Therapeutic Contact Lenses for Comfort Molecules

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Therapeutic Contact Lenses for Comfort Molecules

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The author Maryam Ali was born in Rawalpindi, Pakistan on 25 December 1981. She attended the California Institute of Technology at Pasadena, California from 2001 to 2005 and received a Bachelor of Science in Chemical Engineering in 2005. She began work toward a Master of Science in Chemical Engineering at Auburn University at Auburn, Alabama in the Fall of 2005. She is joining The University of Texas at Austin in the Fall of 2007 for doctoral work in Biomedical Engineering.
Dry eye syndrome affects nearly 15% of the population and can cause extreme discom-fort that interferes with quality of life. Current treatment options include delivering comfort agents such as hyaluronic acid (HA) to the eye via eye drops, but low bioavail-ability continues to be a barrier to effective treatment. We have designed a therapeu-tic hydrogel contact lens that can deliver HA to the eye at a therapeutic rate. Control over the release characteristics is improved through biomimetic imprinting, as functional monomers such as acrylamide,N-vinyl pyrrolidone and (diethylamino)ethyl methacrylate are added to the hydrogel structure. The diffusion coefficients of hyaluronic acid, a long chain molecule, through the hydrogel can be controlled by varying the number of added functional monomers that interact with the drug molecule through memory sites. There is an inverse correlation between the total %-by-mass of functional monomers added to the hydrogel and the diffusion coefficient. Increasing the variety of functional monomers lowered the diffusion coefficient 1.5 times more than including a single type of functional monomer, and 1.6 times more than Nelfilcon without added monomers. By optimizing the
functional monomer content of the hydrogel, we can deliver hyaluronic acid to the eye at a constant therapeutic rate of approximately 6 µg per hour for 24 hours.
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# Table of Contents

**List of Figures**

**List of Tables**

1 Introduction ................................. 1

2 Objective ................................... 4

3 Ocular Drug Delivery ......................... 7
   3.1 Ocular Diseases and Impact ............... 9
   3.2 Barriers to Ocular Drug Delivery .......... 10
   3.3 Strategies to Overcome Drug Removal at the Ocular Surface .......... 17
   3.4 Strategies for Permeation Enhancement through Ocular Membranes .... 26
   3.5 Strategies to Delivery Drugs to the Posterior of the Eye ............. 29

4 Hydrogels and Imprinting ..................... 32
   4.1 Diffusion through a hydrogel .............. 33
   4.2 Theoretical model for diffusion .......... 37
   4.3 Equilibrium swelling theory ............... 40
   4.4 Rubber elasticity theory .................. 41
   4.5 Biomimetic Imprinting .................... 42

5 The Tear Film and Dry Eyes ................. 44
   5.1 The Tear Film ............................ 44
   5.2 Etiology of Dry Eyes ..................... 47
   5.3 Treatment with Hyaluronic Acid ............ 49

6 Lenses for Delivery of Comfort Molecules .... 52
   6.1 Nelfilcon A ................................ 52
   6.2 Hyaluronic Acid Binding Moieties .......... 54
   6.3 Methods and Materials ..................... 58
      6.3.1 Synthesis of hydrogels .............. 58
      6.3.2 Dynamic release studies ............ 59
      6.3.3 Heat stability studies .............. 60
      6.3.4 Tensile strength studies .......... 61
   6.4 Results and Discussion ................... 61
      6.4.1 Molecular transport and diffusion coefficients ............ 61
      6.4.2 Effects of heat sterilization ........ 77
      6.4.3 Structural analysis ................... 77
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Anatomy of eye surface</td>
<td>11</td>
</tr>
<tr>
<td>3.2</td>
<td>Anatomy of anterior chamber</td>
<td>14</td>
</tr>
<tr>
<td>3.3</td>
<td>Anatomy of the retina</td>
<td>16</td>
</tr>
<tr>
<td>3.4</td>
<td>Illustration of drug concentration profile in tear film with application of</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>eye drops</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>Illustration of mesh size within a hydrogel</td>
<td>35</td>
</tr>
<tr>
<td>4.2</td>
<td>Illustration of reptation model for transport through hydrogel</td>
<td>36</td>
</tr>
<tr>
<td>5.1</td>
<td>Tear film and related structures</td>
<td>46</td>
</tr>
<tr>
<td>5.2</td>
<td>Illustration of hyaluronic acid structure</td>
<td>50</td>
</tr>
<tr>
<td>6.1</td>
<td>Synthesis of Nelfilcon A macromer from PVA</td>
<td>55</td>
</tr>
<tr>
<td>6.2</td>
<td>Comparison of functional groups on amino acids and acrylate monomers</td>
<td>57</td>
</tr>
<tr>
<td>6.3</td>
<td>Cumulative release of HA from Nelfilcon hydrogels</td>
<td>63</td>
</tr>
<tr>
<td>6.4</td>
<td>Cumulative release of HA from Nelfilcon hydrogels with different % by mass</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>of functional monomers</td>
<td></td>
</tr>
<tr>
<td>6.5</td>
<td>Diffusion coefficients versus % by mass functional monomer content</td>
<td>68</td>
</tr>
<tr>
<td>6.6</td>
<td>Cumulative release of HA from Nelfilcon hydrogels with different proportions</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>of functional monomers</td>
<td></td>
</tr>
<tr>
<td>6.7</td>
<td>Fractional release of HA from Nelfilcon hydrogels with different proportions</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>of functional monomers</td>
<td></td>
</tr>
<tr>
<td>6.8</td>
<td>Cumulative release of HA from Nelfilcon hydrogels with the same % by mass</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>of DEAEMA</td>
<td></td>
</tr>
<tr>
<td>6.9</td>
<td>24 hour release of HA from Nelfilcon gels versus proportion of DEAEMA</td>
<td>74</td>
</tr>
</tbody>
</table>
# List of Tables

3.1 Major diseases of the eye ................................................. 10

6.1 Diffusion and release order of HA from Nelfilcon hydrogels with varying functional monomer amounts, all in [1:1:2] ratio ......................... 66

6.2 Functional monomer content of hydrogels ............................... 69

6.3 Diffusion and release order of HA from Nelfilcon hydrogels with varying functional monomer proportions ................................. 76

6.4 Equilibrium swelling parameters ............................. 82

6.5 Tensile parameters .......................................................... 84

6.6 Mesh sizes of hydrogels ....................................................... 85

7.1 Varying ketotifen release rates from AA-AM-NVP lenses under infinite sink conditions .................................................. 95

7.2 Summary of ketotifen diffusion coefficients, orders of release and swelling data 100

A.1 Diffusion and release order of Nelfilcon hydrogel with 6.5 and 40 mg HA/g Nelfilcon ...................................................... 120
Chapter 1
Introduction

Dry eye syndrome affects nearly 50 million people in the United States to varying degrees. The disorder occurs when a patient’s eyes are not adequately hydrated by the tears they produce, exposing the epithelia of their cornea and conjunctiva to desiccation. While ocular dryness is not immediately threatening to vision, the desiccation of the epithelia triggers a number of uncomfortable symptoms such as itchiness of the eye, a sensation of grittiness, light sensitivity, excessive watering, blurred vision and inflammation, all of which can significantly affect the quality of a patient’s life.

The customary treatment for dry eyes is the application of artificial tears and comfort agents that hydrate the epithelia. Many artificial tears and comfort agents increase the viscosity of the tears in the eye, preventing their drainage and evaporation, and increasing the moisture of the ocular surface. Artificial tears are commercially available in the form of eye drops that need to be applied every 2 to 4 hours for relief. Unfortunately, eye drops have very low bioavailability in the eye because of the natural turnover of tears. Tear turnover is a barrier to drug delivery, not just for dry eye but for all therapeutics delivered to the surface of the eye, and 95% of the volume of eye drops can get flushed from the eye without reaching their target tissues.

We need to develop a drug delivery technique that can deliver comfort molecules to the eye at a slow constant rate equivalent to the rate at which the eye needs them. Therapeutic contact lenses are devices that behave optically like conventional contact lenses but deliver needed medication at an appropriate rate. They contain drug that is released to the eye
slowly so that the fraction of drug reaching its target is increased. Therapeutic contact lenses also eliminate the need to apply eye drops multiple times a day, as a single lens delivers medication over an extended time period, from several hours to days.

We have developed therapeutic contact lenses that deliver a comfort agent, hyaluronic acid (HA), to the eye at a rate comparable to the dosage regimen of HA eye drops. To control the release rate of the HA from the lens, we turn to a process called biomimetic imprinting that enhances the affinity of the HA for the hydrogel by introducing “memory sites” within the hydrogel that behave similarly to binding sites in HA-binding protein, a protein found naturally in the body. The increased affinity of the HA for the hydrogel slow down its diffusion from the lens, leading to a more linear release rate during the first 24 hours of contact lens wear.

While biomimetic imprinting has previously been used to design contact lenses for delivering ocular allergy medication, the delivered drugs were all relatively small molecules, under 1000 Daltons in size. In contrast HA is a long chain molecule, a polysaccharide with a size of around 1 million Daltons. The application of the biomimetic imprinting process to HA thus introduces new challenges. A small molecule diffusing through the hydrogel material of a contact lens passes through the material with relatively little hindrance. A longer molecule is restrained at multiple points, as the tail end of the chain navigates through a path that the head end of the chain has already passed through. The increased hindrance lowers the HA’s rate of transport through the lens hydrogel material. In this study we explore the extent to which biomimetic imprinting can influence the diffusion of a molecule that is so constrained.

Evaluation of the release characteristics of drug delivery devices, such as therapeutic contact lenses, is usually conducted in vitro before progressing to in vivo studies. Often
the device is seen to behave differently in each study. Conventional *in vitro* evaluation of ocular drug delivery devices immerses the device in an artificial lacrimal fluid environment, with the fluid regularly replaced so that the drug concentration in the fluid outside the device is negligible. In such conditions, the driving force of the concentration flux is the concentration of drug inside the device and the nearly zero drug concentration in the bulk fluid. Within the eye, the volumes of tear fluid are very small and despite the tear turnover rate, there is always a non-negligible concentration of drug in the fluid surrounding the device.

In order to model the flow conditions in the eye and develop an improved technique for evaluation of drug delivery devices in the eye, we developed a microfluidic device that can contain a drug delivery device such as a therapeutic contact lens in a chamber, and flow artificial lacrimal fluid through the device at a rate equal to the flow rate of tears in the eye. Evaluation of a device in an environment that mimics the ocular flow conditions can provide a better understanding of how it will release drug in the eye, and enable researchers to optimize their design prior to conducting *in vivo* studies.
Chapter 2
Objective

The objectives of this research were as follows: (1) to develop a therapeutic hydrogel contact lens for the controlled release of a long-chain drug molecule, specifically hyaluronic acid, to the eye for the amelioration of dry eye related ocular discomfort induced by contact lens wear, and (2) to create a microfluidic device for the in vitro characterization of therapeutic contact lenses under physiological flow conditions.

The specific aims included: (1) the analysis of biological binding proteins in literature to identify amino acids, and functionally similar acrylate monomers, with a potential for affinity with the polysaccharide drug hyaluronic acid (aka HA or hyaluronan or hyaluronate); (2) the design and synthesis of a polymer hydrogel material with incorporated moieties capable of chemical interactions with HA at multiple points; (3) the control of the in vitro release characteristics of HA, such as the diffusion coefficient and cumulative released mass, by varying the number and diversity of functional monomers in the hydrogel; (4) the analysis of the structure of the hydrogel material; (5) the development of a microfluidic device used to release drug from a hydrogel lens in vitro under physiological conditions, and (6) the analysis of release of the drug ketotifen fumarate from a therapeutic lens placed in the microfluidic device.

We hypothesized that increasing the diversity of functional monomers and increasing the chemical similarity of the memory sites with the biological binding site on HA-binding protein (HABP) would enhance the binding between the HA and the hydrogel and decrease the diffusion coefficient. Additionally, increasing the concentration of functional
monomers would increase the number of points of interaction between the hydrogel and the HA molecules, and consequently slow the diffusion of the HA from the hydrogel.

The studies undertaken in this project exemplify how biologically-inspired chemical modification of a hydrogel can tailor the release rate of long-chain molecules in a lacrimal environment. We have developed a polymeric hydrogel material synthesized with HA that can release the drug in the presence of artificial lacrimal solution at physiological temperature. The addition of biomimetically selected functional monomers has allowed us to tailor the delivery of HA to the eye at a constant rate in therapeutic amounts over the course of 24 hours. These modifications do not change the mesh size or mechanical properties of the hydrogel significantly. The HA in the contact lens remains stable and delivers the drug at desired levels after heat sterilization treatment.

In a parallel project, we hypothesized that placing a therapeutic hydrogel lens in an \textit{in vitro} ocular flow environment would decrease the demonstrated rate of drug release. The conventional protocol for drug release measurement immerses the delivery device in an environment with well mixed conditions and significant volumes, where the drug concentration is negligible. Ocular conditions in contrast have small volumes of slow-flowing fluid. These may accumulate the released drug. The increased drug concentration in the fluid surrounding the lens would lower the driving force of the drug transport. The release data collected from such a device can provide us with a better understanding of how therapeutic lenses will behave in the eye.

The studies for this project demonstrate that conducting release studies on ocular drug delivery devices in ocular flow conditions extends their duration of release. Additionally, the release rate is shown to be zero-order, or concentration independent. In contrast, conventional release studies had indicated a Fickian release profile.
Future developments related to the first project will prepare the drug delivering contact lens for commercial manufacture and distribution, and ultimately provide relief to the 5 million people who seek treatment for dry eye syndrome,\(^9\) and the millions more who suffer from ocular discomfort. Developments in the second project will involve the synthesis of the microfluidic device using materials such as silica glass to ensure a more robust design for future studies on the effects of flow. Further refinements in the design can yield a system for superior assessment of drug release from ocular drug delivery devices.
Recent advances in genetics, neuroscience, and molecular biology are leading to unprecedented discovery of mechanisms underlying ocular disease and new therapeutics for treatments that increase quality of life. Equally important is the optimal delivery of therapeutics, which has been the subject of intense research and development that is continually pushing the boundaries currently delineated by traditional topical formulations. Topical formulations such as solutions and suspensions in the form of eye drops have been in use for centuries and are still the most common treatment approach used today.

Effective drug administration rests on getting a sufficient amount or concentration of drug to the site of action within a given time period. For the eye, the majority of drugs are administered topically and the rest are administered in a systemic manner. For anterior or front of the eye therapy, the majority of treatments require non-invasive, topically applied drugs. For posterior or back of the eye therapy, drugs are typically administered via systemic routes and also by intravitreal injection. All of these treatments have their own limitations that primarily involve the body’s natural mechanisms and barriers that impede the transport of molecules. However, it should be distinctly understood that quality of vision, being crucial to our evolutionary survival, translates to the eye doing an excellent job preventing foreign materials from crossing its barriers. Therefore any drug delivery mechanism we use needs to effectively deliver medication without permanently weakening these protective barriers.
The rates at which drugs pass through or interact with the different ocular barriers is of significant interest to the field of ocular drug administration. Systemic delivery of drugs to the eye is impeded by the blood-ocular barriers, which prevent transport from the blood to the eye interior. These barriers along with liver metabolism significantly limit the bioavailability of orally or intravenously administered drug. Drugs delivered topically to the ocular surface also face reduced drug transport, which is influenced by lacrimation and tear turnover, nasolacrimal drainage, spillage from the eye, metabolic degradation, and non-productive adsorption/absorption. These protective mechanisms lead to poor drug absorption on the surface of the eye despite it being a very accessible organ to treat topically. As a result, ocular bioavailability of drugs applied topically to the eye is typically very poor with less than 1-7% of the applied drug being absorbed, and the rest entering the systemic circulation.

From a clinical perspective, the challenge is to provide medication conveniently, non-invasively, and in therapeutically significant concentrations for long times with minimal transfer of drug to the systemic circulation - providing topical, targeted therapy to the eye. This can be best achieved by (i) extending the residence time or duration of drugs on the surface of eye and/or by (ii) increasing drug transport through ocular barriers such as the cornea, sclera, and conjunctiva. The concentration of drug reaching the desired site of action can be significantly improved by altering the kinetics of drug administration, removal, and/or absorption.
3.1 Ocular Diseases and Impact

The US prescription ophthalmic drug market is valued at approximately $4.5 billion and growing at a 7% average annual growth rate.\textsuperscript{35} This is due to a number of factors such as an increase in the overall aging population and subsequent eye issues encountered, an increase in the incidence of disease and needed disease prevention due to an increase in surgical procedures and contact lens use, and an increase in the number of medicines prescribed since optometrists in most states can now directly prescribe most medications.\textsuperscript{94} Highly debilitating diseases such as cataracts, retinal degenerative maladies such as macular degeneration and retinitis pigmentosa, diabetic retinopathy, glaucoma, and uveitis affect a large number of the population and have significant economic impact.\textsuperscript{44} While the aforementioned diseases can lead to partial and complete blindness, other diseases such as dry eye, bacterial conjunctivitis, ocular allergy, and ocular inflammation typically do not lead to complete loss of vision, but they significantly affect quality of life for a larger number of people and also have a considerable economic impact. Posterior drug candidates with a smaller target market have primarily been the work of specialty pharmaceutical companies with subsequent licensing, co-development, and manufacturing from large pharmaceutical companies. In certain respects, many reports highlight that this has led to a lack of ocular drug therapies especially for posterior eye disease.\textsuperscript{101} Table 3.1 outlines the major diseases of the eye, the number of US population affected, as well as the treatment location within the eye.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Ocular Location</th>
<th>US Population Affected or 2007 Ocular Market</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractive Error</td>
<td>Anterior</td>
<td>75 Million (25% of general population)</td>
</tr>
<tr>
<td>Cataracts</td>
<td>Anterior</td>
<td>20.5 Million (54% of people over age 65)</td>
</tr>
<tr>
<td>AMD</td>
<td>Posterior</td>
<td>1.7 Million people over age 50</td>
</tr>
<tr>
<td>Retinal Degeneration</td>
<td>Posterior</td>
<td>5.3 Million (2.5% of people age 18 and older)</td>
</tr>
<tr>
<td>Diabetic Macular Edema</td>
<td>Posterior</td>
<td>500,000</td>
</tr>
<tr>
<td>Diabetic Retinopathy</td>
<td>Posterior</td>
<td>4.1 Million</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>Anterior</td>
<td>2.2 Million (2% of people age 40 and older)</td>
</tr>
<tr>
<td>Uveitis</td>
<td>Anterior/ Posterior</td>
<td>346,000</td>
</tr>
<tr>
<td>Dry Eye</td>
<td>Anterior</td>
<td>50 Million (15% of general population)</td>
</tr>
<tr>
<td>Allergy</td>
<td>Anterior</td>
<td>$740 Million</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Anterior</td>
<td>$630 Million</td>
</tr>
</tbody>
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Table 3.1: Major diseases of the eye

3.2 Barriers to Ocular Drug Delivery

The eye is pharmacokinetically isolated from the rest of the anatomy and the site of drug action ultimately determines the strategy for successful topical delivery. Tear drainage and to some extent the absorption through the eyelids lead to less drug on the surface of the eye available to transport through ocular barriers such as the cornea, conjunctiva, and sclera. The cornea is a transparent, dome-shaped structure covering the front of the eye. It is contiguous with the conjunctiva, a delicate mucous membrane with a highly vascularized stroma that covers the sclera (the tough, opaque, white of the eye) and lines the inner surface of the eyelids. Figure 3.1 presents the anatomy of the ocular epithelia.

The human eye surface holds a tear volume that ranges from 7.0-30.0 $\mu$L with a tear turnover rate of 0.5-2.2 $\mu$L /min. This translates to a therapeutically relevant drug residence time of under 5 minutes with complete exchange of tear volume in approximately...
Figure 3.1: Anatomy of eye surface

[A] is a lipophilic drug which cannot easily penetrate the tear film and is washed away.
[B] is a lipophilic drug in the central cavity of a cyclodextrin molecule. The cyclodextrin solubilizes in the tear film and reaches the ocular epithelium. The lipophilic drug partitions out of the cyclodextrin and into lipid membrane of the epithelium.
[C] is a hydrophilic drug that solubilizes in the tear film and reaches the epithelium. It cannot cross the epithelium transcellularly (because of lipid membrane) or paracellularly (because of tight junctions), and eventually washes away from the eye surface.
[D] is a hydrophilic pro-drug which penetrates the epithelium transcellularly with the aid of a membrane transporter. Once in the ocular tissue, it is converted into the drug by enzymes.

The corneal and conjunctival epithelia are contiguous and contain several layers of cells (not shown), the outermost layer features microvilli that interact with tear film mucins. Drugs that penetrate the epithelia can easily move between ocular tissues such as the corneal and conjunctival stroma, the sclera beneath the conjunctiva, the vascularized choroid, and the leaky endothelium. From there they can diffuse into the anterior chamber or laterally through the sclera to the eye posterior.
14 minutes assuming normal lacrimation and blinking rates since blinking aids in contaminant removal and promotes a well-mixed tear fluid. If the topical medication or the mechanical forces of the instilled drop irritate the eye, lacrimal secretion will increase and further dilute the dosage. The ocular tear system and the tear film play a crucial role in maintaining an optically clear surface in the front of the eye. The bulk of the tear fluid is a 6-7 \( \mu \)m thick aqueous layer with dissolved oxygen, nutrients and proteins. The interface between this layer and the air comprises a 0.1 \( \mu \)m thick layer of lipids that limits evaporative loss of the aqueous film. Between the aqueous layer and the ocular epithelia (which are hydrophobic) exists a layer of hydrophilic mucins that maintain the integrity of the surface by trapping and removing foreign matter, and lubricate against the shearing force applied by blinking. The movement of fluid in the eye depends on the flow of the aqueous phase, which is secreted by the lacrimal glands above the eye, spread over the eye surface through surface tension and blinking, and drains out of the eye through the lacrimal puncta with the aid of a pumping mechanism. Up to 95% of topically applied drug can get washed away from the eye surface within minutes.

The rate determining barriers for transport through the cornea to the aqueous humor are the corneal epithelium, the stroma, and the endothelium. When a drug reaches the corneal or conjunctival epithelium, it needs to find a path through the layers of cells. For a drug to take a transcellular path (i.e., through the cells), it needs to enter the cell either by facilitated transport or by diffusion through the lipid bilayer. The former requires particular chemical interactions with transporters native to the cells while the latter requires lipophilicity and depends on the drug solubility, degree of ionization, and size, and on the cell membrane thickness. Both depend on the drug concentration gradient, and the effective area. Lipophilic drugs can transport quickly through the transcellular pathway.
but hydrophilic drugs, especially larger than 20,000 Daltons, have difficulty. The para-
cellular path (i.e., around the cells) is impeded by the presence of tight junctions. Stromal
transport is approximately equivalent for all ocular drugs and relatively independent of
drug partitioning, and the endothelium is only one cell layer in thickness with transport
depending on partitioning behavior as in the epithelium. We refer the reader to an excellent
review that compiles ocular tissue permeability measurements.

Hydrophilic drugs have been demonstrated to transport through the outer layers of
the conjunctiva more quickly than through the corneal epithelium. After conjunctival
absorption, transport may include lateral diffusion into the corneal stroma and, to a limited
extent, arterial vessel uptake. Drug may also be secreted back to the surface via efflux
proteins in the epithelia.

After passing through the ocular barriers, the drug reaches the anterior chamber be-
tween the cornea and the lens. Typically 3% of the instilled drug reaches this point. The aqueous humor is a clear filtrate of blood that is produced by the ciliary body, circu-
lates through the anterior chamber at 1% per minute, and drains out via the trebecular
meshwork. It delivers nutrients and anti-oxidants to the cornea and lens without interfering
with visual clarity. The aqueous humor poses an additional impediment to topical drugs
targeting the posterior of the eye. Any drug that diffuses through the cornea will be at
risk of dilution and flushing away via the aqueous humor. By this point, drugs delivered
via the corneal route can get diluted to the point of inefficacy even before moving into the
posterior segment. Figure 3.2 demonstrates the movement of aqueous humor through the
anterior segment.

Drug that reaches the sclera has another pathway at its disposal. It may diffuse
laterally through the highly permeable sclera and reach the posterior segment of the eye.
Figure 3.2: Anatomy of anterior chamber

Hydrophilic and lipophilic drugs both pass from the permeable stroma and sclera into the anterior segment the choroid, and the posterior segment. They also penetrate to the ciliary body, transfer to the secreted aqueous humor and circulate around the anterior and posterior chamber before draining away through the trebecular meshwork.
The tissues here support the retina and encase the vitreous humor, a highly viscous fluid. Inside the sclera is a layer of vascularized tissue called the choroid and inside that is the retina, the tissue on which light falls to produce images. The retina consists of several layers of tissue which, relating to their importance to drug delivery, can be classified as neural tissue and the retinal pigmented epithelium (RPE). The choroid nourishes the outermost layers of the retina, including the outer one third of the neural tissue and the RPE. Bruch’s membrane is the innermost layer of the choroid. It also provides the basement membrane of the RPE. The RPE is a significant barrier to the transport of drug from the sclera (or systemically delivered drugs from the choroid) into the neural tissue and the vitreous humor. Another barrier is the endothelial cells of the retinal capillaries that are located among the retinal neural tissue. They prevent drugs from the circulatory system reaching the neural retina. The RPE and endothelial cells also bear efflux proteins that actively remove drugs from the retina. Together the RPE and the retinal endothelial cells form the blood-retinal barrier.\textsuperscript{88,134} Figure 3.3 shows the anatomy.

The common alternatives to reach the posterior segment involve injecting the drug or inserting a drug delivery device into the vitreal cavity of the eye, or using a periocular route of delivery - applying the drug, carrier, or device within the eye surface barriers and relying on transscleral transport. Non-invasive methods are generally preferred because of the relative lack of patient discomfort and surgical complications such as endophthalmitis, hemorrhage, retinal detachment, and cataracts.\textsuperscript{13}

In addition to the ocular barriers, ocular tissues contain metabolic enzymes to break down xenobiotics that manage to penetrate into the tissue. Thus any drugs that reach the interior of the eye are further depleted by the action of enzymes such as esterases, aldehyde and ketone reductases, and many others.\textsuperscript{33}
Figure 3.3: Anatomy of the retina

Topically delivered drugs diffuse through the sclera and systemically delivered drugs diffuse from the choroid vasculature in the posterior segment of the eye. The outermost layer of the retina is called the retinal pigment epithelium, a layer of tight-junctioned cells that prevent drugs from penetrating into the retina. Small lipophilic drugs penetrate the lipid membrane easily but large and hydrophilic drugs require assistance either from permeation enhancers or from transporters. When the drug reaches the neural retina, it acts upon the target cells. The retinal vasculature is lined with endothelial cells bound by tight-junctions to prevent blood borne drugs and pathogens from reaching the neural retina. Together, the retinal vascular endothelium and the RPE form the blood-retinal barrier.
3.3 Strategies to Overcome Drug Removal at the Ocular Surface

The most common method for delivering drugs to the eye is through eye drop solutions administered to the eye surface. They are relatively simple to apply, are non-invasive, and most solutions are easy to prepare with low manufacturing costs. There are over 100 topical eye drop formulations on the market today.

Patient compliance remains one of the biggest drawbacks of topical drop administration with evidence suggesting a large percentage of patients with significant periods of ineffective drug concentration levels. The volume of instilled dose is also highly variable from application to application which depends on the squeeze or pressure force, the angle of administration, and the ability to resist blinking. These issues compound quick drug loss along with tear flow rate which washes the instilled dose from the eye within ten minutes. Also, the tear drainage rate has been shown to linearly increase with instilled volume.

Figure 3.4 highlights these effects on the concentration profile of topically instilled drug in the eye.

Eye drop formulations typically contain preservatives to prevent pathogenic contamination, guarantee sterility, and, in some cases, stabilize the drug. Most multiple-use drops last for approximately one month and the longer the duration of use, the higher the probability for contamination. Preservatives can be toxic to ocular tissue and providers try to optimize the contamination protection/toxicity ratio. In certain cases, preservatives have been shown to have ancillary benefits with antibiotic medications as well as in other formulations acting as permeation enhancers. In preservative-free, single-use containers, the risk for contamination is great and good manufacturing practices must be assured. Typically, preservative-free formulations are single dose containers suited for patients with
The concentration of drug delivered by eye drop to the tear film varies with time. When a drop is placed in the eye, the concentration spikes to its maximum level. The concentration eventually decreases as lacrimation, drainage and (to a lesser degree) absorption deplete the drug. The concentration drops below the level considered therapeutic before the next dose is applied. [A] If a dose is missed, the eye tissues spend excessive time without therapeutic levels of drug. [B] The maximum drug concentration in the eye from one application to another varies because of factors such as squeeze force on dropper and angle of dropper position. Patients may accidentally over-admister the drug as well. These variations in application can push the tear concentration to toxic levels. [C] An excessive volume of drug solution can increase the spillage and drainage from the eye (it is well documented that the drainage rate increases linearly with instilled volume).
allergies or those with significant surgical concerns where preservative toxicity may interfere with healing.

The physiochemical properties of drug such as hydrophilicity/ lipophilicity, degree of ionization, shape, and size affect its ability to transport through ocular barriers. Typically, lipophilic drug properties increase the speed of the molecule through cell membranes, an increased degree of ionization of the drug decreases lipid solubility and subsequent membrane transport, and decreased drug radius or particle size increases transport.

While hydrophilic drugs are formulated in solutions, lipophilic drugs are formulated in suspensions, which typically require resuspension prior to use. Suspensions have a much lower market share compared to solutions and face additional hurdles such as drug precipitation and resuspension, as well as particle size and polydispersity issues, which can limit the amount of drug applied to the eye or the transport through ocular barriers.

In recent years, smaller-sized particles within topical formulations have been studied for their ability to increase transport. These systems will be presented in this section since they have also been hypothesized to increase residence time. Micro or nanoemulsions\textsuperscript{97, 149} are highly stable systems containing hydrophobic organic phases, often in droplet form, dispersed within an aqueous continuous phase with amphiphilic interfacial films. The dispersed phase contains lipophilic drug and the aqueous phase enables the microemulsion to effectively mix with tear fluid. A lipophilic formulation by contrast would wash out of the eye rapidly without reaching the epithelial tissue. Additionally, it is theorized that the lipophilic droplets adhere to the epithelium and increase their residence time.\textsuperscript{39, 135} Particle sizes should be under 10 micrometers in diameter for maximum comfort.\textsuperscript{148} Also, it has been reported that submicron emulsions decrease the susceptibility of drug to degradation.
Liposomes are microscopic vesicles made of concentric phospholipids bilayers with alternating lipophilic and aqueous compartments. Based on their structure, they can be categorized as small unilamellar vesicles, large unilamellar vesicles and large multilamellar vesicles. The cavities within the liposomes, lined by the polar “heads” of the phospholipids, can carry hydrophilic drugs. Lipophilic drugs can be solubilized within the bilayer among the hydrophobic “tails”. The hydrophilic outer surface allows effective dispersion in the tear film. Liposomes also protect the drug from enzymatic degradation, and may have an increased residence time by binding to the epithelium. 34, 87

Nanosuspensions or colloidal suspensions are sub-micron colloidal dispersions of pure drug particles stabilized by surfactants, and have been used in order to increase the solubility of poorly soluble drugs and increase dissolution rates via increased surface area. 107 Recent work highlights nanosuspensions of glucocorticoid drugs in comparison to solutions and micro-crystalline suspensions. In a rabbit model, nanosuspensions exhibited higher intensity of glucocorticoid action and higher extent of absorption with the viscosity of the nanosuspension playing an important role in increasing duration of action. 75

Nano- and microspheres are sub-micron and micron-sized solid particles containing drug dispersed within a polymer. The spheres are suspended in aqueous solution to form eye drops. In one study biodegradable poly(lactide-co-glycolide) (PLGA) microsphere carriers for vancomycin were dispersed in the topical formulation carriers. In vivo results in rabbits measuring the aqueous humor concentration indicated a 2 fold increase in bioavailability over eye drops. Interesting enough, increasing the viscosity of the formulation by adding hydroxypropyl methylcellulose did not increase bioavailability. 46 PLGA microspheres have also been used as carriers for gene delivery, for in vitro studies with human RPE cells and for in vivo studies with rats. In the latter, gene expression was observes in the RPE within
4 to 7 days. Ganciclovir was loaded into albumin protein nanoparticles for intravitreal injection, and no auto-immune response was noted.

To overcome low drug bioavailability, topical formulations have remained marginally effective to a large extent by the administration of small volumes of very high concentrations of drug multiple times on a daily basis. Thus many formulations attempt to deliver more drug and increase the driving force of the flux by delivering highly concentrated drug. This produces only a minor improvement, and can lead to toxic side effects if improperly managed. Various improved methods have focused on increasing the residence time the drug spends on the surface of the eye before it is washed away by normal protective mechanisms.

Viscosity enhancers such as methylcellulose, carboxymethylcellulose, hydroxypropylmethylcellulose, hydroxymethylcellulose, polyvinylalcohol, and polyvinylpyrrolidone have been added to topical formulations to retain the drug on the eye surface for longer periods of time by increasing the viscosity of the tear fluid and decreasing the tear drainage rate. These types of formulations typically are rated more comfortable compared to less viscous or saline based solutions and act as wetting agents lowering surface tension and increasing tear break-up time. Polysaccharides such as chitosan have also been that are mucoadhesive with the negatively charged mucin layer, increasing corneal residence time three fold. A considerable increase in viscosity produces ointments which invoke the smallest rate of drug loss, but significantly interfere with vision, are difficult to apply, and can be quite non-cosmetic. Thus, ointments are used to a much smaller extent than solutions and are typically used at night.

Mucoadhesive polymers interact with the mucin layer of the tear film and adhere to the ocular surface. Hyaluronan and other polymers have been used in this context but their weak interactions prevent true mucoadhesive behavior. A novel set of polymers known as
thiomers are synthesized by modifying polymers with thiol moieties.\textsuperscript{16} Through di-sulfide linkages with the native mucins of the epithelium, they become covalently anchored to the ocular surface. Mucoadhesive polymers can be applied directly to the eye as a vehicle for drug, or they can be used to attach inserts to the eye. A thiolated polyacrylate insert has been shown to deliver fluorescein for 8 hours.\textsuperscript{70} In situ gels and mucoadhesive polymers have both been designed to incorporate microspheres and liposomes for extended release. Timolol maleate encapsulated in chitosan was compared to timolol gel in rabbit eyes and demonstrated similar ability to lower the IOP at half the concentration of drug.\textsuperscript{2} We direct the reader to the following mucoadhesive reviews.\textsuperscript{55, 86}

In-situ gel forming systems are liquid for ease of application, but undergo phase transitions and acquire a gel-like consistency when they encounter the physiological environment of the eye. They are mixed with the desired drug and instilled into the cul-de-sac (i.e., the pocket underneath the lower eye lid) where they gel into a substance that withstands removal by tear circulation without interfering with vision. Currently they can deliver a fairly uniform dosage over the course of about 6 hours.\textsuperscript{84}

Gellation can be triggered by a change in pH (e.g., Carbopol\textsuperscript{®} with methylcellulose, \textsuperscript{126}) by the presence of mono or divalent ions (Gelrite\textsuperscript{®} - a gellan-gum polysaccharide\textsuperscript{125}) and by a change in temperature (e.g., Pluronic\textsuperscript{®} F127 with Pluronic\textsuperscript{®} F68,\textsuperscript{138} poly(N-isopropylacrylamide) and chitosan\textsuperscript{25}). Research has focused on combinations of the aforementioned triggering mechanisms to decrease liquid viscosities, to optimize the phase transition and gain better control over gellation times, to extend drug release, as well as decrease the proportion of polymer needed in a dosage\textsuperscript{83, 144}. We direct the readers to the following reviews.\textsuperscript{51, 113, 127}
Recently, soft hydrogel contact lenses have been demonstrated as extended drug delivery carriers for the eye. New methodologies, greater understanding of polymeric structural properties, as well as network formation have produced a number of developments that are considerably different than past efforts which involved taking a conventional lens and soaking it within a concentrated drug solution. Delivering medications via contact lenses has been a prevailing notion since the inception of using hydrophilic, crosslinked polymer gels on the surface of the eye. In fact, the first patent in the field from Otto Wichterle in 1965 states that “medicinally active substances such as antibiotics may be dissolved in the aqueous constituent of the hydrogels to provide medication over an extended period ...via diffusion.” The biggest obstacle to this rationale is maintaining a significant concentration of drug within the fluid to have a therapeutically relevant effect, which is ultimately limited by the solubility of the drug. This has been the primary reason why drug release from contact lenses has not become a clinical or commercial success. One promising technique is to create contact lenses with therapeutically relevant drug loading and extended release is to produce a macromolecular framework with memory for the drug during polymer synthesis. This technology has roots to a field termed molecular imprinting, which has primarily concentrated on highly crosslinked polymer matrices for separation and sensing.

For molecularly imprinted hydrogel contact lenses, it has been shown that the extension of release for weakly crosslinked systems has a strong dependence on the monomer to template (M/T) ratio as well as the diversity and number of interactions of the recognition site. Drug such as timolol, ketotifen fumarate, and antibiotics have demonstrated in-vitro extension of release using these methods. For example, biomimetic hydrogel contact lenses have been developed for the enhanced loading and extended release of the
anti-histamine, ketotifen fumarate,\textsuperscript{137} which exhibited an extended release profile for duration of 5 days with three distinct rates of release. Multiplicity of monomer-template interactions was achieved with four functional monomers chosen from an analysis of histamine ligand-binding pockets which led to significantly enhanced loading and duration of release compared to less functionalized systems at a constant M/T ratio. Considering these systems maintain the mechanical and optical properties of contact lenses, there is high potential for therapeutic contact lenses based on these types of technology to deliver a number of ocular therapeutics without the need for multiple eye drops. In-vivo validation of the most recent systems is currently under study, and the imprinting process is not as effective with lipophilic drugs due to solubility constraints during hydrophilic gel formation. We direct the reader to the following reviews on hydrogel imprinting.\textsuperscript{5, 23, 62}

Increasing the drug reservoir within contact lenses has also included nanoparticulate and liposomal laden lenses,\textsuperscript{57} and ion exchange hydrogels,\textsuperscript{132} with duration of drug release during in-vitro and in-vivo experiments shown to be less than 1 day. Nanoparticulate laden lenses have shown promise within in-vitro studies and demonstrate 55\% of drug released in 3 days. These techniques have concerns such as inadequate drug loading at therapeutically relevant concentrations for long release times; and for lens dispersed nanoparticles, decreased mechanical stability induced by grain boundaries, reduced optical clarity, and longer and more costly production schemes. Recently lenses have been demonstrated in the literature to deliver PVA chains as a moisturizing agent to counteract ocular discomfort.\textsuperscript{142}

Ocular inserts can also deliver drug to the eye while avoiding the need for repeated eye drops. Soluble inserts such as collagen shields have been used as corneal bandages and drug delivery carriers and are produced from porcine scleral tissue. Typically, they
are soaked in solutions of drug and dissolve in the eye at characteristic rates, but they have had poor control over release and poor comfort since they are not individually fit for patients. They also interfere with vision and cannot be inserted or removed by the patient, and have self-expelled from the eye in many cases. Collagen shields release drug for hours and modification of the collagen film has been shown to produce longer release rates up to several days.\textsuperscript{136}

Ocular inserts are placed in the eye, deliver drug until depleted, and (unless they are biodegradable) are removed at the end of the release period. Ocusert\textsuperscript{®} (Alza Corp., FDA approved in 1974) consists of a small wafer of drug reservoir enclosed by two diffusion controlling membranes, which is placed in the corner of the eye and provides extended release of an anti-glaucoma agent for approximately 7 days with an increased release rate in the first 7 hours.\textsuperscript{10,133} It must be removed at the end of the release period. Lacrisert\textsuperscript{®} (Merck), which is also placed in the lower eyelid, is a cellulose-based polymer insert used to treat dry eyes\textsuperscript{110} administered once-a-day and degradable. However, inserts have not found widespread use due to increased price over conventional treatments, occasional noticed or unnoticed expulsion from the eye,\textsuperscript{115} and potential for fragmentation and membrane rupture with a burst of drug being released.\textsuperscript{72} Gel forming inserts have also been produced from high molecular weight poly(ethylene oxide) (PEO) with drug release controlled by surface erosion. For the delivery of ofloxacin in rabbits, inserts were placed in the lower eyelid and demonstrated a 2 fold increase in drug residence time in the aqueous humor, a 3.8 fold increase in aqueous humor drug concentration, and approximately a 10 fold increase in bioavailability over Exocin\textsuperscript{®} eye drops. The increases were attributed to PEO-enhanced permeability and/or increased tear viscosity.\textsuperscript{31}
Bioadhesive ophthalmic drug inserts (BODI®) are homogeneous extruded mixtures of polymer and drug, shaped into rods 5 mm long a 2 mm in diameter and placed in the cul-de-sac. Animal tests have been conducted in canines,\textsuperscript{11} delivering the antibiotic gentamicin over 7 days. The bacteriological cure rate was similar to that from eye drops, with the added advantage of ease of use - one deposition of the insert as opposed to 21 instillations of eye drops. Another ocular insert under development is the OphthaCoil, a thin coiled stainless steel wire coated with a drug-containing hydrogel. The coiled structure is intended to provide shape and flexibility, the ends are capped to protect the eye from the wire edges, and the coil interior can be used as a drug reservoir. Release of ciprofloxacin has been measured \textit{in vitro} for over five hours. The release time can potentially be increased by modifying the hydrogel coating and the polymer in the drug reservoir.\textsuperscript{108}

The anatomy of the eye has also been altered to increase residence time. For example, a mechanical technique for increasing drug residence time in the eye is to block the lacrimal puncta with punctal plugs. The tears produced in the eye cannot drain, and accumulate in the eye, so any instilled drug is not washed away. This technique has demonstrated results when used with the drug timolol in glaucoma patients.\textsuperscript{13}

\subsection*{3.4 Strategies for Permeation Enhancement through Ocular Membranes}

Drugs that reach the ocular surface need to penetrate the ocular epithelium, but the epithelium presents barriers that few drugs can easily overcome. For hydrophilic drugs transcellular transport is difficult unless facilitated by a limited range of transporters present on the corneal and conjunctival epithelial cells. The intercellular spaces have tight junctions that resist paracellular transport.
Lipophilic drugs can diffuse through the cell membranes with relative ease. However, as mentioned earlier, they cannot pass through the tear film and reach the epithelium as easily as hydrophilic drugs can. We here have a dilemma - drugs that reach the epithelium with ease have trouble penetrating it and vice versa. Very few drugs have a high solubility in water as well as good partitioning in lipids. It would be useful if we could use a hydrophilic vehicle to bring the drug to the cell membrane and then have it diffuse through the membrane through a lipophilic vehicle.

Pairing an ionic drug with its counter-ion has been shown to improve ocular penetration. The cationic timolol, when paired with anionic sorbic acid, has a two-fold higher penetration into the aqueous humor than when delivered alone [80].

Cyclodextrins are ring-shaped oligosaccharides that can sequester lipophilic drugs within their central hydrophobic cavities. The hydrophilic shells solubilize in the tear film and carry the drug to the epithelium, where the latter partitions into the cell membrane and penetrates the epithelium. This strategy has been demonstrated successfully with a number of drugs including pilocarpine, which demonstrated a four-fold increased permeation in rabbit corneas after the addition of hydroxypropyl beta-cyclodextrin. Novel methylated cyclodextrins have lipophilic properties that let them diffuse through the cell membranes in addition to their action as solubilizers of lipophilic drugs in aqueous environments. Dexamethasone has been delivered to the eye posterior as a topical eye drop by complexation with randomly methylated cyclodextrins. Cyclodextrins also have anti-irritant properties.

Another option is to chemically modify the drug into a less therapeutic but more penetrable form, so that after it penetrates the cornea it can be converted into the therapeutic form by enzymes in the eye. The modified form is known as a prodrug. A water-soluble
prodrug of cyclosporine A is produced by esterification of the drug with a moiety containing a phosphate group. The prodrug has improved bioavailability and penetration, and conversion in the eye back to the drug is about 6% in 3 minutes.\textsuperscript{79}

Aside from modifying the drug, researchers have increased permeation by modifying the epithelial cells. The cell membranes can be made more porous by disrupting the lipid bilayers with surfactants such as polyoxyethylene 20 stearyl ether.\textsuperscript{114} Chelating agents like ethylenediamine tetraacetic acid (EDTA) sequester calcium ions and consequently loosen the tight junctions, opening up the paracellular pathway.\textsuperscript{53} Recently studies indicate a cytotoxic effect from many permeation enhancers and absorption promoters, but the use of fetal bovine serum can ameliorate this.\textsuperscript{27} An interesting fact is that absorption promoters have been shown to promote penetration of peptide drugs through the corneal epithelium more than through the conjunctival epithelium. This may allow control over the pathway and extent of drug penetration through the epithelia.\textsuperscript{118}

A third option is to transiently modify the structure of the epithelium so that its permeability increases just long enough to deliver the drug. Iontophoresis, which can be transcorneal or transscleral, delivers drug to the eye close to an electrode with potential equal to the charge of the drug.\textsuperscript{36,95} The circuit is completed by touching the grounded electrode to another part of the body. The resulting electric field forces the drug through the epithelium. Gentamicin has recently been delivered to rabbit eyes through a drug-loaded hydrogel probe.\textsuperscript{37,45}

Phonophoresis is a similar technique that uses ultrasound to transiently increase the porosity of the epithelial membranes. It has been used to enhance the permeability of the drug betaxolol 4.4 times through rabbit corneas \textit{in vitro}.\textsuperscript{146}
3.5 Strategies to Delivery Drugs to the Posterior of the Eye

Drugs delivered to the posterior of the eye can follow a number of routes. While topically applied drug may penetrate the conjunctiva and sclera, it generally gets diluted and eliminated to a sub-therapeutic dosage. The more common topical alternatives to reach the posterior segments involve either injection of a drug, a drug delivery carrier or a drug delivery device into the vitreal cavity of the eye; or by delivering periocularly: following a transscleral route to the back of the eye and allowing it to penetrate the RPE. Additionally, some drugs are delivered through systemic circulation with oral or intravenous sources. Most systemically delivered drugs reach the ocular posterior in minute amounts, and there is a risk of systemic toxicity.

Non-invasive methods are generally preferred because of the relative lack of patient discomfort and surgical complications such as endophthalmitis, hemorrhage, retinal detachment, and cataracts. The least invasive method would be delivery to the ocular surface with eye drops. While there is a tremendous challenge involved in overcoming all the ocular barriers from the tear film to the aqueous humor or RPE, progress is being made in delivering increasing amounts of drug to the posterior from surface delivered sources. A likely drug candidate would have high partitioning in both water and lipids. Methylated cyclodextrins solubilize in both phases and could potentially improve the penetration of any drug sequestered in their central cavities. Their action has been demonstrated for dexamethasone.

More often, intraocular delivery involves repeated injections of the drug directly into the vitreal cavity. The wet form of age-related macular degeneration (AMD) and diabetic macular edema (DME) are commonly treated through intravitreal injections of anti-VEGF
antibody fragments such as ranibizumab or pegaptanib.\textsuperscript{98,119} As the procedure is invasive, there may be side effects such as infection at the injection site, intraocular pressure (IOP) increase,\textsuperscript{43} cataract formation,\textsuperscript{129} or retinal detachment.\textsuperscript{100} In addition, the injections are needed as often as once a month. This not only causes discomfort and inconvenience to the patient, but also increases the chances of developing side effects.

Newer developments include sustained release implants that are inserted into the vitreal cavity, such as Vitrasert (ganciclovir) for CMV retinitis. While this technique is also surgically invasive, the implant many only need to be inserted once every few years. This dramatically reduces the risk of side effects and limits patient discomfort to one surgical procedure annually or less often. Other implants in the market or in late-stage clinical trials are Retisert (Bausch&Lomb) delivering fluocinolone acetonide to treat chronic non-infectious uveitis\textsuperscript{93} and Medidur (Alimera Sciences) delivering the same drug for diabetic macular edema.

While the challenges for delivery to the posterior of the eye are greater than for other parts of the eye, researchers are making progress. One significant area of research is the study of RPE membrane transporters to facilitate penetration through this barrier. The RPE, similarly to the conjunctiva, consists of cell layers bounded by tight junctions. The paracellular transport route is difficult to bypass, so for delivering non-lipophilic drugs transcellularly it can be advantageous to use native membrane transporters. RPE transporters exist for amino acids, peptides, monocarboxylic acids, nucleosides, folate and organic cations.\textsuperscript{88} Studies are currently being done in animal models.

Iontophoresis and sonophoresis are also used to penetrate the sclera near the back of the eye. Coulomb controlled iontophoresis (CCI) deliver specific dosages of drug more accurately. Research on probes has been done to improve efficacy and safety. A probe
coated with a hydrogel containing a gentamicin is being developed and has been tested on rabbits.\textsuperscript{37} In addition to drugs and proteins, iontophoresis can be used to delivery nucleic acids for gene therapy.\textsuperscript{95}

Subconjunctival injections can deliver drug into the sclera while bypassing the epithelial barriers. The drug can diffuse laterally through the sclera and reach the choroid and retina. Cisplatin has been delivered to rabbit retinas successfully by this mechanism. Better results were achieved when the drug was delivered within a collagen matrix rather than in a buffer solution.\textsuperscript{49}

A new technique involves delivering drug systemically, and using light energy to localize the drug in the target tissue. Known as light-targeted delivery (LTD), the procedure begins with liposome encapsulated drug injected intravenously. The encapsulation reduces systemic toxicity. As the liposomes circulate through the body, a light beam is directed into the pupil and directed at ocular tissue such as the choroid neovasculature (CNV) in cases of AMD. The light beam gently warms the RPE, the CNV and the choroid capillaries to 40°C, prompting the liposomes to melt and release the drug to the local area. We suggest a good review discussing the procedure and its applications.\textsuperscript{147}

Scleral plugs are devices that are surgically implanted into the sclera and deliver drug to the sclera for extended time periods. They have the advantage over injections of having a higher capacity, but the implantation procedure is more invasive. There have been successful animal studies involving this device, including the treatment of uveitis in rabbits with a plug that delivered tacrolimus.\textsuperscript{116}
Chapter 4
Hydrogels and Imprinting

Hydrogels are three-dimensional water-swollen networks of polymer chains with crosslinks that are generally covalent or ionic. The chemical nature of the polymer chains determines the unique properties of each type of hydrogel. A hydrogel can be cationic, anionic or neutral depending on the functional groups present on the polymer backbones. The soft material is used in many applications in which soft, flexible and hydrated structures are needed.¹⁰³

Hydrogels are commonly used in drug delivery devices because their high water content makes them biocompatible with tissues in the body. Additionally, they can be designed to be physiologically responsive and alter their properties in response to environmental changes. For instance, oral delivery devices are made of hydrogels that confine insulin while in the highly acidic stomach but release it in the alkaline intestine. The pH change triggers a transition in the mesh size of the hydrogel. Some physical crosslinks weaken in the deprotonated environment of the intestine, the mesh size increases, and the insulin diffuses out. Still other hydrogels are designed to respond to temperature, osmolarity, electromagnetism and other conditions.

The structural characteristics of hydrogels have been modeled extensively in the literature. We are able to conduct tests that reveal macroscopic structural properties of hydrogels, and deduce a number of microscopic properties. A few of the theories describing hydrogel structure are described below.
4.1 Diffusion through a hydrogel

Diffusion of particles is the net transport of particles from an area of high concentration to low concentration, driven by the concentration gradient in order to bring the system to thermodynamic equilibrium. It can be described by Fick’s laws of diffusion.

Diffusion is seen when a soluble drug is added to a solvent such as water. At the microscopic level, each individual drug molecule is undergoing Brownian motion. However because there are more molecules in high concentration areas, they are more likely to move toward low concentration areas than vice versa. The movement of a molecule in water is relatively unconstrained, and we can assume that the separation between drug molecules is great enough that they do not interact with one another. The drug can diffuse through water fairly rapidly, and diffusion coefficients of small molecules in water are in the $10^5 \text{ cm}^2/\text{sec}$ range.

When a molecule is placed within a hydrogel, its random motion is constrained by the presence of the polymer chains. If the length of polymer chain between crosslinks is long, and the spaces within the network large enough relative to the molecule, the latter can move through the solvent without interacting with the hydrogel. In such a case the diffusion coefficient would be similar to that in a free solvent. However as the chain length between crosslinks shrinks, the space between chains or the “mesh” becomes smaller. As the mesh size approaches the diameter of the molecule, the latter is more likely to be hindered sterically by the presence of the hydrogel. Because of this the diffusion coefficient of the molecule through the system decreases. The mesh size of a hydrogel is schematically shown in Figure 4.1.
If a small molecule is constrained in its transport through a hydrogel, a long-chain molecule encounters even more constraint. The long chain can be entangled within the hydrogel and the movement of the front end through the network requires that the rest of the chain be free to move. As longer chains have more length to be entangled with the surrounding hydrogel network, they have lower diffusion coefficients. The pathway that the long-chain molecule follows through the hydrogel can be modeled as a tube whose boundaries are defined by the entangled polymers surrounding the long-chain molecule. It can also be viewed as the sequence of pores that each section of the chain must pass through.

One model that is effective in describing the movement of long-chain molecules through a hydrogel network is the reptation model. Reptation is the movement by which snakes, worms and other legless organism undergo locomotion. In the context of molecular transport, it describes how long-chain molecules can slowly pass through an entangled environment one small segment at a time. A relaxed long-chain molecule has a shorter end-to-end distance, and tube length, than the contour of the chain. If the tube diameter is wide enough, small sections of the chain can undergo movement normal to the tube length. Such “loops” can travel laterally along the length of the chain and, upon reaching the chain “head”, translate it forward by a small distance, as shown in Figure 4.2. If the mesh is larger, the size of the perpendicular loop can be larger and the head can translate by a greater amount leading to faster diffusion. If there is affinity between the chain and the hydrogel because of chemical interactions, the free energy needed for dissociation leads to slower traveling loops and decreased diffusion. Increasing the number of affinity points between the two can decrease diffusion.
Figure 4.1: Illustration of mesh size within a hydrogel

The space within a hydrogel network between crosslinked polymer chains is known as the mesh size (ξ). Molecules significantly smaller than ξ diffuse easily through the hydrogel. Molecules with size on the same order as ξ may be slowed by the steric hindrance they encounter from the polymer chains. Molecules significantly larger than ξ cannot penetrate through the hydrogel.
A long-chain molecule within a hydrogel or other entangled polymer can be said to be confined within a “tube” whose boundaries are defined by the surrounding polymer which restricts the movement of the long-chain molecule. When the long-chain molecule is in a relaxed state, its contour does not exactly match the tube. Rather, it has loops that can slide or “reptate” along the length of the tube. As the loop reaches the “head” of the long-chain molecule, the latter moves forward by a small length. This process enables the transport of the long-chain molecule through the hydrogel.
4.2 Theoretical model for diffusion

We can mathematically model the transport of a particle through a hydrogel. We consider the case in which the hydrogel is shaped like a slab. The aspect ratio of the exposed surface diameter to the thickness is greater than 10 so we can assume diffusion is occurring in one dimension. The hydrogel is immersed in an aqueous environment when the concentration of the diffusing particle is negligible in the bulk fluid outside the hydrogel. By application of Fick’s Second Law, and assuming the given initial and boundary conditions,

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial t^2} \quad (4.1)
\]

\[
C(x, t) = C_0 \text{ when } t = 0 \quad (4.2)
\]

\[
\frac{\partial C}{\partial t} = 0 \text{ while } t > 0 \text{ and } x = 0 \quad (4.3)
\]

\[
C = C_s \text{ while } t \geq 0 \text{ and } x = \pm L/2 \quad (4.4)
\]
We describe a system in which a planer hydrogel undergoes one-dimensional diffusion over time in an environment in which the drug concentration is always effectively zero, where \( C_0 \) represents the initial drug concentration (assumed to be uniform) in the homogeneous gel, \( x \) represents the distance from the central length-wise axis of the hydrogel to the surface, \( C \) is the concentration of the drug within the gel at any given position and time, \( C_s \) is the concentration at the surface of the gel, \( D \) represents the constant diffusion coefficient which is independent of position and concentration, \( t \) is time, and \( L \) is the thickness of the gel. At \( x = 0 \), the flux of the particle is effectively zero.

The solution of the PDE is given by,

\[
\frac{C - C_0}{C_b - C_0} = 1 - \frac{4}{\pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{2n+1} e^{-\frac{(2n+1)^2\pi^2D}{4L^2}} t \cos \frac{(2n+1)\pi x}{2L} \tag{4.5}
\]

An effective way of comparing the release kinetics from different gels is to compare the fractional release of the drug at time \( t \) relative to the total drug released at infinite time, or \( M_t/M_\infty \). \( M_t \) is the total cumulative mass of therapeutic released at time \( t \), and \( M_\infty \) is the total cumulative mass of therapeutic released at infinite time.

\[
\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} e^{-\frac{(2n+1)^2\pi^2D}{4L^2} t} \tag{4.6}
\]

The above expression can be expressed in terms of error functions.
\[
\frac{M_t}{M_\infty} = 4 \left[ \frac{D}{L^2} \right]^{\frac{1}{2}} \left[ \frac{1}{\pi^2} + 2 \sum_{n=1}^{\infty} (-1)^n erfc \left( \frac{nL}{2\sqrt{Dt}} \right) \right]
\] (4.7)

At short times ( \( M_t / M_\infty < 0.65 \)) the expression can be simplified to

\[
\frac{M_t}{M_\infty} = 4 \left[ \frac{D}{\pi L^2} \right]^{\frac{1}{2}}
\] (4.8)

By plotting the fractional release of HA versus \((t^{0.5}/L)\), we can calculate the diffusion coefficient from the slope.

We can also measure how well the data matched a Fickian release profile by the empirical Power Law equation:

\[
\frac{M_t}{M_\infty} = kt^n
\] (4.9)

By plotting the log of fractional release versus the log of time and calculating the slope, we can determine the order of release of the particle from the hydrogel. The order of release and the slope of the plot, \(n\), are related by \(\text{order} = |n - 1|\). For Fickian release, the order is 0.5. For time-independent release, the order is 0.
### 4.3 Equilibrium swelling theory

Hydrogels in water are subject to a number of thermodynamic influences. As the polymers solvate in water they tend to elongate, but the chemical bonds making up the chain resist the elongation. The free energy of mixing and the opposing free energy of elasticity both contribute to the total change in free energy.

\[
\Delta G = \Delta G_{\text{mix}} + \Delta G_{\text{el}}
\]  

(4.10)

Based upon this relationship, Peppas and Merrill\textsuperscript{104} have developed a model describing the relationship between the average molecular weight between crosslinks ($M_c$) and the polymer volume fraction in the swollen state ($v_{2,s}$) in an swollen network of crosslinked polymers synthesized in the presence of a solvent. The model allows us to calculate the $M_c$, a microscopic parameter, from experimentally determined values such as the $v_{2,s}$, polymer volume fraction in the relaxed state ($v_{2,r}$) and specific density of the polymer ($\bar{\rho}$), and known quantities such as $V_1$ (molar volume of water), molecular weight of uncrosslinked polymer chains ($M_n$) and the Flory polymer-solvent interaction parameter ($\chi_1$).

\[
\frac{1}{M_c} = \frac{2}{M_n} - \frac{\bar{\rho}/V_1}{v_{2,s}} \left[ \ln(1 - v_{2,s}) + v_{2,s} + \chi_1 v_{2,s}^2 \right] 
\]

\[
\frac{v_{2,s}}{v_{2,r}} \left[ \frac{v_{2,s}^2}{v_{2,r}^2} \right] - \frac{1}{2} \left( \frac{v_{2,s}}{v_{2,r}} \right)
\]

(4.11)
The mesh size \( (\xi) \) can be calculated from the \( \overline{M}_c \) by the following equation 4.12. \( C_n \) is the rigidity factor \( (8.3, 56) \), \( M_r \) is the molecular weight of the polymer repeating unit \( (44 \text{ mol/g}) \) and \( l \) is the length of the carbon-carbon bond \( (1.54 \text{ Å}) \).

\[
\xi = \nu_{2,s}^{1/3} \left( \frac{2C_n \overline{M}_c}{M_r} \right)^{1/2} l
\]  

(4.12)

Lower \( \overline{M}_c \), and higher \( \nu_{2,s} \), corresponds to a smaller mesh size. In general, a hydrogel with a smaller mesh size will manifest a lower diffusion coefficient for solvent or other molecules diffusing through it. When all other factors are held constant, gels with lower \( \overline{M}_c \) and higher \( \nu_{2,s} \) tend to display lower diffusion coefficients and vice versa.

### 4.4 Rubber elasticity theory

Hydrogels demonstrate elastomeric behavior. When a constant stress is applied to a hydrogel, it undergoes deformation and reaches an equilibrium strain. Under such conditions, the sample is undergoing changes in the Helmholtz free energy

\[
A = U - TS
\]

(4.13)

Under isothermal conditions, assuming the volume of the sample does not change, the relationship in equation 4.14 exists.
\[ f = \left( \frac{\partial A}{\partial L} \right)_{T,V} \]  

(4.14)

From this relationship, Sillman and Peppas and Merrill\textsuperscript{104} deduced for a hydrogel prepared in the presence of solvent, a relationship between the normal stress applied (\(\tau\)), the elongation ratio (\(\alpha\)), the ratio of polymer volume fractions of swollen and relaxed gels (\(v_{2,s}/v_{2,r}\)), the ideal gas constant (\(R\)), the temperature of experimental conditions (\(T\)), specific polymer volume (\(\bar{v}\)), the molecular weight of uncrosslinked polymers (\(M_n\)), and the molecular weight between crosslinks (\(M_c\)). This model allows us to measure the relationship between \(\tau\) and \(\alpha\) experimentally and calculate the \(M_c\).

\[
\tau = RT \left( \frac{1}{\bar{v}M_c} \right) \left( 1 - \frac{2M_c}{M_n} \right) \left( \alpha - \frac{1}{\alpha^2} \right) \left( \frac{v_{2,s}}{v_{2,r}} \right)^{\frac{1}{3}}
\]  

(4.15)

The \(M_c\) enables us to calculate the mesh size according to equation 4.12.

### 4.5 Biomimetic Imprinting

Biomimetic imprinting is a technique for synthesizing hydrogels and conferring certain unique physical properties to it in the process.\textsuperscript{62} It is based on the premise that synthesizing a hydrogel by crosslinking its constituent monomers in the presence of template molecules can alter the microstructure of the gel. Consequently, we can change the way the hydrogel interacts with the environment and with molecules diffusing through it.
To prepare a hydrogel by biomimetic imprinting, the constituent monomers are mixed with a selected molecule and allowed to reach equilibration. The monomers complex with the template molecules to lower the free energy of solvation in the mixture. If the hydrogel is crosslinked with the monomers in these optimum configurations, then the hydrogel retains a molecular “memory” of the template molecule which persists even if the template is washed away. This molecular memory enhances the affinity of the hydrogel for the drug used as the template.

The selection of monomers involved in the biomimetic imprinting process is critical for effective imprinting. This involves identifying binding molecules and other biological agents that have evolved to bind with the template molecule. The active site of the binding molecule is examined for amino acids that are critical for binding to the template molecule. Analogous acrylate monomers are selected by comparing the chemistry of the acrylates to the critical amino acids. The acrylate monomers with functional groups most similar to the critical amino acids are likely to form the best memory sites within the hydrogel. This technique has been demonstrated for the molecule ketotifen fumarate, a small molecule.
5.1 The Tear Film

The tear film covers the exposed surface of the eye, and it comprises distinct layers. The epithelium of the eye secretes mucins, both membrane-bound and free floating. They form a dense layer over the epithelial microvilli called the glycocalyx (0.01-0.07 $\mu$m), and these perform a number of functions - providing the hydrophobic epithelium a hydrophilic surface, lubrication against the strong shearing effects of blinking, and regular cleanup of the eye surface. The concentration of mucins decreases toward the anterior section of the aqueous-mucin layer (4-9 $\mu$m thick), which keeps the eye surface bathed in water with dissolved oxygen and antibacterial enzymes (Tear fluid does not supply glucose. This is provided by interstitial fluid for conjunctiva, and aqueous humor for cornea). At the interface between the tear film and the atmosphere, there is a thin (0.1 $\mu$m) layer or lipid molecules - non-polar lipids form a coating that retards the evaporation of tear water to the air, and polar lipids form an interface between the aqueous layer and the hydrophobic non-polar lipids. The lipids are secreted by the Meibomian glands in the eyelids.

Tear fluid turnover can be classified into three phases: secretory, distributional and excretory. In the secretory phase the aqueous component and some mucins of tear fluid are produced by the lacrimal glands via the lacrimal ducts above the eye at a baseline rate of 1 $\mu$L/min. The tear-film turnover rate is 16% per minute, and the film’s thickness is 4-9 $\mu$m. The cul-de-sac behind the lower lid can hold 7-9 $\mu$L normally, and up to 30 $\mu$L if
blinking is avoided. It creates a reservoir of tear fluid increasing the residence time of tears in the eye. The pH of tears ranges between 6.5 and 7.6.

The distributional phase consists of the blink mechanism, which compresses the lipid layer within the palpebral fissure and redistributes the mucins across the epithelial surface. When the eye opens after the blink, the tear film is spread out across the eye surface. The high surface tension between the aqueous surface and the air prompts the lipid to spread over the aqueous layer, forming a stable tear film.

The excretory phase involves the removal of tear fluid through the puncta or openings of the lacrimal duct. The drainage process is rapid and highly efficient - 90% of all tear fluid drains through the lacrimal duct at 25-50 µL per 90 seconds. The fluid flows through the puncta into lacrimal sacs behind the nose, encouraged by the negative pressure in the sac and the ”pumping” effect of blinking. It passes via the lacrimal duct into the vascularized nasal meatus in the nose, where the fluid and any drugs it contains are absorbed. The remaining 10% of fluid evaporates between blinks, at a rate of 7% per minute.¹²¹ If the eye is held open and immobilized, the time taken for the tear film to break up (BUT) is 15-50 seconds for a normal eye, and less than 10 seconds for a dry eye. The tear film and the related anatomical structures are shown in Figure 5.1.

Air at the surface of the eye may also be considered an ”ocular fluid” when we consider the transport of oxygen. Diffusion from the atmosphere is the only mechanism for the delivery of respiratory oxygen to corneal tissue, because optical clarity requires that the tissue be non-vascularized. The rate of diffusion of oxygen to the eye is estimated to be 7.8 µL/cm² per hour.²¹
The tear film on the eye surface consists of roughly three layers: the mucus layer secreted by goblet cells, the watery layer secreted by the lacrimal gland and the oily layer secreted by the Meibomian glands. Tears secreted from glands flow over the eye. They are spread over the eye surface through blinking mechanisms, accumulate under the lower lid, and drain from the eye via the lacrimal puncta. They pass through the lacrimal duct into the lacrimal sac and are absorbed into the body through vasculature in the nose.
5.2 Etiology of Dry Eyes

Keratoconjunctivits sicca, commonly referred to as “dry eyes”, is a condition where the conjunctiva and cornea are not enclosed with a healthy amount or quality of tear fluid. Hyperosmolarity of the tear fluid and inadequate hydration of the epithelial surface produce symptoms of itching, burning and excessive watering, and can lead to inflammation, corneal ulcers, bacterial conjunctivitis, corneal perforation, and loss of vision. A wide range of factors can be held responsible for dry eyes from congenital malfunction of tear glands, to autoimmune disorders such as Sjogren’s syndrome, to environmental risk factors such as contact lens wear or air quality. Nonetheless, many of the causes share mechanistic pathways. Furthermore, multiple forms of dry eye syndrome can coexist in one patient, with or without causal links.

In normal eyes a thin aqueous film is confined within the boundaries of the eyelids over the hydrophilic mucin-covered epithelial surface. The film is stable because the surface tension of the fluid film plus the interfacial tension at the solid-fluid boundary is lower than surface tension of solid surface. Between blinks the surface tension increases as the tear film thins from evaporation. The film becomes unstable and breaks up in spots, exposing the epithelium to air. The blinking process replenishes the tear film, redistributes mucins over the eye surface and removes any foreign particles. During each blink while the eye is closed, the surface tension at the lipid-air interface drops as the exposed surface area decreases. When the eye opens, the tension increases rapidly and the film thins and spreads over the eye surface. Over time, because of evaporation and drainage, the film thickness continues to decrease until the film destabilizes and breaks. The interval from the blink to the film destabilization is called the break-up time (BUT), and the exposure to air triggers the next
blink. In normal eyes, the BUT is 15 to 50 seconds long. In dry eyes, the BUT decreases to less than 10 seconds\textsuperscript{121} as the eye attempts to compensate for the rapid evaporation.

Dry eye syndromes can be classified as aqueous tear deficient (ATD) or evaporative dry eye (EDE). The former occurs when insufficient volumes of aqueous tears are produced by the lacrimal glands. ATD is further classified as Sjogren’s syndrome related (where abnormal autoantibodies attack the glands) or non-Sjogren’s syndrome related (generally milder, with etiology not fully determined but usually multifactorial).\textsuperscript{106} EDE occurs when the tear film evaporates from the eye faster than it is secreted, leading to hyperosmolarity and desiccation. It is caused by thin lipid layers (from malfunctioning Meibomian glands), reduced blink rate, poor lid-globe congruity, unfavorable environments and disruptions in the tear film continuity.\textsuperscript{41} Dry eyes are also developed by users of contact lenses and people who have undergone Laser-Assisted in Situ Keratomileusis (LASIK surgery).\textsuperscript{130}

Many contact lens users complain of ocular discomfort that makes contact lens wear irritating or even painful.\textsuperscript{40} This discomfort has been linked to EDE. The tear film is disrupted by the edges of the lens and the increased surface tension at the boundary. It has also been demonstrated that\textsuperscript{50} continued contact lens wear leads to decreased blinking rate. However, some studies show that the aqueous layer becomes more stable if the lens gets coated by mucins.\textsuperscript{69} The mucins make the lens surface more hydrophilic, lowering the aqueous-lens interfacial tension. Ocular dryness triggered by lens-wear is known as contact lens-induced dry eye (CLIDE). In one survey 76.8% of current contact lens users reported ocular dryness, with 26.8% reporting frequent to constant symptoms.\textsuperscript{26} There are economic impacts of this phenomenon - firstly the productivity lost by people who need to spend time and resources to manage the condition, and secondly the loss of sales by contact lens manufacturers when people stop using contact lenses.\textsuperscript{145}
Current techniques for managing dry eye symptoms vary depending on the etiology. Most people with non-Sjogren-related dry eye and contact lens induced dry eye apply rewetting drops and artificial tears to their eyes via eye drops. Some deliver lipids\textsuperscript{41} to make the lipid layer more substantial. Others apply viscosity enhancers to slow tear drainage and hold moisture close to the epithelia. But eye drop usage is inconvenient when needed multiple times a day, because it is followed by a time interval in which vision is blurred and activities such as driving and reading must be interrupted. Nonetheless eye drop formulations such as hyaluronic acid and carboxymethyl cellulose are commonly used as artificial tears. Patients of Sjogren’s syndrome need anti-inflammatory drugs to control their dry eye.

5.3 Treatment with Hyaluronic Acid

Hyaluronic acid (HA) is a polysaccharide that is fast becoming a preferred treatment for dry eye syndrome as an artificial tear solution. It is an unbranched non-sulfated glycosaminoglycan composed of units of the saccharides D-glucuronic acid and D-N-acetylglucosamine, linked together via alternating $\beta$-1,4 and $\beta$-1,3 glycosidic bonds, as shown in Figure 5.2. Its conjugate base is hyaluronate, with a deprotonated carboxylic group on the glucuronic acid. The dimer unit has a molecular weight of about 415 Da, but the entire chain can be 25,000 dimer units in length. HA is polydispersive, and depending on the location of origin the average size can range from 5000 Da to 20 Million Da \textit{in vivo}.

HA is found throughout the body as a natural lubricant, predominantly in the synovial fluid in skeletal joints and in the vitreous humor of the eye. It is also a major component of many extracellular matrices. Where it does not constitute the bulk of the material, it
Hyaluronic acid is a long-chain molecule, specifically a polysaccharide consisting of repeating units of glucuronic acid and N-acetylglucosamine. The glucuronic acid has a carboxylic groups that deprotonates and interacts with cationic monomers such as (diethylamino)ethyl methacrylate and amino acids such as lysine and arginine. HA is found in various lengths depending on the source, usually in the $10^6$ Da range.
acts as a structural scaffold for other materials such as chondroitin sulfate proteoglycans. The largest amount exists in the skin tissue, more so in the epidermis than the dermis.

Concentrated HA has strong viscoelastic properties and displays shear-thinning behavior. When subjected to fast and short flow, the chains tangle and demonstrate elasticity with low viscosity. In contrast during slow and extended flow, the chains partially separate and align, and their interactions lead to more viscous behavior. The viscosity enhances the tear stability\textsuperscript{60} and slows tear removal\textsuperscript{131} by slowing the flow rate. The shear thinning behavior has a lubricating effect. In the eye in particular, it prevents the shearing force of the eyelid from damaging the epithelium during blinking.\textsuperscript{1} In normal eyes, the healthy composition of the tears is enough to prevent this damage.

HA is also mucoadhesive and interacts with ocular mucins when delivered to the surface of the eye. The mucins are proteoglycans, and the glycan components are similar to HA in structure and function. The glycans interact with the HA, and the HA behaves as part of the mucin layer, effectively increasing the mucin layer’s thickness. When a contact lens is present in the eye, HA may cover the lens as an “artificial mucin” and counteract the tear film destabilization that occurs in the presence of the lens.

Because HA is hygroscopic, it retains water close to the ocular surface and reduces dehydration.\textsuperscript{96} It also encourages corneal wound healing\textsuperscript{24} by promoting epithelial cell migration.\textsuperscript{52}

For the above reasons and its high biocompatibility, HA has been used as a topically delivered artificial tear solution for over twenty years.\textsuperscript{124} Because of its proven track record we have selected HA as a comfort molecule for delivery to the eye from a therapeutic contact lens for the treatment of ocular discomfort and some cases of dry eye syndrome.\textsuperscript{8, 9, 22}
Chapter 6
Lenses for Delivery of Comfort Molecules

There is a strong unmet need for the sustained delivery of dry eye treatments without recourse to the use of eye drops. Dry eye syndrome causes great discomfort to people and compels many sufferers to abandon the use of contact lenses until symptoms are alleviated. Currently patients manage their symptoms through the use of topical delivery of artificial tears, moisturizers and comfort agents, with or without concomitant use of contact lenses. Instillation of eye drops is imprecise in untrained hands, and can be a nuisance for people who have to interrupt daily activities to apply them. The recommended amount of eye drops is not enough for some people, and patients have been known to apply artificial tears 12 or more times a day.\textsuperscript{29}

The development of a device that can deliver comfort agents in a sustained manner over the course of several hours to days without interfering with contact lens use would greatly ease the suffering of dry eye patients. Furthermore, a contact lens that delivers the comfort agent to the eye directly would simplify the procedure considerably. In this study we design such a therapeutic contact lens that delivers the comfort molecule hyaluronic acid (HA) to the eye in a sustained and tailorable fashion.

6.1 Nelfilcon A

This study was sponsored by CIBA Vision, Inc., a major producer of contact lenses and lens care products, to formulate a version of their flagship daily disposable contact lens
Focus®DAILIES™ that alleviates ocular discomfort via HA release and can be worn by patients with some types of dry eye syndrome.

Daily disposable lenses are one class of contact lenses currently in the market. They are inexpensive and designed to forego the need for daily cleaning and storage of contact lenses by the user. A new lens is worn daily while the old lens is discarded. Daily disposable lenses are recommended for patients with ocular allergies, to prevent the accumulation of dust and other allergens on the lens. Other classes of lenses include extended wear lenses that can be worn continuously in the eye for thirty days without removal, and are gaining popularity for their ease of use. In contrast annual and quarterly lenses, which need to be removed for cleaning every night, are growing less popular.

The material that we have chosen for the fabrication of the contact lens is a commercial formulation known as Nelfilcon A. It is primarily used for the synthesis of daily-wear disposable contact lenses, although such lenses have been known to be worn for multiple days. Nelfilcon A is a hydrogel material consisting of biocompatible polyvinyl alcohol (PVA) polymers in aqueous solution, photocrosslinked into a network structure. A novel feature of Nelfilcon A is the lack of monomers in the prepolymerization sol, eliminating the need to purify the prepared lens. In fact, almost every component required for polymerization is tethered to the PVA chains so that small unreacted monomers do not remain in the final hydrogel. This includes the crosslinkers, the initiator, and the tint where applicable.

PVA contains hydroxyl (OH⁻) groups attached to a repeating polymeric backbone in the 1,3 position. It is synthesized by the acid hydrolysis of polyvinyl acetate. The 1,3 hydroxyl groups are perfectly positioned to undergo cyclic acetal formation upon reaction with aldehydes, and this is a highly useful mechanism for the attachment of components necessary for hydrogel formation.
The synthesis of Nelfilcon A macromer was performed by CIBA Vision, Inc. (Duluth, GA) according to a two step procedure.\textsuperscript{142} First a diacetal with acrylate functionality, N-acryloylaminoacetaldehyde-dimethylacetal (NAAADA), was synthesized by reacting aminoacetaldehyde with acryloyl chloride in a low-temperature alkaline aqueous solution. After neutralization and extraction, the crude product was purified through molecular distillation. In the second step, PVA was transacetylated with NAAADA resulting in PVA chains with a well controlled number of pendant crosslinking acrylate groups per macromer chain.\textsuperscript{17} A number of reactions were taking place at this acid catalyzed stage: the crosslinker’s acetal was hydrolyzed to aldehyde, the aldehyde reacted with the PVA, and some acetate groups remaining on the PVA from its synthesis were converted to hydroxyl groups, as shown in Figure 6.1. The added crosslinker was also modified by attaching the initiator Irgacure 2959. Atmospheric oxygen was used as the stabilizer for the acrylate. To make tinted lenses, a commercial dye such as Remazol Brilliant Blue R\textsuperscript{TM} was also activated and attached to PVA through acetylation. The reaction was quenched by neutralizing with alkali. The photopolymer was then purified by ultrafiltration to the desired purity and concentration.

6.2 Hyaluronic Acid Binding Moieties

Within the human body, HA binds to various receptors, the most significant is the cell-surface glycoprotein CD44. Using molecular modeling and site-specific mutagenesis, researchers have identified the amino acid residues most responsible for the binding of CD44 to HA.\textsuperscript{12} Residues deemed critical for HA binding were tyrosine-42, arginine-78 and
Figure 6.1: Synthesis of Nelfilcon A macromer from PVA

Nelfilcon A is synthesized using poly(vinyl alcohol) (PVA) as a starting material. N-acryloyl-aminoacetaldehyde-dimethylacetal (NAAADA) is reacted with the PVA through transacetylation under acidic aqueous conditions. The product is a PVA macromer with pendant acrylate groups at well-defined intervals. The trans-acetylation process can be used to attach initiator and tint to the macromer. The product is purified by diafiltration.
tyrosine-79. Residues considered important for HA binding were lysine-38, arginine-41, lysine-68, asparagine-100, asparagine-101 and tyrosine-105.

Based on this analysis we sought acrylate monomers with functional groups that bore similarities to tyrosine, arginine, lysine and asparagine. Tyrosine contains a 4-hydroxy-phenyl group which features aromatic behavior with some hydrogen bonding capability. Arginine and lysine have amine groups which bear positive charges when protonated. Asparagine possesses an amide group for additional hydrogen bonding.

Acrylate monomers with similar chemical behavior are acrylamide (AM), N-vinyl pyrrolidone (NVP) and (diethylamino)ethyl methacrylate (DEAEMA). AM shares an amide group with asparagine. NVP, an aromatic lactam, can be seen as an analog to tyrosine for its aromaticity and hydrogen-binding capability. Finally, DEAEMA is a cationic acrylate, and is similar to arginine and lysine because of its positive charge. The structures are shown in Figure 6.2.

We hypothesized that these monomers, if incorporated into the Nelfilcon A network, would non-covalently interact with HA and increase the affinity of the molecule for the hydrogel, thereby giving us an additional level of control over the release rate. The DEAEMA was expected to form an ionic bond with the carboxylic groups on the glucuronic acid units, and the AM and NVP to form general hydrogen-bonds with varied groups on both glucuronic acid and acetylglucosamine. The increased ability to tailor the release kinetics would enable us to design the optimum formulation for the desired product.

We also employed the principle of biomimetic imprinting, as described in Section 4.5. We hypothesized that when the functional monomers are added to the HA-Nelfilcon mixture and allowed to equilibrate with HA, the monomers would prefer to be spatially arranged in a low energy configuration. Such a configuration would favor electrostatic and polar
For the biomimetic imprinting of hyaluronic acid, we select acrylate monomers that bear chemical similarity to the amino acids found on the binding site of hyaluronic acid binding protein CD44. Acrylamide and asparagines both have amide moieties, N-vinyl pyrrolidone and tyrosine have hydrogen bonding capability while (diethylamino)ethyl methacrylate is positively charged, like arginine and lysine.

**Figure 6.2: Comparison of functional groups on amino acids and acrylate monomers**
interactions between the monomers and the HA, much like the interactions between amino acids and HA in the CD44 binding site. When the gel is crosslinked the monomers would be immobilized in these favorable configurations, creating sites within the network with a stronger affinity for HA than areas with the same chemical composition and random configuration.

6.3 Methods and Materials

6.3.1 Synthesis of hydrogels

To prepare 6.5 mg HA/g Nelfilcon hydrogels, 5 g of Nelfilcon A macromer (CIBA Vision, Inc.) was mixed with 32.5 mg of hyaluronic acid sodium salt (Streptococcus equi, Fluka) in a 15 mL centrifuge tube. Functional monomers acrylamide (Aldrich), N-vinyl pyrrolidone (Polysciences, Warrington, PA) and (diethylamino)ethyl methacrylate (Aldrich) were added to prepare imprinted hydrogels. The mixture was repeatedly stirred, centrifuged (30 minutes to 1 hour at a time, a minimum of 4 times), and rested overnight to dissolve the HA in the prepolymer until homogeneous. The mixture was finally centrifuged for 5 to 10 minutes to remove air bubbles.

For the benchmark studies in the appendix, the concentration and size of HA were varied to study release characteristics. For the former, hydrogels were prepared at concentrations of 2 mg HA/g Nelfilcon, and 40 mg HA/g Nelfilcon. For the latter, 6.5 mg HA/g Nelfilcon hydrogels were prepared using HA of molecular weights 50 kDa and 100 kDa (Genzyme Pharmaceuticals).

Moulds for the hydrogel lenses were prepared. PTFE Teflon® (Scientific Commodities Inc., Lake Havasu, AZ) spacers of 5 mil thickness were constructed by cutting sheets into 2”
by 1.5” frames with a 1” by 1” central space. Spacers were affixed to 2” by 1.5” microscope slides. Between 125 to 200 mg of the prepolymer mixture was pipetted into the central space carefully to avoid the introduction of air bubbles, and the mould was closed by placing a second microscope slide on top, sandwiching the prepolymer between the slides and within the spacer. The mould is clamped with binder clips reserved for this purpose.

The mould was exposed to ultra-violet light from a UV light source (Novacure 2100, Exfo, Mississauga, Canada). The intensity of delivered light was 10.5 mW/cm$^2$ (8.5 mW/cm$^2$ for the benchmark studies included in the appendix) measured by radiometer (International Light IL400A). Duration of exposure was 15 seconds for hydrogels without functional monomers, and 45 seconds for hydrogels with functional monomers. The exposure times were determined with a Q-100 modulated differential photo calorimeter (TA Instruments, New Castle, DE), measuring the reactio progression.

The mould was opened and the hydrogel was covered with a small volume (2 to 5 mL) of water to soften and release the material. After 5 minutes, the hydrogel was peeled from the mould and cut into a disk with a cork borer (size no. 4, 14 mm diameter).

To prepare strips for tensile studies, the Teflon spacer was cut with an inner space of dimensions (6 cm by 3 cm). Crosslinking took place in a light source (Dymax UV flood light) at an intensity of 10 to 12 mW/cm$^2$. The hydrogel was cut with a clean blade to strips 6 to 10 mm wide.

### 6.3.2 Dynamic release studies

Dynamic release studies were conducted on the hydrogels to measure the release of HA. Prepared lenses were placed in 50 mL centrifuge tubes (in triplicate) with 20 mL of artificial lacrimal solution 6.78 g/L NaCl, 2.18 g/L NaHCO$_3$, 1.38 g/L KCl, 0.084 g/L
CaCl$_2$.2H$_2$O, pH 8$^{59}$), and incubated at 35°C on an orbital shaker (Stovall Life Sciences, Greensboro, NC) at a rotation speed of 20 to 30 rpm. After measured time intervals, the lenses were extracted and deposited into fresh lacrimal solution. The samples with released HA were stored at 4°C until assayed with a sandwich ELISA assay kit (Corgenix, Denver, CO). The assay kit had a detection range between 20 to 800 ng/mL, and some samples were diluted to prevent signal saturation.

Benchmark release studies in the appendix were carried out using the protocol described previously.$^{142}$

6.3.3 Heat stability studies

The hydrogels were subjected to simulated sterilization conditions to determine if the release characteristics would be affected. Nelfilcon hydrogels were prepared with 6.5 mg HA/ g Nelfilcon, no added monomers, and placed in 2 mL microcentrifuge vials with 6.5 mg/mL solution of HA in DI water to prevent partitioning out of the HA. The pH of the solution was adjusted to 11. The vials were heated to 120°C for 40 minutes and then cooled in a room temperature water bath. The lenses were removed from the vials, blotted to remove excess HA from the surface, and studied for their release kinetics.

The effect of heat conditions on HA solution was also assessed. In 2mL microcentrifuge vials, 1 mL samples of HA solutions of 500 ng/mL and 10µg/mL were heated to 105°C and 121°C respectively. The 500 ng/mL samples were heated for 0 min, 5 min, 30 min and 60 min while the 10µg/mL samples were heated for 15 min, 30 min, 45 min and 60 min. The sample vials were quenched in a room temperature water bath and assayed with an ELISA assay kit for HA.
Hydrogel swelling studies  After release in lacrimal solution, hydrogel lenses were dried in air for a minimum of 24 hours and then in a vacuum oven (VWR) at 30°C and 28 in.Hg to remove moisture until the weight change in the lenses was less than 0.1% (at least 5 days). The gels were then weighed in air and in heptane, a non-solvent, using a microbalance (Sartorius). The lenses were equilibrated in DI water overnight and the fully swollen lenses were weighed in air and heptane.

Swollen lenses without HA were synthesized and weighed after equilibrating in water overnight. Hydrogels in the relaxed state were synthesized and removed from mould without exposure to water, then weighed immediately. These were again weighed after dehydration.

6.3.4 Tensile strength studies

Hydrogels prepared in strips (in triplicate) were mounted on a dynamic mechanical analyzer (RSA III, TA Instruments) at a gauge length of 30 to 35 mm, and extended at a rate of 4 mm/min. The gels were fully hydrated through the experiment, and hydration was maintained with an aerosol diffuser.

6.4 Results and Discussion

6.4.1 Molecular transport and diffusion coefficients

Hydrogels made with 6.5 mg HA/g demonstrated a concentration dependent release profile. Figure 6.3 shows the cumulative mass of HA released from the hydrogel over a 5 day period. The release rates can be classified into three general zones. Initially HA is released over the first 2 hours at a rate of around 24 µg per hour. Over a 24 hour period...
after that, we see a linear release profile delivering 4 µg per hour. After that the release rate gradually decreases until very low amounts are releasing for the last 3 days.

We can subject the release profile to the analysis described in Section 4.2 and determine the order of release and diffusion coefficient of the HA from this hydrogel. From equation 4.8 we can plot \( \frac{M_t}{M_\infty} \) versus \( (t/L^2)^{\frac{1}{2}} \) and calculate the diffusion coefficient \( D \) by setting the slope \( (k) \) equal to \( 4\sqrt{D/\pi} \). The order of release is \( n - 1 \) when \( n \) is equal to the slope of \( \text{Log}[M_t/M_\infty] \) versus \( \text{Log}[t] \). The calculated diffusion coefficient is \( 5.69 \times 10^{-10} \), and the order of release is 0.61, close to the order of Fickian release, 0.5.

The decrease in diffusion coefficient is influenced by two factors. First, the hydrogel network presents a steric barrier to the Brownian movement of particles through the solvent. For a particle to diffuse through a hydrogel, it needs to pass through the open space or mesh between crosslinked polymer chains. The smaller the mesh, the less space the particle has to diffuse.

Second, our drug is not a simple particle but a long-chain molecule, behaving like a series of particles joined together. The chain-like nature of HA restricts the path that each constituent “particle” can pass through to diffuse through the chain - each must follow the one in front of it. The motion of a long-chain molecule through a hydrogel mesh can be described by the reptation model as described in Section 4.1. The HA does not slide through the mesh in one smooth motion. Rather the “tail” end of the polymer moves slightly forward to form a loop, and it is the loop that travels along the length of the chain, with none of the individual units traveling a large distance. When the chain reaches the “head” of the chain the loop disappears and as a result the entire chain undergoes a small displacement through the hydrogel.
The prepolymer formulation used to make this hydrogel contained only the Nelfilcon macromer and hyaluronic acid. The release profile demonstrates Fickian kinetics over 5 days, with three distinct release rates. The initial rate lasts about 6 to 10 hours with a release rate of 12 μg/hr. The intermediate region demonstrates a nearly linear release profile, delivering 4 μg/hr. After about 2 days, the release rate tapers off until it is negligible.
To compare the release rate of HA from these hydrogel lenses with the therapeutic regime of HA eye drops, we considered the therapeutic regime of topically delivered HA artificial tear eye drops. In particular, we examined AQuify\textsuperscript{®} Long-Lasting Comfort Drops, a 0.1% solution of HA. According to the package insert, the recommended dosage is 2 drops up to 3-4 times daily. The volume of a typical eye drop is 20 $\mu$L\textsuperscript{122} so if drops are delivered four times a day, the delivered dosage would be 40 $\mu$g of HA every 6 hours or 6.67 $\mu$g per hour. As the bioavailability of eyedrops is always less than 100% because some drug is always lost through lacrimal drainage, it appears the hydrogel lens can deliver therapeutic or near therapeutic amounts of HA over the first couple of days. However, by incorporation of functional monomers to the system we may be able to exert an additional level of control over the release rate.

In Section 4.5 we discussed the process of biomimetic imprinting, which allows tailoring of the release properties of a hydrogel by adding functional monomers and crosslinking in the presence of the drug molecule. This phenomenon has been demonstrated previously\textsuperscript{137} for ketotifen fumarate, a relatively small molecule, in a formulation containing added functional monomers as 5% of the total prepolymer mixture. Using this percentage of functional monomers as a starting point, we modified the basic Nelfilcon A macromer sol by adding acrylamide (AM), N-vinyl pyrrolidone (NVP) and (diethylamino)ethyl methacrylate (DEAEMA) as functional monomers and crosslinking the gels in the presence of HA. The monomers AM, NVP and DEAEMA were added in a ratio of 1:1:2 by moles, and together comprised 5% by mass of the prepolymer before addition of HA.

Release studies carried out with hydrogels of this formulation resulted in a negligible amount of HA released in a 24 hour period. Theorizing that the functional monomer
content was too high, we reduced the monomer content to 1% by mass of the prepolymer. Again, negligible HA was released.

To understand the mechanism by which the functional monomers were immobilizing the HA, we produced hydrogels with 1% by mass functional monomers and placed them in release solutions of lacrimal solution at different pH conditions. We found that the 1% imprinted hydrogels released negligible HA in pH 8 solution but released significantly increased amounts in pH 12 solution. It appeared that the excessive OH$^-$ ions in the alkaline solution would deprotonate the DEAEMA, reducing the electrostatic interactions with HA (in hyaluronate form) and allow the HA to release from the hydrogel. However we are designing our system for ocular drug delivery and it needs to release HA at physiological pH.

With evidence indicating that electrostatic (and potentially other non-covalent) interactions were responsible for the immobilization of HA in the hydrogel, we further reduced the functional monomer content of the hydrogel. We hypothesized that if each HA chain were interacting with fewer functional monomers, it would undergo reptation at an increased rate. Our hypothesis can be illustrated by making an analogy with the fabric hook-and-loop fasteners such as Velcro$^\text{TM}$. Velcro$^\text{TM}$ consists of two fabric surfaces, one with minute loops and the other with hooks. If one isolated hook is connected to an isolated loop, the bond between them can be severed with the application of a very small force. But if large fabric surfaces are brought together, the combined bonds between thousands to hooks and loops require much more applied force to dissociate. Similarly, if the HA (with a carboxylate functional group on each dimer) is in contact with fewer functional groups, it encounters less resistance while diffusing through the hydrogel.
Table 6.1: Diffusion and release order of HA from Nelfilcon hydrogels with varying functional monomer amounts, all in [1:1:2] ratio

As we tailored down the functional monomer content of the prepolymer, we were successful in attaining HA release from a hydrogel containing 0.25% functional monomers by mass. Release studies were also carried out on hydrogels with 0.125% and 0.025% monomers by mass. The cumulative release rates are presented in Figure 6.4. For all other hydrogel compositions, we see a clear trend that increasing the functional monomer content in the prepolymer reduces the cumulative mass of HA released. We calculate the diffusion coefficients and orders of release for these profiles according to the method described in Section 4.2.

The decrease in total mass released indicates that as more monomers are included, more HA is prevented from diffusing out of the hydrogel. That is, a fraction of the HA is immobilized inside the hydrogel. The HA that does diffuse from the hydrogel has diffusion coefficients that depend on the %-by-mass of monomer content in the hydrogel. The monomer content and diffusion coefficient are strongly correlated, as shown in Figure 6.5.

The graph indicates that hydrogels with monomer content less than 0.36% are likely to allow HA chains to diffuse through the mesh, while diffusive release is not expected from hydrogels with monomer content much higher than 0.36%. This agrees with our data from the 1% and 5% monomer content hydrogels, which released negligible amounts of HA.
Dynamic release studies were conducted on a series of hydrogels prepared from prepolymer containing different %-by-mass of functional monomers: 0.05% (■), 0.125% (▲), 0.25% (●), 1% (×) and 5% (○). All were made with the same ratio of functional monomers: [AM : NVP : DEAEMA] ~ [1:1:2]. A hydrogel made with prepolymer containing no functional monomer is also shown (◆). It appears from the release profiles that increasing the %-by-mass of functional monomers in the hydrogel decreases the release rate and the cumulative released mass of HA.
There is a strong inverse correlation between the diffusion coefficients of HA from hydrogels against the %-by-mass functional monomer content in the prepolymer. The diffusion coefficient is expected to decrease to minute levels as the functional monomer content approaches 0.361%. This agrees with our data that demonstrates negligible release of HA from hydrogels with 1% and 5% functional monomer content.
The above analysis demonstrates that the presence of the functional monomers tailors the diffusion coefficient of HA releasing from the hydrogel. It is unclear at this point in the analysis whether the HA interacts with the hydrogel solely because of the electrostatic interaction between HA carboxylate groups and the protonated DEAEMA, or if the AM and NVP contribute to the interaction. To explore this, we produced a series of hydrogels containing 0.125% functional monomers by mass in the prepolymer mixtures, but containing varying proportions of AM, NVP and DEAEMA. The compositions are summarized in Table 6.2.

<table>
<thead>
<tr>
<th>Relative proportion of monomers</th>
<th>Functional monomer as % by mass of prepolymer mixture</th>
<th>All monomers</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.125% [AM:NVP:DEAEMA]</td>
<td>AM</td>
<td>NVP</td>
</tr>
<tr>
<td>0.125% [0:0:1]</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.125% [1:1:2]</td>
<td>0.0313%</td>
<td>0.0313%</td>
</tr>
<tr>
<td>0.125% [1:1:0]</td>
<td>0.0625%</td>
<td>0.0625%</td>
</tr>
</tbody>
</table>

Table 6.2: Functional monomer content of hydrogels

Release studies were conducted on these hydrogels as described in Section 7.2, and the cumulative release profiles are presented in Figure 6.6.

The cumulative mass released tends to decrease as the proportion of DEAEMA is increased. This suggests that DEAEMA has a strong affinity for HA and immobilizes a larger fraction within the hydrogel. However if we compare the fractional release of HA from these hydrogels, a different phenomenon with a distinct trend is revealed, as seen in Figure 6.7.

During the first 0.6 fraction of the cumulative release, the diffusion of HA from both 0.125% [1:1:0] and 0.125% [0:0:1] hydrogels is faster than diffusion from the 0.125% [1:1:2] hydrogel. In other words, even if the mass of functional monomers in the prepolymer is kept...
Dynamic release studies were conducted on a series of hydrogels prepared from prepolymer containing different proportions of functional monomers [AM : NVP : DEAEMA], all at 0.125%-by-mass of prepolymer: [1:1:0] (■), [1:1:2] (▲) and [0:0:1] (×). For comparison we also plot the release profiles of hydrogels with 0.25% [1:1:2] (●) and hydrogels with no functional monomers (♦). Cumulative mass released seems to decrease as the proportion (and hence total amount) of DEAEMA increases.

Figure 6.6: Cumulative release of HA from Nelfilcon hydrogels with different proportions of functional monomers
Figure 6.7: Fractional release of HA from Nelfilcon hydrogels with different proportions of functional monomers

Dynamic release profiles were normalized with the total amount of HA released by hydrogels prepared from prepolymers containing different proportions of functional monomers [AM : NVP : DEAEMA], all at 0.125%-by-mass of prepolymer: [1:1:0] (□), [1:1:2] (▲) and [0:0:1] (×). For comparison we also plot the fractional release profiles of hydrogels with 0.25% [1:1:2] (●) and hydrogels with no functional monomers (Crystal). The diffusion coefficient appears to decrease with greater diversity of functional monomers in the prepolymer.
constant, increasing the variety of functional monomers reduces the diffusion coefficient of the HA.

For comparison we also juxtapose the cumulative release profiles of 0.25% [1:1:2] and 0.125% [0:0:1] in Figure 6.8. They both contain the same amount of DEAEMA, but the former contains an additional 0.0625%-by-mass each of AM and NVP. The cumulative released mass from both hydrogels is the same, but the diffusion coefficients vary.

We can conclude from this that the release rates of HA can be controlled in two distinct ways - we can vary the cumulative mass released by varying the total amount of functional monomers added, and we can vary the diffusion coefficient by varying the diversity of incorporated monomers. The two trends are made more apparent in Figures 6.9 and 6.10.

The cumulative mass released for all 0.125% monomer compositions is compared in Figure 6.9. The composition with no DEAEMA, 0.125% [1:1:0], releases a high cumulative mass of HA, almost as much as is released by the Nelfilcon hydrogel without any added monomers. As the proportion of DEAEMA is increased, the release amount decreases to the level released by 0.25% [1:1:2], which contains all functional monomers.

The diffusion coefficients of HA from all 0.125% monomer compositions are compared in Figure 6.10. The compositions with no DEAEMA (0.125% [1:1:0]) and with only DEAEMA (0.125% [0:0:1]) have diffusion coefficients close to that of Nelfilcon without added monomers. The composition containing all monomers (0.125% [1:1:2]) has a significantly lower diffusion coefficient: 1.5 times lower than the 0.125% [0:0:1], and 1.6 times lower than the Nelfilcon without monomers. Also compare the diffusion coefficients of 0.125% [0:0:1] and 0.25% [1:1:2], which contain the same amount of DEAEMA. Although the release equivalent cumulative masses of HA, their diffusion coefficients differ by a factor
Figure 6.8: Cumulative release of HA from Nelfilcon hydrogels with the same % by mass of DEAEMA

The hydrogels of composition 0.125% [0:0:1] (×) and 0.25% [1:1:2] (○) contain the same amount of DEAEMA. They release similar cumulative masses of HA, but their diffusion coefficients are different. The composition with greater diversity of functional monomers has the lower diffusion coefficient.
Figure 6.9: 24 hour release of HA from Nelfilcon gels versus proportion of DEAEMA

The amounts of HA released over 24 hours by various hydrogels containing % by-mass of functional monomers 0.125% (▲) and 0.25% (●) were compared, along with hydrogels with no functional monomers (◆). The three data points for 0.125% correspond to different proportions of DEAEMA. The 0 on the x-axis indicates [AM : NVP : DEAEMA]=1:1:0, the 0.5 refers to [1:1:2] and the 1 refers to [0:0:1]. It is clear that increasing the amount of DEAEMA in the hydrogel decreases the cumulative mass of HA released. Furthermore, the 0.125% [0:0:1] and 0.25% [1:1:2] hydrogels contain the same amount DEAEMA, and release the same cumulative mass of HA.
Figure 6.10: Diffusion coefficients versus proportion of DEAEMA

The diffusion coefficients of HA through various hydrogels containing % by mass of functional monomers 0.125% (▲) and 0.25% (●) were compared, along with hydrogels with no functional monomers (●). The three data points for 0.125% correspond to different proportions of DEAEMA. The 0 on the x-axis indicates [AM : NVP : DEAEMA]~[1:1:0], the 0.5 refers to [1:1:2] and the 1 refers to [0:0:1]. We can see that increasing the diversity of the functional monomers, by incorporating all of AM, NVP and DEAEMA in the hydrogel, lowers the diffusion coefficient.
of 3. The diffusion coefficients and orders of release of compositions discussed in relation to the monomer diversity comparison are summarized in Table 6.3.

<table>
<thead>
<tr>
<th>Hydrogel [AM:NVP:DEAEMA]</th>
<th>Diffusion coefficient (cm^2/s)</th>
<th>Std. dev. (cm^2/s)</th>
<th>(R^2)</th>
<th>Release order</th>
<th>Std. dev. (0.1)</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nelfilcon only</td>
<td>5.689 (\times 10^{-10})</td>
<td>0.005 (\times 10^{-10})</td>
<td>0.99</td>
<td>0.61</td>
<td>0.02</td>
<td>0.99</td>
</tr>
<tr>
<td>0.125% [1:1:0]</td>
<td>4.824 (\times 10^{-10})</td>
<td>0.010 (\times 10^{-10})</td>
<td>0.99</td>
<td>0.57</td>
<td>0.03</td>
<td>0.97</td>
</tr>
<tr>
<td>0.125% [1:1:2]</td>
<td>3.553 (\times 10^{-10})</td>
<td>0.004 (\times 10^{-10})</td>
<td>0.99</td>
<td>0.47</td>
<td>0.02</td>
<td>0.99</td>
</tr>
<tr>
<td>0.125% [0:0:1]</td>
<td>5.306 (\times 10^{-10})</td>
<td>0.015 (\times 10^{-10})</td>
<td>0.96</td>
<td>0.66</td>
<td>0.01</td>
<td>0.99</td>
</tr>
<tr>
<td>0.25% [1:1:2]</td>
<td>1.797 (\times 10^{-10})</td>
<td>0.001 (\times 10^{-10})</td>
<td>0.99</td>
<td>0.50</td>
<td>0.021</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Table 6.3: Diffusion and release order of HA from Nelfilcon hydrogels with varying functional monomer proportions

We can explain the changes in diffusion coefficients in the above experiments by referring to the biomimetic imprinting process. In the prepolymer mixture containing the Nelfilcon macromer, functional monomers and HA, the functional monomers position around the HA so that the interactions between the functional monomers and HA moieties decreases the free energy of the system. When the mixture is crosslinked, the functional monomers are incorporated into the hydrogel in these favorable configurations through the pendant acrylate groups on the PVA chains. In this manner, the hydrogel is synthesized with memory sites that have an affinity for HA. While interactions do occur between the HA and each functional monomer individually, the presence of all three monomers allows multiple moieties on the HA to interact with the hydrogel at one site. The multiple functional interactions are believed to increase the similarity of the interaction sites with the binding sites on HA-binding protein CD44, leading to enhanced affinity and lower diffusion coefficients.

The possibility remains that the addition of monomers resulted in a hydrogel network with a tighter mesh structure, and the diffusion coefficient decreased because reptation takes longer as steric hindrance increases. To examine this possibility fully, we performed hydrogel swelling studies to obtain information about the mesh size.
6.4.2 Effects of heat sterilization

In the manufacture process of Nelfilcon A contact lenses, the crosslinked lenses are immediately sealed in foil blister-packs with 0.85 mL of buffer solution at pH 9. The sealed packs are then autoclaved at 121°C for 40 minutes. As HA is known to undergo denaturation when heated to high temperatures, we needed to assess the effect of the high temperature sterilization procedure on the HA incorporated in the lenses. We heated aqueous solutions of HA (500 ng/mL and 10 µg/mL) to temperatures above the boiling point of water for various time intervals. When the samples were assayed, the concentration of 1 million Da HA chains was lower for samples that had been heated longer.

Interestingly, the % change in concentration over time was lesser for the more concentrated solution (10 µg/mL) than the more dilute solution (500 ng/mL). The change in concentration in the 10 µg/mL solution was 30% over 60 minutes while the change in signal strength in the 500 ng/mL solution was 50% over 60 minutes. The percentage change in concentration versus time of heating is shown in Figure 6.11. This suggests that higher concentrations of HA may have protective effects on the stability of the long-chain HA molecule. We conducted a dynamic release study to assess the heat effects on the Nelfilcon-HA hydrogel lenses. Comparing the heat-treated lenses with the control untreated lenses we measured similar release profiles, suggesting that heat-treatment does not denature the HA within the hydrogel. The release profiles are shown in Figure 6.12.

6.4.3 Structural analysis

There are many theoretical models that enable us to obtain structural and configurational information about a hydrogel from experimental data obtained by swelling and
Figure 6.11: Comparison of stability of HA solutions under heat sterilization conditions

When solutions of HA in water are heated to above 100°C, they undergo some heat degradation. In the less concentrated solution (500 ng/mL, ■) nearly 50% of the large HA degrades to shorter HA over 60 minutes, whereas in the higher concentration solution (10 µg/mL, ▲), the degradation is only 30% in the same time. This suggests that higher concentrations have a protective effect on the large HA molecule.
Figure 6.12: Cumulative release of HA from Nelfilcon hydrogels before and after heat-sterilization

When hydrogels containing HA with Nelfilcon were heated to 121°C, the dynamic release profile of HA for such lenses (△) was similar to the release profile from hydrogels that did not undergo heat treatment (●).
tensile studies. In particular we can obtain information about the mesh size of the hydrogel and determine whether a tighter mesh is responsible for the decrease in the diffusion coefficient of HA through imprinted hydrogels.

The mesh size is related to the molecular weight between crosslinks according to equation 4.12.

The Nelfilcon macromer mixture contains water as a solvent. Based on the discussion in Section {sec:EquilibriumSwellingTheory, the Peppas-Merrill model describes the relationship between the average molecular weight between crosslinks ($M_c$) and the equilibrium polymer volume fraction ($v_{2,s}$) for a swollen hydrogel crosslinked in the presence of a solvent.

Equilibrium swelling studies were performed on the hydrogels to determine the weight swelling and volume swelling ratios ($q$ and $Q$) and the polymer volume fraction ($v_{2,s}$). $Q$ is the ratio of swollen to dry volumes of the hydrogel. $q$ is the ratio of swollen to dry weights. $v_{2,s}$ is the ratio of the dry polymer volume to swollen polymer volume, and $(1- v_{2,s})$ gives us the fractional water content of the hydrogel. These parameters were calculated for all hydrogels and are summarized in Table 6.4. We also illustrate some of the values in Figure 6.13.

The hydrogels synthesized with HA generally have slightly higher $Q$ and $q$ than the hydrogels synthesized without HA. The former also have lower $v_{2,s}$, indicating higher water content. Two factors influence this difference. First of all, the presence of HA in the prepolymer mixture can influence the formation of polymer chains and associated crosslinking points, making the polymer chains more mobile and increasing the hydrogels’ capacity to hold water. Secondly, the residual HA in the hydrogels increases the hydrogels’ capacity to hold water.
Figure 6.13: Comparison of polymer volume fractions for various hydrogels

All hydrogels have an equilibrium polymer volume fraction that lies in the narrow range between fractions 0.23 and 0.3, and the hydrogels with released HA generally have a lower polymer volume fraction than hydrogels synthesized without HA.
Table 6.4: Equilibrium swelling parameters

In general, all the hydrogels have $v_{2,s}$ falling within the range of 0.23 and 0.29 suggesting that the mesh size is similar for all the gels synthesized. In particular, we note that the swelling parameters of Nelfilcon hydrogel with 1%-by-mass functional monomers remain the same at pH 7 and pH 12, indicating that the pH dependent increase in HA release described in Section 6.4.1 did not result in change in mesh size. Furthermore, the swelling parameters do not change for Nelfilcon gels synthesized with HA despite heat sterilization.

In Figure 6.14, we illustrate the relationship between the diffusion coefficient and polymer volume fraction in gels with various %-by-mass of functional monomers.
Figure 6.14: Diffusion coefficients versus polymer volume fraction for Nelfilcon hydrogels with different %-by-mass of functional monomers

The diffusion coefficients of hydrogels made with different %-by-mass of functional monomers are plotted against their polymer volume fractions: 0.05% (■), 0.125% (▲), 0.25% (●), and no added functional monomers (◆). The diffusion coefficients vary significantly while the polymer volume fractions are limited to a narrow range. This indicates that changes in mesh size are not responsible for the changes in diffusion coefficients.
The figure clearly reveals the narrow range of polymer volume fraction of the hydrogels. In contrast, the diffusion constants of HA through these networks varies dramatically. The highest diffusion coefficient (from the gel without functional monomers) is nearly 4 times higher than the lowest diffusion coefficient (from the gel with 0.25% functional monomers). This is strong evidence that the diffusion coefficients do not vary because of structural parameters such as the mesh size.

To determine mesh size, further structural analysis was done through tensile testing of the sample, in which the hydrogel samples were extended at a constant rate and the tension on the sample was recorded. The extension versus applied force were plotted for four types of hydrogel samples: Nelfilcon gels without added functional monomers or HA, Nelfilcon with HA, Nelfilcon with 0.25% by mass functional monomers, and Nelfilcon with HA and 0.25% by mass functional monomers. Structural parameters for the hydrogels were obtained and summarized in Table 6.5.

<table>
<thead>
<tr>
<th>Hydrogel</th>
<th>Young's Modulus (MPa)</th>
<th>Std. dev.</th>
<th>Shear Modulus (MPa)</th>
<th>Std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nelfilcon</td>
<td>0.557</td>
<td>0.023</td>
<td>0.201</td>
<td>0.011</td>
</tr>
<tr>
<td>Nelfilcon with HA</td>
<td>0.423</td>
<td>0.060</td>
<td>0.153</td>
<td>0.022</td>
</tr>
<tr>
<td>Nelfilcon with 0.25% f.m.</td>
<td>0.535</td>
<td>0.032</td>
<td>0.195</td>
<td>0.015</td>
</tr>
<tr>
<td>Nelfilcon with 0.25% f.m. and HA</td>
<td>0.550</td>
<td>0.015</td>
<td>0.203</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Table 6.5: Tensile parameters

Of the four gels, three have very similar structural parameters. Nelfilcon with HA (no functional monomers) has moduli that are lower than the other hydrogels. This suggests that the presence of HA in the prepolymer without added functional monomers results in a network with longer chains between crosslinks. We base this on equation 4.15.
From this equation, we can calculate the molecular weight between crosslinks ($M_c$) in a hydrogel. The normal stress applied is $\tau$, the elongation ratio is $\alpha$, the ratio of polymer volume fractions of swollen and relaxed gels is $v_{2,s}/v_{2,r} \approx 1$, the ideal gas constant is $R = 8.314472 \text{ cm}^3\text{MPa.K}^{-1}\text{mol}^{-1}$, the temperature of experimental conditions is $T = 298 \text{ K}$, specific polymer volume is $\sigma = 0.909$, the molecular weight of uncrosslinked polymers is $M_n \approx 50,000 \text{ Da}$, and the molecular weight between crosslinks, $M_c$, remains to be calculated. This model allows us to measure the relationship between $\tau$ and $\alpha$ experimentally and calculate the $M_c$.

The slope of $\tau$ versus $\alpha - 1/\alpha^2$, obtained from the tensile tests, is the Shear Modulus and enables us to calculate $M_c$. From $M_c$, we can calculate the size of the mesh between crosslinked hydrogel chains. The $M_c$ and the mesh sizes are summarized in Table 6.6.

<table>
<thead>
<tr>
<th>Hydrogel</th>
<th>$M_c$ (g/mol)</th>
<th>std.dev</th>
<th>$\xi$ (Å)</th>
<th>std.dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nelfilcon</td>
<td>$8.79 \times 10^3$</td>
<td>$0.325 \times 10^4$</td>
<td>133</td>
<td>3</td>
</tr>
<tr>
<td>Nelfilcon with HA</td>
<td>$10.5 \times 10^3$</td>
<td>$0.876 \times 10^3$</td>
<td>155</td>
<td>7</td>
</tr>
<tr>
<td>0.25% without HA</td>
<td>$8.98 \times 10^3$</td>
<td>$0.438 \times 10^3$</td>
<td>140</td>
<td>4</td>
</tr>
<tr>
<td>0.25% with HA</td>
<td>$8.74 \times 10^3$</td>
<td>$0.250 \times 10^3$</td>
<td>144</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 6.6: Mesh sizes of hydrogels

This confirms that the mesh of Nelfilcon crosslinked with only HA has a more open network than the other hydrogels. The presence of HA along with Nelfilcon macromer in the prepolymer in the absence of functional monomers appears to produce a hydrogel with a greater molecular weight between crosslinks than Nelfilcon macromer does alone. The HA seems to influence the formation of polymer chains and associated crosslink points in the hydrogel resulting in a larger mesh size. This agrees with the results of the swelling studies, in which Nelfilcon alone has a greater polymer volume fraction than Nelfilcon with HA. However, the addition of functional monomers leads to a decrease in the molecular weight between crosslinks, indicating that the presence of the functional monomers decreases the
mesh size to an extent comparable with Nelfilon hydrogel with no added HA or functional monomers.
In a Fickian model of release kinetics, the release rate of a drug from the delivery device is proportional to the concentration gradient between the drug source and the surroundings. In practical terms this means that as the finite drug source is depleted, the rate of drug release decreases. A zero-order (i.e., independent of time) release rate is preferable because it would deliver medication at a constant rate for an extended time. The challenge is to use a finite drug source to achieve an extended zero-order release, and a number of strategies have been attempted in hydrogel drug delivery systems.

Therapeutic contact lenses are swollen when inserted into the eye, and cannot exploit the solvation-transition rate to control drug delivery. The other strategies also have drawbacks and cannot be easily applied to produce contact lenses. Our lab has recently demonstrated with molecular imprinting methods that one can also restrict and delay the transport of drug from the matrix via interaction of numerous functional groups with the template drug. In biomimetic imprinting, monomers chosen to mimic residues in the drug’s biological binding molecule are complexed non-covalently to the drug and crosslinked into a hydrogel matrix. The drug’s heightened interaction with these residue pockets slows its release from the hydrogel, exploiting a programmable memory within the polymer chains and not the free volume available for drug transport. This type of network formation - with a proper optimization of drug affinity relating to number and strength of functional monomer interactions, crosslinking structure, and mobility of polymer chains - has a strong
potential to influence a number of hydrogel systems and add to the variables one can alter to control the release profile.

In efforts to understand the mechanisms behind release kinetics, various mathematical models of solvent penetration and solute release have been developed. Typically, in both modeling and experimental work, infinite sink conditions are assumed and accumulation of drug in the solution surrounding the hydrogel is considered to be negligible. This is appropriate for the majority of studied systems but for ocular drug delivery, considering the small tear volume and flow rates encountered in vivo, it does not realistically describe drug release kinetics. In these types of physiological situations, it is imperative that microfluidic models be used to characterize the release profiles.

7.1 Microfluidic Platforms for Evaluating Drug Delivery Devices

Microfluidic platforms, typically dealing with $10^{-9}$ to $10^{-19}$ liters of small fluid amounts, interface engineering, chemistry, and biology for conducting experiments at very small scales. For instance, solid-state silicon microchips can provide controlled release of single or multiple chemical substances on demand or with multi-pulse drug release from resorbable matrices. While there have been an increasing number of cases in the last few years of controlling drug release by the application of micro- and nanotechnology for drug administration, there has been very little to no use of micro- or nanofluidic platforms in the evaluation of drug release devices.

This paper describes the in vitro drug release kinetics of imprinted hydrogel contact lenses within in vivo physiological flow by developing and implementing a microfluidic chip that matches the steady state tear volume of the eye with flow rates within the physiological
range. This has not been demonstrated to date with any other contact lens drug delivery systems. We hypothesized that the physiological flow model of drug release would show that the therapeutic lens, under flow conditions similar to those in the human eye, would increase the release time and may provide a more linear and sustained release profile.

7.2 Materials and Methods

Acrylic acid (AA), acrylamide (AM), 2-hydroxyethylmethacrylate (HEMA), N-vinyl 2-pyrrolidone (NVP), azobisisobutyronitrile (AIBN) and ketotifen fumarate were purchased from Sigma-Aldrich (Milwaukee, WI). Polyethylene glycol (200) dimethacrylate (PEG200-DMA) was purchased from Polysciences, Inc. (Warrington, PA). All chemicals were used as received. Polymer and copolymer networks were made using various mixtures of above monomers (e.g. poly (AA- co- AM- co- HEMA- co- PEG200DMA), poly (AA- co- HEMA- co- PEG200DMA), poly (AM- co- HEMA- co- PEG200DMA), poly (AA- co- AM- co- NVP- co- HEMA- co- PEG200DMA)).

7.2.1 Synthesis of molecularly imprinted hydrogel networks

Hydrogels of differing compositions were synthesized in a temperature controlled, non-oxidative environment using free-radical UV photopolymerization. Polymer compositions consisted of 5 mole% crosslinking monomer and 95 mole% functional monomer (92 mole% of the backbone functional monomer, HEMA, and the balance 3 mole% as combinations of other functional monomers). The monomer to template ratio was optimized to achieve desired amount of drug loading.

Typically, the reaction solutions consisting of monomers and template molecule were sonicated to produce a homogeneous mixture before adding the initiator. Solutions were
then allowed to equilibrate in darkness in order to facilitate non-covalent complexation at the molecular level. The solutions were transferred to a MBRAUN Labmaster 130 1500/1000 glovebox, where they were purged with nitrogen until oxygen levels were 0.1 to 10 ppm. The solutions were pipetted into trichloromethylsilane-treated glass moulds (6” × 6”), separated by a Teflon frame of varying thicknesses. The polymerization reaction was allowed to run for ten minutes with light intensity, measured using a radiometer (International Light IL1400A), equal to 40 mW/cm² (Dymax UV flood light) at a constant temperature of 36°C. Control gels were prepared without the template molecule, following similar steps. The polymerized gels were removed from the nitrogen atmosphere, submerged into a deionized water bath (Millipore, 18.2 mΩcm, pH 6), and carefully peeled from the glass surface. Circular discs of 13.5 mm and 400 µm or 700 µm were cut with a cork borer. Polymer discs were then washed for several days with deionized water until ketotifen fumarate could no longer be detected by spectroscopic monitoring (Biotek UV-Vis Spectrophotometer). The lenses were then loaded with drug by equilibrating in ketotifen fumarate solution.

7.2.2 Dynamic Therapeutic Release Studies

Kinetic release studies were conducted in artificial lacrimal fluid (6.78 g/L NaCl, 2.18 g/L NaHCO3, 1.38 g/L KCl, 0.084 g/L CaCl2·2H2O, pH 8.59). In the infinite sink studies, gels which had been reloaded with drug were placed in 30 ml of fluid which was continuously agitated with a Servodyne mixer (Cole Palmer Instrument Co.) at 120 rpm. Preliminary experiments were conducted to determine the amount of fluid needed to approximate infinite sink analysis by comparing release rates for a fixed amount of fluid versus release rates when refreshing the fluid at specific various time intervals. Release of drug was monitored
at 268 nm by drawing 200 µL of fluid into a 96-well Corning Costar UV-transparent microplate, and measurements were taken in a Synergy UV-Vis/Fluorescence/Luminescence Spectrophotometer (Biotek). Absorbances were recorded for three samples and averaged. Solutions were replaced after each reading. In the physiological flow studies, the drug-loaded disk was placed within the chamber of the microfluidic device. A KDS101 Infusion Pump from KD Scientific (Holliston, MA) injected lacrimal fluid into the chamber at 3 µL/min, while an outlet line removed fluid from the chamber at the same rate for collection at regular time intervals. Release of drug for two samples was monitored similarly to the infinite sink case.

7.2.3 Microfluidic Chip Design and Fabrication

The microfluidic chips were fabricated by soft lithography.\textsuperscript{67,68,66} Masks defining microfluidic features were designed with AutoCAD 2006 (Auto Desk) and photopotted on transparencies at the resolution of 4000/5000 dots per inch (DPI) through a commercial printing company (CAD/ART Services, Bandon, Oregon). The transparent masks were used to fabricate microstructures of photoresists on a four-inch silicon wafer with photolithography, an established method for semiconductor production. Two different photoresists, SU-8 2025 (Microchem Co., Newton, MA) for 50µm thick fluidic channels and SU-8 2100 (Microchem Co.) for a 550µm very-thick chamber, were used. For the third step, based on the microstructures on a silicon wafer, the microfluidic layer was made out of transparent silicone polymer, polydimethylsiloxane (PDMS). A drug loaded lens was placed in the central chamber and the chip was sealed against a glass plate. To ensure reproducible flow rates and to limit non-specific adsorption to the device, each device had a lifetime of three-five runs or less, as determined by monitoring fluid leakage (e.g., the
surface free energy decreases with time and the forces holding the chip and plate together weaken) and adsorption in separate experiments.

All the processes for manufacturing the devices, including the chip design, mold fabrication, and chip fabrication were carried out in the Alabama Micro-electronics Science and Technology Center (AMSTC) and Nano/Microfluidics Laboratory. The structure is shown in Figure (7.1).

The device is designed to mimic the flow rate of tears but does not fully reproduce other ocular conditions. While the device was operated at ambient temperature, ocular physiological temperature is 35°C and will increase the diffusional transport. In the human eye, the mixing and flow of tears is complicated by the presence of contact lenses. The tear film is thinner and varies in thickness by evaporation between blinks and tear breakup. These factors may also affect drug release, but relative to tear flow rate these effects are small.\textsuperscript{115,123} In future versions of the device, we plan to reproduce more of these conditions \textit{in vitro}.

\subsection*{7.2.4 Dynamic Weight/Volume Swelling Studies and Partition Coefficients}

Recognitive and control gels were dried at room temperature for 24 hours, followed by vacuum drying (T=30 °C, 28 in. Hg vacuum) until no change in dry weight was observed (i.e., less than 0.1 weight percent difference). Dry samples manufactured with and without drug (n=3) were placed in a constant volume of deionized water at 25°C. The gels were weighed by removing the gels from the swelling media at specific time points and blotting with absorbent, lint-free tissue to remove excess surface solvent. When the samples reached equilibrium water uptake the weight swelling ratio at equilibrium (\(q\)) (the weight of the swollen polymer divided by the weight of the dry polymer at equilibrium) was calculated.
Figure 7.1: Structure of microfluidic device for drug delivery evaluation

(a) Schematic of the experimental set-up for contact lens drug delivery evaluation. The hydrogel is placed in the microfluidic chamber between the four posts, lacrimal fluid is flowed through the chip and drug release is measured. (b) Example of microliter/nanoliter flow control and near-plug flow profile in the microfluidic chip (two different food dyes were used). (c) Length of scale of microfluidic chip.
The equilibrium volume swelling ratio \((Q)\) was calculated as the ratio of the swollen gel volume at equilibrium to the volume of the dry polymer. The volume of the gel in the swollen or dry state was obtained by determining its weight in air and in n-heptane, a non-solvent for the polymer, and calculated using Archimedes buoyancy principle. The partition coefficients of the gels (calculated as the ratio of the drug concentration in the gel to the equilibrium drug concentration in solution) were obtained by immersing gels in ketotifen fumarate solution and obtaining the concentrations via mass balances.

Aqueous solubility was measured by saturating ketotifen fumarate in deionized water and stirring overnight. The solution was adjusted to pH 7 and filtered. The concentration was measured by absorbance at 268 nm against a series of ketotifen standards.

Log P octanol/water was calculated as the logarithm of the ratio of the equilibrium concentrations of ketotifen in octanol to ketotifen in water. A volume of 4 mL of a known concentration of aqueous ketotifen solution was shaken with 4 mL of octanol for 24 hours and then let rest for 24 hours. Concentration of ketotifen in octanol was obtained by mass balance.

7.3 Results and Discussion

Among the infinite sink dynamic release studies, the most structurally functional network, poly(AA-co-AM-co-NVP-co-HEMA-PEG200DMA), exhibited an extended release profile for a duration of 5 days within artificial lacrimal solution (80% of drug was released in approximately 4 days) (Figure 7.2). This system also demonstrated the highest loading. Other less functionalized systems demonstrated controlled release for approximately one day. The partition coefficients are 5.65, 7.13, 18.06 and 45.05 for poly
(AA-co-HEMA-PEG200DMA), poly (AM-co-HEMA-PEG200DMA), poly (AA-co-AM-co-HEMA-PEG200DMA) networks and poly (AA-co-AM-co-NVP-HEMA-PEG200DMA) networks, respectively when the gels are placed in solutions of ketotifen fumarate in deionized water of concentration 0.4 mg/mL. Additionally, the bound concentrations of ketotifen within the gels are $5.1 \times 10^{-3}$ mmol/g, $7.4 \times 10^{-2}$, and $4.9 \times 10^{-2}$ mmol/g for poly (AA-co-HEMA-PEG200DMA), poly (AM-co-HEMA-PEG200DMA), poly (AA-co-AM-co-HEMA-PEG200DMA) networks and poly (AA-co-AM-co-NVP-HEMA-PEG200DMA) networks respectively. Ketotifen fumarate is hydrophilic with log$_{10}$ octanol-water partition coefficient of -0.3, and an aqueous solubility of 3.4 mg/mL at pH 7 ± 0.2 and 20°C. This indicates that molecular imprinting and multiplicity of interactions have a greater influence on binding than general hydrophobic interactions.

Table 7.1: Varying ketotifen release rates from AA-AM-NVP lenses under infinite sink conditions

<table>
<thead>
<tr>
<th>Phase</th>
<th>Release Rate (mg/min)</th>
<th>Duration of Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>$3.04 \times 10^{-3}$</td>
<td>120 min</td>
</tr>
<tr>
<td>B</td>
<td>$2.97 \times 10^{-4}$</td>
<td>120 - 2280 min (2 hrs to 2 days)</td>
</tr>
<tr>
<td>C</td>
<td>$1.34 \times 10^{-4}$</td>
<td>2880 - 10620 min (2 days to 7.3 days)</td>
</tr>
</tbody>
</table>

Within a Fickian diffusion process, the fractional mass released depends linearly on $t^{0.5}/L$ at short times or fractional release less than 0.67 with a slope directly proportional to the diffusion coefficient. Poly (AA-co-AM-co-NVP-co-HEMA-PEG200DMA) networks exhibited a ketotifen fumarate diffusion coefficient of $5.57 \times 10^{-10}$ cm$^2$/sec, which was a factor of 9, 7.2, and 13.8 less than poly (AA-co-HEMA-PEG200DMA), poly (AM-co-HEMA-PEG200DMA), and poly (AA-co-AM-co-HEMA-PEG200DMA) networks, respectively (Figure 7.3, Table 7.2). These results show that therapeutic release can be tailored via the memory within the polymer chains, through the arrangement, type, and amount
Figure 7.2: Cumulative release of ketotifen fumarate from HEMA lenses in infinite sink conditions

Cumulative mass released from the poly (AA-co-AM-co-NVP-co-HEMA-co-PEG200DMA) lens. Release rates were calculated for the three phases of release under infinite sink conditions. A was calculated $3.04 \times 10^{-3}$ mg/min over the first 120 minutes, B was $2.97 \times 10^{-4}$ mg/min from 120 minutes to 2 days, and C was $1.34 \times 10^{-4}$ mg/min from 2 days to 7.3 days.
of functionality. This is significant considering all hydrogels exhibited equilibrium volume swelling ratios that were in close agreement with one another suggesting similar structures available for free volume transport. Also, the monomers PEG200DMA and HEMA make up 97% of the feed monomers in each gel, and reaction analysis indicated that most of the double bonds within the systems reacted.\textsuperscript{137}

Because the hydrogels were produced without solvent, the Flory-Rehner equation\textsuperscript{105} can be used with the experimentally derived equilibrium swollen polymer volume fraction to determine network structural parameters such as the molecular weight between adjacent crosslinks, and also the correlation length or network mesh size. Therefore the polymer volume fraction can be used as an indicator that correlates with structural parameters. Figure 7.4 highlights the significant difference in the diffusion coefficient of the different gels as compared to the equilibrium polymer volume fraction in the swollen state. Once again, the equilibrium polymer volume fraction in the swollen state was not statistically different between all the gels.

In order to gauge the appropriateness of the fit to a Fickian mechanism, the log of the fractional drug release was plotted against the log of time. The exponents of all gels in the infinite sink release indicated that they were in agreement with a Fickian diffusion mechanism, where the values of $n$ are approximately equal to 0.5 (Table 7.2).

The fractional release at physiological flow rates for the poly (AA-co-AM-co-NVP-co-HEMA-PEG200DMA) networks shows that under physiological flow conditions, drug is released in a linear manner and at much lower concentrations than conventional infinite sink release studies suggested, indicating that such hydrogel lenses have the capacity to deliver sustained amounts of drug in a constant manner over an extended time period as shown in Figure 7.5. Results demonstrate a slower release of drug with a constant, zero-order rate
Figure 7.3: Fractional release of ketotifen from various HEMA lenses under infinite sink conditions

Fractional release profiles of therapeutic contact lenses for poly (n-co-HEMA-co-PEG200DMA) networks in artificial lacrimal fluid at 25 °C, where n is AA (●), AM (■), AA-co-AM (▲) or AA-co-AM-co-NVP (♦) imprinted networks. The abscissa is time normalized to the square of thickness, (due to slab geometry) as the thicknesses of the gels differed; 700 μm (■, ▲, ●) and 400 μm (♦). (n=3).
Figure 7.4: Diffusion coefficients versus polymer volume fraction in hydrogels releasing ketotifen

The change in transport characteristics is related to the imprinting process rather than a change in overall polymer mesh structure for poly (n-co-HEMA-co-PEG200DMA) networks where n is AA (●), AM (■), AA-co-AM (▲) or AA-co-AM-co-NVP (♦).
Table 7.2: Summary of ketotifen diffusion coefficients, orders of release and swelling data of release for approximately 3 1/2 days (i.e., independent of concentration or time). For the physiological release case, using the empirical power law equation indicates the order of release $n - 1$ is equal to 0.019 ± 0.006 for the physiological flow case. Therefore, zero-order release is achieved by reducing the concentration gradient through the accumulation of ketotifen in the slow moving fluid at simulated physiological tear turnover rates. The importance of matching physiological flow is crucial to the characterization of this delivery system.

Compared to the infinite sink release profile, the cumulative mass released under physiological conditions is reduced by a large amount and is dependent on the volumetric flow rates. In 3.5 days, 45 µg is released at a constant rate of 12.9 µg/day compared to approximately 1,200 µg in the infinite sink release study which shows decreasing rates of release. This is a decrease of a factor of 27. These lenses are about 3-4 times thicker than conventional contact lenses and normalizing for the difference in thickness, with fractional mass released being proportional to the inverse of the square of the thickness, yields 0.8 to 1.4 µg/day release. Conventional ketotifen topical drops deliver approximately 1-1.5 µg/day based on the recommended dosage regimen (assuming the maximum 7% bioavailability).
Figure 7.5: Fractional release of ketotifen fumarate from HEMA lenses under physiological flow

The fraction of ketotifen fumarate released from poly(AA-co-AM-co-NVP-co-HEMA-co-PEG200DMA) lenses in artificial lacrimal fluid at 25°C via steady in vitro physiological flow at 3 μL/min using the microfluidic device (sample size n=2).
within a typical topical peak and valley profile (i.e., Zaditor™ ophthalmic solution concentration is 0.345 mg ketotifen fumarate/mL; assuming a typical eyedrop volume of 20µL per drop at the recommended frequency of administration of 2-3 drops per day yields 1-1.5 µg/day at 7% bioavailability). Therefore, there is strong potential to release therapeutically relevant concentrations of drug from a contact lens platform. However, it is important to note that these calculations should be taken as estimates since the lens release studies were conducted at a temperature of 25°C and not 35°C, the temperature of the eye. At physiological temperature the release of drug will be faster from the lens.

This long, constant duration of release of a therapeutic dosage from a contact lens has not been demonstrated previously and is inherently linked to finite tear flow rates. Considering we know the total amount that is delivered from these lenses with the infinite sink study (depending the initial loading concentration), we have delivered less than 5% of the loaded drug in 3.5 days in the physiological flow case. Thus the potential to deliver for extended time periods much greater than a week could be possible with a contact lens platform, depending on the tolerability of the lenses in the eye. Furthermore, the slowed depletion of the drug concentration in the gel and the non-zero concentration of the drug in the surrounding fluid produce effectively zero-order release kinetics. A near zero-order release profile could be maintained for a much greater amount of time, for at least a week.

These studies demonstrate that it is imperative to evaluate hydrogel release kinetics with a non-zero bulk concentration if finite turnover conditions hold true for the in vivo environment. The results clearly demonstrate that release from imprinted lenses is further delayed in an in vitro environment by matching the ocular volumetric flow rates. This effect may be due to two reasons - finite tear turnover rates which lead to significant boundary layers compared to the finite sink case, and molecular imprinting strategies which lead
to delayed release kinetics despite equivalent effective volume of transport through the polymer chains. Within the device, both lens surfaces are in contact with the flowing fluid and the lens rests on the bottom surface of the device. The average velocity in the device is $5.5 \times 10^{-4} \text{m/s}$ considering a cross-sectional area of $8.69 \times 10^5 \text{µm}^2$ (560 µm height by 1,600 µm width) and a volumetric flow rate of $3 \text{µL/min}$. This translates to a Reynolds number of approximately 2-8 along the length of the lens (using physical properties of water at 25°C), indicating laminar flow. At these velocities mass transfer effects in the fluid cannot be neglected and significant boundary layers will exist.

The microfluidic release rate is lower and more constant compared to infinite sink conditions. Within infinite sink conditions there is sufficient fluid volumes producing a maximum concentration driving force and stirring which disrupts boundary layers. It is clear that boundary layer effects are important to the differences in release comparing the microfluidic and the infinite sink cases. It is premature to ascertain the effect of imprinting on these results, but the infinite sink case and polymer volume fraction analysis highlights a potential mechanism for delayed transport via imprinting.

The mechanism of delayed transport due to imprinting is hypothesized to consist of multiple on-off binding interactions between ketotifen and the memory “sites” consisting of multiple functionality within the network. Thus as ketotifen moves through the network its transport is slowed down due to interactions with the polymer chains. Also, molecular imprinting strategies pull the technology into a clinically significant reality by enhancing the therapeutic loading, which is crucial for achieving therapeutically relevant delivery levels. Further control of the tear flow rate with a partially non-wetted surface, mixing similar to the flow profiles induced by the lid blinking process, appropriate tear film, and addition of protein and lipid to the artificial lacrimal fluid are warranted for further study in the
near future, and will ultimately confirm the high potential for drug releasing contact lenses. However the results from the device, as presented here, are a much better approximation to the actual ocular conditions than the infinite sink model.
The release data of hyaluronic acid from imprinted Nelfilcon hydrogels reveals interesting relationships between the amount and variety of functional monomers added to the prepolymer. The cumulative mass of HA released and the diffusion coefficient decrease with increasing functional monomer composition. The cumulative mass released under no functional monomer addition is 200\( \mu \)g, with a diffusion coefficient of 5.689 \( \times 10^{-10} \) cm\(^2\)/s. When the functional monomer content increases to 0.25\% of the prepolymer by mass, the mass released is 17.5\( \mu \)g with a diffusion coefficient of 1.797 \( \times 10^{-10} \) cm\(^2\)/s. The HA mass released decreases as the proportion of DEAEMA increases, but the diffusion coefficient is lowest in the presence of all functional monomers. The hydrogel containing DEAEMA exclusively has a cumulative release mass of 16.2 \( \mu \)g and a diffusion coefficient of 5.306 \( \times 10^{-10} \) cm\(^2\)/s. The hydrogel containing AM and NVP with no DEAEMA has a cumulative released mass of 138 \( \mu \)g and a diffusion coefficient of 4.824 \( \times 10^{-10} \) cm\(^2\)/s. The hydrogel with all three functional monomers has a cumulative released mass of 54\( \mu \)g and diffusion coefficient of 3.553 \( \times 10^{-10} \) cm\(^2\)/s. Additionally, the diffusion coefficient is negligible when functional monomer \%-by-mass greater than 0.36\%.

The optimum composition for functional monomers would release the maximum amount of HA, so that less of the HA initially added to the system would be permanently entrapped. It would also have a low diffusion coefficient so that the release rate over the first 24 hours would be more linear.
Future work on the system could potentially optimize the amounts and proportions of the various functional monomers to determine what hydrogel composition would release higher amounts of HA with lower diffusion coefficients in order to extend the release over longer periods of time. The optimum monomer content would be between 0.25% and 0.361% by mass of the prepolymer, while the fraction of DEAEMA among all functional monomers would be between 0 and 0.5.

Before practical implementation of the HA releasing imprinted hydrogels, assurances need to be made that the monomers, particularly acrylamide, will not have a toxic effect on the ocular tissues. One potential strategy is to synthesize oligomers of the required functional monomers, and then attach these to the PVA chains of the Nelfilon macromer. In keeping with the technique used to functionalize PVA, the oligomers could be modified with an aldehyde or ketone moiety on one of the oligomer terminals. A transacetylation reaction could attach the oligomers to the PVA as pendant groups at defined intervals. The new macromer structure could be optimized by varying the intervals between oligomer attachments. Furthermore, the oligomers could be varied by length of the chain, and the nature and size of the repeating unit. A useful design goal would be to synthesize an oligomer that folds to form a binding site similar to that found in hyaluronic acid binding protein.

Future improvements in the microfluidic device for simulation of ocular environment for drug delivery devices would bring the device closer to ocular conditions in terms of temperature, blink forces, protein content in the tear fluid and other factors.
Bibliography


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The first objective is to ensure that the lens is capable of delivering a therapeutic dosage of HA over an acceptably long time period. Too low a dosage would be insufficient for treatment, while an excessive dosage would be economically unfeasible.

We selected three concentrations of HA in Nelfilcon, made lenses, and conducted release studies to help us benchmark the appropriate amount of HA to add to the formulation. Figure A.1 shows the release curves obtained from lenses made with formulations containing 2 mg/g, 6.5 mg/g and 40 mg/g of HA in Nelfilcon.

Release was measured over 24 hours. The 2 mg/g formulation released 15 µg over the first 6 hours, and then ceased to release any more. The 6.5 mg/g formulation released 41 µg over the first six hours, and then decreased its release rate. The 40 mg/g formulation released 581 µg for the first 6 hours, with irregular release thereafter. The 6.5 mg/g and 40 mg/g lenses released HA for at least 24 hours. In contrast, lenses with the formulation 2 mg/g released the maximum amount of HA within the first 6 hours.

<table>
<thead>
<tr>
<th>Hydrogel</th>
<th>Diffusion coefficient</th>
<th>Std dev.</th>
<th>$R^2$</th>
<th>Order</th>
<th>Std. dev.</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nelfilcon with 6.5 mg/mL HA</td>
<td>$5.689 \times 10^{-10}$</td>
<td>$0.005 \times 10^{-10}$</td>
<td>0.99</td>
<td>0.609</td>
<td>0.018</td>
<td>0.99</td>
</tr>
<tr>
<td>Nelfilcon with 40 mg/mL HA</td>
<td>$6.370 \times 10^{-10}$</td>
<td>$0.054 \times 10^{-10}$</td>
<td>0.91</td>
<td>0.599</td>
<td>0.109</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Table A.1: Diffusion and release order of Nelfilcon hydrogel with 6.5 and 40 mg HA/g

It appears that the diffusion coefficient does not vary significantly with the concentration of HA in the Nelfilcon lens, and the released amount of HA depends on the concentration incorporated into the hydrogel. The 6.5 mg/g formulation gave the closest match to the desired HA delivery rate, thus we selected it as the basis for all future experiments.
Figure A.1: Cumulative release of different concentrations of HA from Nelfilcon hydrogels
Release studies were done on Nelfilcon lenses containing 2 mg/g (▲), 6.5 mg/g (♦) and 40 mg/g (■) of HA in the prepolymer mixture. No functional monomers were added.
APPENDIX B

DYNAMIC RELEASE OF HA OF VARIOUS SIZES

As HA is a long-chain molecule, we predicted that it would encounter difficulty in diffusing from the hydrogel. A small molecule would encounter relatively little hindrance from the polymer network as it diffuses out of the hydrogel. The diffusion coefficient would therefore be relatively high. As the molecular weight of the drug increases and the effective radius becomes comparable to the polymer mesh size, it faces more steric hindrance and the diffusion coefficient decreases. We conducted dynamic release studies to determine the effect of the size of HA on the diffusion coefficient using HA of different molecular weights. We synthesized HA-Nelfilcon lenses with 6.5 mg of HA per g of Nelfilcon, using HA of three different sizes: 1 million Da, 100,000 Da and 50,000 Da. The fractional release profiles are shown in Figure B.1.

Figure B.1 is a plot of the release profiles of HA of different sizes normalized to the maximum amount of HA released. It is evident that the smaller HA molecules diffused out of the hydrogel lenses rapidly, compared to the largest HA molecule which was diffusing out of the lens for up to 3 days.
Figure B.1: Fractional release of various sizes of HA from Nelfilcon hydrogels

Release studies were done on Nelfilcon lenses containing 50 kDa (▲), 100 kDa (■) and 1 MDa (♦) of HA in the prepolymer mixture. No functional monomers were added.
Appendix C
Tensile testing of Hydrogels

To evaluate the tensile strength of the Nelfilcon based hydrogels, the synthesized hydrogels were cut into strips and extended uniaxially. The hydrogel sample made with Nelfilcon without added HA or functional monomers was tested in duplicate, while the 6.5 mg/g HA in Nelfilcon sample, the 0.25% functional monomers in Nelfilcon sample, and the 0.25% functional monomers and 6.5 mg/g HA in Nelfilcon sample were all measured in triplicate as shown in Figures C.1 to C.11.
Figure C.1: Tensile test Nelfilcon– sample 1

$y = 193120x - 3905.8$

$R^2 = 0.9955$

Figure C.2: Tensile test Nelfilcon– sample 2

$y = 209335x - 7547.9$

$R^2 = 0.9997$
Figure C.3: Tensile test Nelfilcon with HA– sample 1

Figure C.4: Tensile test Nelfilcon with HA– sample 2
Figure C.5: Tensile test Nelfilcon with HA– sample 3

Figure C.6: Tensile test Nelfilcon with functional monomers– sample 1
Figure C.7: Tensile test Nelfilcon with functional monomers– sample 2

Figure C.8: Tensile test Nelfilcon with functional monomers– sample 3
Figure C.9: Tensile test Nelfilcon with functional monomers and HA – sample 1

\[ y = 192832x - 1585.4 \]
\[ R^2 = 0.9976 \]

Figure C.10: Tensile test Nelfilcon with functional monomers and HA – sample 2

\[ y = 206752x - 4173.3 \]
\[ R^2 = 0.9951 \]
Figure C.11: Tensile test Nelfilcon with functional monomers and HA – sample 3