound was measured at excitation / emission wavelengths of 380 / 480 nm, respectively.

The decomposition of metformin in the solid state is seen only at temperatures exceeding 230°C [25]. This is important to note, as during this device production process, the solid drug is exposed to elevated temperatures. Likewise, ciprofloxacin maintains its antimicrobial activity at elevated temperatures and during autoclaving (typically 121°C) [27]. No degradation of tobramycin is seen at temperatures up to 224°C [28].

5.4.4 Statistical Analysis

For effects of PEG, a Student's T-test was used to analyze the drug release data in comparison to PDMS. For effects of PEG concentration, a one-factor analysis of variance was used, followed by a post-hoc Tukey when significant differences were identified ($p<0.05$). Each experimental formulation was repeated three times. All error bars represent standard deviation of the three replicates samples when measured using spectrophotometric methods.

5.5 Results

5.5.1 Physical appearance of materials

Materials were opaque white in colour after blending and after cross-linking. This is due to the insolubility of PEG in PDMS, with the slow cure rate allowing time for the components to physically separate. In the heat-cured procedure used in this study, the two components remained physically entangled and phase separation was dispersed throughout, reducing the material opacity. Increasing the concentration of PEG to above 10% did not result in consistently, mechanically strong or solid materials, likely due to the physical interference between the PEG and the PDMS during crosslinking. These formulations were thus not included in the study. These mechanical changes above 10% w/w of PEG are consistent with observations in described in the literature [29], [30].

5.5.2 Ciprofloxacin release

The influence of PEG molecular weight on ciprofloxacin release is shown in Figure 5.1, where the drug release curves for 5% ciprofloxacin are presented. In the presence of PEG_{400}, the cumulative release of 5% ciprofloxacin is significantly greater than the release from PDMS alone (Figure 5.1). This was observed for all concentrations of PEG_{400} tested (1, 5, 10%). However, release from materials containing $PEG_{20,000}$ was not statistically significantly different than from PDMS-only discs, except with 10% $PEG_{20,000}$. When comparing the release rates from the PEG_{400} disks with that from $PEG_{20,000}$, only at 10 was the release from PEG_{400} statistically significantly greater.

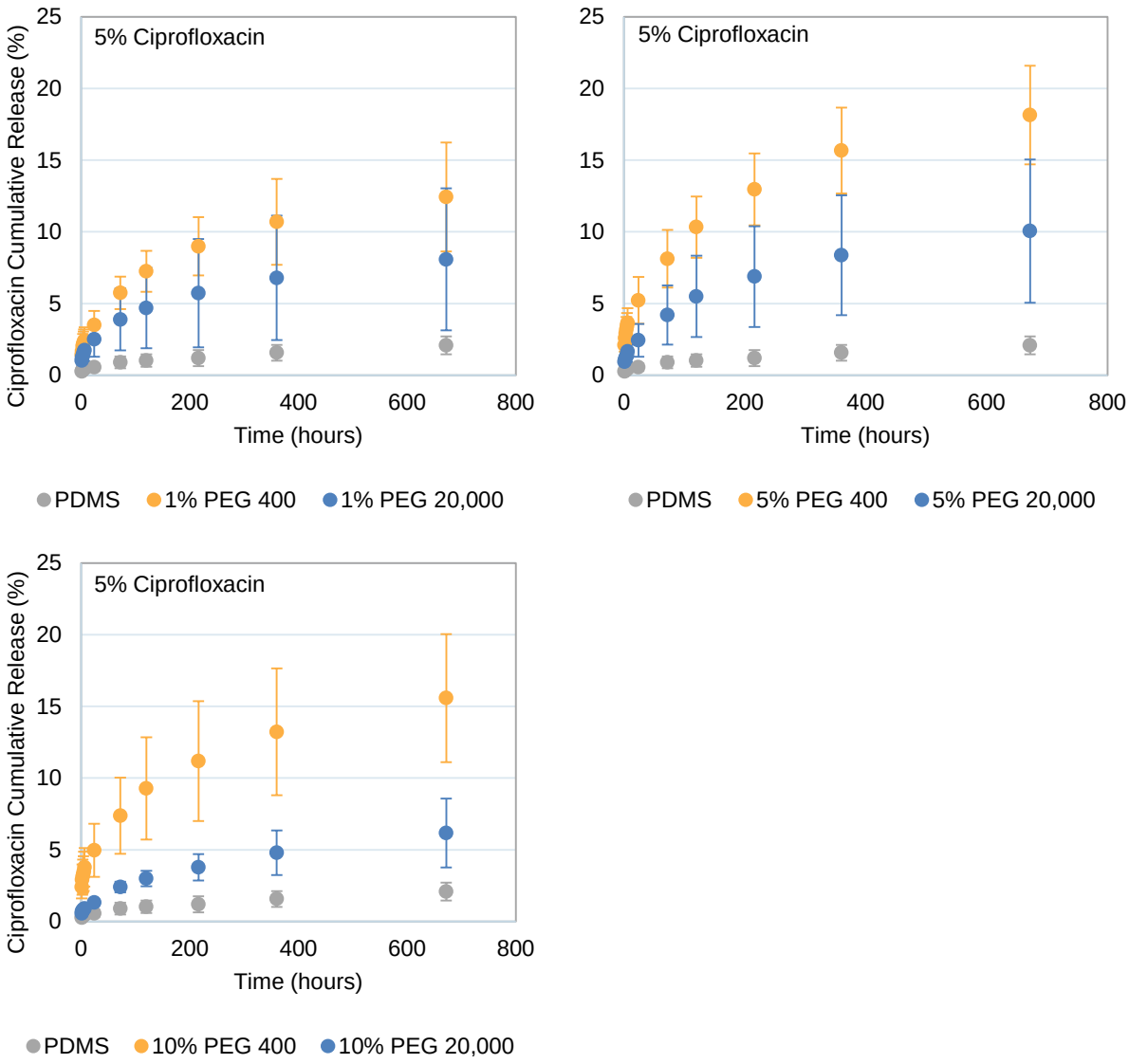

Figure 5.1. Cumulative release of ciprofloxacin loaded at 5% in PEG_{400} is statistically significantly greater than PDMS at all PEG concentrations tested. Release from materials containing $PEG_{20,000}$ was only statistically different from PDMS with 10% $PEG_{20,000}$.

The release of ciprofloxacin from PEG-PDMS materials was measured at two ciprofloxacin loadings of either 5% or 10%. These two loadings were investigated in order to determine whether the drug itself impacts the drug release rate. Given that ciprofloxacin is hydrophobic, this study design allowed for observation of the effect of increasing hydrophobic content in the material.

In Figure 5.2, when a higher ciprofloxacin loading (10%) was examined, the release of ciprofloxacin was seen to be statistically significantly higher only with 10% PEG_{400}. 1% and 5% PEG_{400} materials and materials with all concentrations of $PEG_{20,000}$ did not

produce statistically significant differences in drug release in comparison to PDMS-only discs.

Overall, it can be seen from Figures 5.1 and 5.2 that PEG molecular weight impacts ciprofloxacin release rate, as does the ciprofloxacin loading.

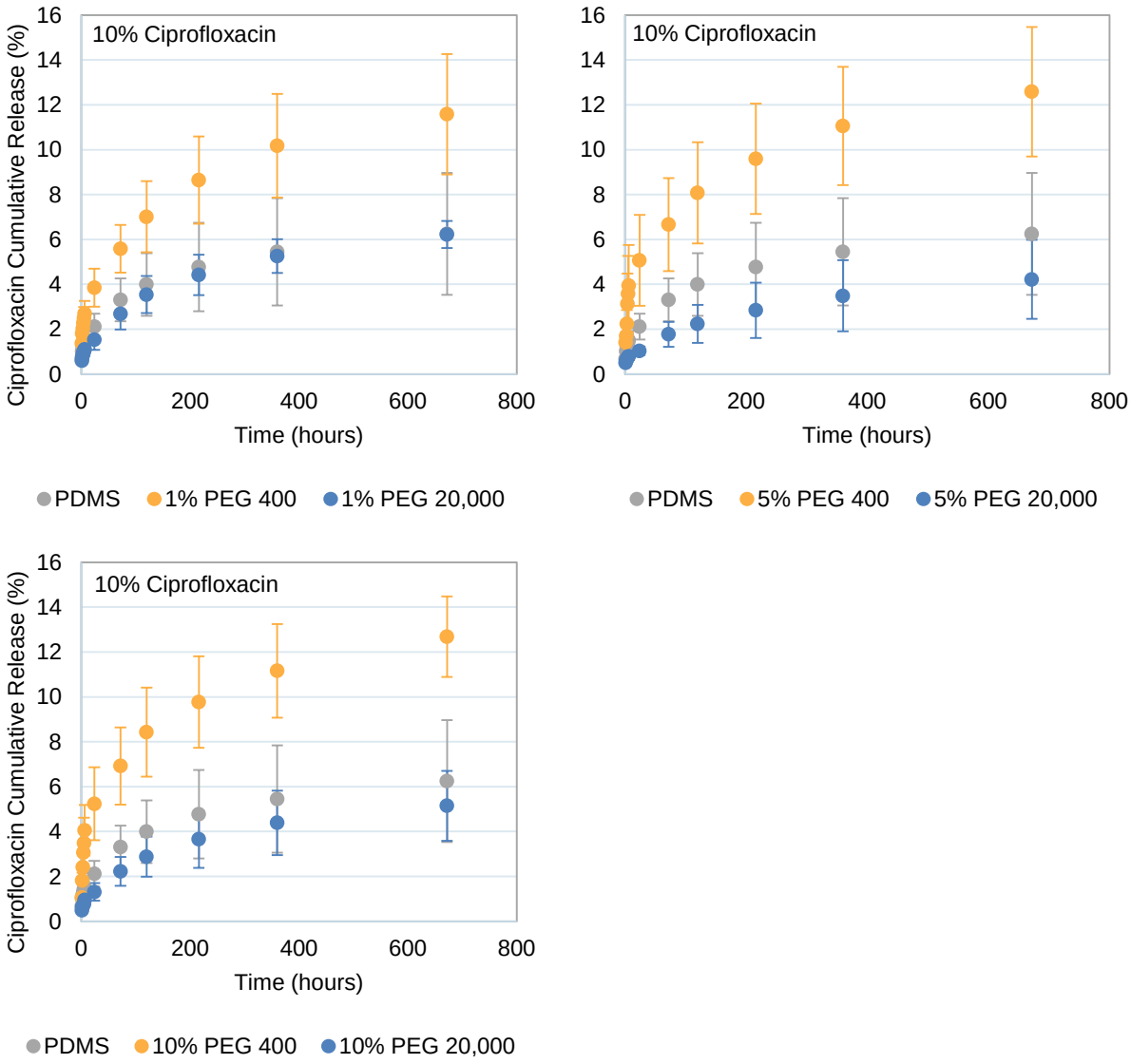

Figure 5.2. The effect of PEG with varying molecular weight on ciprofloxacin release when 10% ciprofloxacin is loaded. Only PEG_{400} statistically increased cumulative drug release in discs containing 10% PEG_{400}.

The influence of PEG concentration on ciprofloxacin release is shown in Figures 5.3 and 5.4, with loadings of 5% or 10% ciprofloxacin, respectively. For all of the PEG-PDMS materials, the drug release did not statistically significantly differ. The data suggest that ciprofloxacin release from PDMS-PEG discs is independent of PEG concentration over the 1-10% w/w range measured.

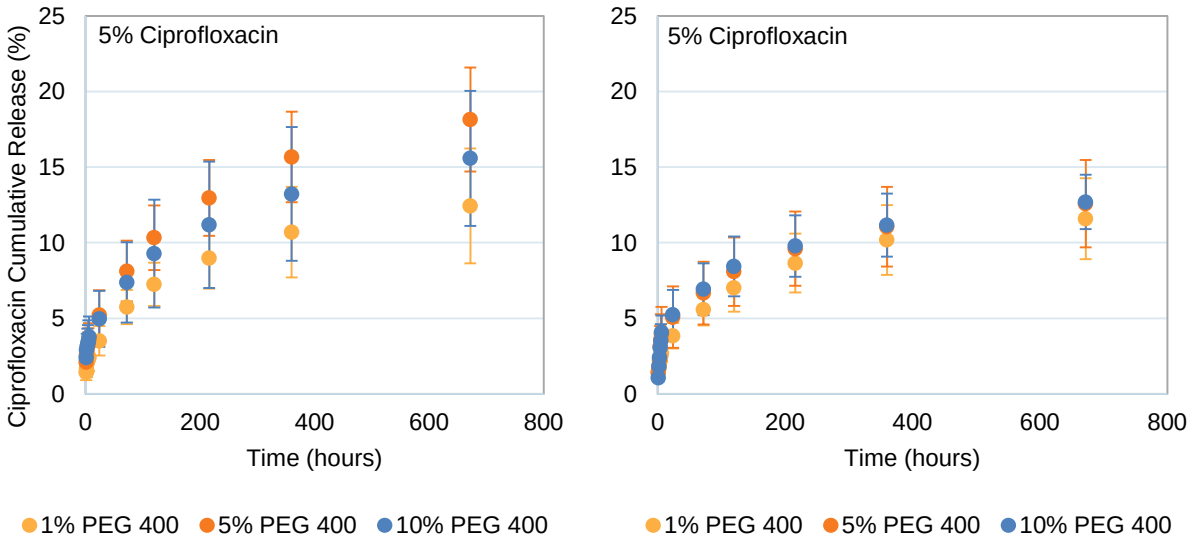

Figure 5.3. Effects of increasing PEG_{400} concentration on ciprofloxacin release. Data indicate that the release curves are not statistically significant different.

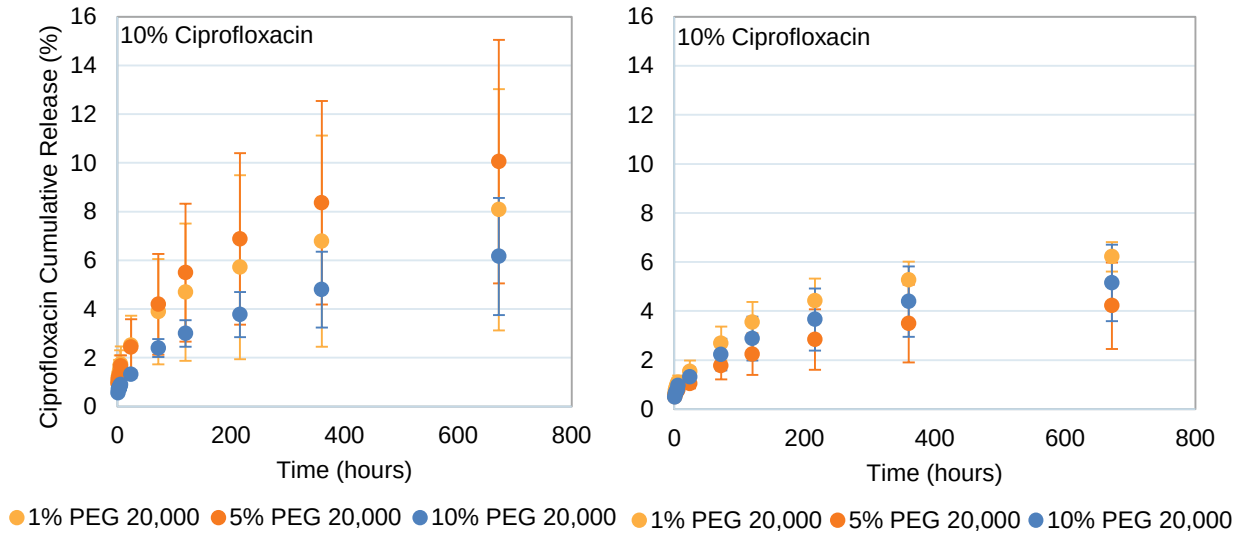

Figure 5.4. Effects of increasing $PEG_{20,000}$ concentration on ciprofloxacin release. Data indicate the release curves are not statistically significant.

5.5.2.1 Release kinetics of ciprofloxacin from PDMS-PEG materials.

The release of ciprofloxacin from PDMS-PEG materials was examined by fitting the release curves against common kinetic models to better understand the interaction of the drug with the material. Briefly, good fit to first-order release kinetics indicates the release is directly proportional to the amount remaining inside the release vehicle. The polymer may play a role in the diffusion of drug but the main influence on drug release is the drug concentration [31]. On the other hand, a good fit to the Higuchi model indicates that the drug release is more influenced by the diffusivity of the drug in the polymer [32]. In both cases, diffusion is the driving force for drug release from the materials, however the main influencing factor on the rate of diffusion (drug release) differs.

Ciprofloxacin release kinetics (Table 5.2) fit the Higuchi model very well ($R^2 \geq 0.93$) for all formulations. Thus, the data demonstrate that the material composition and drug diffusivity in the material influence ciprofloxacin release.

Table 5.2. Release kinetics of ciprofloxacin show excellent fit to the Higuchi model for drug release.

Material Formulation		
Ciprofloxacin Concentration	PEG Molecular Weight and Concentration	Higuchi (R^2)
5%	PDMS only	0.98
	1% PEG_{400}	1.00
	5% PEG_{400}	1.00
	10% PEG_{400}	1.00
	1% $PEG_{20,000}$	0.99
	5% $PEG_{20,000}$	0.93
	10% $PEG_{20,000}$	1.00
10%	PDMS only	0.99
	1% PEG_{400}	1.00
	5% PEG_{400}	1.00
	10% PEG_{400}	0.99
	1% $PEG_{20,000}$	0.99
	5% $PEG_{20,000}$	0.99
	10% $PEG_{20,000}$	0.99

5.5.3 Tobramycin release

In Figure 5.5, the effect of PEG molecular weight on drug release in materials containing 5% tobramycin is seen. The cumulative drug release is statistically significantly reduced in the presence of PEG_{400} and $PEG_{20,000}$ at all loadings tested (1, 5, 10%) in comparison to PDMS alone. There is no statistically significant change in release when comparing materials with PEG_{400} and $PEG_{20,000}$.

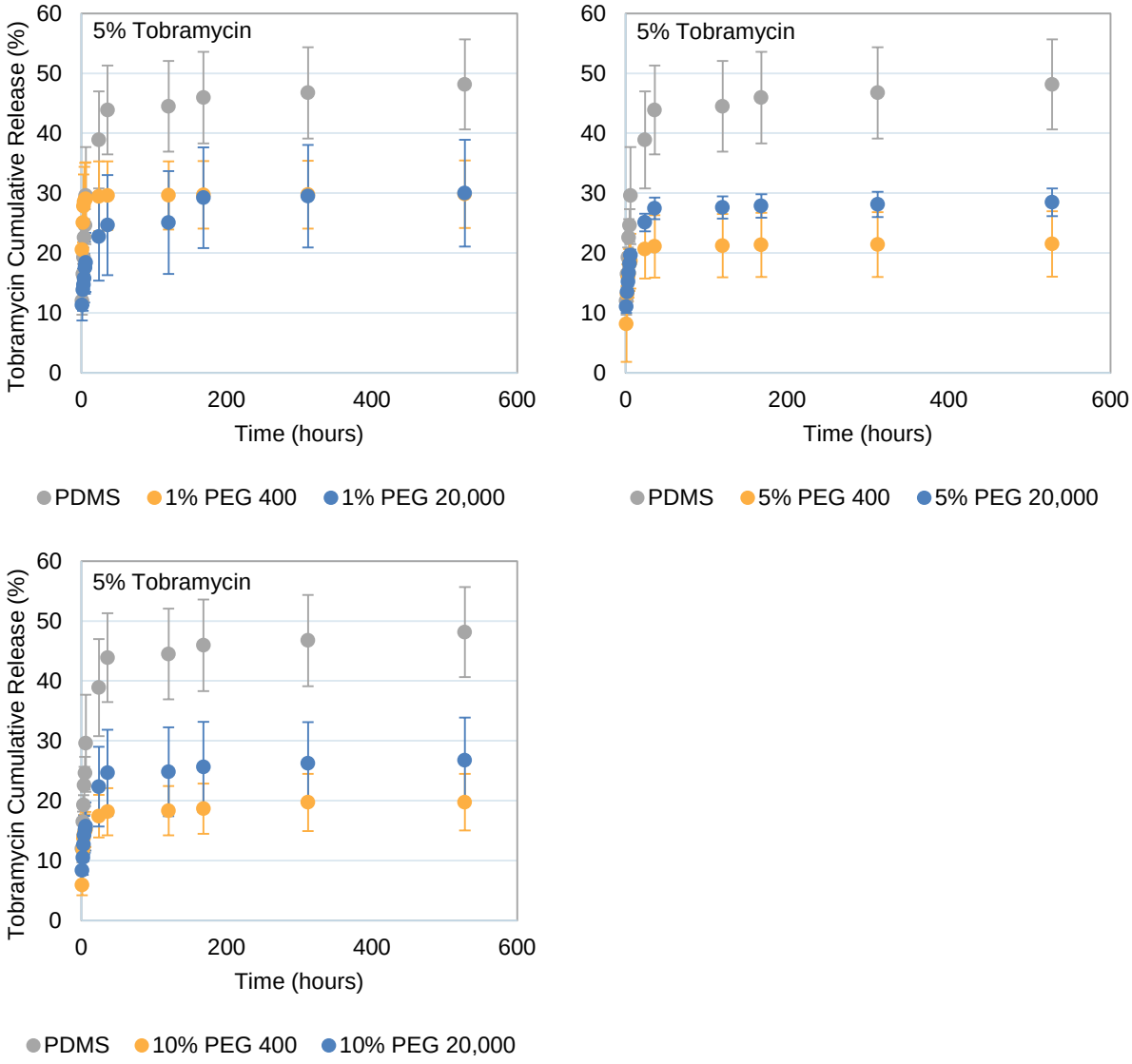

Figure 5.5. The presence of PEG_{400} or $PEG_{20,000}$ statistically significantly reduces tobramycin drug release in comparison to PDMS-only discs.

In Figure 5.6, the release curves for tobramycin are seen. The presence of PEG_{400} or $PEG_{20,000}$ in PDMS-PEG materials at any concentration (1, 5, 10%) did not alter the release of tobramycin in comparison to PDMS-only materials. There was no statistically significant difference in the cumulative drug release observed.

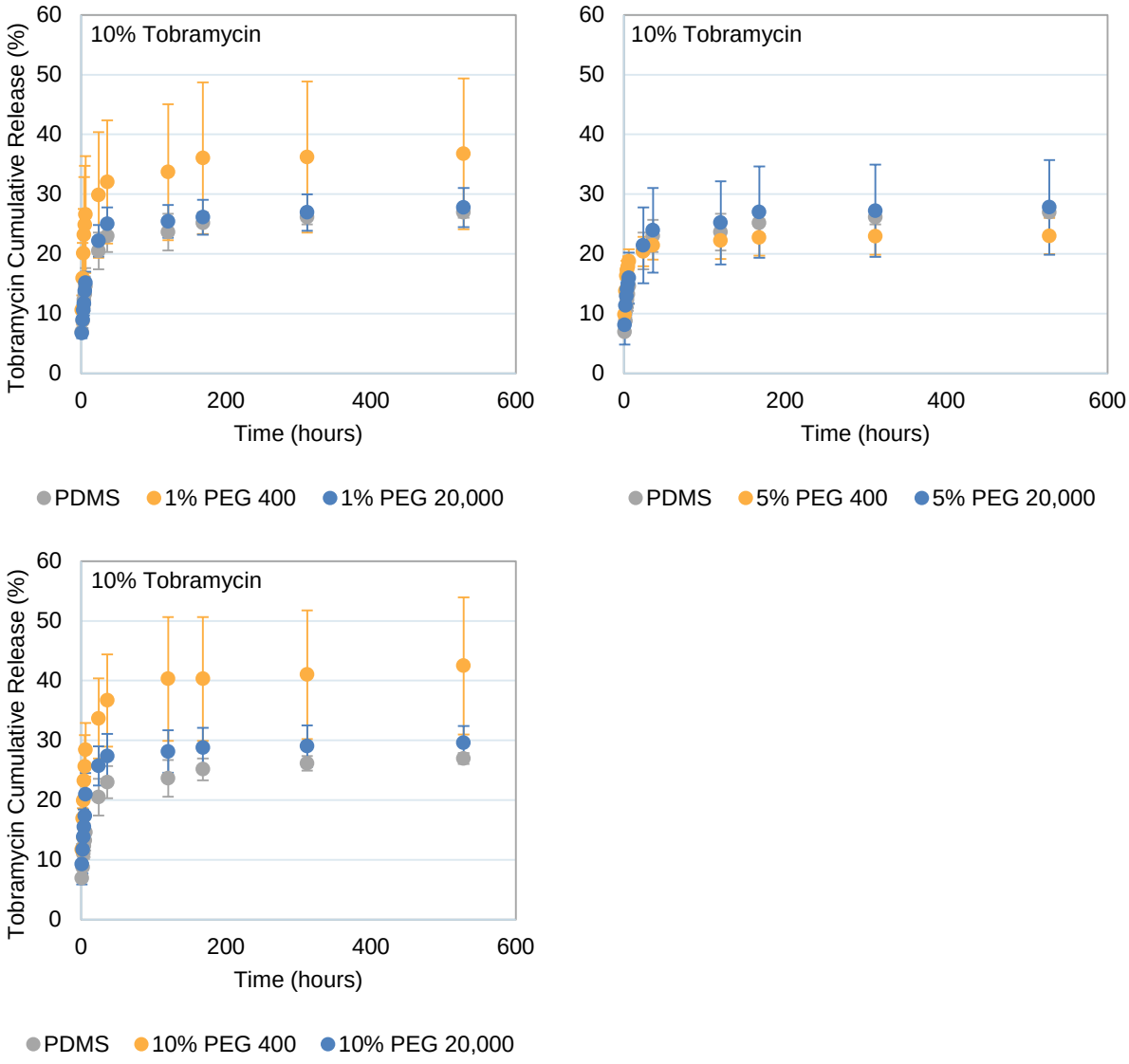

Figure 5.6. The presence of PEG_{400} or $PEG_{20,000}$ does not significantly alter tobramycin release in comparison to PDMS-only discs.

The effect of increasing PEG concentration on tobramycin release is seen in Figures 5.7 and 5.8. At loadings of 5% and 10% tobramycin, there was no statistically significant change in release with increasing PEG concentration for both PEG molecular weights studied. Thus, the data suggest that tobramycin release is independent of PEG concentration.

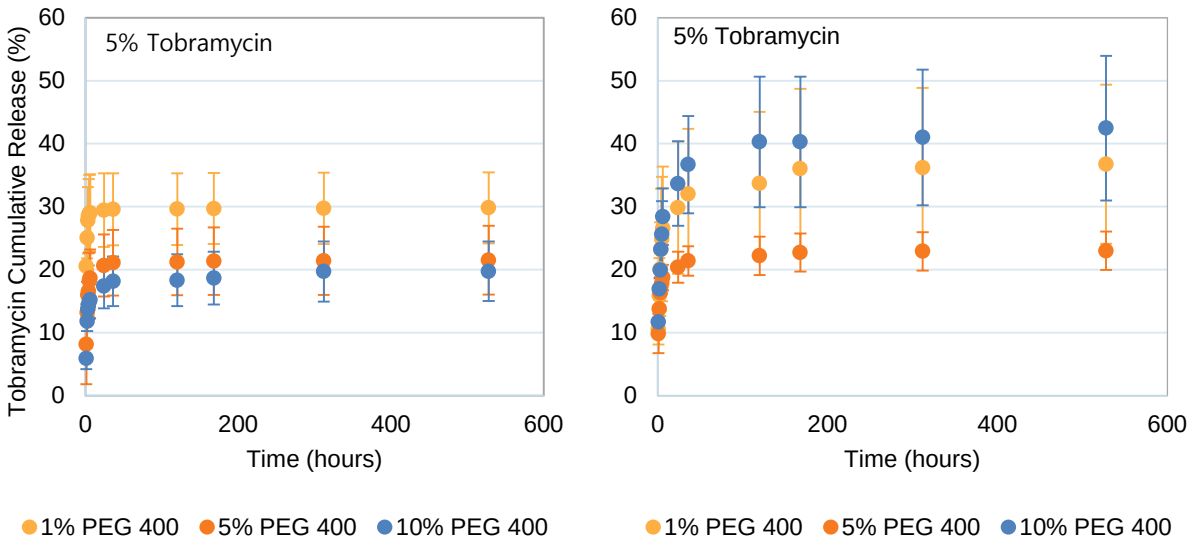

Figure 5.7. Effect of increasing PEG_{400} concentration on tobramycin release from PDMS-PEG discs. No statistically significant change is observed for either PEG molecular weight (400 or 20,000).

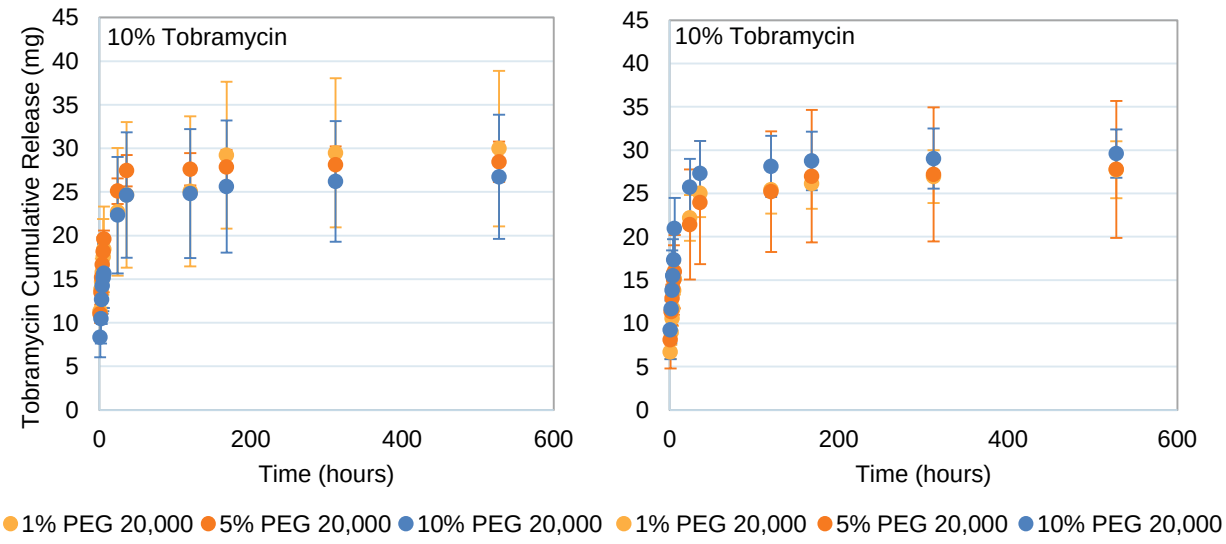

Figure 5.8. Effect of increasing $PEG_{20,000}$ concentration on tobramycin release when PDMS-PEG discs are loaded with 10% tobramycin. No statistically significant change is observed for either PEG molecular weight (400 or 20,000).

5.5.3.1 Release kinetics of tobramycin

Overall, the release kinetics of tobramycin do not fit Higuchi kinetic models (Table 5.3). However, the release data fit the Higuchi model well ($R^2 \geq 0.90$) over the first 6 hours from all formulations (containing PEG_{400} or $PEG_{20,000}$). The data were also fitted to the Korsmeyer-Peppas model but showed poor fit (data not shown). Thus, the data indicates release follows the Higuchi model early in the release, while later release is influenced by other factors.

Table 5.3. Release kinetics of tobramycin show excellent fit to the Higuchi model for drug release over the first 6 hours. Long-term release does not fit the First order or Higuchi models well.

Material Formulation		Higuchi (R^2)	
Tobramycin Concentration	PEG Molecular Weight and Concentration	Overall	Hours 1-6
5%	PDMS only	0.69	0.98
	1% PEG_{400}	0.25	0.86
	5% PEG_{400}	0.42	0.93
	10% PEG_{400}	0.53	0.84
	1% $PEG_{20,000}$	0.65	0.99
	5% $PEG_{20,000}$	0.57	0.94
	10% $PEG_{20,000}$	0.69	1.00
10%	PDMS only	0.75	0.99
	1% PEG_{400}	0.80	0.99
	5% PEG_{400}	0.64	1.00
	10% PEG_{400}	0.69	0.99
	1% $PEG_{20,000}$	0.70	0.99
	5% $PEG_{20,000}$	0.75	0.98
	10% $PEG_{20,000}$	0.64	0.96

5.5.4 Metformin release

The influence of PEG on hydrophilic drug release was further examined by measuring the release of the hydrophilic drug metformin hydrochloride. This variation in drug structure but similar hydrophilicity allows for the comparison of tobramycin, an inherently hydrophilic molecule, with a pharmaceutical salt.

Only at 5% metformin hydrochloride and 1% PEG was a statistically significant change in overall release noted compared to release from PDMS-alone as shown in Figure 5.9. For all other formulations (Figures 5.9 and 5.10), no statistical significance across the release curve time points was seen (data not shown). Gradual release was uniquely seen up to 99 hours for formulations with 10% metformin-HCl and $PEG_{20,000}$.

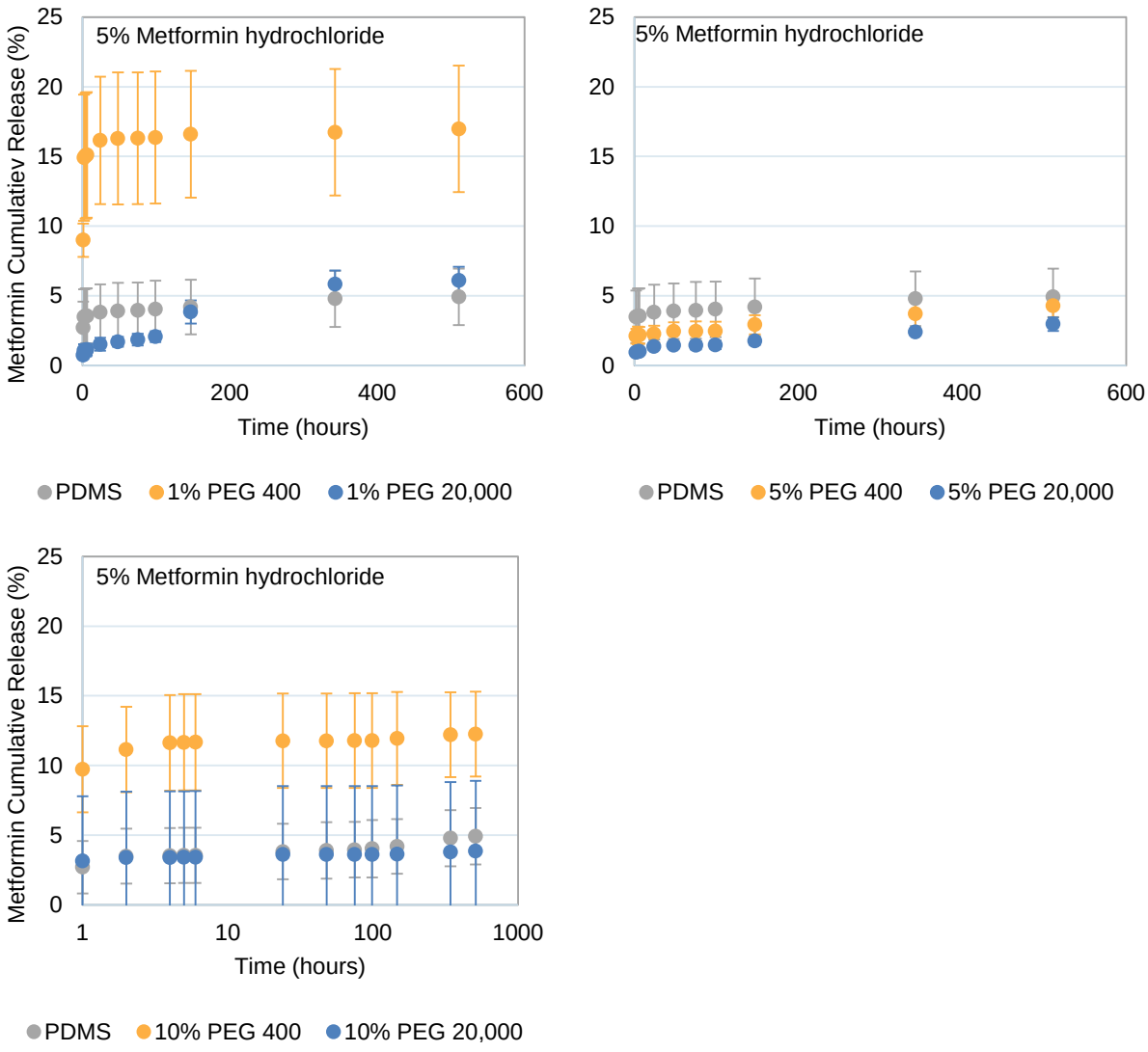

Figure 5.9. Release of metformin hydrochloride does not significantly differ in the presence of PEG_{400} or $PEG_{20,000}$ in comparison to PDMS-only control. 1% PEG_{400} showed statistically significant difference, however this value looks to be an outlier due to a larger variation between experimental replicates.

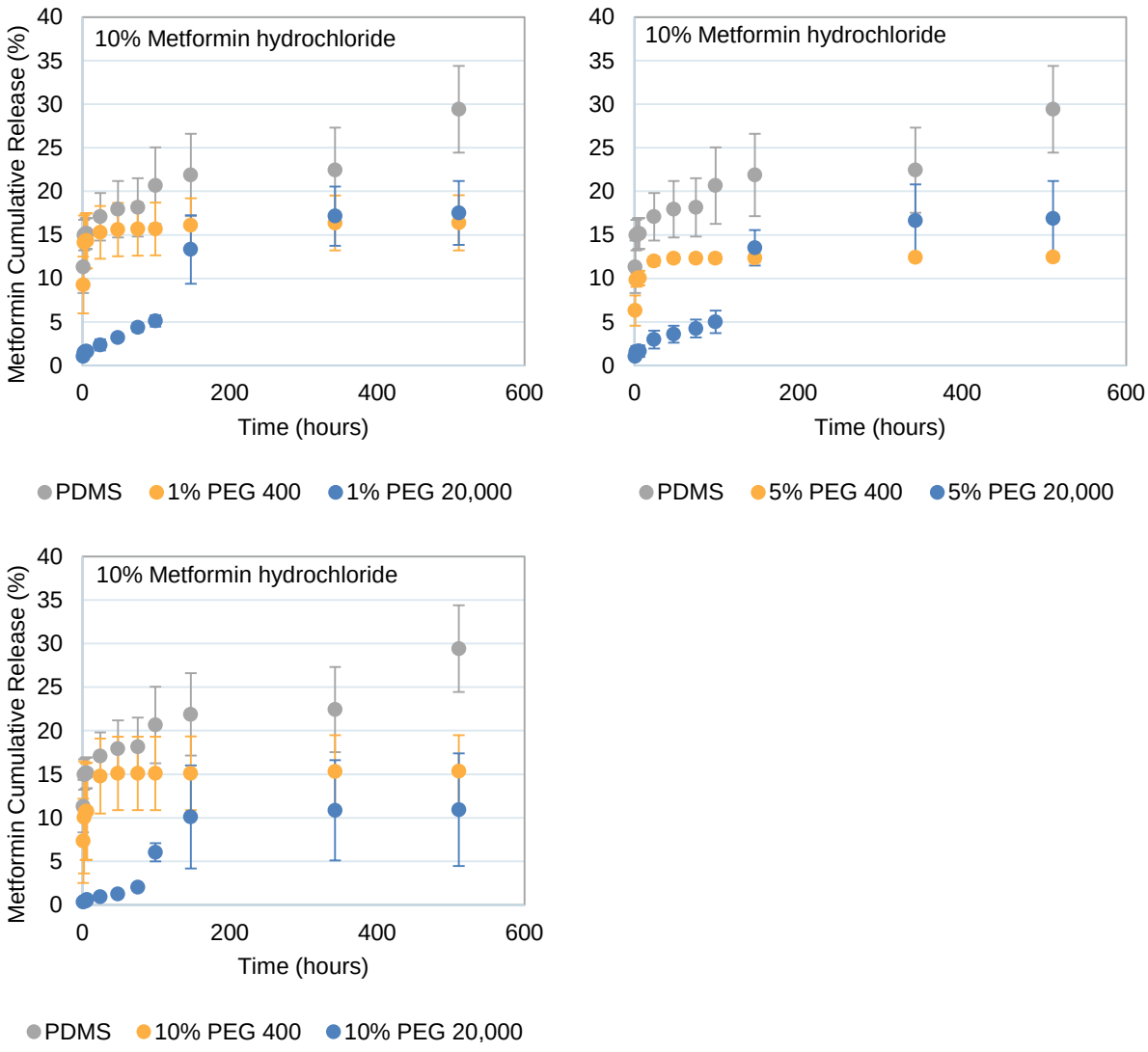

Figure 5.10. Release of metformin hydrochloride does not significantly differ in the presence of PEG_{400} or $PEG_{20,000}$ in comparison to PDMS-only control.

The release curves in Figures 5.9 and 5.10 were also investigated for their fit against common kinetic models as with ciprofloxacin and metformin. However, the overall data fit very poorly (R^2<0.3) to each of model including First-order and Higuchi.

5.6 Discussion

This study was undertaken in order to better understand the contrasting trends observed in the literature. This was hypothesized to be in part due to the variation in the polymerization procedure, variation in the type of silicone used, and the varying solvents used across the various model systems.

To better understand the relationship and influence of various factors in these drug release systems, a novel PEG-PDMS material was a solvent-free, heat-cured system that incorporated the commercially available Sylgard 184® siloxane. This material design provided the opportunity to directly infer relationships between the material composition, without the influence of artifacts from the presence of solvent, or active changes in material composition (phase separation) over time due to the use of a slow curing method. Further, the use of PEG and PDMS inherently provides potential for this novel material to be used in biomedical applications as a drug delivering device. This is because PEG and PDMS are already FDA-approved. In addition, the system is solvent-free and uses a platinum-based curing method, reducing downstream toxicity concerns.

The more focused objective of this study was to elucidate the influence of the unique osmotic agent poly(ethylene glycol) – which can exhibit amphiphilic properties – when incorporated as part of a hydrophobic drug delivering system. The relationship between the structure of PEG and three types of small molecule therapeutics – hydrophobic, hydrophilic, drug salts – was investigated. It was determined that (1) hydrophobic drug release is dependent on PEG molecular weight, (2) hydrophilic drug release can be influenced by the presence of PEG, and (3) drug salts produce inherently unique release mechanisms, distinct from hydrophilic drugs.

5.6.1 Ciprofloxacin release is dependent on PEG molecular weight

As described earlier, hydrophobic drug release from silicone elastomers has been shown to occur for periods of time up to years. However, release rates are often very low, given that hydrophobic drugs have low solubility in the surrounding aqueous/biological medium, and also remain preferentially in the hydrophobic, elastomeric device. Thus, despite the ability to attain long-term release using silicone systems, controlled release rates of hydrophobic drugs remains desirable. Indeed, in Figures 5.1 and 5.2, the release amount of ciprofloxacin from PDMS-only materials was seen to be only ~2% when 5% ciprofloxacin was loaded, and ~6% when 10% ciprofloxacin was loaded after ~650 hours of drug release.

It was expected that the presence of PEG would increase the release rate of ciprofloxacin from all PEG-PDMS materials. This was because the high affinity of osmotic agents for water has been shown in some cases to produce water channels throughout the material [17]. Then, given that ciprofloxacin is hydrophobic and is preferentially partitioned in PDMS, PEG channels could allow for more deeply embedded ciprofloxacin to be dissolved through the penetrating PEG-water channels and then released out [30], [33]. In Figures 5.1 and 5.2, this statistically significant increased release was seen to only occur in the presence of PEG_{400} (in Figure 5.2, only in the presence of 10% PEG_{400}). In comparison with release from PDMS-only materials, at

~650 hours, PEG_{400} resulted in ciprofloxacin release of 12-15%. Release from materials containing $PEG_{20,000}$ was not statistically significantly different from release from PDMS-only materials.

This molecular-weight dependent increase in ciprofloxacin release is hypothesized to be the result of the unique ability for short-chain PEG to readily adopt both polar and non-polar conformations. Specifically, it has been previously shown that short-chain PEGs exhibit less order in their structure and can adopt non-polar conformations due to high conformational freedom around each of C-C and C-O bond of PEG [34]. In contrast, the long chain $PEG_{20,000}$ is more likely to maintain its helical secondary structure, where more bonds have a polar conformation. As a result, this would allow PEG_{400} to have a higher diffusivity than $PEG_{20,000}$ while trapped in the PDMS elastomer. Together, the more amphiphilic nature of PEG_{400} (ability to alter bond conformation) could allow more water to enter the material, while also encouraging the release of hydrophobic ciprofloxacin, leading to the increased release seen in Figures 5.1 and 5.2.

When observing the effect of PEG concentration on release rates, there was generally no statistically significant difference between release in the presence of 1, 5 or 10% PEG_{400} or $PEG_{20,000}$. This result was surprising, given that in the presence of more PEG, it would be expected that water enters the material and more ciprofloxacin is released. However, it is likely that the inherent hydrophobicity and strong, crosslinked PDMS chains are able to physically prevent the materials from swelling / absorbing additional water. Alternatively, the PEG concentration range tested may be too narrow to observe a statistically significant effect of PEG concentration on release rates. Given that materials with greater than 10% PEG exhibited poorer mechanical strength or incomplete polymerization it may not be possible to test this hypothesis under similar conditions. Rather, alternative characterization of the materials (such as SEM, swelling studies) may be required.

The release rates of ciprofloxacin from all PEG-PDMS formulations was found to fit the Higuchi drug release model (Table 5.2). This was generally expected, given that ciprofloxacin is hydrophobic and thus has good diffusivity in the bulk PDMS polymer, which results in a concentration gradient of drug as ciprofloxacin is released. Surprisingly, formulations with PEG_{400} continued to maintain excellent Higuchi fit, despite the change in drug release that was observed. This continued fit supports the hypothesis that ciprofloxacin is able to be released through PEG_{400} channels as a result of the ability for PEG_{400} to adopt a favorable non-polar conformation. For $PEG_{20,000}$ formulations, given that drug release is not statistically significantly different from the control, the Higuchi data suggests that $PEG_{20,000}$ does not promote ciprofloxacin release. Rather, ciprofloxacin only diffuses out through the PDMS component.

Overall, the data indicates that hydrophobic drug release, as observed using the model drug ciprofloxacin, can be controlled and increased using low-molecular weight PEG_{400}, while maintaining predictable, Higuchi release.

5.6.2 Tobramycin release can be influenced by the presence of PEG

For tobramycin release, it was expected that there would be rapid burst, followed by more minimal, gradual release over time. This is because the highly hydrophilic tobramycin has low diffusivity in hydrophobic PDMS. As a result, tobramycin located close to the material surface is able to be released rapidly ('burst'), while more deeply embedded tobramycin will remain trapped in the material. This drug release profile was indeed observed for all formulations loaded with 5% or 10% tobramycin (Figures 5.5 and 5.6).

It was further hypothesized that the presence of PEG would create water channels that would increase the release of the more deeply embedded tobramycin. In contrast however, for materials loaded with 5% tobramycin, all formulations with PEG demonstrated statistically significant reductions in tobramycin release. Given the ability for both PEG and tobramycin to form hydrogen bonds, it is logical to hypothesize that hydrogen bonds may form between PEG and tobramycin which lead to the reduced release rates. Literature data further supports this theory. It has been shown that active pharmaceuticals can hydrogen-bond with poly(ethylene glycol) when drugs are formulated as solid dispersions [35], [36]. For example, data have demonstrated that each PEG ether oxygen can hydrogen-bond with two indomethacin molecules [36]. The hydrogen bonding between the drug and PEG additionally leads to structural changes in PEG which influences the properties of the dispersion system [36]. Specifically, PEG is normally found in a semi-crystalline state due to its ability to hydrogen bond intra-molecularly, forming a broken helix (short helical sections connected by random coils) [37]. In the presence of drugs with hydrogen-bonding capability, PEG becomes less crystalline (more amorphous), leading to increased free volume around PEG and increased rotational / translational motion [36]. This change in state is important in drug delivery, as it alters the physical properties of solid dispersion systems and can change the rates of release of the active compounds [36]. In the model PEG-PDMS system studied herein, similar changes to PEG structure are likely. However, given that PEG is entrapped within cross-linked PDMS chains, there is far less free volume and rotational/translational motion available to PEG to expand / relax upon hydrogen bonding with tobramycin or water. As a result, there is minimal 'bulk' water available for release of tobramycin, and in contrast, tobramycin is tightly bound to PEG through hydrogen-bonding – a state of tobramycin that can be referred to as "stationary" [38].

With 10% tobramycin loaded, the reduction in tobramycin release was not observed in the presence of PEG. However, studies of PEG as an inactive binder in solid dispersion systems have shown that there is an inverse relationship between drug loading and PEG crystallization [36]. This supports the expectation that lower drug release would be expected with higher drug loading. However, the study data indicate that there may be an upper concentration of loaded drug above which the reduced drug release that 1-10% PEG produces is no longer statistically detectable. Specifically, the loading of additional tobramycin (which is evenly distributed throughout the PEG-PDMS material) results in more tobramycin available in PDMS located close to the material surface. As a result, there is a two-fold increase in tobramycin that is released at the surface, which may

eclipse the reduction in drug release that results from the presence of 1-10% PEG. In addition, the measurement accuracy across some replicate samples may further eclipse any small alteration in release rate. To better understand this underlying mechanism, future studies examining the drug loadings in the range of between 5-10% may define the transition point at which the PEG reduction is no longer detectable. This would thus clarify the relationship between the drug, PEG and the PDMS system, expanding the foundation for predictable release established herein.

In addition, it may be of interest to test materials where tobramycin and PEG are blended together prior to dispersion throughout PDMS. This may lead to one of two results. Firstly, more loosely-bound tobramycin may be found near PEG. As the loosely bound tobramycin (closest to the surface) a free volume would remain. This free volume (or enlarged PEG 'channel') could allow more water to enter, leading to the release of more deeply embedded, loosely-bound tobramycin, and thus a longer duration of drug release. Alternatively, a stronger reduction in tobramycin release may also be possible. As the drug is found closer to PEG (and less in PDMS) PEG can tightly hydrogen bond with the drug and the quantity and rate of tobramycin release could decrease. Combining these studies with additional modifications to the amount of PDMS crosslinking will provide valuable data into understanding the ability of PEG to control drug release rates, and the ability of the PEG-PDMS system to allow for long-term release of hydrophilic compounds such as tobramycin.

In this PEG-PDMS-tobraycin delivering system, the fit of the data to kinetic models was poor against the Higuchi model (Table 5.3). However, given the observation that most of the release occurs in the first few hours, this early release was tested for fit as well. Over the first 6 hours of release, strong fit was seen against the Higuchi model. This data supports the hypothesis that the majority of the release occurs through diffusion of shallowly embedded tobramycin, by a concentration gradient that is dependent on the diffusivity of tobramycin in the polymer and slowing over time. The relatively small decrease in drug release seen in PEG-containing formulations (Figure 5.5), may not be sufficiently significant to detect through kinetic modelling. This data is contrasted from that seen by Panou et al, who identified Higuchi drug release kinetics for all drug solubilites and consistently increased release when PEG was loaded into PDMS [50]. Similar to their work, in future studies for all the drug types tested, it will be beneficial to understanding drug release mechanism further by confirming that crystalline drug is present – to fit the Higuchi assumption that drug inside is loaded at a higher concentration than its solubility in the polymer. Calculating the percolation thresholds of PEG in PDMS will also help to understand these release kinetics further.

Thus, this work indicates that, as seen in solid-dispersion drug release models, PEG is able to control the release of the highly hydrophilic model drug tobramycin. It notably expands on this work by demonstrating that PEG is able maintain this property when blended in a novel PEG-PDMS system, and when present at concentrations as low as 1%. However, there may be an upper limit above which the reduction is no longer detectable within the boundaries of the formulations in this study. This upper limit is dependent on the concentration of the drug loaded, and possibly the distribution of the

drug throughout the material. The release was demonstrated to follow the Higuchi model for the majority of the early time-period. This provides the foundation for predictable hydrophilic drug release from PEG-PDMS materials for up to 100 hours (based on the current formulations and material production method). Further studies will build the ability to accurately predict the release of more deeply-embedded tobramycin at later timepoints.

5.6.3 Metformin hydrochloride shows a unique release mechanism, distinct from hydrophilic drugs.

The release of metformin hydrochloride from the PEG-PDMS materials was distinct from tobramycin and ciprofloxacin. A very rapid burst release reaching a maximum within 2 hours was seen with most formulations (Figures 5.9 and 5.10). In addition, some experimental replicates were seen to swell over time, while others did not, leading to larger measurement variation than was seen with the tobramycin or ciprofloxacin containing materials. The inconsistent physical properties are likely the result of the presence of the salt. Literature data has shown that the presence of salt as an osmotic agent in PDMS results in irregular pores that produce very high local stresses on the crosslinked PDMS network and the formation of cracks. These cracks can lead to altered drug release rates. In the case of the materials tested herein, variability in the size and location of cracks may contribute to the variation that was seen between replicates.

Given the rapid release and physical variability of the materials, the release kinetics were also variable and did not fit well against kinetic models (data not shown). This was unexpected because metformin can hydrogen-bond similarly to tobramycin, and some literature data has shown that salts can increase drug release rates. However in this study, the release quantity is generally lower than tobramycin, and the presence of PEG does not statistically significantly influence overall release rates (as was seen with tobramycin). Thus, the data suggest these distinct release curves are likely due to the drug properties, specifically, the ionizable nature of metformin hydrochloride and/or the presence of the hydrochloride salt.

Literature data further support the hypothesis that the salt influences release from PEG-PDMS materials. Salts are regularly used in research to disrupt hydrogen bonds intra- and inter-molecularly, which could disturb the proposed semi-crystalline structure of PEG. The addition of electrolytes has further been shown to decrease PEG solubility by decreasing PEG hydrophilicity [39] and disrupting PEG-water interactions [40]. This research provides a rationale for the observation that PEG-PDMS release curves were not statistically significantly different from PDMS-only release curves. Specifically, the ionizable metformin and hydrochloride salt are likely able to maintain the non-polar conformation of PEG, which prevents the absorption of water into the material, minimizing the creation of PEG water channels, and leading to release that is similar to PDMS alone.

With $PEG_{20,000}$ and higher metformin hydrochloride loading (10%), this salt-effect may also drive the gradual release that is seen in Figure 5.10. Specifically, the release from this formulation has high linearity across the first 99 hours with 1% and 5% $PEG_{20,000}$

(R^2>95%) and the first 75 hours with 10% $PEG_{20,000}$. This result was novel and unexpected, given that it demonstrates that zero-order drug release can be attained using $PEG_{20,000}$ and a drug salt. Additional experiments are required to fully elucidate the mechanism by which this controlled release occurs. In particular, differential scanning calorimetry measuring the diffusivity of metformin hydrochloride with PEG_{400} or $PEG_{20,000}$ may be able to confirm the disruption in PEG conformation due to the presence of the ionizable compounds, and provide better understanding of the structure-effect relationship that allows $PEG_{20,000}$ to control the release, while PEG_{400} does not at equal weight percent concentration.

Overall, the data demonstrate that the presence of salt as part of a pharmaceutical agent alters the release in contrast to that observed with a hydrophilic molecule, increases the variability in physical properties of the materials, and consequently the drug release across timepoints. However, at higher drug concentrations and in the presence of long-chain $PEG_{20,000}$, zero-order drug release can be attained for periods of between 75 and 99 hours. This suggests the potential for PEG-PDMS materials to act as drug releasing materials superior to PDMS alone. ,

5.6.4 Potential applications of PEG-PDMS drug releasing materials

Given the wide application of siloxanes, the possibilities are broad for a predictive, small-molecule releasing siloxane material. However, as both PEG and PDMS are FDA approved and have decades of data indicating their general safety in the body, a high potential translation of PEG-PDMS materials is to the field of medical drug delivery is possible.

The most direct route of translation is to the use of PEG-PDMS materials as medical device coatings. Whether the devices are implanted or used surgically, a thin coating of a drug releasing silicone-based material could provide added protection to the patient, by delivering anti inflammatory or anti-microbial therapeutics at the site of action, and for a prolonged duration. Given that the ability to control the release using PEG, formulations could be adjusted using modelling data. Potential sites of action for PEG-PDMS materials could include infections of the inner ear, intra-vaginal long-term therapeutic delivery (whether hormonal or other therapeutics), transdermal delivery, and (if formulated to be degradable) inside the vitreous of the eye to prevent interior infections during surgery or to release therapeutic compounds long-term to the difficult-to-reach eye posterior.

The ability to simply adjust the drug loading, to predict the release through kinetic models, and to create the materials using simple laboratory equipment, provide an additional source of strong potential of the materials for use in biochemical studies. For example, given the depth and breadth of *in-vitro* models that have been developed in all therapeutic areas, the long-term exposure of cells to a therapeutic could be simply studied using a single material disc, in high-throughput format.

5.7 Conclusion

A novel drug delivery platform based on a simple manufacturing method using PEG and PDMS was developed, providing the potential for better understanding the role of PEG in the release of small molecules. This delivery device was shown to demonstrate controlled release of hydrophobic, hydrophilic and salt therapeutics (influenced by PEG structure) that could be fitted to kinetic models. Specifically, where hydrophobic materials often remain entrapped within PDMS, the presence of low molecular weight PEG_{400} increased the release rate and provided sustained release for over 600 hours. Where hydrophilic drugs often exhibit rapid burst release, the presence of PEG_{400} or $PEG_{20,000}$ reduced the burst and provided more gradual release when 5% tobramycin was loaded. Similarly, where hydrophilic pharmaceutical salts are prone to burst release, the presence of $PEG_{20,000}$ provided tempered, gradual release for up to 100 hours. Further expansion of the study including increasing the range of PEG molecular weights tested, or altering crosslinking as well as characterization of PEG directly will strengthen and expand the predictability of the release to other therapeutic compounds.

5.8 References

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Literature Appendix

Metformin is an orally administered, hydrophilic molecule, highly prescribed for the treatment of type-II diabetes [41]. These patients do not respond normally to insulin produced by the body upon a glucose increase, or are not able to produce enough insulin on their own [42]. Metformin acts by reducing the glucose amount produced by the liver, reducing the glucose amount taken in by food, and activating the production of insulin [42]. In addition to it being a model for the release of a pharmaceutical salt from the device, the current oral administration method has a number of drawbacks that could be improved with an alternate release method. A high oral dosage is required, which after approximately 6.5 hours for some formulations, is completely eliminated from the body [43]. Moreover, low bioavailability and high incidence of gastrointestinal side effects further support the need for an improved delivery method [43]. Improving its poor absorption [44] and maintaining steady release rates could potentially reduce adverse effects and improve treatment.

Tobramycin is an aminoglycoside antibiotic [45]. This type of antibiotic is too hydrophilic to diffuse across cell membranes and is thus readily excreted from the body. Ciprofloxacin is a synthetic fluoroquinone with moderate bioavailability of 55-75% when administered orally [46]. However, the effectiveness of all antibiotics is sensitive to maintenance of drug concentrations within the therapeutic window [47]. In particular, concentrations which are too low will fail to eliminate all pathogens and may lead to the selection for drug-resistant bacteria [48]. Concentrations above the therapeutic dose are also an issue, as toxicity to mammalian cells has been of concern with some fluoroquinones [49]. It is therefore imperative that the drug dosage is maintained within this window for all antibiotics.

6 Conclusions

6.1 Overall Conclusions

The motivation for the work in this thesis began with the observation that there exist only a few types of advanced biomaterials and drug delivering devices on the market. At the same time, academic research in this area is vast, complex and holds exciting potential for solving many medical needs, improving the lives of patients. This dichotomy has existed for decades and medical breakthroughs with small molecules, biologics and even gene therapy are now successfully on the market or in late-stage clinical trials. However, given the years of research that were required to translate in vitro work to the clinic, the price of recovering the cost of developing of a single novel therapeutic today is exorbitant and in many cases, leads to patients not being able to obtain life-saving therapies.

Throughout the development process, lead therapeutic candidates are often chosen based on their potential pharmacokinetic profiles in order to reduce the risks of translation and to ensure the adequate safety and efficacy profiles will be attained. Optimization of pharmacokinetics is often performed through altering the dose and rate of dosing. Thus, despite the vast variety of drug delivering systems and polymers showing great promise in academic settings, their use has not been integrated as part of industrial drug development processes.

Taken together, it has become clear that the cost, time and approach to PK optimization currently used does not allow for rapid translation of novel technologies to the clinic. Thus, the overall objective of this thesis was to develop and demonstrate an approach to material design that considers the downstream requirements for clinical translation. This involved:

- Strategically defining which polymers would be of interest to the industry and acceptable to the regulatory bodies.
- Developing manufacturing procedures that are streamlined and reduce the need for complex and costly removal of solvents and potential toxins.
- Designing studies that provide a deeper understanding and control of the relationship between the polymers and their function/properties.

It was hypothesized that poly(ethylene glycol), based on a long history of use as a biomaterial, would be acceptable to industry thus reducing the risk of translation that was defined as a critical success factor for meeting the objective of this thesis work.

The unique properties of PEG coupled with the strategic experimental designs produced studies that demonstrated achieving these objectives is indeed possible. In addition, two novel drug delivery systems were developed, and the activity of PEG as a melanoma viability inhibitor was discovered. The key findings and contributions are reviewed within this chapter.

Overall, the work within this thesis demonstrated additional potential for PEG in biomedical applications than being used as simply a hydrophilic additive. Greater understanding of the abilities and limitations of PEG in various states (conjugated, entrapped and free) were developed and shown to be connected through the simple structure of PEG that allows for conformational freedom, and the ability to form multiple hydrogen bonds. By maintaining a forward looking material development strategy, multiple clinical and research needs were identified and were able to be met using systems containing PEG. Finally, the outcomes described in this thesis challenge the acceptance of PEG as a simple polymer, expand the knowledge of controlled drug delivery, and it is hoped that this demonstrated approach to material development may contribute to more successful translation of materials to the clinic.

6.2 Key Findings and Contributions of Each Chapter

Chapter 3: Examination of the influence of free PEG on extracellular medium and melanoma cell activity.

In Chapter 3, free PEG was explored in the presence of cultured melanoma cells to determine how the structure of PEG may influence its environment and thus cell activity. Key findings and contributions of this chapter included:

- Demonstration that PEG exhibits biological activity indirectly on multiple cell types.
    - Apoptosis was identified for 10% PEG_{200} through detection of caspase 3/7 activation.
- PEG activity on cells is directly correlated with its structure of repeating ethylene oxide units.
    - The reduction in cell viability mediated by PEG is correlated with changes in osmolality of the cell medium.
    - The relationship is specific to PEG, as experiments using the hydrophilic polymer poly(vinyl pyrrolidone) indicate an alternative mechanism of activity.
- The strength of activity (impact on cell viability) can be tuned by adjusting the PEG molecular weight and concentration
    - Osmolality is inversely correlated with cellular viability.

Future Work to Achieve Biomedical Application

For the thesis work, this study was designed to improve the knowledge of the unique PEG structure-function relationships in cellular applications. Due to the design of the study, the potential of polymers as pharmaceutical agents could also be explored. The ability of PEG to act so strongly as a chemotherapeutic agent towards melanoma cells was unexpected and provides support for future work involving PEG in cancer treatment. Exploring modifications to PEG may be required in order to reduce toxicity towards normal cells. An additional area of interest is the identification that PVP also induces cell death, however the mechanism is not due to changes in cellular osmolarity. Future work

should include expanding the study outside of only PEG, because as hypothesized, seemingly inert polymeric compounds may have been overlooked for their ability to act as pharmaceutical agents with unique cellular effects.

Chapter 4: Examination of the influence of PEG on its environment when directly conjugated as part of a silicone hydrogel

In Chapter 4, the ability of PEG to control its environment when conjugated as part of a silicone hydrogel was explored. Key findings and contributions of this chapter included:

- The establishment of a simple manufacturing method for direct co-polymerization of TRIS and PEG macromers, creating silicone hydrogels based on regulatory-approved polymers.
- Development of a novel silicone hydrogel with advantageous properties for biomaterial applications across a range of formulations.
    - No phase separation – Excellent transparency, low refractive index and high transmittance.
    - Inherent wettability – low contact angle and protein repulsion across all TRIS-PEG formulations with up to 70% TRIS.
- Finding that PEG is able to control its environment based on its structure.
    - Equilibrium water content was directly correlated with the number of ethylene oxide units of PEG.
- Finding that PEG is able to control the release of the highly hydrophilic antibiotics.
    - Surprisingly, release rates showed excellent fit to Higuchi kinetics. This was believed to be possible due to drug-PEG hydrogen-bonding.

Future Work to Achieve Biomedical Application

The results in this chapter suggest that PEG-TRIS silicone hydrogels may fare well as model contact lens materials. As described in the research chapter, additional material properties such as the mechanical strength and oxygen transport must first be optimized to meet the standards for this application. However, given the ability to tune the TRIS / PEG content with low impact on advantageous properties such as wettability, there is high potential for the development of hydrogels to meet these requirements. In addition, the use of TRIS and PEG in the material provide high potential for excellent cellular compatibility in vivo, which may lead to a reduced need for lengthy optimization of the material for ocular biocompatibility.

The ability of PEG to control the release of tobramycin further broadens the potential application of this material to ocular drug delivery. Investigating the physics of the interactions of tobramycin with PEG, the changes to the conformation of PEG and the influence of water content will provide a deeper foundation for future optimization of these and possibly other PEG-containing materials for drug delivery applications.

Chapter 5: Examination of the influence of PEG on drug release when entrapped in a PDMS elastomer

In Chapter 5, the ability of PEG to control drug delivery when entrapped as part of an elastomeric system was explored. Key findings and contributions of this chapter included:

- Development of a novel PEG-PDMS elastomer based on a solvent-free, rapid curing method.
- Kinetic modelling of release rates to provide deeper understanding of the relationship between PEG structure and its influence on drug release.
- The identification that the amphiphilic characteristics of PEG are able to influence the release of three types of therapeutics with varying solubility.
    - Hydrophobic ciprofloxacin
        - Low molecular weight PEG_{400} increased drug release, where $PEG_{20,000}$ present at an equal concentration did not. By applying the understanding of the ability of PEG to adapt both non-polar and polar conformations, this provided deeper insight into the controlled, Higuchi-based release kinetics that were observed.
    - Hydrophilic tobramycin
        - PEG is able to influence the release of tobramycin at concentrations as low as 1%, however there is an upper limit of drug that can be loaded for the effect to be observed. Based on the understanding of PEG-drug interactions in the literature and from Chapter 2, the data suggest that the altered release rates are due to hydrogen-bonding between PEG and tobramycin. Predictable release of hydrophilic compounds may be possible using this PEG-PDMS system as a result of the excellent fit to the Higuchi model for up to 100 hours.
    - Pharmaceutical salt metformin hydrochloride
        - $PEG_{20,000}$ in the presence of higher drug loading (10%) demonstrated high linearity in early drug release, whereas other formulations demonstrated burst release. This controlled zero-order release of a pharmaceutical salt has not been previously shown, as studies in the literature have generally examined overall drug release and found that salts are able to create their own channels (and are thus not able to be well controlled).

Future Work to achieve Biomedical Application

The PEG-PDMS system developed in this chapter provides a foundation for the improvement and understanding of drug delivering systems. In order to strengthen the relationships discovered between PEG, the therapeutic, and the drug release kinetics, expansion of the study is recommended. For example, a limitation of the long-term and large study designed herein was that the smaller sample size resulted in larger variation

in some drug release curves. Increasing the sample size, the number of drugs tested, the number of PEG molecular weights tested and PEG concentrations will build an even better understanding of the strength and limitations of the relationships observed within the current study design. Following this work, input of the data into a statistical program which uses multivariate analysis will provide a more rapid and robust technique for analyzing relationships and drug release. Then, input of long-term characterization of the material properties in vitro, and in vivo will ultimately allow for the strategic development of polymeric systems for improved translation to the clinic.