To my family and friends
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Currently millions of people in the United States suffer from eye ailments such as glaucoma, infections, cataract, macular degeneration, dry eyes etc. and the number is likely to increase due to the continuous increase in the mean age in the country. Most current ophthalmic drugs are delivered through eye drops, which are relatively inefficient due to rapid clearance from the ocular surface. Improvements in ophthalmic drug delivery require a thorough understanding of various transport mechanisms in the eyes so that optimal delivery vehicles and devices could be developed. This thesis focuses on understanding and modeling various transport processes in the eyes to predict the effectiveness of various drug delivery approaches. The thesis also focuses on development of novel devices for drug delivery, and modeling release from the devices in vitro and predicting the release behavior in vivo.

Ophthalmic drugs that are instilled via eye drops get cleared through tear drainage from eyes to the nose, and through transport across various ocular epithelia. We focused on understanding each of these pathways individually, and then combining these into a comprehensive model that can be used to predict the bioavailability of drugs delivered through eye drops or through any other device. While permeability
values are reported in literature for transport of drugs across cornea and conjunctiva, these are based on assumptions of pseudo-steady state which are likely not accurate. To understand the detailed transport mechanisms in the eyes, we exposed an excised rabbit cornea to fluorescent drug analogs and measured the time and position dependent fluorescence through a confocal microscope. The profiles were than fitted to mechanistic multiscale diffusion-binding models to obtain various transport parameters. These studies were conducted for a hydrophobic molecule Rhodamine B and a hydrophilic molecule fluorescein. In future, similar studies will be conducted to obtain the detailed models for various ophthalmic drugs. Based on our models and previous existing tear drainage models, we demonstrate that the bioavailability increases when drugs are delivered through extended release devices rather than through eye drops. We thus also focused on developing some extended release devices for ocular applications particularly for treatment of dry eyes. We developed conjunctival inserts and puncta plugs that can deliver dry eye drug cyclosporine A for about 3 months at therapeutic doses. These devices will likely increase bioavailability and also improve patient compliance. These devices were prepared by thermal polymerization in presence of drug at high loadings to create inserts and plugs containing particles of drug dispersed in the matrix. The drug release rates were measured to explore the effect of length, drug loading, crosslinking, and mixing in the release medium. Mathematical models were developed and simulated to understand the mechanism of transport of drug molecules inside these polymeric devices. A model combining tear drainage and tear balance is used to predict that our plugs can deliver similar amount of drug as Restasis® eye drops, the only approved medication for dry eyes.
The results from this thesis will hopefully lead to a better understanding of various transport mechanisms in eyes, and to development of better drug delivery vehicles. While the main focus of this work is in ophthalmology, the mechanisms for transport across epithelia and also from drug delivery devices may find applications in other areas of biomedical engineering.
CHAPTER 1
INTRODUCTION

More than 100 million people in the United States suffer from mild or severe eye ailments. Eye drops are the most common treatment for most of the ocular disease [1,2]. But, it has been well established that drug delivered through eye drops have very less bioavailability (<5%). When a drug is delivered through eye drops, it leaves the body through two routes. One route is that it gets absorbed across the outer most membrane of the eye named as cornea and other route is getting eliminated along with the tears through the tear drainage. Most of the ocular drugs have to traverse through cornea to reach the target tissues. Therefore bioavailability of drugs delivered through eye drops or any other ocular device would be determined by the amount absorbed across the cornea relative to the amount eliminated through tear drainage. Therefore, there is a need to understand the transport of drugs or eye drops in front surface of the eye and based on this develop better ophthalmic treatments. In this research we have mainly focused on developing drug delivery devices for dry eye treatment which is a medical condition in which there is a lack of moisture or tear film on the corneal surface. Devices such as conjunctival inserts and punctal plugs have been proposed which could solve the problem of low bioavailability and improve patient compliance.

Eye drops have low bioavailability because more than 90% of the drug instilled is lost from the ocular surface by tear drainage towards the nose and only a small fraction gets absorbed across the cornea, sclera, and/or conjunctiva. The rate of tear drainage is determined by the blink action, which pumps the tears from the ocular surface into the nasolacrimal duct. Consequently, the topically applied drug is absorbed across the nasal mucosa into systemic circulation. This fraction of the applied drug along with the
drug absorbed across the conjunctival epithelium may provoke systemic side/adverse effects. Thus, topical administration, besides having the potential for systemic toxicity, results in a rapid decay of the drug concentration on the ocular surface[1-5]. Since half-life of the drug on the ocular surface is only 4-6 min [1,2][3,4] and because of potential for systemic toxicity, there is a need for rational design of topical drugs such that kinetics of their penetration into the intraocular structures of the eye is maximized.

As indicated above, most topical drugs access the intraocular structures by penetration across the cornea. Trans-scleral penetration is known to play a role only for a few drugs [1,2,4,5]. The cornea is a transparent structure with a central matrix of connective tissue called the stroma bounded by cellular layers. The anterior cellular layer is the epithelium. This layer is stratified into ~5-6 layers and is ~40 μm thick in humans. The superficial epithelium forms the main barrier to the penetration of topically applied hydrophilic drugs as exhibits multi-stranded tight junctions [6]. Accordingly, lipophilic drugs penetrate the epithelium readily presumably by dissolving in the lipid bilayers of the plasma membrane and subsequent movement by the transcellular route. For further transport towards the anterior chamber, however, the ability to partition into the stroma would be an important consideration for lipophilic drugs. In fact, the stroma being hydrophilic with > 80% water, offers no more resistance than an equivalent thickness of water layer for most topical drugs [7]. Thus, although the drugs may diffuse readily in the stroma, poor partitioning of lipophilic drugs would be a determinant for transport across the cornea. Finally, the posterior layer of the cornea is a monolayer of leaky endothelium, and thus does not offer much resistance to the paracellular movement of solutes. This multi-laminate structure (i.e., oil:water:oil layers) of the
cornea, shown by the schematic in Figure 1-1, strongly suggests the importance of lipophilicity of the drug for its trans-corneal penetration.

Pharmacokinetics of topical drugs has been frequently described by compartmental models [8, 9,10,11] which assume that drug concentration becomes uniform throughout the cornea instantaneously after topical administration. Hence, the compartmental models disregard the heterogeneity (i.e., multi-laminate structure) of the cornea and diffusive nature of transport in each of its layers as described above. This over-simplification has led to a poor understanding of the dynamics of trans-corneal drug transport and consequently to empirical approaches for design of dosage regimen. Several attempts have been made to model the transport across the cornea that takes into consideration of the multi-laminate structure [12-14]. In the absence of measurements of trans-corneal concentration profiles, the distributed parameter models developed to date are based on general considerations, and accordingly, assumptions used in such models have never been tested for robustness of the models. In essence, it is difficult to determine which transport step among those involved in the drug penetration forms a key determinant of the bioavailability and rate of penetration.

The overall trans-corneal penetration of drug could be broken down into elementary steps consisting of drug-cell binding/partition dynamics, interfacial resistance to transport, and/or transport dynamics inside the cells. Since measurement of concentration vs. depth profiles of real drug molecules is not easily achieved, in this study we have employed a fluorescent dye Rhodamine B (RhB) as a lipophilic drug analog [15]. and hydrophilic fluorescein as hydrophilic drug analaog, and determined their concentration profiles across the cornea by a custom-built confocal fluorescence
microscope. The transient concentration profiles have been used subsequently to develop a phenomenological non-compartmental pharmacokinetic model. Thus, for the first time, the model reported herein exemplifies a microscopic approach to correlate the physico-chemical properties of drug analogs to their transport properties across the cornea.

As discussed above, this thesis also focuses on developing drug delivery devices for dry eye treatments. Currently about one in seven Americans suffer from dry eyes, which is an ocular condition characterized by a dryness sensation in the eye accompanied by a foreign-body sensation, discomfort, tearing, burning, and blurred vision. Furthermore, many of the contact lens wearers suffer from mild or severe dry eyes [16-20]. The dryness in the eyes is potentially caused by tear imbalance that could lead to a loss of proper lubrication, leading to discomfort. The dry eye symptoms can ultimately lead to inflammation of ocular surface and epithelial cell damage, which in turn reduces the production of tears or mucus leading to a further decrease both the quality and quantity of tears. Dry eyes symptoms are commonly alleviated by instillation of artificial tears [21] which results in an increase in tear volume. The tear volume slowly returns to its steady state value within a few minutes [22] due to tear drainage through the canaliculi, and fluid loss through other means such as evaporation or transport across the ocular epithelia. Therefore only a temporary relief is achieved through the use of eye drops or artificial tear formulations. The residence time and thus the efficacy of the artificial tears could be increased by addition of viscosity enhancers [23-25]; however a high viscosity leads to increased shear on the ocular surface, which causes discomfort.
In 1935, it was first proposed that the tear volume could also be increased through occlusion of the tear drainage route by inserting a punctal plug into the canaliculi [26] which connects eyes to the nose. Currently, occlusion of puncta through punctal plugs is the most common non medical treatment of dry eyes [27]. Insertion of puncta plugs has been reported to improve disorders related to inflammation of conjunctival and corneal epithelial cells [28], and also improve vision for dry eye patients [29]. Also, insertion of punctal plugs has been reported to improve tear film stability and tear osmolarity [30].

Another potential treatment of dry eyes is administration of cyclosporine A, which is an immuno-suppressant that is often used for prevention of transplant rejection [31,32] and has shown promising results in the treatment of dry eyes [33,34]. When applied on the ocular surface, cyclosporine A is believed to inhibit T cell activation [35,36] that are responsible for production of inflammatory substances. These inflammatory substances apart from performing tissue damage also lead to activation of more T cells which in turn produce more inflammatory substances [37]. Due to low solubility of cyclosporine A in water, its delivery through aqueous eye drops will lead to very low bioavailability and potential side effects due to systemic uptake of the drug. To increase bioavailability and residence time on the ocular surface, other delivery methods such as ointments have also been used, but they may cause discomfort due to increased shear on the corneal epithelium [38,39]. Optimmune® [Schering Plough], 0.2% USP ophthalmic ointment has been approved for veterinary use [40] but is not being used for human beings because of its poor acceptability by patients [41] Effect of several permeation enhancers on transcorneal permeation of cyclosporine A has been
studied [42], but in spite of showing promising results their low corneal tolerance limit their usage. Also PLGA implants loaded with cyclosporine A have been developed to deliver the drug through subconjunctival route [43], but these are invasive leading to reduced patient acceptability. Currently, cyclosporine A is delivered through 2-drops per day of oil-in-water emulsion (Restasis®, Allergan) that deliver about 28 μg (assuming a drop volume of 28 μl) of drug to the eye [44]. While delivery of cyclosporine A through Restasis® is therapeutically effective, it still suffers from issues of low bioavailability and low residence time due to rapid clearance of the eye drops from the ocular surface, perhaps leading to limited efficacy. Also, cyclosporine A levels delivered by Restasis® are not sufficient to prevent rejection after corneal allograft, therefore a in situ gelling microemulsion formulation has been proposed in [45] which claims to have higher concentrations and higher duration of cyclosporine A in cornea as compared to Restasis®

Since both punctal plugs and cyclosporine A improve dry eye symptoms, albeit due to different reasons, it was speculated and then shown in a clinical study that a combination therapy including punctal plug and instillation of cyclosporine A could have additive therapeutic effect [46]. In the study by Roberts et al., dry eye patients were randomized to 1 of 3 treatments: cyclosporine A 0.05% ophthalmic emulsion (RESTASIS®) twice daily, lower-lid punctal plugs (PARASOL), or a plugs-cyclosporine A combination. Results showed that the combination therapy produced the greatest improvement. While the combination therapy was useful, it still suffers from issues related to rapid clearance of cyclosporine A and difficulties in eye drop instillation particularly for elderly subjects. Here we propose a novel approach of providing the
combination therapy by designing punctal plugs which also release cyclosporine A for extended periods of time.

**Plug Design:** A punctal plug is inserted into the canaliculi to block drainage of tears from the ocular surface to the nose. According to anatomical studies, each canaliculus has a vertical part that is about 2 mm long and a horizontal part that is about 10 mm long [47]. The diameter of the vertical and the horizontal parts are about 0.3 mm and 0.5 mm, respectively. The joint between these two parts is called the ampulla and its diameter can be up to 2 to 3 mm. The commercial punctal plugs range in length from 1.1 to about 2 mm and in diameter from 0.4 to 1.1 mm. The punctal plugs are inserted into the vertical portion of the canaliculi. Typical drug eluding puncta plug designs described in patent literature consist of cylindrical cores coated with an impermeable shell to minimize the drug loss into the canaliculus tissue [48-50]. In such devices, the drug essentially diffuses out from the circular cross-section in contact with the tears.

The punctal plug design proposed here and shown in Figure1-2. is novel as only a fraction of the plug length is covered with an impermeable shell. The diameter of the shell is 0.94 mm to ensure a snug fit into the canaliculus and diameter of the core is 0.51 mm. Since only a fraction of the total plug length is covered with the shell, the drug will diffuse out from the circular cross-section and also the exposed curved surface. The drug molecules released from the curved surface will diffuse axially through the tear filled canaliculus into the tears in about 15-30 minutes due to the short axial distance of about 1 mm that molecules travel to reach the ocular tear volume. The uptake of the canalicular tissue during this time may not be significant but if this is an issue, the device can be further modified by including an impermeable sleeve that covers the
entire device. The advantage of the design proposed here compared to prior designs proposed in literature is that the release from the curved surface presents another variable that can be optimized to obtain suitable release rates. Additionally, if the release occurs only from the circular cross-section in contact with the tears, the release rates may depend strongly on the degree of mixing in the tear fluid in the canthus region, and could also be significantly impacted by protein binding to the cross-section. To our knowledge, there are no prior publications on release of ophthalmic drugs from punctal plugs. Also the design described above and shown in Figure 1-1 is novel and significantly different from those in patent literature for drug eluding puncta plugs.

Also, we propose to deliver cyclosporine A through conjunctival inserts which can be placed in the inferior conjunctival sac of the eyes. Several researchers have explored ophthalmic drug delivery through inserts [51-56] and some commercially available inserts such as Ocusert and Lacrisert® have been used to treat glaucoma and dry eyes, respectively. Lacrisert®, the conjunctival insert prescribed for treating dry eyes, is a 3.5 mm long and 1.27 mm diameter insert made of hydroxypropyl cellulose [57]. The insert dissolves over a period of a day after insertion leading to increased tear viscosity and lubrication. The inserts proposed here were chosen to be geometrically similar to lacrisert® and thus have a length ranging from 4 mm to 10 mm and a diameter ranging from 1.02 to 1.47 mm. Also, according to a few authors the cylindrical shaped inserts are best for retention in the conjunctival sac [58-60]. Moreover, the inserts proposed here are prepared from HEMA and EGDMA, which are both commonly used in ocular applications such as contact lenses.
In addition to the goal of development of punctal plugs and conjunctival inserts, this research also aims to focus on the mechanisms of drug transport in the cylindrical inserts and plugs, particularly for situations in which the drug loadings are so high that the drug is dispersed in the gel as particles. The drug transport in such systems involves a combination of particle dissolution, diffusion in the gel, and diffusion in the surrounding fluid. Also, drug transport in the gel is impacted by drug binding on the polymer. This research focuses on developing a comprehensive model that accounts for each of these issues.

Furthermore, the pharmacokinetic models proposed here are novel and are very helpful in preliminary evaluation of the efficacy of the devices at treating dry eyes. Also, chapter 5 in thesis deals with evaluating bioavailability of Restasis® eye drops to compare the effectiveness of our devices with commercially existing solutions for dry eyes.
Figure 1-1. Cornea as a Oil:Water:Oil multi-laminate. This is a well accepted pharmacokinetic view of the cornea. However, most pharmacokinetic models to date assume either the whole cornea as a single compartment or three well-stirred compartments. The innovation of the model in this study assumes the three layers of the cornea to be uniform and their transport across each layer to occur by diffusion.
Figure 1-2. Schematic and Image of the drug loaded punctal plug (not to scale).
TRANSCORNEAL PENETRATION OF RHODAMINE B ACROSS RABBIT CORNEA

2.1 Introduction

This chapter focuses on understanding transport of Rhodamine B, a lipophilic drug analog, across rabbit cornea. Cornea consists of three layers namely epithelium, stroma and endothelium. A comprehensive model is developed to explain the transient concentration profiles in all the three layers when tear side of cornea is exposed to a fixed concentration of Rhodamine B dye.

2.2 Materials and Methods

RhB (Cat # R6626; MW: 479; CAS Number 81-88-9) and all other reagents for Ringers solution were obtained from Sigma Chemical Company (St. Louis, MO). Eyes were obtained from freshly killed albino (New Zealand White) rabbits of either sex. All procedures for animal handling were in accordance with the guidelines set by the Association for Research in Vision and Ophthalmology (ARVO) and approved by Laboratory Animal Care Committee in the laboratory of (late) Prof. David Maurice, Ophthalmology at Stanford University (SP. Srinivas). The corneas were isolated and mounted as previously described [61,62]. They were maintained at 34°C by circulating water through the jacket and perfused with HCO₃⁻ Ringers (containing reduced glutathione, glucose, adenosine, 40 mM HEPES, and 40 mM NaHCO₃) at the anterior and posterior surfaces [63].

The trans-corneal profiles of RhB were obtained using a custom-built confocal scanning microfluorometer, as described previously [61,62]. About 30 minutes after mounting the cornea, the epithelial surface was exposed to RhB dissolved (1 μg/mL) in the Ringers. Depth scanning was performed through a stepper motor mechanically
coupled to the fine focus knob of the microscope. Depth resolution was ~ 8 μm at a sensitivity of 10^{-6} gm/mL of fluorescein (SNR > 20) using a 40x water immersion objective of 0.75 NA (Zeiss Inc) [62,64]. Scanning was performed at ~ 600 μm/min over 800 μm depth. Scatter and fluorescence scans were measured to obtain corneal thickness and trans-corneal concentration profile of RhB, respectively. More than 6 experiments were performed and data from one typical experiment is considered in this study for analysis. The experimental data alone were presented in an abstract form by Srinivas and Maurice previously at the Association of Research in Vision and Ophthalmology [65].

2.3 Results

2.3.1 Transcorneal Penetration of Topical Rhodamine B

The implications of the multi-layer oil:water:oil structure of cornea, as well as the shortcomings of the compartmental models, are illustrated by our experimental observations of the penetration of RhB, as shown in Figure 2-1. It is clearly evident that RhB distributes across the cornea with distinct fluorescence discontinuities at the cellular boundaries between the epithelium and stroma as well as between stroma-endothelium. Furthermore, in the epithelium and stroma, the concentration is non-uniform. Also noteworthy is that the RhB fluorescence is elevated in the lipophilic cellular layers (epithelium and endothelium) relative to its level in the hydrophilic stroma. The concentration discontinuities and the increased levels in the cellular layers are indicative of the preferential partitioning of RhB into the lipophilic structures across the cornea. The time- and position-dependent fluorescence gradients, apparent in the epithelium and stroma, indicate diffusional resistance for RhB transport. These
observations, in turn, suggest that the epithelium and stroma are not well-stirred and cannot be construed as homogenous compartments let alone the entire cornea. Therefore, penetration of RhB cannot be described by conventional compartmental models.

In addition to the above general observations on the penetration characteristics of RhB, the fluorescence profiles in Figure 2-1 also suggests the following: (a) The fluorescence at the epithelial surface reaches a high value within 6 minutes, and then increases at a decreasing rate, (b) The fluorescence gradient in the epithelial layer shows a sharper gradient at ~ 140 min compared to earlier times. (c) A peak in the epithelial fluorescence profile begins to evolve for t > 30 min, and is quite distinct at 140 min at the epithelium-stroma interface. (d) The gradient of fluorescence in the stroma reaches a near constant value at 30 min. (e) The fluorescence peak in the endothelium appears after 30 min, and the peak concentration increases slowly. Key observations that should be included in modeling include: (a) accumulation of RhB in the epithelium and endothelium, (b) negligible accumulation in the stroma, and (c) appearance of the peak at the epithelium-stroma interface.

2.3.2 Model for Representing the RhB Penetration Kinetics

In this section, we develop a general mathematical model for trans-corneal penetration of a lipophilic solute. Its parameters will be estimated subsequently from the unsteady state trans-corneal profiles of RhB shown in Figure 2-1. In accordance with the physio-chemical properties of RhB, we assume that RhB is not metabolized during transport across the cornea.
2.3.3 Transport across the Cellular Layers

The first step in the transcorneal penetration of a topically administered lipophilic solute is its partitioning into the lipid bilayers of the plasma membrane (referred to as the epithelial or lipid bilayer) of the superficial corneal epithelium that is in contact with the tears. Depending on the octanol-water partition coefficient, a fraction of the solute in the epithelial bilayer will partition into the hydrophilic cytoplasm. Once in the cytoplasm, the solute could also partition into putative intracellular lipophilic domains, such as the lipid membranes of the intracellular organelles (e.g., endoplasmic reticulum) (Figure 2-2). We model these steps as follows:

The rate of transport from the epithelial bilayer to intracellular domains is expressed as the product of a rate constant ($k_1$) and the net driving force for transport $(C_1 - \frac{C_1^b}{K_1})$, where $C_1$ is the average concentration of the solute in the epithelial bilayer, $C_1^b$ is the average concentration in the intracellular lipophilic domains, and $K_1$ is the ratio of $C_1^b$ and $C_1$ at equilibrium. More precisely, $C_1$ is the concentration in the lipid bilayers based on total cell volume, i.e., it is the product of the actual concentration in the bilayers and the volume fraction of the bilayers in the epithelial cells. Similarly, $C_1^b$ is the product of the concentration in the internal hydrophobic regions and the volume fraction of such regions in the cell. The parameter $k_1$ is the permeability of cytoplasm that separates the epithelial bilayer from intracellular lipophilic domains.
Based on the above model for accumulation in the intracellular domains, transient
mass balance of the solute in the corneal epithelium and its association with the
intracellular lipophilic domains can be written as

\[
\frac{\partial C_1}{\partial t} = D_1 \frac{\partial^2 C_1}{\partial y^2} - k_1 \left( C_1 - \frac{C_1^b}{K_1} \right) \\
\frac{\partial C_1^b}{\partial t} = k_1 \left( C_1 - \frac{C_1^b}{K_1} \right)
\]

(2-1)

In the above and subsequent equations, the \( y \)-coordinate refers to the depth
across the cornea; \( y = 0 \) is at the tear-epithelium interface and \( t \) refers to time (Figure 2-3). The parameter \( D_1 \) is the effective diffusion coefficient in the lipid bilayer. For ease of
reference, we have compiled all the parameters of the model in Table 2-1. The second
term on the right side of the mass balance can also be interpreted as a first order-
reversible binding of the solute. Since the expression for the rate-limiting binding is
mathematically identical to that for diffusion limited rates of transport into the internal
hydrophobic domains, we combine the two rate mechanisms together.

Since the posterior surface of the cornea is a cellular layer, we assume that the
model for transport across the endothelium is mathematically similar to the epithelium.
Thus, the trans-endothelial solute transport is described:

\[
\frac{\partial C_3}{\partial t} = D_3 \frac{\partial^2 C_3}{\partial y^2} - k_3 \left( C_3 - \frac{C_3^b}{K_3} \right) \\
\frac{\partial C_3^b}{\partial t} = k_3 \left( C_3 - \frac{C_3^b}{K_3} \right)
\]

(2-2)
In the above formulation, we use subscript 3 to refer endothelium. Thus, the parameters $D_3$, $K_3$, $k_3$, $C_3$, and $C_3^b$ have same definitions to the corresponding variables for the epithelium (Table 2-1).

### 2.3.4 Transport across the Stroma

The corneal stroma is composed of ~300 lamellae of collagen fibrils bound with glycosaminoglycans (GAGs) [66]. In general, a solute could bind to collagen and GAGs. We assume that binding-unbinding reactions occur at a faster time scale compared to that of diffusion. This assumption is consistent with the experimental data on diffusion of small molecules in artificial collagen networks, which suggests that the binding-unbinding events are rapid and transport of small solutes is governed by diffusion alone [67]. Therefore, the bound and the unbound forms are always at equilibrium so that we need to address the mass balance for the total solute only. Accordingly, the following describes solute transport across the stroma:

\[
\frac{\partial C_2}{\partial t} = D_2 \frac{\partial^2 C_2}{\partial y^2}
\]  

(2-3)

where $D_2$ and $C_2$ are effective diffusivity and total concentration of the solute in the stroma, respectively (Table 2-1).

### 2.3.5 Boundary Conditions

At the tear-epithelium interface, the solute concentration in epithelium is at equilibrium with concentration in tear fluid. This can be written as:

\[
C_1 = \Phi_{10} C_0
\]

(2-4)
where $\Phi_{10}$ is the partition coefficient between the epithelium and the tears and $C_0$ is the drug concentration in tears (Table 2-1 and Figure 2-3). At the epithelium-stroma interface, we expect concentration equilibrium and flux continuity. We model condition of concentration equilibrium as

$$D_1 \frac{\partial C_1}{\partial y} = k_{\text{perm}} \left( C_1 - \frac{C_2}{\Phi_{21}} \right) \quad (2-5)$$

where $k_{\text{perm}}$ is the permeability of epithelium-stroma interface, and $\Phi_{21}$ is the equilibrium partition coefficient between the stroma and the epithelium (Table 2-1 and Figure 2-3). Inclusion of the permeability at the epithelium-stroma interface in Equation 2-5 is consistent with the observed increasing concentration gradient with time in the epithelium and the development of concentration peak at the epithelium-stroma interface. In Equation 2-5, $1/k_{\text{perm}}$ represents mass transfer resistance at the interface. When $k_{\text{perm}}$ is large, Equation 2-5 correctly reduces to the concentration equilibrium requirement. As discussed later, we have evaluated the model with and without the $k_{\text{perm}}$ parameter. For flux continuity, we impose the following boundary condition at the interface:

$$D_1 \frac{\partial C_1}{\partial y} = D_2 \frac{\partial C_2}{\partial y} \quad (2-6)$$

We now specify similar boundary conditions for the interface between the stroma and endothelium, but we assume that the interface concentrations to be in equilibrium so that
\[
D_2 \frac{\partial C_2}{\partial y} = D_3 \frac{\partial C_3}{\partial y}
\]  
(2-7)

\[C_3 = \Phi_{32} C_2\]

Finally, at the endothelium-aqueous humor interface, the diffusing drug is swept away rapidly from the interface, and thus a reasonable representation of this is to set the concentration to zero (i.e., sink conditions)

\[C_3 = 0\]  
(2-8)

The initial conditions correspond to zero concentration across the entire cornea and the known initial concentration of the solute in the tears.

### 2.3.6 Parameter Estimation

We have employed unsteady state concentration profiles of RhB in Figure 2-1 to estimate the 11 parameters (Table 2-1) in the above model. We first note that the model has no non-linear terms so that the concentration at any point across the cornea would be proportional to the solute concentration in tears. Secondly, at the low concentration used, fluorescence of RhB is linearly proportional to concentration so that measured fluorescence value represents concentration of diffusing RhB. Therefore, we use the fluorescence and the solute concentration across the cornea interchangeably in the following estimation procedures.

### 2.3.7 Initial Guess for Parameter Estimation

For the case of constant concentration in the tears \((C_0)\), the concentration at the tear-epithelium interface \((y = 0)\) can be given by
\[
C_t(y = 0) = C_1(y = 0) + C_0^t(y = 0) = C_0 \left( \Phi_{10} + K_1 \Phi_{10} e^{\frac{k_1}{K_1}} \right)
\]

(2-9)

Since \(C_0\) was constant in our measurements, a plot of experimental \(C_t(y = 0)\) vs. \(t\) can be fitted to Equation 2-9 for estimating \(\Phi_{10}, K_1\) and \(k_1\). At \(t \sim 0\) or at small times, at the epithelium-stroma interface, the ratio of the total concentration at the stroma to the epithelium is the partition coefficient \(\Phi_{21}\). The diffusivity of the solute in stroma \(D_2\) is assumed to be that in free solution, and the diffusivity in epithelium \(D_1\), is approximated by multiplying \(D_2\) with the ratio of average slopes of concentration profiles in the stroma and epithelium. The value of \(D_3\) is expected to be equal to \(D_1\), recognizing that both consist of similar biological cell layer(s). Also, the values of \(k_3\) and \(K_3\) are expected to be equal to \(k_1\) and \(K_1\), respectively. With these initial constraints imposed on the parameters of Equation 2-1 to 2-9, we developed a MATLAB program that estimated model parameters by minimizing the sum of the residual errors (denoted by \(E\)) between the calculated and measured fluorescence (i.e., concentration) values.

The measured fluorescence at a given depth \(y\) arises from the excitation of all fluorophores at the neighborhood, and is obtained by convoluting the concentration profiles with the instrument response function (IRF) given by

\[
IRF(y - y') = e^{\frac{-(y-y')^2}{2\sigma^2}}
\]

(2-10)

where \((2.36 \sigma)\) represents full width of the Gaussian. This was measured to be \(\sim 10 \mu m\) for the 40x objective (Zeiss, 0.75 NA; Water immersion) and a defined excitation and emission slit widths employed during the measurements [61,62,64]. Further, since the
measured fluorescence arises from RhB present both in the cellular bilayers as well as intracellular lipophilic domains, we write

\[ C_{\text{total}} = C_i + C_i^b \]  \hspace{1cm} (2-11)

where \( C_i \) and \( C_i^b \) represent concentrations in the cellular bilayers and intracellular lipophilic domains. Note \( C_i^b \) and \( C_3^b \) represent the bound RhB in the epithelium and endothelium, respectively.

To compare the model prediction (\( C_{\text{Model}} \)) with measured fluorescence values, we performed convolution of \( C_{\text{total}} \) with IRF as

\[ C_{\text{Model}}(y') = \int_{y}^\infty C_{\text{total}}(y) \text{IRF}(y - y') \, dx \]  \hspace{1cm} (2-12)

We define the following objective function for estimating the model parameters

\[ E = \sum_{i=1}^{3} \sum_{k=1}^{N_i} \left[ C_{\text{Model}}^{\text{Exp}} - C_{\text{Exp}}^{\text{Exp}} \right] \]  \hspace{1cm} (2-13)

where \( C_{\text{Exp}} \) is the measured concentration at a given position and time \( t \), \( N_i \) is the total number of data points in the \( i^{th} \) layer, with \( i = 1 \) to 3 (representing the three corneal layers), and \( C_{\text{Model}} \) is RhB concentration predicted by the model. The number of points in each layer \( N_i \) is the product of the total number of data points at each time step and the total number of time instants at which data were recorded. The error function (\( E \)) was minimized using the \textit{fminsearch} program of the MATLAB, to obtain optimal parameter values. The values of the parameters thus estimated are given in Table 2-2 and the model predicted fluorescence profiles are compared with the experimental data in Figure 2-4. We also examined cases with and without inclusion of the \( k_{\text{perm}} \) parameter.
The parameters given in Table 2-2 are only provided for the case with $k_{perm}$ since the profiles without this term did not match the data well, and in particular the model without $k_{perm}$ cannot capture the characteristics (b) and (c) of the transient concentration profiles listed above.

2.4 Parameter Sensitivity Analysis

2.4.1 Contour Plots and Correlation Coefficients

In order to check for the robustness of the model, we examined the identifiability of parameters by constructing sensitivity contour plots and also by calculating parameter correlation coefficients [68]. The sensitivity contour plots consisted of contour lines of $E$ (i.e., iso-$E$ lines; calculated using Equation 2-13) for variations in parameters taken one pair at a time, keeping all other parameters at their estimated values. Thus, for any parameter pair to be uncorrelated and be true/robust estimates (i.e., based on global minimum of $E$), we expect that the $E$ contours would show a single minima that would converge towards small circles or small line segments. Contours that manifest as long lines should imply that the optimal values of the parameters are not unique, i.e., different sets of parameters could yield the same minimum error, and thus the parameters are not identifiable. Specifically, long horizontal lines imply that the parameters on the $x$-axis are not identifiable and similarly, long vertical lines imply that the $y$-axis parameter is not identifiable. Long slanted lines imply that the parameters on the $x$ and the $y$ axes are correlated.

In order to examine the kinetic model, contour plots for 55 possible pairs of parameters pairs ($^{11}$C$_2$ combinations) were constructed around the minima using MATLAB defined by the parameter estimates in Table 2-2 (Col. 3). Each of the parameters was varied $\pm$ 50% around the estimate. Illustrative cases of $E$ contour plots...
are shown in Figs. 2-5 and 2-6. For $\Phi_{10}$-$D_2$ and $\Phi_{10}$-$K_1$ parameter pairs, the error contours are shown in Figure 2-5A and 2-5C, respectively. The error minima converges along small, vertical line segments which eventually converge to a point. These observations confirm, as expected, negligible correlation between $\Phi_{10}$ and $D_2$ as well as between $\Phi_{10}$ and $K_1$. Similarly, the limiting contours for $k_1$-$D_2$ and $D_1$-$k_{\text{perm}}$ pairs presented in Figure 2-5B and Figure 2-5D, respectively, represent small horizontal lines segments converging to a point. Again, these plots indicate a negligible correlation between $k_1$ and $D_2$ as well as $D_1$ and $k_{\text{perm}}$. In contrast to these representative plots representative of robust parameter estimates, we also found that certain estimates that appear to be ill-identified. For example, E contours for $D_3$-$D_1$ pair shown in Figure 2-6A where two contours have same values of error. This implies that the parameter estimation might depend on the initial guesses of the parameters. However, the minima are relatively close to each other. Similarly, for the $k_3$-$K_3$ pair, the parameter estimation might depend on starting parameter values (Figure 2-6B). The plots involving parameter $\Phi_{21}$ in Figs. 2-6C and 2-6D show that the y-axis parameter $\Phi_{21}$ is not well defined because the contour plots are vertical lines.

We have next quantified the information implied in contour plots above in terms of correlation coefficients between all the parameters of the model. The calculated values for the possible 55 combinations of parameters pairs are given in Table 2-3. For the purposes of emphasizing the importance of E-contours vis-à-vis Table 2-3, the coefficients for the parameter pairs employed E contours shown in Figs. 2-5 and 2-6 are highlighted (shaded and bold text). A parameter is well identified if its regression coefficient with all other parameters lies between $-0.9 < r < 0.9$ [68]. As per this criterion,
it is evident from Table 2-3 that most of the parameters are well-identified, with the exception of $\Phi_{21}$, $\Phi_{32}$, and $K_3$. This conclusion is consistent with those drawn on the basis of the contour plots in Figures 2-5 and 2-6.

### 2.4.2 Single Parameter Sensitivity Analysis

We next determined the sensitivity of each parameter vis-à-vis total error $E$ defined by Equation 2-13. Since all parameters except one are fixed in this calculation, the error function is expanded as a Taylor series at the point of minimum error,

$$E = E_{\min} + \frac{1}{2} \left. \frac{d^2E}{du^2} \right|_{u_{\min}} (u - u_{\min})^2$$

(2-14)

where $E_{\min}$ is the minimum error or the error corresponding to the optimum parameter. The parameter $u$ in the above denotes any one of the eleven model parameters. $u_{\min}$ is the value of $u$ at which the error is minimum. In Equation 2-14, the linear term is absent as the first derivative ($dE/du$) is zero at the minimum. We further define a dimensionless sensitivity parameter $\alpha$ derived from Equation 2-14:

$$\alpha = \frac{\left[ \frac{E - E_{\min}}{E_{\min}} \right]}{\left[ \frac{u - u_{\min}}{u_{\min}} \right]^2} = \frac{u_{\min}^2}{2E_{\min}} \left. \frac{d^2E}{du^2} \right|_{u_{\min}}$$

(2-15)

where the index $\alpha$ quantifies the changes in $E$ due to changes in $u$. For instance, a value of 5 for $\alpha$ implies that 10% change in the parameter from its optimal estimate (i.e., $u - u_{\min} = 0.1 u_{\min}$) gives a 5% change in error $(E-E_{\min})$. To calculate $\alpha$ for a specific value of $u$, we determined the second derivative in Equation 2-15 computing $E$ around $u_{\min}$.
and then fitting the resulting data to a quadratic polynomial. The calculated values of $\alpha$
shown in Table 2-2 indicates that $\Phi_{21}$ and the parameters involving endothelium (i.e., $\Phi_{32}$, $K_3$, $k_3$, and $D_3$) are $<< 5$, suggesting that the model is insensitive to these parameters indicating that they may be poorly identified. This is consistent with the conclusions drawn from the contour plots (Figs. 2-5 and 2-6) and the correlation matrix (Table 2-3). The lack of sensitivity of the parameters involving endothelium could be attributed to limited sampling of RhB in the monolayer given its smaller thickness compared to other layers; sampling interval was 0.5 μm. The lack of sensitivity to $\Phi_{21}$ occurs because the transport from the epithelium to the stroma is limited by the barrier offered by the epithelium-stroma interface and so small changes in the stromal concentration have a negligible impact on the flux at the epithelium-stroma interface.

### 2.5 Prediction of In Vivo Pharmacokinetics

Guss et al. [15] instilled a drop of 0.1% RhB on surface of the cornea of rabbits and measured the concentration transients in the epithelium, stroma, and aqueous humor. However, the study did not report the concentration profiles across each of the layers due to lack of high depth resolution on their measurements. Therefore, the measured values represent spatial averages in different layers. The model described in Equations 2-1 to 2-10 can be employed to predict the in vivo pharmacokinetics with the caveat that RhB concentration in tears decreases exponentially after administration of a drop [69]. Therefore, we assume tear RhB concentration as

$$C_{\text{tear}} = C_0 e^{-\frac{t}{\tau}}$$

(2-16)
where \( C_0 \) is the initial concentration of RhB (after accounting for dilution in the tears) and \( \tau \) is the time constant of elimination from the corneal surface. This modifies the boundary condition at \( y = 0 \) as

\[
C_1 = \Phi_{10} C_{\text{tear}} = \Phi_{10} C_0 e^{-\frac{t}{\tau}} \tag{2-17}
\]

Additionally, we incorporate the anterior chamber dynamics into the *in vitro* model to account for RhB clearance *in vivo*, which occurs largely through aqueous humor outflow. Thus, the mass balance in the anterior chamber can be given by

\[
V_{\text{aq}} \frac{\partial C_{\text{aq}}}{\partial t} = -D_3 A_{\text{cornea}} \frac{\partial C_3}{\partial y} - K_{\text{clearance}} C_{\text{aq}} \tag{2-18}
\]

where \( C_{\text{aq}} \) is the concentration of RhB in the anterior chamber (which is assumed to be well-mixed), \( V_{\text{aq}} \) is the volume of anterior chamber, \( K_{\text{clearance}} \) is the total clearance from anterior chamber, and \( A_{\text{cornea}} \) is the surface area of cornea. The values of \( V_{\text{aq}} \) and \( A_{\text{cornea}} \) for rabbits were assumed to be 0.311 mL and 1.54 cm\(^2\), respectively [70]. \( K_{\text{clearance}} \) is approximated as the aqueous humor outflow in rabbits, which is reported as 0.411 \( \mu l/min \) [71], while the drop volume is assumed to be 30 \( \mu l \) [64]. The value of \( \tau \) was taken to be 1 min based on the fact that the tear concentration drops to negligible values a few minutes after instillation [1,4,64,69,72]. Based on these considerations, we computed the concentration transients for spatially averaged concentration in epithelium. The comparison of the predicted to the values reported by Guss et al.,[15] is presented in Figure 2-7. The comparison is not exact but is comparable, despite the variability in the physiological parameters and the uncertainty in the tear concentrations. The value of \( K_{\text{clearance}} \) is also uncertain because of binding to various tissues and
diffusion through the lens (shown significant RhB accumulation in lens [15]); however, its uncertainty has very little impact on the concentration transients in epithelium as the concentration in anterior chamber is sufficiently close to perfect sink conditions for most of the time.

2.6 Discussion

In this study, we established a kinetic model for characterizing transport of a lipophilic solute across the cornea. Most models on the trans-corneal penetration kinetics reported to date are based on the method of compartmental modeling [8-10]. A few studies that attempted to characterize the diffusive transport across each of the corneal layers were lacked experimental evidence of trans-corneal concentration profiles [12-14]. The model developed in this study accounts not only for the multi-laminate structure of the cornea (Figure 1-1) but also is based on temporal and spatial experimental concentration profile of a fluorescent dye, RhB, as a lipophilic drug analog. Because of corneal transparency, we measured unsteady state trans-corneal profiles of RhB. This novel data provide experimental evidence for a mechanistic description of the trans-corneal transport, and as a result enabled development of a physiology-based pharmacokinetic model. It is worthy to note that the fluorescent dye RhB has been previously used as a model drug surrogate for characterizing transport across the skin [73,74], cornea/lens [15,75] conjunctiva/sclera [76].

2.6.1 Modeling Interphase and Intraphase Transport

A novel aspect of our model is the integration of the multi-laminate view of the cornea illustrated in Figure 1-1. Although this model is well known [1,4,5,77], models for topical pharmacokinetics, in general, have failed to include the existence of heterogeneous nature (i.e., presence of three distinct phases) across the cornea. RhB,
being a lipophilic molecule (octanol-water partition coefficient: 100-310) [15,74], turned out to be very useful in mimicking a lipophilic drug analog. Its trans-corneal distribution not only highlights interphase transport (i.e., lipophilic layer → hydrophilic layer → lipophilic layer) but also demonstrated a unique mechanism of intra-phase transport within the cellular layers, namely the epithelium and endothelium. To accommodate inter-phase transport, we formulated partitioning at the interface (Equation 2-4, 2-5, and 2-7) and also modeled interfacial resistance (Equation 2-5) for transport across the epithelium-stroma interface. For describing intra-phase transport in the stroma, we assumed homogeneous transport properties but the solute transport is by diffusion (Equation 2-3). For transport across the cellular layers, we incorporated an additional length scale in describing the diffusive transport. In this approach, the transport is modeled as diffusion across the bilayers of plasma membrane and partitioning into intracellular lipophilic domains. Since the concentration in the cytoplasm is expected to be small for lipophilic solutes, partitioning into such domains is likely to be prolonged (as discussed previously in the model section). We believe that the slow accumulation of RhB in the cellular layers (Figure 2-1) and a continuous increase in the observed RhB fluorescence at the tear-epithelium interface shown in Figure 2-1 support our reasoning. Therefore, we conclude that the transport of lipophilic molecules across the cellular layers is rate limited by transport across the hydrophilic cytoplasm.

2.6.2 Model Validation

In terms of model validation, uncertainties in the model from the point of view of estimated parameters have been analyzed extensively. However, model verification with multiple fluorescent dyes was beyond the scope of current study and need to be considered in further studies. As a first step in the success of our model, we note that
the parameters Table 2-2 predicted the experimental data accurately. Secondly, the calculated parameters exhibited high sensitivity and the estimated values were independent of starting initial estimate. This conclusion is based on the sensitivity analysis presented in error (E) contour plots (Figure 2-5 and 2-6) and the correlation coefficients between the parameters (Table 2-3). As noted earlier, three of the parameters, namely $\Phi_{21}$, $\Phi_{32}$, and $k_3$, showed poor sensitivities, indicating a need for additional experiments to accurately determine their values. As noted earlier, it is possible that this can be overcome by overcoming the inadequate sampling across the endothelium given its small thickness (4 $\mu$m) [78].

A principal set of parameters relating to transport and phase equilibrium at tear-epithelium, epithelium-stroma, and stroma-endothelium interphase are given in Table 2-1. To establish the validity of these parameters, it is instructive to compare the values reported in Table 2-1 with values previously reported in literature. The parameters $K_1$ and $K_3$ have never been measured or estimated previously so we cannot compare our fitted values to any prior measurements. The parameter $1/k_1$, the time constant of transfer of drug from lipid bilayer to other hydrophobic domains in epithelium, also has not been reported, and thus a direct comparison with measurements is not feasible. A scaling analysis shows that this parameter is about $5 \Phi_{10} R^2/D_{1C}$, where $R$ is the radius of the epithelial cell and $D_{1C}$ is the diffusivity of RhB in the cytoplasm of the epithelial cells. Setting $R = 8.3 \mu$m , $D_{1C} = 4 \times 10^{-12}$ m$^2$/s (based on Stokes Einstein equation [79]) and $\Phi_{10} = 10$, we get $1/k_1 = 800$ s, which is significantly lower than the fitted value of 2500 s (Table 2-2). None the less, the value obtained from the scaling analysis is of the same order of magnitude as the fitted value, which is encouraging, particularly
considering the assumptions implicit in the scaling analysis and the uncertainty in the values of the parameters utilized in the scaling calculations. The time constant of transfer of drug from lipid bilayer to other hydrophobic domains in endothelium is about $5\Phi_{30} R^2/D_{3C}$, which should be approximately equal to the value obtained for the epithelium, i.e., 2500 s due to similarities in the structure of the epithelium and endothelium cells. This value is in reasonable agreement with the fitted value of 3330 s (Table 2-2). Based on a model reported by Zhang et al., the diffusivity of a 0.55 nm size lipophillic molecule in epithelium should be $\sim 2 \times 10^{-12}$ m$^2$/s. Our fitted value of $7.9 \times 10^{-12}$ m$^2$/s is in reasonable agreement with the value predicted by the model. Similarly, based on Zhang et al. [14], the diffusivity in endothelium should be $\sim 2 \times 10^{-12}$ m$^2$/s, which is in good agreement with the fitted value of $1.5 \times 10^{-12}$ m$^2$/s. Zhang et al.,[14] also reported a value of diffusivity in stroma as $2.2\times10^{-10}$ m$^2$/s, which is an order of magnitude higher than the fitted value of $2.3 \times 10^{-11}$ m$^2$/s. The discrepancy between the value estimated from the model of Zhang et al.,[14] and that obtained by fitting the transient profiles is likely due to significant binding of the drug in stroma to collagen and proteoglycans.

The value of the partition coefficient between the stroma and tears $\Phi_{20} = \Phi_{21} \times \Phi_{10}$ is $\sim 100$, which is much larger than 1, even though stroma is almost water-like, supporting the hypothesis that a large fraction of RhB in stroma is bound. The bound and the free concentrations are in stroma are likely in equilibrium due to fast binding-unbinding kinetics, but the bound fraction does not diffuse leading to a reduction in the value of the diffusivity by the ratio of the free drug to the total drug present locally [80]. To our knowledge, there is no reported value of the barrier at the epithelium-stroma interface, and so a direct comparison with experiments is not feasible. The experimental
profiles for fluorescence show a slight shoulder/peak at the interface between the epithelium and stroma, which appears to support the hypothesis that a thin layer at this interface presents a barrier to transport. Alternatively, the resistance could also be due to an additional transport step of RhB from inside the lipid bilayers of the epithelium to the stroma. Further investigations are needed to examine this issue.

2.6.3 Asymptotic Behavior

In order to obtain further insights on the penetration kinetics, we examined the pseudo-steady state behavior after a prolonged time after topical instillation. For this purpose, we have the time derivatives in the model to zero and then calculated concentration profiles. Under these conditions, the concentration in each layer is linear so that the transport across the cornea can be modeled by a lumped model with an overall permeability coefficient given by

\[
K_{\text{eff}} = \frac{1}{L_{\text{stroma}} + L_{\text{epi}} + L_{\text{endo}}} = \frac{1}{\Phi_{21} \Phi_{10} D_2 + \Phi_{10} D_1 + \Phi_{10} k_{\text{perm}} + \Phi_{32} \Phi_{21} \Phi_{10}}
\]  

(2-19)

where \(L_{\text{epi}}, L_{\text{stroma}}, \) and \(L_{\text{endo}}\) are the thicknesses of epithelium, stroma, and endothelium, respectively. To determine the time after which this lumped model can be employed, we calculated the diffusive time scales of each layer \((L^2/D)\) and also for transport into the interior lipophilic domains given by \(1/k_1\) and \(1/k_3\). These characteristic times for the estimated parameters (Table 2-2) are given in Table 2-4. The time required to reach pseudo-steady state was found to be \(~ 60\) min. This implies that for \(t < 60\) min, the lumped model is unsuitable for assessing the trans-corneal penetration. In other words, for time up to 60 min, the unsteady terms in the mass balances must be
included. Since the residence time of topical drugs is only a few minutes, it is clear that lumped model is a poor representation of clinical relevant pharmacokinetics. However, the lumped model could be employed for describing transport in vitro experiments with diffusion chambers as the topical concentration can be maintained constant over an extended time period.

Additional insights on trans-corneal transport can be obtained by comparing the transport resistance of each layer. Thus, using the estimated parameters, the transport resistance of stroma, epithelium, and endothelium and the interfacial resistance at the epithelium-stroma interface, respectively, are:

\[
\frac{L_{\text{stroma}}}{\Phi_{21} \Phi_{10} D_2} = 0.128 \times 10^6 \text{s/m}
\]

\[
\frac{L_{\text{epi}}}{\Phi_{10} D_1} = 0.58 \times 10^6 \text{s/m}
\]

\[
\frac{L_{\text{endo}}}{D_3 \Phi_{32} \Phi_{21} \Phi_{10}} = 0.03 \times 10^6 \text{s/m}
\]

\[
\frac{1}{\Phi_{10} k_{\text{perm}}} = 1.73 \times 10^6 \text{s/m}
\]

We note that each layer, with the exception of the endothelium, offers significant resistance. The effective permeability of the cornea to RhB, based on Equation 2-19, is \(0.41 \times 10^{-6} \text{ m/s}\), and is in reasonable agreement with permeability values reported in the literature for molecules with similar size and hydrophobicity [11]. However, it should be emphasized that the overall permeability coefficient is not relevant for predicting
pharmacokinetics of drugs delivered through eye drops because the residence time of drug is far less than the time required for reaching pseudo-steady state.

### 2.7 Conclusions

The mathematical model developed here accurately characterizes the transient solute transport through the cornea. The fitted values are reliable with a low level of uncertainty for all parameters except the endothelium parameters and the partition coefficient $\Phi_{21}$. The model can predict the in vivo pharmacokinetics of RhB with reasonable accuracy. The model developed here is a significant improvement over conventional approaches using a lumped permeability approach because the drug residence time is much smaller than the time needed for establishing pseudo-steady. Thus, the lumped overall permeability is not a useful measure of total transport resistance. The experimental data shows a slow accumulation of hydrophobic solutes in epithelium and endothelium, and we ascribe this to the slow transport from the bilayers to interior hydrophobic sites.
Figure 2-1. Trans-corneal penetration of RhB after topical administration across rabbit cornea mounted *in vitro*; Y-axis represents fluorescence in arbitrary units (AU). The fluorescence scans were obtained with a custom-built scanning microfluorometer (see Methods) with a depth resolution of ~8 μm using a 40x objective (Zeiss, Inc. 0.75 NA; water immersion).
Figure 2-2. Transport across the cellular layers: The main mechanism for transport of lipophilic solutes is through the lipid bilayers of the plasma membrane (dark arrow). Another important but slower mechanism leads to continued accumulation of hydrophobic solute in the intracellular hydrophobic domains (e.g., membrane associated with endoplasmic reticulum). This mechanism can be resolved into following steps in sequence: (1) transport by partition into the bilayer of the plasma membrane from tears, (2) partitioning of the drug into cytoplasm from the bilayer, and (3) partitioning into intracellular hydrophobic domains. Hydrophilic solutes can pass through paracellular pathways, independently of their partition coefficient and degree of ionization (dotted arrow). Steps 2’ and 3’ represent steps analogous 2 and 3 required, which permit an alternative route for trans-cellular movement of the lipophilic solutes.
Figure 2-3. Partition equilibrium of a lipophilic topical drug: Suppose $C_0$ is the concentration of the topical drug in the tears at an instant $t$. Then, partitioning of the drug into the epithelium results in a concentration $C_1$ (at $y = 0$) at its outer boundary given by $(PC \times C_0)$, where $PC$ is the partition coefficient between tears (equivalent to a buffer) and lipid-rich epithelial layer (equivalent to octanol). Once the drug is in the epithelium, it diffuses down along its concentration gradient in the epithelium. Abbreviations. $C_1$: Concentration in epithelium; $C_2$: Concentration in stroma; $C_3$: Concentration in endothelium; $C_a$: Concentration in the anterior chamber; $y$: Depth across cornea.
Figure 2-4. Comparison of model predictions (solid lines) and experimental measurements (circles) for transient fluorescence profiles in cornea at t = 6, 30, 60, and 140 min.
Figure 2-5. Contour plots of error, i.e., square of the difference between the model predictions and experimental values for Rhodamine B concentration in cornea (eq 13). The parameters on the x and the y axes are varied in a range of ±50% around the optimal values, while keeping all other variables fixed at the optimal values. The contours in each case show a single minimum and contours converge proving that the fitting is robust and the pair of parameters \([\Phi_{10}, D_2]\) in (a); \([k_1, D_2]\) in (b) \([\Phi_{10}, K_1]\) in (c) \([K_{perm}, D_1]\) in (d)] are uncorrelated [a-d are clockwise starting from top left]
Figure 2-6. Contour plots of error, i.e., square of the difference between the model predictions and experimental values for Rhodamine B concentration in cornea (eq 13). The parameters on the x and the y axes are varied in a range of ± 50% around the optimal values, while keeping all other variables fixed at the optimal values. The contour plots in Panels A and B show closely spaced multiple minima, implying that the value of the optimal parameters predicted by the error minimization (D_3-D_1 in Panel A) and k_3-K_3 in Panel B) would vary slightly depending on the initial guess of the parameters. The contours in Panels C and Panel D are straight lines, proving that variation of \( \Phi_{21} \) has a negligible effect on error if \( \Phi_{21} > 10 \).
Figure 2-7. Comparison of experimental data by Guss et al., [15] and model prediction for spatially averaged transient epithelium concentration of RhB in rabbit cornea. In the experiment, a single drop of 1% RhB was instilled in the rabbit eye.
Table 2-1. Description of the model parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Phi_{10}$</td>
<td>-</td>
<td>Ratio of the average concentration in the epithelium bilayers (based on total cell volume) and the concentration in tears at equilibrium.</td>
</tr>
<tr>
<td>$K_1$</td>
<td>-</td>
<td>Ratio of the average concentration in the epithelial bilayers (based on total cell volume) and that in the internal hydrophobic regions (based on total cell volume) at equilibrium</td>
</tr>
<tr>
<td>$k_1$</td>
<td>s$^{-1}$</td>
<td>Permeability of the cytoplasmic medium separating the lipid bilayers and the internal hydrophobic regions in the epithelium</td>
</tr>
<tr>
<td>$D_1$</td>
<td>m$^2$/s</td>
<td>Diffusion coefficient of RhB in the lipid-bilayers in the epithelium</td>
</tr>
<tr>
<td>$k_{perm}$</td>
<td>m/s</td>
<td>Permeability coefficient of epithelium-stroma interface</td>
</tr>
<tr>
<td>$\Phi_{21}$</td>
<td>-</td>
<td>Ratio of the concentration in stroma and the average concentration in the epithelium bilayers (based on total cell volume) at equilibrium</td>
</tr>
<tr>
<td>$D_2$</td>
<td>m$^2$/s</td>
<td>Diffusion coefficient of RhB in stroma</td>
</tr>
<tr>
<td>$\Phi_{32}$</td>
<td>-</td>
<td>Ratio of the average concentration in the endothelium bilayers (based on total cell volume) and the concentration in stroma at equilibrium</td>
</tr>
<tr>
<td>$K_3$</td>
<td>-</td>
<td>Ratio of the average concentration in the endothelium bilayers (based on total cell volume) and that in the internal hydrophobic regions (based on total cell volume) at equilibrium</td>
</tr>
<tr>
<td>$k_3$</td>
<td>s$^{-1}$</td>
<td>Permeability of the cytoplasmic medium separating the lipid bilayers and the internal hydrophobic regions in the endothelium</td>
</tr>
<tr>
<td>$D_3$</td>
<td>m$^2$/s</td>
<td>Diffusion coefficient of RhB in the lipid bilayers of the endothelium</td>
</tr>
</tbody>
</table>
Table 2-2. The optimal values of the model parameters obtained by minimizing the total error between the model prediction and experimental data for RhB concentration in cornea at various times. Sensitivity analysis of the transport model. Values of the sensitivity index larger than 5 indicates that error between the experimental data and the model fit increased by less than 5% for a 10% change in the model parameter, and implies a robust fit and a reliable value of the fitted parameter.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimated value</th>
<th>Units</th>
<th>Sensitivity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Phi_{10}$</td>
<td>9.8</td>
<td>-</td>
<td>100.27</td>
</tr>
<tr>
<td>$K_1$</td>
<td>1.6</td>
<td>-</td>
<td>6.39</td>
</tr>
<tr>
<td>$k_1$</td>
<td>$4 \times 10^{-4}$</td>
<td>s(^{-1})</td>
<td>4.48</td>
</tr>
<tr>
<td>$D_1$</td>
<td>$7.9 \times 10^{-12}$</td>
<td>m(^2)/s</td>
<td>3.01</td>
</tr>
<tr>
<td>$k_{perm}$</td>
<td>$6 \times 10^{-8}$</td>
<td>m/s</td>
<td>10.36</td>
</tr>
<tr>
<td>$\Phi_{21}$</td>
<td>10.6</td>
<td>-</td>
<td>0.11</td>
</tr>
<tr>
<td>$D_2$</td>
<td>$22.8 \times 10^{-12}$</td>
<td>m(^2)/s</td>
<td>4.92</td>
</tr>
<tr>
<td>$\Phi_{32}$</td>
<td>2.8</td>
<td>-</td>
<td>0.54</td>
</tr>
<tr>
<td>$k_3$</td>
<td>$3 \times 10^{-4}$</td>
<td>s(^{-1})</td>
<td>0.01</td>
</tr>
<tr>
<td>$K_3$</td>
<td>1.2</td>
<td>-</td>
<td>0.02</td>
</tr>
<tr>
<td>$D_3$</td>
<td>$1.5 \times 10^{-12}$</td>
<td>m(^2)/s</td>
<td>0.78</td>
</tr>
</tbody>
</table>
Table 2-3. Coefficient of correlation between all the model parameters obtained by fixing all parameters except the two chosen parameters. Correlation coefficient is calculated for the limiting contours encompassing the minima. Coefficient of correlation should lie between -0.9 and 0.9 for variables to be uncorrelated. High values of correlation coefficients have been marked with an asterisk in the table. The blank entries in the table correspond to the cases of vertical or horizontal contours for which the correlation coefficient is undefined, ex., contour plots in Fig 2-6 (c-d).

<table>
<thead>
<tr>
<th></th>
<th>$\Phi_{10}$</th>
<th>$K_1$</th>
<th>$k_1$</th>
<th>$D_1$</th>
<th>$k_{perm}$</th>
<th>$\Phi_{21}$</th>
<th>$D_2$</th>
<th>$\Phi_{32}$</th>
<th>$K_3$</th>
<th>$k_3$</th>
<th>$D_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Phi_{10}$</td>
<td>1</td>
<td>-0.13</td>
<td>-0.32</td>
<td>-0.42</td>
<td>-0.05</td>
<td>-</td>
<td>0.06</td>
<td>0.12</td>
<td>-0.2</td>
<td>-0.33</td>
<td>0.22</td>
</tr>
<tr>
<td>$K_1$</td>
<td>1</td>
<td>-0.53</td>
<td>-0.14</td>
<td>0.12</td>
<td>-0.32</td>
<td>0.12</td>
<td>0.04</td>
<td>0.35</td>
<td>0.34</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>$k_1$</td>
<td>1</td>
<td>-0.54</td>
<td>-0.02</td>
<td>0.91*</td>
<td>-0.09</td>
<td>-0.25</td>
<td>0.61</td>
<td>0.35</td>
<td>-0.08</td>
<td></td>
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</tr>
<tr>
<td>$D_1$</td>
<td>1</td>
<td>-0.19</td>
<td>-</td>
<td>0.55</td>
<td>0.82*</td>
<td>0.01</td>
<td>-0.14</td>
<td>-0.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{perm}$</td>
<td>1</td>
<td>-0.19</td>
<td>0.61</td>
<td>0.16</td>
<td>0.11</td>
<td>0.03</td>
<td>0.19</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>$\Phi_{21}$</td>
<td>1</td>
<td>0.03</td>
<td>0.60</td>
<td>-0.15</td>
<td>-0.16</td>
<td>-0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$D_2$</td>
<td>1</td>
<td>-0.28</td>
<td>0.98*</td>
<td>0.03</td>
<td>-0.2</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Phi_{32}$</td>
<td>1</td>
<td>0.30</td>
<td>0.31</td>
<td>-0.90*</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>$K_3$</td>
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<td>-0.1</td>
<td>0.71</td>
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</tr>
<tr>
<td>$k_3$</td>
<td>1</td>
<td>0.81*</td>
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<tr>
<td>$D_3$</td>
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<td></td>
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</tbody>
</table>
Table 2-4. Characteristic time scales for the principal mechanisms included in the model.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Time Scale</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffusion in the Epithelium</td>
<td>( (L_{\text{epithelium}})^2 / D_1 )</td>
<td>4.3</td>
</tr>
<tr>
<td>Diffusion in the Stroma</td>
<td>( (L_{\text{stroma}})^2 / D_2 )</td>
<td>68.2</td>
</tr>
<tr>
<td>Diffusion in the Endothelium</td>
<td>( (L_{\text{endothelium}})^2 / D_3 )</td>
<td>2.2</td>
</tr>
<tr>
<td>Transport from bilayers of the plasma membrane into intracellular lipophilic domain in the epithelium</td>
<td>( 1 / k_1 )</td>
<td>41.6</td>
</tr>
<tr>
<td>Transport from bilayers of the plasma membrane into intracellular lipophilic domain in the endothelium</td>
<td>( 1 / k_3 )</td>
<td>55.6</td>
</tr>
</tbody>
</table>
CHAPTER 3
TRANSCORNEAL PENETRATION OF FLUORESCEIN ACROSS RABBIT CORNEA

3.1 Introduction

Most of the eye medications delivered topically have to have to transport across cornea to reach the target tissues. Fluorescein is used as a drug analog for hydrophilic drugs to understand the transport mechanism of these molecules across cornea. This chapter focuses on developing a mechanistic model to explain the transient kinetics of fluorescein, when endothelium side is exposed to a fixed concentration of sodium fluorescein.

3.2 Materials and Methods

Sodium fluorescein and reagents for ringers solution were obtained from Sigma Chemical Company (St. Louis, MO). Eyes were obtained from freshly killed albino (New Zealand White) rabbits of either sex. All procedures for animal handling were followed in accordance with the guidelines set by the Association for Research in Vision and Ophthalmology (ARVO) and were approved by Laboratory Animal Care Committee in the laboratory of (late) Prof. David Maurice, Ophthalmology at Stanford University (SP. Srinivas). The corneas were isolated, mounted and were maintained at 34°C by circulating water through the jacket and perfused with HCO$_3^-$ Ringers (containing reduced glutathione, glucose, adenosine, 40 mM HEPES, and 40 mM NaHCO$_3$) at the anterior and posterior surfaces. The trans-corneal profiles of fluorescein were obtained using a custom-built confocal scanning microfluorometer, as has been described previously [61,62]. About 30 minutes after mounting the cornea, the endothelium was exposed to fluorescein dissolved (0.01 µg/mL) in the Ringers. Depth scanning was performed through a stepper motor mechanically coupled to the fine focus knob of the
microscope. Depth resolution was ~ 8 \( \mu \text{m} \) at a sensitivity of \( 10^{-6} \text{gm/mL} \) of fluorescein (SNR > 20) using a 40x water immersion objective of 0.75 NA (Zeiss Inc) [62,64].

Excitation wavelength used in the experiments was 485\( \pm \)10 nm while emission wavelength was 530 \( \pm \)10 nm. Scanning was performed at \( \sim 600 \mu \text{m/min} \) over 800 \( \mu \text{m} \) depth. Scatter and fluorescence scans were measured to obtain corneal thickness and trans-corneal concentration profile of fluorescein, respectively. More than 6 experiments were performed and data from one typical experiment is considered in this study for analysis. The experimental data alone were presented in an abstract form by Srinivas and Maurice previously at the Association of Research in Vision.

### 3.3 Results and Discussion

#### 3.3.1 Fluorescence Profiles

The spatially resolved fluorescence profiles in cornea are plotted in Figure 3-1 for various times. It is reiterated that the cornea is exposed to fluorescein (FL) on the anterior chamber side, and fluorescein then diffuses across the three layers of the cornea. Since FL is hydrophilic, it is expected to face significant barrier to transport in the endothelium and epithelium. The profiles show elevated concentration of FL in stroma, which is expected because of hydrophilic nature of FL and stroma. Additionally, there are a few key aspects of the experimental data which are important to understanding the mechanisms of transport through each of the cornea layers: (i) The concentration of FL in endothelium is only slightly smaller than that in the stroma; (ii) The fluorescence at the stroma-endothelium interface increases with time; (iii) The fluorescence boundary layer in stroma is about 175 \( \mu \text{m} \) thick at 41 minutes and it
reaches the stroma-epithelium boundary, which is about 350 μm from the endothelium, in less than 120 min.

The first observation suggests that FL is able to diffuse across the lipid bilayers of the endothelial cells and accumulate in the cytoplasm of the endothelial cells. The fluorescence at the stroma-endothelium interface is plotted as a function of fluorescence from the endothelium (cytoplasm) with time as a parameter in Figure 3-2. This figure suggests that the ratio is relatively constant suggesting that the concentration in the endothelium and that in stroma at the stroma-endothelium boundary are in equilibrium. The first and the second observation suggest that the dominant mechanism for transport of FL across the endothelium is transcellular transport through the lipid bilayers.

The thickness of a purely diffusive boundary layer changes as square root of time, and thus the time taken for the boundary layer to reach 350 μm depth should be about 4 times the time that it took to reach the 175 μm depth. The third observation listed above clearly shows that the time to reach epithelium is less than 4 times the time for the boundary layer to traverse half of the stroma, suggesting that FL transport in stroma must be due to a combination of diffusion and convection. The convection in stroma is generated due to the pressure difference created across the cornea during the experiment. The fluid is continuously pumped in and out of the anterior chamber and thus the pressure in the aqueous chamber is larger than the atmospheric pressure, whereas the pressure on the epithelium-tear interface is atmospheric. This pressure gradient leads to convection through the cornea.
3.3.2 Swelling of Cornea

Water transport in cornea can occur due to a number of driving forces including pressure, osmosis, electro-osmosis, and water channels aquaporins. The thickness of various corneal layers particularly stroma is controlled by a balance between water fluxes from various mechanisms. These driving forces persist even after a cornea is excised, but their balance likely gets disturbed, and thus various layers of cornea could swell during the experiment. The thickness of various layers of cornea can easily be determined by utilizing the dynamic concentration profiles because each layer has a different affinity for fluorescein and thus the fluorescence is expected to be discontinuous at the interface between various layers. The discontinuity in fluorescence implies infinite slope at the interface. The discontinuities are smoothened in the experimental profiles (Figure3-1.) because the fluorescence measurement at a spatial location includes contributions from the fluorophores in the vicinity, with the contribution from each fluorophore decreasing as a Gaussian away from its location. This effect, also known as the instrument response function (IRF) smoothes the profiles; yet, the slopes at the interfaces is larger than those in the vicinity. Thus, the location of each of the interfaces can be detected by calculating the slope of the concentration profiles and determining the local maxima of slope. After the interfaces are located, the thickness of each layer can easily be determined as the distance between the pertinent interfaces. The thicknesses of epithelium, stroma, and endothelium are denoted by \( L_e, L_s, \) and \( L_{en} \), respectively. The thickness of stroma and epithelium are plotted as a function of time in Figure 3-3A and Figure 3-3B respectively. The thickness of stroma is relatively unchanged in the first 200 minutes, after which it swells at a relatively constant rate of \( \frac{1}{2} \) micron per min. The thickness of epithelium is also relatively constant for the first 200
minutes, after which it swells rapidly by about 10 microns in an hour, followed by a more gradual swelling. The swelling of cornea could be due to the changes in water in flow and outflow, but it could also be a manifestation of degradation of the physical integrity of the cornea. Since the details of the mechanisms of the corneal swelling are not the focus of this paper, we only utilize the data for time less than 3 hours to model transport in stroma and endothelium. However, during this period the fluorescence in the epithelium is negligible, therefore data from longer times are utilized. These details are discussed in the next section on model development.

3.3.3 Mathematical Model for Fl Transport in Cornea

3.3.3.1 Transport in stroma

As discussed above, Fl transport in cornea occurs through a combination of diffusion and convection due to the fluid movement from endothelium towards the epithelium (Equation 3-1). The corneal stroma is composed of ~ 260 lamellae of collagen fibrils bound with glycosaminoglycans (GAGs). The solute molecules could bind to collagen and GAGs. However, the binding-unbinding events typically occur on a faster time scale compared to that of diffusion, and thus we assume that the bound and the unbound forms are in equilibrium so that one only needs to write the mass balance for the total solute amount. Therefore, the governing equation for Fl transport in stroma can be written as

\[
D \frac{\partial^2 C_2}{\partial x^2} - v \frac{\partial C_2}{\partial x} = \frac{\partial C_2}{\partial t}
\]  

(3-1)

where \(C_2\) is the total concentration of Fl (or equivalently fluorescence) in stroma, \(D\) is the average diffusivity in stroma, and \(v\) is the velocity of fluid. The above differential
equation needs to be supplemented by boundary conditions at the interfaces with endothelium and epithelium.

### 3.3.3.2 Transport in endothelium

Transport across endothelium can occur through two routes - paracellular i.e., transport through the space in between the cells; or transcellular i.e., transport across the cells. Due to the hydrophilic nature and relatively small molecular weight of 332 Da, the permeability of molecules through the paracellular route is expected to be larger as compared to the permeability through the transcellular route which requires the molecules to traverse the lipid bilayers of the endothelial cells. However, the area of the space in between the cells available for paracellular transport is much smaller than the area available for transcellular transport, and thus the net transcellular flux could be the leading transport mechanism. While measuring permeability of endothelium through macroscopic experiments using diffusion cells, it is not feasible to determine the dominant mechanism. However, accumulation of FL in the endothelium evident in the profiles in Figure 3-1 shows that the solute must be crossing the lipid bilayers to accumulate inside the cytoplasm because the paracellular volume in the endothelium is not sufficient to contribute to the measured fluorescence in endothelium. Also, as mentioned earlier, the fact that the ratio of the fluorescence in endothelium and that in stroma at interface with endothelium is relatively constant suggesting that the two concentrations are in equilibrium due to transport through the lipid bilayers of the cells. Further evidence regarding the dominance of the transcellular transport is that if a constant permeability is assumed for the endothelium, the models fits do not agree with the experimental data (fits not shown).
To model the endothelium transport, we propose the following mass balance on the endothelial cells,

$$V \frac{\partial C_1}{\partial t} = kS(C_0 - \frac{C_1}{\Phi_{10}}) - kS(\frac{C_1}{\Phi_{12}} - C_2) \quad (3-2)$$

where $C_1$ is the concentration in endothelium, $C_0$ is the concentration in anterior chamber, $\Phi_{10}$ is the partition coefficient of drug between cytoplasm in endothelium and aqueous humor, $\Phi_{12}$ is the partition coefficient between cytoplasm in endothelium and stroma, and $k$ is the permeability of the endothelial lipophillic membranes. In the above equation, the LHS represents the accumulation; the first term on the RHS accounts for net transport from anterior chamber to endothelium, and the last term accounts for the net transport from endothelium to stroma. The surface area $S$ is the transcellular area available for transport, and $V$ is the volume of epithelial cells. The endothelium is coupled to stroma through the following boundary condition

$$-D \frac{\partial C_2}{\partial x} = k(\frac{C_1}{\Phi_{12}} - C_2) \quad (3-3)$$

The diffusion resistance in stroma ($D/L_s$) is much smaller than that in endothelium ($k$) because of the large thickness of the stroma in comparison to that of endothelium (this statement will be tested after values for the parameters are determined). Accordingly, the above boundary condition can be simplified to

$$C_2 = \frac{C_1}{\Phi_{12}} \quad (3-4)$$

This simplification implies that the stroma concentration at the interface is in equilibrium with the endothelium concentration, which is clearly supported by the data in Figure 3-2. This simplified boundary condition can now be applied in the governing equation for
endothelium (Equation 3-2) to yield the following simplified mass balance for endothelium

\[ V \frac{\partial C_1}{\partial t} = kS(C_0 - \frac{C_1}{\Phi_{10}}) \]  

(3-5)

The above equation can be solved by using \( C_1 = 0 \) as an initial condition to give the following expression for the endothelium concentration

\[ C_1 = \Phi_{10} C_0 (1 - e^{kS \Phi_{10}}) = \Phi_{10} C_0 (1 - e^{-k_1 t}) \]  

(3-6)

where \( k_1 = \frac{kS}{V \Phi_{10}} \). By utilizing the above equation into boundary condition (Equation 3-4), we get the following boundary condition for stroma at the stroma-endothelium interface,

\[ C_{21} = \Phi_{21} \Phi_{10} C_0 (1 - e^{-k_1 t}) = \Phi C_0 (1 - e^{-k_{12} t}) \]  

(3-7)

where \( \Phi_{21} (= 1/ \Phi_{12}) \) is the partition coefficient of drug between stroma and cytoplasm in endothelium. It is to be noted that \( \Phi = \Phi_{21} \times \Phi_{10} \) is the effective partition coefficient of drug between stroma and aqueous humor.

3.3.3.3 Transport in epithelium

The epithelium consists of 5-6 layers of cells with multi-stranded tight junctions. Based on the mechanism for FL transport in endothelium, the likely mechanism in epithelium is transcellular transport through the cell layers. The cornea transport can thus be modeled as 6 layers of cells with transport barriers between each layer. This model is described in details later, but a simpler approximation is utilized to model the fluorescence profiles in stroma at short times. Due to the multilayer nature of epithelium, it is expected to offer significantly larger resistance to transport, and thus, it
can be assumed that in the short time regime, the flux from the stroma to the epithelium is essentially zero, i.e.,

$$-D \frac{\partial C}{\partial x} + v C = 0$$

(3-8)

This simplified model cannot predict the epithelium concentrations, and the detailed epithelial model that will be utilized to fit the epithelium data is presented later.

The governing equation for transport in stroma (Equation 3-1) along with the boundary conditions (Equation 3-7 and Equation 3-8) can be solved numerically by finite difference to obtain the concentration profiles as a function of time in stroma, and also the concentration transients in endothelium.

It is noted that the model presented above has no non-linear terms and so the response is linearly proportional to the solute concentration in the tears. Furthermore, if the fluorescence is linearly proportional to concentration with the same constant of proportionality in each layer, the concentration can simply be expressed in fluorescence units. Based on these assumptions, below we interchangeably use the terms fluorescence signal and the solute concentration.

### 3.3.3.4 Parameter estimation

The fluorescence at any location x is contributed by fluorophores located in the vicinity of x, with the contribution from any fluorophore decaying away from its location as a Gaussian. Thus, the measured fluorescence profiles are a convolution of the concentration profiles and the Instrument Response Function (IRF) which given by the following expression,
\[
\text{IRF}(x - x') = \frac{e^{-\frac{(x-x')^2}{2\sigma^2}}}{\sqrt{2\pi\sigma^2}}
\]  

(3-9)

where \((2.36 \sigma)\) represents full width of the Gaussian, which is 20 µm for the experiments reported here. The predicted concentration profiles can be convoluted with the IRF to predict the fluorescence profiles, i.e.,

\[
C_{\text{Model}}(x') = \int_0^\infty C(x) \text{IRF}(x - x') dx
\]  

(3-10)

The four unknown model parameters \((D, v, \Phi, k_1)\) can be obtained by matching the model prediction to the experimental data by minimizing the following objective function \(E\), which is the sum of squares of the difference between model and the experimental results.

\[
E = \sum_{i=1}^{N} \left[ C_{\text{Model}} - C_{\text{Exp}} \right]^2
\]  

(3-11)

where \(C_{\text{exp}}\) and \(C_{\text{Model}}\) are the measured and predicted concentrations at given position and time, and \(N\) represents the total number of data points. The four unknown parameters can be obtained by fitting the entire data in stroma and endothelium, or alternatively the parameter \(k_1\) can first be determined by fitting the endothelium data, and the remaining three parameters can be obtained by fitting the stromal data. We adopt the second approach as the endothelium data is highly sensitive to the parameter \(k_1\), and thus more reliable values of \(k_1\) can be obtained by fitting endothelium concentration, or equivalently the stroma concentration at the stroma-endothelium interface. The remaining three parameters were obtained by minimizing the sum of squares (Equation 3-9) in stroma. The values of the fitted parameters are presented in
Table 3-1 and the best fit fluorescence profiles are compared with the experimental data in Fig 3-4.

3.4 Sensitivity Analysis

Since the model has a relatively large number of parameters it is important to determine whether the parameter values listed in Table 3-1 are unique or a number of different combinations of the parameters can fit the data. We adopted various approaches to test the reliability of the three parameters (D, v, ϕ) including constructing contour plots, obtaining correlation coefficients, and sensitivity indices.

3.4.1 Contour Plots and Correlation Coefficients

The contour plots were constructed by picking any two parameters, varying them within ±50% of their estimated values, and plotting contours along which the error is constant. The shapes of the contour plots reflect the correlation between the two parameters that are varied for that contour plot. For instance, if the contour plots around the minimum are concentric and culminate in a point or a small line segment, it implies that parameters are not correlated around the estimated values of the parameter. However if the contours are elliptic culminating in a line, it implies that the parameters are correlated, i.e., changes in one parameter can be compensated by changes in the other parameter, and thus accurate determination of the parameters is not possible. If one the other hand, the contours are vertical (or horizontal lines), the error is independent of the parameter on the x axis (or y axis for horizontal contours). The contour plots in Figure 3-5A and 3-5B show that the contours for ϕ-D pair and ϕ-v pair converge towards a single point, which mean that these parameters are well identified. The contour plots for the D-v pair (Figure 3-5C) show that close to the global minimum, there are multiple contours that have the similar values of error, which implies
existence of multiple local minima. In this case, the parameter estimation might depend on the initial guesses of parameters. However the three minima are relatively close to each other, and thus the best-fit value is reliable.

The qualitative information visible in the contour plots can be quantified by computing the correlation coefficients between various pairs of parameters. The correlation coefficients for each pair of parameters are presented in Table 3-2. All the correlation coefficients between the parameters lie between -0.9 and 0.9 which proves that parameters are not correlated.

### 3.4.2 Sensitivity Index

To further examine the sensitivity of each model parameter, we obtain a sensitivity index which is a measure of the relative increase in error for changes in that parameter. A large value of the index implies that small changes in the parameter lead to relatively large changes in the error, and thus the parameter value is robust. Since all parameters except one are fixed in this analysis, the error can be expanded as a Taylor series around the point of minimum error,

$$ E = E_{\text{min}} + \frac{1}{2} \frac{d^2 E}{du^2} |_{u_{\text{min}}} (u - u_{\text{min}})^2 $$  

(3-12)

where $E_{\text{min}}$ is the minimum error or the error corresponding to the predicted parameter, $u$ is the specific parameter (any one of the eleven model parameters), and $u_{\text{min}}$ is the value of $u$ at which the error is minimum or the estimated value of $u$. It is noted that the linear term is absent from the above expansion because the first derivative $dE/du$ is zero at the minimum. As discussed in the previous chapter (chapter 2), the sensitivity index $\alpha$ is defined by the following equation
\[ \alpha = \frac{E - E_{\min}}{E_{\min}^2} \frac{E_{\min}^2}{u - u_{\min}} = \frac{u_{\min}^2}{2E_{\min}} \frac{d^2E}{du^2} \]

(3-13)

The second derivative of \( E \) was obtained by computing \( E \) around \( u_{\min} \) in the range 
-0.05 \( E_{\min} < E - E_{\min} < 0.05 \) \( E_{\min} \) and then fitting the data to a quadratic polynomial (without the linear term). Equation 3-13 was then used to calculate the value of \( \alpha \) for all the parameters and results are presented in Table 3-1. All sensitivity indices are about 5 or larger, which again implies that the model is sensitive to each of the parameters.

### 3.5 Discussion

The sensitivity analysis suggests that parameters are well identified and the model clearly explains the important characteristics of the experimental data. However, to further test the validity of the model it is useful to compare the fitted model parameters with prior values reported in literature.

Diffusivity, \( D \), in stroma has been measured to be \( 1.21 \times 10^{-10} \) m\(^2\)/s [81] which is an order higher than our estimated value. Discrepancy can be explained on the basis of very different route of conduction of experiments. Also, \( \Phi \), partition coefficient between stroma and aqueous humor has been measured to be 1.33 [81], while our model predicts a very similar value of 1.79. Specific hydraulic conductivity of stroma has been measured to be between \( 0.5 \times 10^{-14} \) cm\(^2\) and \( 10 \times 10^{-14} \) cm\(^2\) [82]. Based on predicted velocity and 20 cm of water head at endothelium surface with epithelium surface being exposed to atmosphere, we calculate the value of hydraulic conductivity to be \( 2.4 \times 10^{-14} \) cm\(^2\), which belongs in the range of experimentally measured values. A very low value of
epithelium permeability reported in [83] justifies our assumption of negligible permeability to solve the model for short time scales.

3.6 Conclusion

A mechanistic pharmacokinetic model has been developed to explain the transient concentration profiles of FL in rabbit cornea. Apart from traversing through paracellular route, FL molecules also transports itself across the cells through transcellular route. Model developed could play a major role in developing better ocular formulations and is a significant improvement over previously existing compartmental models.
Figure 3-1. Transient concentration profiles of Fluorescein in rabbit cornea, when endothelium side was exposed to a fixed concentration of the dye.
Figure 3-2. Plot of concentration of fluorescein at stroma-endothelium interface vs concentration in endothelium (cytoplasm in endothelium) with time as parameter.
Figure 3-3. Transient swelling of corneal layers specifically, (A) Stroma and (B) Epithelium.
Figure 3-4. Comparison of model predictions and experimental profiles of fluorescein concentration in stroma for short times (t < 3 hours). Here x=0 represents endothelium-stroma interface while x=334µm represents stroma-epithelium interface.
Figure 3-5. Contour plots are plotted by picking two parameters and fixing all other parameters. Value of the objective function, error, is the third dimension. Along a particular contour error remains constant.
Table 3-2. Values of parameter in the model and their sensitivity to the model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimated value</th>
<th>Units</th>
<th>Sensitivity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\phi$</td>
<td>1.79</td>
<td>-</td>
<td>132.8</td>
</tr>
<tr>
<td>$k_1$</td>
<td>0.1176</td>
<td>hr$^{-1}$</td>
<td>94.0</td>
</tr>
<tr>
<td>$D$</td>
<td>$7.76 \times 10^{-12}$</td>
<td>m$^2$/s</td>
<td>17.8</td>
</tr>
<tr>
<td>$V$</td>
<td>$1.38 \times 10^{-6}$</td>
<td>m/s</td>
<td>4.32</td>
</tr>
</tbody>
</table>

Table 3-3. Correlation coefficients are calculated for limiting contours encompassing the minima.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$\phi$</th>
<th>$D$</th>
<th>$V$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\phi$</td>
<td>1</td>
<td>-0.58</td>
<td>0.087</td>
</tr>
<tr>
<td>$D$</td>
<td></td>
<td>1</td>
<td>-0.3274</td>
</tr>
<tr>
<td>$V$</td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>
CHAPTER 4
DRUG TRANSPORT IN HEMA CONJUNCTIVAL INSERTS CONTAINING PRECIPITATED DRUG PARTICLES

4.1 Introduction

This chapter focuses on development of polymeric conjunctival inserts for delivery of cyclosporine A for dry eye treatment. Inserts will be placed in conjunctival sac of the eyes and will release drug in tears which will go towards cornea or conjunctiva. Drug exists as particles in these devices which is verified using imaging techniques. Also a mechanism of transport of drug in these HEMA inserts has been discussed. Although the mathematical equations describing the model have been proposed for cylindrical geometry, similar mechanism could be used to propose a model for any polymeric system containing drug as particles.

4.2 Materials and Methods

Hydroxyl ethyl methacrylate (HEMA), Ethylene glycol dimethacrylate (EGDMA), and Azobisisobutylonitrile (AIBN) were purchased from Sigma-Aldrich (St. Louis, MO); Cyclosporine A was purchased from LC Labs (Woburn, MA); and Silastic® laboratory tubing of two different sizes (ID 1.02 mm and 1.47 mm) were purchased from Dow Corning (Midland, MI). The Dulbecco’s phosphate buffered saline (PBS) used in the drug release experiments was purchased from Sigma-Aldrich (St Louis, MO).

4.2.1 Fabrication of Inserts

The inserts were fabricated in two different designs using p-HEMA and p-EGDMA, which are common materials for commercial contact lenses. The basic structure of the design I inserts is shown in Figure 4-1A, with a p-HEMA core, which is loaded with cyclosporine A using “direct entrapment” procedure described below. For making the core, about 1.35 mL of HEMA monomer was mixed with EGDMA monomer in various
ratios (5μL of EGDMA in 1.35 ml HEMA is referred as “1X” crosslinking), and the mixture was purged with N₂ for about 10 minutes to remove the O₂ in the monomer. Next, 10 wt% of cyclosporine A and about 0.03 g of AIBN (initiator) were added and the mixture was stirred for about 5 minutes. The resulting solution was filled into Silastic® tubing with 1.02mm ID that served as the molds for the polymerization. The filled molds were sealed at both ends and submerged into a water bath maintained at 80 °C for 20 minutes for polymerization. After the polymerization, the p-HEMA core was taken out of the mold to obtain a drug loaded design I insert of 1.02 mm diameter with 1X crosslinking and 10% drug loading. The degree of crosslinking was increased or decreased by changing the amount of EGDMA in the polymerization mixture to obtain inserts with 0X, 10X or 20X crosslinking, which contain 0, 10 and 20 times the EGDMA utilized in the procedure described above. Also the amount of drug loading was altered to prepare inserts with drug loading varying from about 0 to 30%. The inserts without drug (0% drug loading) were soaked in drug-PBS solutions to load drug into the inserts. Subsequently, drug release studies were conducted with these inserts, and fitted to a diffusion model to obtain diffusivity and partition coefficient of cyclosporine in the inserts.

The design II insert shown in Figure 4-1B consists of drug loaded 1.02 mm dia p-HEMA core and a concentric 0.225 mm thick EGDMA shell. To prepare the monomer mix for the shell, 0.03 g of AIBN was added to purged 1.35 mL of EGDMA, and the mixture was stirred for 5 minutes. The 1.02 mm diameter p-HEMA core loaded with cyclosporine A (design I insert) was first inserted into Silastic® tubing with a 1.47 mm ID, and the gap between the core and the tube was filled with the EGDMA solution, while ensuring that the p-HEMA core was centered. The tubing was sealed with office
clamps, and submerged into a water bath at 80˚C for around 20 minutes for polymerization. The end product had a substantially cylindrical-shaped core-shell structure, with a 1.02 diameter p-HEMA core loaded with cyclosporine A, surrounded by a 0.225 mm thick shell of p-EGDMA. In some cases, the shell of the design II insert was prepared with a mixture of HEMA and EGDMA to reduce the degree of crosslinking in the shell.

4.2.3 Drug Release

After the inserts were fabricated, they were cut into cylinders of variable lengths using a surgical blade, and then used in drug release studies. The drug release was measured in 3.5ml of phosphate buffer saline (PBS), which was replaced every 24 hours to simulate perfect sink conditions. The concentration of cyclosporine A in the release medium was determined with an HPLC (Waters, Milford, MA) equipped with a reverse phase C18 column (Waters, Milford, MA) and a UV detector. The mobile phase was 70% acetonitrile and 30% deionized water at a flow rate of 1.2 mL/min. The column temperature was maintained at 60 ˚C and the UV detector wavelength was 210 nm.

4.3 Results and Discussion

The diffusivity of cyclosporine A through the p-HEMA inserts will play an important role in the drug release dynamics. Accordingly, before discussing the drug release profiles from the inserts, we report results for diffusivity obtained by theoretical models and by release studies from inserts in which drug was loaded by soaking in drug-PBS solution.

4.3.1 Estimation of Diffusivity in the p-HEMA Inserts

A number of researchers have developed theoretical models for estimating diffusivities of solutes through porous hydrogels such as p-HEMA. Brinkman developed
the following relationship between the hydraulic permeability of the hydrogel and the solute diffusivity given [84],

$$D_{th} = \frac{k_r T}{6\pi \mu r_i} \left( 1 + \left( \frac{r_i^2}{k} \right)^{1/2} + \frac{1}{3} \left( \frac{r_i^2}{k} \right)^{3/2} \right)^{-1}$$

(4-1)

where $D_{th}$ is the theoretically determined diffusivity of the molecule through a porous medium of hydraulic permeability $k$, $r_i$ is the solute radius, $\mu$ is the viscosity of water, $T$ is the temperature and $k_B$ is the Boltzmann’s constant. Philips et al. confirmed the validity of Brinkman’s equation by Stokesian dynamics and also by experiments [85]. It has however been suggested that Equation 4-1 is valid only for diffusion in pressure driven flows and in its absence, the coefficient of $r_i^2 / k$ should be 1/9 instead of 1/3 [86]. Due to the absence of pressure driven flow in our experiments, we use the modified form of Brinkman’s equation by replacing 1/3 with 1/9. The parameters required for estimating cyclosporine A diffusivity through a p-HEMA gel are listed in Table 4-1. Based on Equation 4-1, the cyclosporine A diffusivity in the p-HEMA gels was estimated to be $10.2 \times 10^{-12} \, \text{m}^2/\text{s}$.

**4.3.2 Measurement of Diffusivity in the p-HEMA inserts**

To measure diffusivity of cyclosporine A in inserts, we prepared inserts without drug, and then loaded drug by soaking the inserts in drug-PBS solution. Subsequently, drug release profiles were measured and fitted to the diffusion equation to determine the diffusivity. Specifically, 7.5 mm long inserts were fabricated and three such inserts were soaked together in 7 ml of drug-PBS solution with a concentration $C_0^*$ 9.5μg/ml for a period of 6 days. The three drug loaded inserts were then removed from the drug solution and soaked in 2 ml of PBS which was changed at regular intervals to maintain
perfect sink conditions. The concentration of drug in the release medium was measured every time the fluid was replaced. The cumulative drug release from these experiments is plotted as a function of time in Figure 4-2A.

Drug transport from the cylindrical inserts can be modeled as a 1-dimensional diffusion because the length of the inserts is much longer than the radius, i.e.,

$$D \frac{\partial^2 C}{\partial r^2} + \frac{D C}{r} \frac{\partial C}{\partial r} = \frac{\partial C}{\partial t}$$  \hspace{1cm} (4-2)$$

where $C(r,t)$ is the total drug concentration in the insert at position $r$ and time $t$, and $D$ is the diffusivity of the drug in the insert. The drug release profiles showed that the loading duration of 6 days was not sufficiently long to reach equilibrium, and thus dynamics of both loading and release phase need to be simulated. The drug uptake can be simulated by solving the differential equation (Equation 4-2.) with the following boundary and initial conditions:

$$C = KC^*_o \quad \text{at} \quad r = R$$  \hspace{1cm} (4-3)$$

$$D \frac{\partial C}{\partial r} = 0 \quad \text{at} \quad r = 0$$  \hspace{1cm} (4-4)$$

$$C = 0 \quad \text{at} \quad t = 0$$  \hspace{1cm} (4-5)$$

where $K$ is the partition coefficient of drug in HEMA, $C^*_o$ is concentration of drug in solution in which uptake experiments were conducted and $R$ is the radius of the insert. The boundary conditions are based on the negligible change in concentration during the uptake due to large volume of fluid used (Equation 4-3) and symmetry about the centerline (Equation 4-4). The differential equation (Equation 4-2) along with conditions Equations 4-3 to 4-5 can be solved analytically to yield the following solution for the concentration profiles $C(r,t)$ in the insert.
\[ C(r, t) = KC_0^* - \sum_{n=1}^{\infty} \frac{2KC_0^*}{\lambda_n J_1(\lambda_n)} J_0\left(\frac{\lambda_n r}{R}\right)e^{-\frac{\lambda_n^2 D}{R^2}t} \]  
(4-6)

where \( J_0 \) and \( J_1 \) are Bessel functions of zero and first order respectively, and \( \lambda_n \) are the zeroes of \( J_0 \) i.e., roots of the equation \( J_0(\lambda_n) = 0 \). Based on the above solution, the concentration in the insert at the end of the loading duration, i.e., \( t = t_1 = 6 \) days is given by the above equation with \( t = 6 \) days.

The drug release from the inserts can be simulated by solving the differential equation (Equation 4-2) with the following boundary and initial conditions

\[ C = 0 \quad \text{at } r = R \]  
(4-7)

\[ D \frac{\partial C}{\partial r} = 0 \quad \text{at } r = 0 \]  
(4-8)

\[ C = C_1(r) \quad \text{at } t = 0 \]  
(4-9)

where \( C_1(\ r\ ) \) is defined by Equation 4-6 with \( t = 6 \) days. The boundary conditions are based on the perfect sink (Equation 4-7) and symmetry about the centerline (Equation 4-8). Equation 4-2 along with Equations 4-7 to 4-9 can be solved analytically to yield the following solution for the concentration profiles \( C(r,t) \) in the insert during release experiments

\[ C = \sum_{n=1}^{\infty} \frac{2KC_0^*}{\lambda_n J_1(\lambda_n)} \left(1 - e^{-\frac{\lambda_n^2 D}{R^2}t}\right) J_0\left(\frac{\lambda_n r}{R}\right)e^{-\frac{\lambda_n^2 D}{R^2}t} \]  
(4-10)

The cumulative mass of drug released into the solution \( M(t) \) can be related to flux of drug at the boundary, i.e. at \( r = R \) by the following equation

\[ \frac{dM}{dt} = -D \left(\frac{\partial C}{\partial r}\right)_{r=R} 2\pi RL. \]  
(4-11)
Substituting Equation 4-10 in Equation 4-11 and integrating with respect to $t$ yields the following equation for $M(t)$

$$M = 4Kc_0V_{\text{insert}} \sum_{m=1}^{\infty} \left( 1 - e^{-\frac{\lambda_n^2 D}{R^2} t} \right) \left( 1 - e^{-\frac{\lambda_n^2 D_f}{R^2} t} \right) \frac{\lambda_n^2}{\lambda_n^2}$$

(4-12)

where $V_{\text{insert}}$ is the volume of inserts soaked in pbs. Diffusivity, $D$, and partition coefficient, $K$, are unknown and are therefore used as a fitting parameter to fit the experimental data for cumulative amount released $M(t)$ to Equation 4-12 to yield values of $(0.95 \pm 0.29) \times 10^{-13} \text{m}^2/\text{s}$ and $41.8 \pm 4.73$ for $D$ and $K$, respectively. The simulated profile with average values of $D$ and $K$ is plotted in Figure 4-2B along with the experimental profile.

The value of the measured diffusivity $D = (0.95 \pm 0.29) \times 10^{-13} \text{m}^2/\text{s}$ is more than an order lower than the value of $D_{\text{th}} = 10.2 \times 10^{-12} \text{m}^2/\text{s}$ estimated from the Brinkman’s equation. This difference between the diffusivity values could potentially be attributed to the binding of the drug molecules on the p-HEMA polymer chains. A majority of the cyclosporine A that is absorbed into the p-HEMA inserts during soaking is absorbed on the polymer as evident from the high partition coefficient. The free cyclosporine A molecules can diffuse through the pores in the p-HEMA hydrogel, and the bound molecules could potentially diffuse along the polymer chains. Additionally, there is a very rapid exchange of the bound and the free cyclosporine A molecules. Assuming that the exchange between the bound and free molecules is more rapid than diffusive time scales, an effective diffusivity, $D$, can be defined taking into account both surface and free diffusion of the bound and the free forms [87]

$$D = \frac{f D_f + (K - f) D_{su}}{K}$$

(4-13)
where f is volume fraction of water in hydrated HEMA insert, $D_I$ is the bulk diffusivity of cyclosporine A inside the gel i.e., through the porous structure in the polymer, and $D_{su}$ is the diffusivity of cyclosporine A on the surface of polymer. The value of f was determined to be approximately 40% by water swelling experiments as discussed later (Figure 4-12). Assuming negligible surface diffusion, the expression for effective diffusivity simplifies to $D = \frac{fD_I}{K}$. Using the value of estimated diffusivity based on Brinkman’s equation and measured values of f and K yields a value of $D = (0.98 \pm 0.11) \times 10^{-13} \text{ m}^2/\text{s}$, which is in reasonable agreement with the measured value of $(0.95 \pm 0.29) \times 10^{-13} \text{ m}^2/\text{s}$. Using a t-test analysis, we can say that the difference in diffusivities obtained from two different methods is statistically insignificant. This reasonable agreement suggests that the discrepancy between the measured and predicted values for diffusivity arose due to drug binding to the polymer, and that this effect can be taken into account by defining an effective diffusivity through Equation 4-13.

Below we discuss cyclosporine A release from inserts in which the drug was loaded by direct addition to the polymerizing mixture.

4.3.3 Design I-Insert

4.3.3.1 Effect of length on drug release

Figure 4-3A shows the cumulative amount of drug released as a function of time from three 1.02 mm diameter inserts of different lengths (4, 7.5 and 10 mm for the short, medium and long inserts, respectively) with 20% drug loading and 0 X crosslinking. The release profiles appear to be approximately zero order for the first 15 days for all the three cases. The release rates increase with increasing length and in fact are linear in length for longer inserts as shown in Figure 4-3B in which the % release (cumulative
release/initially loaded amount x 100) overlap for the medium and the long inserts. The 7.5 mm insert releases approximately 20μg/day for 15 days. The linear dependence of released amount of drug on length is expected because the curved surface areas of the longer (7.5 and 10 mm long) devices are much larger than their cross-sectional areas; therefore, majority of the drug flux is in the radial direction. Accordingly, the axial transport can be neglected while modeling drug release from these systems, which is described in a later section.

4.3.3.2 Effect of drug loading

To explore the effect of drug loading on the drug release profiles, we prepared 1.02 mm diameter and 7.5 mm long inserts with 0 X crosslinking and several different drug loadings such as 5%, 10%, 20% and 30%. The results in Figure 4-4A show that the cumulative release curves are linear in time and independent of drug loadings for certain period. The instant of time at which a curve deviates from the other overlapping curves depends on the drug loading, with higher loadings inserts exhibiting linear, loading-independent behavior for longer times. We propose that the independence of the release rate on drug loading at short times arises from the fact that the drug release rates are initially controlled by the mass transport resistance on the fluid side. The resistance in the gel increases with time due to the thickening of the mass transfer boundary layer, and beyond certain time, the release is controlled by the gel, and subsequently the drug release rates begin to depend on the drug loading. The details of this mechanism will be described and validated later in the section on model development.
4.3.3.3 Effect of crosslinking

To explore the effect of crosslinking on release profiles, inserts were prepared with crosslinking of 0X, 10X and 100X respectively, with 0X, 10X and 100X crosslinking referring to 0%, 2.25% and 22.5% by wt of EGDMA in the monomer mix, respectively. These inserts were each 7.5 mm long, 1 mm in diameter and contained 20% drug. The results in Figure 4-4B show that increasing the crosslinking from 0X to 10X does not have a significant effect on the drug release, but increasing crosslinking to 100X decreases the drug release rate from 20 to about 14μg/day. It is noted that the fact that the drug release rates are relatively similar for the 0X and 10X inserts does not imply that the drug diffusivity is the same for both systems. As mentioned above, at short times the drug release rates are controlled by the fluid, and accordingly the release rates are independent of both drug loading and gel crosslinking. For the 100X crosslinking, the gel begins to control release at an earlier time and so its release rates are different than those for 0X and 10X systems. In fact at longer times, the release rates from the 10X systems are expected to be less than that for the 0X systems.

The above results show that the drug release rates from these devices can be controlled by manipulating the geometry, crosslinking, and drug loading or a combination of these parameters.

4.3.3.4 Effect of convection

The experiments described above were performed without forced stirring to simulate the limited mixing conditions in the conjunctival sac. To explore the transport mechanisms, drug release experiments were conducted with 7.5 mm long, 1mm diameter plugs with 5%, 10%, 20% and 30% drug in presence of forced stirring. Comparison of the cumulative release profiles in presence of convection shown in
Figure 4-4C with those in absence of convection (Figure 4-4A) clearly show that stirring increases the drug release rates, further supporting the hypothesis that that limited mixing in the release medium is impacting the release from the inserts and it must be taken into account while modeling the drug release.

4.3.3.5 **SEM imaging of inserts**

The drug solubility in the monomer mixture is much higher than that in the HEMA polymer and thus the drug molecules are likely to form precipitates during the polymerization process. The presence and sizes of the precipitates were verified directly through imaging of the cross-sections of the drug loaded inserts in JEOLJSM-6400 scanning electron microscopy (X-SEM). The X-SEM images for pure HEMA insert (0% drug loading) along with the images for inserts with 5%, 10% and 15% drug loading are presented in Figure 4-5. The imaging clearly proves the existence of precipitates, which are fairly non-uniform in size, with the largest aggregates about 5-10 microns in size for all drug loadings. Although the precipitate sizes are in the micron range, the particles are much smaller than the device size, which is about 1-mm, and thus in the model developed below, the particles can be considered as point-sources uniformly dispersed in the gel matrix.

4.3.3.6 **Model**

The X-SEM images prove that cyclosporine A drug is present as particles inside the conjunctial inserts. Also, prior experimental studies and models for materials that contain drug as particles show that the plots of % drug released as function of $\sqrt{t/C_p}$, where t is the time and $C_p$ is the drug loading, overlap for different drug loadings. The plots of % release from the inserts as a function of $\sqrt{t/C_p}$ (Figure 4-6) do overlap for
conjunctival inserts, which is consistent with release rates from devices that contain drugs dispersed as particles [87]. Therefore conjunctival inserts can be modeled as cylinders which contain drug embedded in a polymer matrix as particles. Additionally, the curves in Figure 4-4 are linear at long times, which is expected [87] but each curve has a non-linear curved portion at short times which is quite interesting. In fact, in the non-linear portion, the rate of drug released from each insert is the same, and is thus independent of the drug loading. Furthermore, the amount released in this non-linear portion increases with increased mixing, suggesting that the short-time behavior is caused because of the mass transfer resistance on the fluid side. In general, drug transport from inside the insert to the bulk fluid faces two mass transfer resistances in series – resistance due to diffusion in the gel and that due to diffusion in the fluid. The resistance in each phase is directly proportional to the boundary layer thickness. The boundary layer thickness in the fluid depends on the extent of mixing and is independent of time, whereas, the boundary layer thickness in the insert is zero at initial times, and it then grows as square root of time. Accordingly, at short times the resistance in fluid will dominate because of very small mass transfer boundary layer, and hence negligible resistance in the gel, and at long times the resistance in gel will dominate because of thickening of the boundary layer in the gel. Below, we develop a mathematical model for drug release from the inserts that takes both resistances into account and explains all the observed trends in the drug release profiles.

Since the drug loading is above the solubility limit, the hydrated conjunctival inserts contain drug in three forms [87] (i) free drug dissolved in the fluid that hydrates the gel, (ii) drug adsorbed on the polymer surface, and (iii) drug present as
aggregates/particles. On exposing the inserts to PBS, the un-aggregated drug diffuses out of the insert to reduce the free concentration of the drug below the solubility limit, which leads to dissolution of the drug particles. The breaking up of aggregates will form a depletion zone near the surface, whose length is denoted by $\delta$, which will increase in time. To model the problem, we assume that the concentration profile in the depletion zone is in pseudo-steady state, i.e., the time scale for transport is controlled by the time scale for the growth of the depletion zone. This is a reasonable assumption because the total drug loading is significantly above the solubility limit, and so a very large fraction of the drug exists as aggregates. To develop a mass balance equation, consider the interface between the region that is depleted of all the drug particles due to drug transport into the PBS and the core of the insert that still has drug as particulates. The thickness of the depletion zone is denoted by $\delta$, thus this interface is located at a distance $(R-\delta)$ from the axis of cylinder, where $R$ is the radius of the insert. A graphical representation of the model is presented in Figure 4-7. The radial diffusive flux of the drug from this surface leads to a growth of the depletion region by $\Delta \delta$, i.e.

$$-D \frac{\partial C}{\partial r} 2\pi r L = C_p 2\pi (R-\delta)L \frac{\Delta \delta}{\Delta t} \quad \delta < r < R$$

(4-14)

where $C = C(r, t)$ is concentration at time $t$ and distance $r$ inside the depletion layer $\delta < r < R$; $D$ is the effective diffusivity of cyclosporine A inside the gel, $L$ is the length of the cylinder, and $C_p$ is the initial loading of drug inside the cylinder. Equation 4-14 can be simplified to yield

$$r \frac{\partial C}{\partial r} = \frac{(R-\delta)}{D} \frac{d \delta}{dt} C_p \quad \delta < r < R$$

(4-15)

The above governing equation is subjected to the following boundary conditions:
at \( r = (R - \delta) \), \( C = KC^* \) \hspace{1cm} (4-16)

at \( r = R \), \( C = KC_f \) \hspace{1cm} (4-17)

at \( r = R \), \( -D \frac{\partial C}{\partial r} = \frac{D_{\text{fluid}} (C_f - C_b)}{\delta_f} \) \hspace{1cm} (4-18)

where \( K \) is the partition coefficient for the gel, i.e., the ratio between the equilibrium concentrations of drug in the gel and that in the aqueous phase. Furthermore, \( C^* \) is the solubility limit of cyclosporine A in the aqueous phase, \( D \) and \( D_{\text{fluid}} \) are the effective diffusivities of cyclosporine A in the insert and in the release medium (PBS), respectively, \( C_t \) is the drug concentration at any instant \( t \) in the fluid at the boundary with the insert, i.e., at \( r = R \), and \( \delta_t \) is the boundary layer thickness of the mass transfer boundary layer in the fluid that depends on the extent of mixing. \( C_b \) is the concentration of cyclosporine A in the bulk in PBS (release medium) which would be zero if perfect sink conditions were assumed. The boundary conditions in Equation 4-16 defines equilibrium at \( r = R - \delta \) between the concentration in the aqueous phase in the gel, which must be the solubility limit \( C^* \), and the total drug concentration in the gel, which is accordingly \( KC^* \). Similarly, at \( r = R \), i.e., on the surface of the cylinder in contact with the release medium, the gel concentration \( C \) will be in equilibrium with the fluid concentration at \( r = R \), which is denoted by \( C_t \) (Equation 4-17). It is noted that the drug concentration in the mass transfer boundary layer on the fluid side decreases from \( C_t \) at \( r = R \) to the bulk concentration \( C_b \) at \( r = R + \delta_t \), where \( \delta_t \) is the mass transfer boundary layer thickness in PBS. Accordingly, the diffusive flux in the mass transfer boundary layer in fluid is \( D_{\text{fluid}} \frac{C_f - C_b}{\delta_f} \). This expression assumes that the boundary layer
thickness is much smaller than the insert radius. A slightly more accurate expression that accounts for the curvature can be easily include, if desired. The diffusive flux in fluid at \( r = R \) must equal the diffusive flux inside the gel at \( r = R \), which is given by the expression \(-D \frac{\partial C}{\partial r}\). By equating these two fluxes, we obtain the boundary condition given by Equation 4-18. The above sets of equations are consistent with the observation that the release profiles for all drug loadings overlap when plotted as a function of \( \sqrt{t/C_p} \). The diffusion of drug into the fluid leads to an increase in concentration of cyclosporine A in the bulk in PBS (release medium) \( C_b \), which can be determined from the following mass balance

\[
V_b \frac{dC_b}{dt} = 2\pi(R-\delta)L\rho \frac{d\delta}{dt} - \frac{d}{dt} \log(C_p) \eta \tag{4-19}
\]

where \( V_b \) is the volume of PBS in which release experiments were conducted. The concentration \( C_b \) increases with time but it is reset to be zero every time the pbs is replaced.

Equation 4-15 can be integrated with respect to \( r \) to get Equation 4-20, where \( \eta \) is a constant that needs to be determined.

\[
C(r,t) = -\frac{(R-\delta)}{D} \frac{d\delta}{dt} C_p \log r + \eta \tag{4-20}
\]

By utilizing the three boundary conditions (Equation 4-16 to Equation 4-18) in Equation 4-20, we obtain the following ordinary differential equation for \( \delta(t) \),

\[
\frac{d\delta}{dt} = \frac{C^* - C_b}{(R-\delta)C_p \left( \frac{\delta_f}{R D_f} - \log\left(\frac{R-\delta}{R}\right) \right)} \tag{4-21}
\]
Equations 4-19 and Equation 4-21 now represent a set of coupled ordinary differential equations that can be solved simultaneously to obtain $\delta$ and $C_b$ as a function of time. As stated above $C_b$ is assigned to be zero every time the fluid is replaced. The values of the known parameters that are needed to solve the model are listed in Table 4-2. The diffusivity of cyclosporine A in aqueous phase, $D_{\text{fluid}}$, was obtained by using Stokes–Einstein equation for cyclosporine A using a radius of 9.5 Å [77] and temperature 298 K. The rate of the drug diffusing from the insert into the release medium $N$ is given by the following expression

$$N = 2\pi(R - \delta)LC_p \frac{d\delta}{dt}$$

(4-22)

The cumulative mass of cyclosporine A released into the release medium, $P$, is related to $N$ by integrating the release rate $N$ with respect to time. The values of $P$ obtained as a function of time from the model can be fitted to experimental data to obtain the unknown parameters $K_D$ and $\delta_f$. Since the effective diffusivity $D$ was earlier shown to be equal to $\frac{fD_f}{K}$, the parameter $K_D$ can be simplified to $fD_f$. It is noted that while the amount of drug loaded into the insert is known, a fraction of it was lost during fabrication, and some fraction may be irreversibly trapped. Accordingly, the total amount of drug released from an insert was also utilized as a parameter to fit the data. The fit was done by minimizing the least square error between the predicted and measured cumulative release profiles. A few sample comparisons between the predicted and the experimental profiles for the cumulative release are presented in Figure 4-8A (without convection) and Figure 4-8B (with convection). The model predictions match the data well proving the validity of the proposed mechanisms and the model.
We estimate $\text{KD} = fD_f$ by fitting the model to the release data with low drug loadings (for the case of without convection) and use that value to fit all the release profiles (Figure 4-8A.). We estimate a value of $\text{KD} = fD_f = (10.93 \pm 2.37) \times 10^{-12} \, \text{m}^2/\text{s}$ and value of mass transfer boundary layer thickness inside the fluid $\delta_f$, to be $0.47 \pm 0.06 \, \text{mm}$. The values of the diffusivity in an insert with a particular drug loading should be independent of degree of mixing and must be same for the case of convection and without convection. Also, mass transfer boundary layer in fluid which is a representation of amount of convection in fluid should be different for the cases of convection and without convection. Therefore we use the same value of diffusivity and different value of thickness of the mass transfer boundary layer to fit the data with convection (Figure 4-8B.). The value of $\delta_f$ obtained from the fits for the case of convection is $0.154 \pm 0.05 \, \text{mm}$. A t-test analysis showed that the boundary layer thicknesses for the case of convection and without convection are significantly different statistically ($p<0.01$) and that the values decrease on increasing mixing.

Utilizing a value of $41.8 \pm 4.73$ for $K$ and the fitted value of $\text{KD} = fD_f = (10.93 \pm 2.37) \times 10^{-12} \, \text{m}^2/\text{s}$ yields $D = \frac{KD}{K}$ to be $(2.62 \pm 0.27) \times 10^{-13} \, \text{m}^2/\text{s}$, which is of the same order but more than twice the values of effective diffusivity obtained from the direct measurements described previously. This difference could potentially be due to the impact of drug on the polymerization. Alternatively, the value of $C^*$ in the gel could be different from the solubility limit of the drug in bulk fluid reported in Table 4-2.
4.3.4 Design II Insert

4.3.4.1 Effect of length

The design II insert comprises of a 1.02 mm diameter HEMA core with a concentric 0.235 mm thick EGDMA shell. Figure 4-9A shows drug release profile from 3 inserts of different lengths (4mm, 7.5mm and 10mm) containing 20% drug. The drug release is approximately zero order for first 10 days and its magnitude increases linearly with length as clearly evident in Figure 4-9B in which the % Release ((cumulative release/ initially loaded amount) x 100) overlap for the 7.5 and 10 mm inserts. The 7.5 mm insert releases 8μg/day of cyclosporine A, which is about 50% of the drug release rate from the design I insert of same length and drug loading. The linear dependence of released amounts on length is expected because the curved surface of the devices is much larger than the cross-section area for the 7.5 and 10 mm inserts.

4.3.4.2 Effect of drug loading

Figure 4-10A shows the release profiles of three 7.5 mm long design II inserts with drug loadings of 5%, 20% and 30%. The release profiles overlap within error bars for first few days and start deviating as time increases suggesting that here again at short times the drug release rates are controlled by the transport in the fluid.

4.3.4.3 Effect of convection

The drug release experiments reported above were conducted without stirring or mixing to mimic the limited mixing in the conjunctival sac. The effect of convection was explored by conducting release experiments with stirring from 7.5 mm long design II conjunctival inserts of diameter 1.47mm with core diameter of 1.02mm (Figure 4-10B). On comparing the results in Figure 4-10B and in Figure 4-10A, one can conclude that
convection increases the amount of drug release showing that the mass transfer in the fluid controls the release rates in early part of drug release.

4.3.4.4 Mechanisms

A 0.235 mm thick EGDMA shell is expected to provide significant resistance to drug transport and so only a 60\% reduction in drug release rates (reduced from 20 \mu g/day to 8 \mu g/day) may be surprising. However the drug that is released from the design II inserts with pure EGDMA shell does not diffuse through the shell but through the cracks that form in the shell due to stress developed in the shell because of differences between the swelling behavior of the EGDMA shell and the HEMA core. Image of the cracks formed in a design II conjunctival insert with 20\% drug loading taken from a sony DSC-T700 digital camera is presented in Figure 4-11A. An enlarged image of the largest crack in insert taken from an optical microscope at a resolution of 10X is presented in Figure 4-11B. Cracks are formed in the radial direction and have varying sizes, largest one having a size of about 0.2mm. The EGDMA water content is almost negligible and the HEMA water content is about 40\%. Thus swelling of the HEMA core leads to stresses in the EGDMA shell leading to cracks that occupy roughly 40\% of the surface. Accordingly, the design II inserts are expected to release drug at a rate of about 40\% of the Type I insert, which is close to the experimentally observed value. Furthermore, the drug release rates are independent of drug loading and are zero order in time because the rate limiting step is drug transport in the fluid. At longer times, the drug release rates will depend on drug loading and will cease to be zero order. A mathematical model for the design II inserts is more complex than that for the design I insert because the drug transport occurs both in radial and axial directions.
around the cracks, which are of complex shapes distributed randomly along the length, and is not presented here.

4.3.4.5 Effect of crosslinking in shell

Figure 4-10C shows release profiles for four design II inserts of length 7.5 mm, overall diameter 1.47 mm, and drug loading of 20% in the core, with different degrees of crosslinking in the shell. The ratio of HEMA and EGDMA was varied to observe the effect of degree of crosslinking in the shell on the drug release. We observe that as we increase HEMA fraction in shell from 0% to 25%, the rate of drug release decreases, but on further increasing the HEMA fraction to 50%, the release rates begin to increase. This behavior occurs because of dual transport mechanisms in parallel: diffusion through the shell and diffusion through the cracks. As explained above, the inserts with pure EGDMA shell release drug through the cracks that form on the surface due to differential swelling between the HEMA core and the EGDMA annulus. The drug transport through the EGDMA shell is negligible because of the negligible drug diffusivity through the EGDMA matrix. As the HEMA content in the shell increases from 0 to 25%, the swelling difference between the core and the annulus decreases leading to a reduction in the crack formation, and a consequent reduction in the drug release rates. As the HEMA fraction in the shell increases beyond 25%, the crack formation further reduces, and thus the amount of drug that diffuses through the crack likely reduces. However this reduction in drug transport through the cracks is compensated by the increased drug diffusion through the shell due to the increased pore size of the HEMA + EGDMA matrix, and thus the total transport rates increase with HEMA addition to the core.
To quantitatively validate the proposed mechanism on the effect of HEMA addition to the shell, we measured the water content of HEMA + EGDMA gels with HEMA fraction varying from 0 to 100% (Figure 4-12). The data shows that the water content (based on increase in weight due to hydration) increases from about 0% for the pure EGDMA gels to about 45% for pure HEMA gels. The differential swelling defined as the difference in water content of the core material (pure HEMA) and the shell material is about 45% for the pure EGDMA shell and about 40% for the 25% HEMA shell. Since the crack formation is expected to be proportional to the differential swelling, the inserts with 0 and 25% HEMA in the shells are expected to have about 45% and 40%, respectively of the surface occupied by cracks. Consequently, the 0% HEMA and 25% HEMA shell inserts should release drug at rates that are about 45% and 40%, respectively of the rates for the design I insert with same length, core diameter and drug loading, which is consistent with the data shown in Figure 4-10C. With further increase in the HEMA fraction, the water content in the shell becomes high enough (~ 10% for the 50% HEMA shells) to allow diffusion of the drug through the shell, thus leading to an increase in the release rates with increasing HEMA fraction in the shell.

4.3.5 Bioavailability of Conjunctival Inserts

The inserts developed here can release about 10-20 µg/day depending on time, length, loading, coating, and degree of mixing in the fluid surrounding the insert. The mixing in the inferior conjunctival sac is expected to be small, and thus the in vivo release rates may be smaller than the values obtained in the in vitro experiments. The exact release rates and the therapeutic efficacy of these devices can only be
established through in vivo experiments, but a simple pharmacokinetic model could be useful to obtain a rough estimate of the efficacy of these systems.

To develop the pharmacokinetic model, we perform a mass balance on the drug released from a device placed in the inferior conjunctival sac of an eye. A human eye has a tear volume $V$ of about 7-10 μl, and this volume is maintained through a balance between tear secretion from the lacrimal glands and conjunctiva and tear elimination through drainage through the canaliculi into the nasal cavity and also through evaporation. The tear volume increases after eye drop instillation and then decreases to the baseline value in about 5-10 minutes depending on the viscosity of the instilled fluid. The drug released from the conjunctival insert into the tear film can either diffuse into the ocular tissue through the cornea and the conjunctiva, or exit the tear volume with tears that drain into the nasal cavity. Thus, a mass balance on the drug released from the insert gives the following equation,

$$V \frac{\partial C}{\partial t} = F - q_{\text{Drainage}}C - kAC$$

(4-23)

where $V$ is total volume of fluid on ocular surface, $F$ is the drug release rate by conjunctival inserts (~ 10-20 μg/day), $kA$ is the sum of the product of the area and the permeability for the cornea and the conjunctiva, and $C$ is the dynamic drug concentration.

Since the time scale for release from the inserts is a few days, which is much longer than the time scale for concentrations in the eyes to reach a steady state, one can assume that the drug release from the inserts could be considered to be at a pseudo-steady state, i.e.,

$$F = q_{\text{Drainage}}C + kAC$$

(4-24)
The details of the mechanism of the therapeutic action of cyclosporine A and the exact site of action are not exactly known and so for simplicity we assume that both the drug that enters the cornea and also the conjunctiva can lead to therapeutic benefits. Accordingly, we define bioavailability as the ratio of the drug that enters the ocular tissue (cornea or conjunctiva) and the amount released by the insert, i.e.,

\[
\text{Bioavailability} = \frac{kAC}{F} = \frac{kA}{kA + q_{\text{drainage}}}
\]

We assume corneal and conjunctival permeability to be similar and equal to 1.1×10^{-6}\, \text{cm/s} [88] and corneal and conjunctival area to be 1.04 cm² and 17.65 cm², respectively [89]. Using \(q_{\text{drainage}}\) ranging from 1×10^{-11}\, \text{m}^3/\text{s} to 4×10^{-11}\, \text{m}^3/\text{s} [22], we obtain bioavailability values of conjunctival inserts ranging from 34\% to 67\%.

The typical dry eye treatment based on cyclosporine A involves delivery of two drops of Restasis® each day. Restasis® contains 0.05\% drug, and thus two 28 \(\mu\)l drops of this formulation deliver about 28\(\mu\)g cyclosporine A to the eye. The bioavailability of cyclosporine A delivered via eye drops is expected to be small due to the small residence time of about 10 minutes in the eyes [90] and thus a release of about 10-15 \(\mu\)g each day from the inserts may be adequate, particularly considering the increased bioavailability for inserts. This simple pharmacokinetic model suggests that the release from the inserts developed here may be therapeutically efficacious.

### 4.4 Conclusion

We have explored the mechanisms of cyclosporine transport in HEMA rods for developing conjunctival inserts. The diffusivity of the drug in the inserts can be correctly predicted by using Brinkman’s equation but only after drug binding to the polymer has been taken into account. The release of the drug from the inserts exhibits some
interesting trends that cannot be explained by simple diffusive transport in the gel. For instance, the release rates are zero-order in time and are independent of drug loading and crosslinking for certain duration, beyond which the rates decrease in time and are lower for lower loadings and higher crosslinking. All of these effects arose due to limited mixing in the fluid that resulted in creation of a mass transfer boundary layer in release medium. In general, drug transport from inside the insert to the bulk fluid faces two mass transfer resistances in series – resistance due to diffusion in the gel and that due to diffusion in the fluid. The resistance in each phase is directly proportional to the boundary layer thickness. The boundary layer thickness in the fluid depends on the extent of mixing and is independent of time, whereas, the boundary layer thickness in the insert is zero at initial times, and it then grows as square root of time. The boundary layer thickness scales as $\sqrt{Dt}$ in inserts in which the drug is below the solubility limit but scales as $\sqrt{Dt} \sqrt{\frac{C_p}{KC^*}}$ in inserts in which the drug is dispersed as particles (all notation used here is described previously in the text). In inserts or other gels that do not contain drug particles, the boundary layer in the device grows rapidly and thus control the overall release behavior except for extremely short times in which fluid mixing may play a role. However, the very slow growth of the mass transfer boundary layer in gels that contain drug particles ensures that the mass transfer resistance in the fluid will be rate limiting for a larger duration of time. Enhanced mixing reduces the mass transfer barrier in the fluid, but cannot completely eliminate it. Thus, the behavior of zero-order release will likely be observed from all gels in which drug concentration is so high such that drug is dispersed as particles. In all such cases, the simple diffusion model will not
match the data but the modified model proposed here that incorporates the presence of the particles and the mass transfer boundary layer in the fluid will be needed.

The proposed model can be used to determine the release rates for any degree of mixing. The extent of mixing is not yet established in eyes; therefore *in vivo* experiments would have to be conducted to determine the release profiles under physiological mixing. The diffusivity of drug in the polymer was also estimated from Brinkman’s equation and also directly measured by loading and releasing drug into inserts that were prepared without any drug. The values of diffusivity measured agreed with the value estimated from the Brinkman’s equation if only the free form of cyclosporine A was assumed to diffuse, i.e., the drug adsorbed on the polymer was assumed to be in equilibrium with the free form but not diffusible along the surface. Both, the directly measured value and that estimated from the Brinkman’s equation were about half of the value obtained from fitting the model to drug release from the design I insert. The difference could be due to the effect of the drug directly added to the monomer mixture on the polymerization, and/or the differences between the solubility limit of the drug in the pores of the gel and solubility limit in bulk fluid.

The inserts developed here deliver cyclosporine A at 20μg/day for about a month compared to about 28 μg/day delivered by Restasis. The duration of release and the amount released each day can be increased by increasing the drug loading and/or crosslinking, and length, respectively. The release depends on the extended of mixing, which is limited in the lower conjunctival sac, but not yet established definitively; therefore *in vivo* experiments would have to be conducted to determine the release profiles under physiological mixing. It is noted that although the devices proposed here
seem promising, but their potential for medical applications needs to be demonstrated through *in vivo* animal and human trials to determine the degree of comfort, biocompatibility, *in vivo* release profiles, and therapeutic efficacy.
Figure 4-1. Schematic representation of Design I (A) and Design II (B) inserts (not to scale).
Figure 4-2. Estimation of diffusivity of cyclosporine A in p-HEMA insert (A) Drug Release from the inserts which were soaked in a cyclosporine A solution for 4 days to load the drug. (B) Comparison of model and experimental results for release experiments using the mean value of fitted diffusivity.
Figure 4-2. Effect of length on release profiles from design I conjunctival inserts (drug loading =20%) (A) Cumulative release. (B) % Release (amount released / total drug loading).
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Figure 4-6. Model for drug-release from a cylindrical rod (conjunctival insert) that contains drug at concentrations $C_p$, which is above the solubility limit, and so a fraction of the drug precipitates as particles. The model combines mass transfer in the insert and that in the surrounding fluid boundary layer of thickness $\delta_f$. The insert contains drug particles distributed uniformly at $t = 0$. Dissolution of the particles and subsequent diffusion of the drug creates a particle-free zone near the periphery, whose thickness $\delta$ growth with time.
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Figure 4-10. Continued
Figure 4-10. Image of a soaked design II insert with (A) a sony T 700 digital camera and an (B) enlarged image of the crack taken at higher resolution by an optical microscope.
Figure 4-11. Dependence of % change in weight of gels due to swelling in water on HEMA % in HEMA:EGDMA gels.
Table 4-1. Values of parameters used to estimate theoretical diffusivity inside HEMA

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<td>$r_1$</td>
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Table 4-2. Values of parameters used in fitting the model to experimental results for release of drug from design I insert

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<tr>
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</tr>
<tr>
<td>L</td>
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</tr>
<tr>
<td>$D_{\text{fluid}}$</td>
<td>$2.29 \times 10^{-10}$ m²/s</td>
</tr>
<tr>
<td>$C^*$</td>
<td>25 gm/m³ [92]</td>
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</tbody>
</table>
CHAPTER 5
DRUG DELIVERY BY PUNCTAL PLUGS

5.1 Introduction

This chapter focuses on delivery of cyclosporine A drug via punctal plugs that are to be placed in canaliculi which connects eyes to the nose. A novel design of punctal plugs has been proposed and mechanism of transport of drug in these systems has been discussed. A pharmacokinetic model has been developed to compare the effectiveness of Restasis® emulsion (commercially existing eye drop) and the punctal plugs.

5.2 Materials and Methods

Cyclosporine A was bought from LC Labs (Woburn, MA); and Silastic® laboratory tubing of size (ID 0.51 mm and OD 0.94mm) and size (ID 1.47mm and OD 1.96mm) were purchased from Dow corning (Midland, MI). Hydroxyl Ethyl Methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), and Azobisisobutylonitrile (AIBN) were purchased from Sigma-Aldrich (St. Louis, MO); while Acetonitrile (HPLC grade) and deionized water (HPLC grade) used as mobile phase in HPLC were bought from Fisher-Scientific. The Dulbecco’s phosphate buffer saline (PBS) used in the drug release experiments was purchased from Sigma-Aldrich (St Louis, MO).

5.2.1 Punctal Plug Fabrication

Punctal plugs were made of HEMA, EGDMA and silicone, all of which are biocompatible. The basic structure of the plugs is shown in Figure 1-2., with a p-HEMA core, which is loaded with cyclosporine A using “direct entrapment” procedure described below. For making the core, 1.35 mL of HEMA monomer was mixed with 5μL of EGDMA monomer referred as “1×” crosslinking, and the mixture was purged with N₂ for
10 minutes to remove the O₂ in the monomer. Subsequently, 20 wt% of cyclosporine A and 0.03 g of AIBN (initiator) were added and the mixture was stirred for 5 minutes. The resulting solution was filled into Silastic® tubing with 0.51 mm ID and 0.94 mm OD. After the tubing was sealed at both ends with office clamps, it was submerged into a water bath at 80 °C for 20 minutes for polymerization. Polymerization of the monomer inside the tubing leads to formation of a compound rod with a p-HEMA core surrounded by an annulus of the silicone tubing. The compound rod was cut into segments of desired length and then the silicone annulus was cut off from a part of the plug to create the designs illustrated in Figure 1-2. The degree of crosslinking was increased by increasing the amount of EGDMA in the polymerization mixture to obtain plugs with 100X crosslinking, which contains 100 times the EGDMA utilized in the procedure described above. Also the amount of drug loading was altered to prepare plugs with drug loading of 20% and 40%.

5.2.2 Drug Release

In vitro drug release profiles were measured by soaking a plug in 3.5 ml of phosphate buffer saline (PBS) which was replaced at regular intervals. The concentration of cyclosporine A in the release medium was determined with an HPLC (Waters, Milford, MA) equipped with a reverse phase C18 column (Waters, Milford, MA) and a UV detector. The mobile phase was 70% acetonitrile and 30% deionized water at a flow rate of 1.2 mL/min. The column temperature was maintained at 60 °C and the UV detector wavelength was 210 nm.

5.3 Results

Commercial plugs are about 1 mm in diameter and 2-mm in length. The plugs described here comprise of a 0.51-mm diameter p-HEMA core with a fraction of the
core coated with a silicon shell of outer diameter 0.94 mm. The length of the plug was chosen to be 3.2 mm with about 50% of the length covered with the shell, which is larger than the commercial plugs, but the length can easily be reduced to 2-mm while keeping other design parameter same. The solubility of cyclosporine A in the polymer is less than that in the monomer mixture; therefore the drug is expected to form precipitates during the polymerization. The cross-section of punctal plugs with 20% drug loading and 0X crosslinking were imaged through JEOL JSM-6400 Scanning electron microscope (X-SEM) at different magnifications (Figure 5-1.). The drug clearly forms particles with a non-uniform size distribution with largest particles of size ~10 μm.

The drug release profile from a plug with a drug loading of 20% and 0X crosslinking is shown in Figure 5-2. The core of the plug weighs about 0.67 mg, and thus it contains about 0.134 mg drug. The plug releases drug at a rate of 3.5 μg/day for about a month without any initial burst, and a relatively zero-order release for the first 10 days. Since, typical plugs are worn for a period much longer than a month; it is desirable to increase the duration of release from the plugs, possibly by increasing the crosslinking. An increase in crosslinking from 0X to 100X results in a decrease in the release rate to about 1.6 μg/day, and the profile is non-zero-order for the entire duration (Figure 5-2). Thus increased crosslinking has the desired effect of increased release duration but the daily release amount decreases, and the duration of zero-order release also decreases. To increase the daily release, plugs were prepared with 40% drug loading while keeping 100 X crosslinking. The effect of increased drug loading while keeping crosslinking at 100X is shown in Figure 5-3. The plugs with 40% drug loading release at a constant rate of about 3 μg/day for about a month and then the rate
decreases slightly. The plugs contain about 225 μg of drug so these are expected to release for about 3 months, which is the typical duration of plug wear.

5.3.1 Mechanisms of Release

The results described above show that the cumulative release curves are linear in time for certain duration. We propose that the independence of the release rate on drug loading at short times arises from the fact that the drug release rates are initially controlled by the mass transport resistance on the fluid side. In general, drug transport from inside of the plug to the bulk fluid faces two mass transfer resistances in series – resistance due to diffusion in the core and that due to diffusion in the fluid. The resistance in each phase is directly proportional to the boundary layer thickness. The boundary layer thickness in the fluid depends on the extent of mixing and is independent of time, whereas, the boundary layer thickness in the core is zero at initial times, and it then grows as square root of time. Accordingly, at short times the resistance in fluid will dominate because of very small mass transfer boundary layer and hence negligible resistance in the gel, and at long times the resistance in gel will dominate because of thickening of the boundary layer in the gel. The resistance in the gel increases with time due to the thickening of the mass transfer boundary layer, and beyond certain time, the release is controlled by the gel, and subsequently the drug release rates begin to depend on the drug loading. These mechanisms suggest that an increase in convection in the release medium will lead to an increase in the release rates. This hypothesis was tested by conducting release experiments with mixing in the release medium.
5.3.2 Effect of Convection

Figure 5-4A shows the effect of convection on drug release profiles from plugs with 20% drug loading and 100X crosslinking. Similarly, the effect of convection on plugs with 40% drug loading and 100X crosslinking is shown in Figure 5-4B. In both cases, increased convection clearly increases the drug release rates proving that at short times, the release rates are controlled by the mass transfer resistance in the fluid. These results support the mechanisms proposed above. The mixing in the canaliculus in the tears surrounding the exposed section of the plug is expected to be very small, and thus the results provided in previous sections may be close to the physiological conditions. However in vivo experiments are necessary to determine the release profiles after insertion of plugs in the canaliculi.

5.4 Model for the Zero Order Release

In the previous section, the zero order profiles at early times were attributed to the diffusion through the mass transfer boundary layer in the fluid. To prove this hypothesis, we predict the release profiles based on this hypothesis and compare the results with the measurements. If mass transfer is limited by diffusion in the fluid, the rate of drug released from the device is given by

\[
\frac{\partial N}{\partial t} = \frac{D_f C^* A}{\delta}
\]  

(5-1)

where \(C^*\) is the concentration of drug in fluid at solid-liquid interface and is assumed to be equal to solubility limit of drug (=25µg/ml [92]) in the release medium. Also, \(D_f\) (=2.29×10\(^{-10}\) m\(^2\)/s [88]) is the drug diffusivity in the fluid, \(\delta\) refers to boundary layer thickness in the fluid, and \(N\) and \(A\) refer to cumulative amount of drug released from the
insert and total surface area for drug release respectively. It is to be noted that drug is released from the plugs through two distinct surfaces, the cylindrical curved surface and the circular cross-section surfaces. Since streamlines of convection in the fluid greatly depend on surface geometry, the boundary layer thicknesses in the fluid for the two surfaces would be independent of each other and have to be evaluated separately. As a result, Equation 5-1 can be modified as

\[
\frac{\partial N}{\partial t} = D_j C^* \left( \frac{S_1}{\delta_1} + \frac{S_2}{\delta_2} \right)
\]

where \( S_1 \) and \( S_2 \) are the curved surface and cross-section areas, respectively, and \( \delta_1 \) and \( \delta_2 \) are the respective boundary layer thickness. Each of the boundary layer thicknesses could be determined by designing the plug such that one of the surface areas for diffusion is blocked. For example, if a plug is designed such that the silicon shell covers the entire length of the plug, the drug can diffuse out only from the circular cross-sections. Equation 5-2 can then be simplified by eliminating the term corresponding to \( S_1 \). The release data from such a plug could then be fitted to the simplified Equation 5-2 to determine \( \delta_2 \). Similarly, a plug with no shell and circular faces covered, or a long plug in which the curved surface area far exceeds the cross-section area could be utilized to determine \( \delta_1 \).

To determine \( \delta_2 \), drug loaded p-HEMA cores of diameter 1.02 mm were first coated with an EGDMA shell of thickness 0.225 mm, and were then surrounded by a silicon shell of thickness 0.25 mm to eliminate any possibility of drug release from the curved areas. Results not reported here showed that only a silicon shell was not sufficient to completely eliminate all radial transport of drug. Plugs were prepared with several different drug loadings to ensure that the release rates were independent of the
drug loadings which is expected on the basis of the model proposed above (Equation 5-2). The data in Figure 5-5 clearly shows that the release profiles are independent of loadings and is zero order for first 8-10 days. The solid line shows the fitted release profiles based on Equation 5-2 with $S_1 = 0$ and $S_2 = 2\pi(0.5)^2$ mm$^2$ and a fitted value of 0.2 mm for $\delta_2$. The value of the boundary layer thickness in fluid surrounding the curved surface of a cylindrical device of radius 0.5 mm, $\delta_1$, has been determined to be 0.47mm in chapter 4. The values of $\delta_1$ and $\delta_2$ can now be utilized in Equation 5-2 with the appropriate values of $S_1$ ($=5.65 \times 10^{-6}$ m$^2$) and $S_2$ ($1.56 \times 10^{-6}$ m$^2$) to predict rate of drug release from the plugs. The predicted profiles are plotted as the solid lines in Figure 5-2 and Figure 5-3. The prediction is in good agreement with the release from the systems with 20% drug loading and 0X crosslinking (Figure 5-2) and 40% drug loading and 100 X crosslinking (Figure 5-3.). The release from 20%, 100X cannot be compared with the model because the profiles are non zero-order as the gel resistance is comparable to the resistance in fluid at early times because of high crosslinking in gel leading to non zero-order behavior. A general model that includes both fluid and gel resistance can be developed in a similar way as discussed in previous chapter (chapter 4) but is not presented here as we are primarily interested in plugs with high drug loadings, and long durations of zero-order release due to their suitability for therapeutic use as drug eluding punctal plugs.

5.5 Pharmacokinetics of Cyclosporine A Delivery via Restasis® and Punctal Plugs

The typical dry eye treatment based on cyclosporine A involves instillation of two drops each day of 0.05 % Restasis® delivering about 25μg cyclosporine A to the eye.
The plug with 40% drug loading and 100X crosslinking release cyclosporine A at a rate of about 3 μg/day. Since the length of the commercial plugs are about half of those developed here, and only one of the circular cross-sections will contact tears, the release rates from a plug inserted in the canaliculus could be about 1.5 μg/day, which is only about 5% of the rate of drug delivered through Restasis® emulsion. However, the bioavailability of cyclosporine A delivered via eye drops is expected to be small due to the small residence time of about 5 minutes in the eyes and thus a release of about 1.5 μg each day from the plugs may be adequate. While clinical trials can determine the therapeutic release rates from plugs, we obtain a rough approximation of the desired rates by developing a pharmacokinetic model for ocular delivery of cyclosporine A both through Restasis® and plugs.

5.5.1 Pharmacokinetics of Cyclosporine A Delivery via Restasis®

This pharmacokinetic model is based on the tear balance model [22] and the tear drainage model [93], which predicts the dynamic aqueous concentration of drugs after instillation through eye drops. After an eye drop is instilled in the eyes, the components in the drop are eliminated through three routes: evaporation, transport into the cornea and the conjunctiva, and tear drainage into the lacrimal sac. The Restasis® formulation is an emulsion that contains 0.625% castor oil and 0.05% cyclosporine A by weight, and surface active agents that are neglected in this analysis. Two drops (25μl each) of this formulation are typically instilled in each eye every day. To develop a pharmacokinetic model for Restasis®, one has to perform mass balances for the three main components of the Restasis® eye drop: drug cyclosporine A, castor oil, and water. The drug cyclosporine A is present both in the oil and the water phase, and the concentrations in
the two phases are expected to be in equilibrium due to the large surface area of the oil drops. In the model presented below, it is assumed that the oil phase only acts as a reservoir of the drug and does not directly penetrate into the corneal or the conjunctival epithelia, which is reasonable due to the relatively large size of the drops. The oil phase also cannot evaporate and so it is eliminated only through the tear drainage pathway.

5.5.1.1 Aqueous phase mass balance

A mass balance on the aqueous fraction of the tears yield

$$\frac{dV_{aq}}{dt} = q_{\text{production}} - (q_{\text{evaporation}} + q_{\text{absorbed}}) - q_{\text{drainage}}\Phi_{\text{aqueous}} \quad (5-3)$$

where $V_{aq}$ refers to total volume of aqueous phase on ocular surface, $q_{\text{production}}$ is rate of tears produced, $q_{\text{evaporation}}$ is rate of tears evaporated, $q_{\text{absorbed}}$ is the rate of absorption of tears by conjunctiva and $q_{\text{drainage}}$ is the rate of tears drained from the eyes towards the nose and $\Phi_{\text{aqueous}}$ is the fraction of aqueous phase in tears. Here it is assumed that the tear production rate, the evaporation rate and absorption rate are not affected by the instillation of eye drops.

Based on the tear drainage model [93], if the viscosity of the eye drop formulation is less than about 10 cp, which is the case for Restasis®, the drainage rate is given by the following expression,

$$q_{\text{drainage}} = \frac{\pi L}{t_c} \left[ \left( \frac{bE \times R_o}{bE + \sigma R_m} \right)^2 - \left( \frac{bE \times R_o}{bE + (p_a - p_sac)R_o} \right)^2 \right] \quad (5-4)$$

where $L$ is the canaliculus length, $t_c$ is the duration of a blink-interblink cycle, $R_0$ is the radius of the undeformed canaliculus, $\sigma$ is the surface tension of the instilled fluids or tears, $R_m$ is the radius of curvature of the tear meniscus, $b$ and $E$ are the thickness and
the modulus of the canaliculus respectively, \( p_o \) is imposed on the canaliculus during a
blink and \( p_{\text{sac}} \) is pressure in the lacrimal sac, which can be taken as zero (atmospheric
pressure). The details of the derivation of the expressions and the values of all the
parameters are available in Ref. [93].

### 5.5.1.2 Oil phase mass balance

A mass balance for oil phase in the tear film yields

\[
\frac{dV_{\text{oil}}}{dt} = -q_{\text{Drainage}} \Phi_{\text{oil}}
\]  

(5-5)

where \( V_{\text{oil}} \) is the volume of oil present on ocular surface at any instant of time and \( \Phi_{\text{oil}} \)
represents the fraction of oil present.

### 5.5.1.3 Drug balance

The mass balance for drug on the ocular surface yield

\[
\frac{d(C_{\text{oil}} V_{\text{oil}} + C_{\text{aq}} V_{\text{aq}})}{dt} = -q_{\text{Drainage}} (\Phi_{\text{oil}} C_{\text{oil}} + \Phi_{\text{aq}} C_{\text{aq}}) - \sum_{i=1}^{2} k_i A_i C_{\text{aq}}
\]

(5-6)

where \( C_{\text{oil}} \) is concentration of drug in oil phase and \( C_{\text{aq}} \) is concentration of drug in
aqueous phase. The parameters \( k_1 \) and \( k_2 \) are permeabilities of cornea and conjunctiva,
respectively for cyclosporine A, and \( A_1 \) and \( A_2 \) are the areas of cornea and conjunctiva,
respectively. Finally, the aqueous and oil fractions are defined by Equation 5-7.

\[
\Phi_{\text{oil}} = \frac{V_{\text{oil}}}{V_{\text{oil}} + V_{\text{aq}}}, \quad \Phi_{\text{aq}} = \frac{V_{\text{aq}}}{V_{\text{oil}} + V_{\text{aq}}}
\]

(5-7)

Also, the oil concentration and aqueous phase concentration at any instant of time are
assumed to be in equilibrium, i.e.,

\[
C_{\text{oil}} = K_{\text{Partition}} C_{\text{aq}}
\]

(5-8)
Due to lack of available data for partition coefficient of cyclosporine A in castor oil $K_{\text{partition}}$, its value is assumed to equal the octanol-water partition coefficient for the drug.

5.5.1.4 Geometric relationship

The radius of meniscus, $R_m$, required in Equation 5-4 to obtain the drainage rates is a function of total ocular volume $V_{\text{total}}$ ($= V_{\text{aq}} + V_{\text{oil}}$) at any instance of time and can be related to the meniscus curvature through the following geometric relationship [93].

$$V_{\text{total}}(R_m) = V_{\text{film}} + (1 - \frac{\pi}{4}) R_m^2 L_{\text{lid}}$$  \hspace{1cm} (5-9)

Here $V_{\text{total}}$ is total volume including oil volume and aqueous phase volume of fluid, $V_{\text{film}}$ is fluid in the exposed and unexposed tear film, and $L_{\text{lid}}$ is perimeter of the lid margin. Total tear volume at steady state is assumed to be 10 $\mu$l [94] and using a steady state radius of meniscus of 0.37 mm, $V_{\text{film}}$ is determined to be 8.3 $\mu$l.

By solving the above equations simultaneously using finite difference method we can obtain the ocular volume, oil and aqueous volume fractions, and the drug concentration as a function of time. The parameters used to solve the above set of equations are available in [22,93] and are also presented in Table 5-1. The initial condition for the simulations corresponds to addition of a 25 $\mu$l drop of Restasis® emulsion to the ocular film that has the steady state radius of meniscus of 0.37 mm, which is equivalent to a initial tear volume of 10 $\mu$l.

5.5.1.5 Bioavailability

After determining the aqueous and oil volume fractions, and the drug concentration as a function of time, one can determine the bioavailability ($F$) of cyclosporine A. The details of the mechanism of the therapeutic action of cyclosporine A and the exact site of action are not exactly known and so for simplicity we assume
that both the drug that enters the cornea and also the conjunctiva can lead to therapeutic benefits. Accordingly, we define bioavailability as the ratio of the drug that enters the ocular tissue (cornea or conjunctiva) and the amount administered into the eye. It can thus be defined as

$$F = 1 - \frac{\int q_{\text{Drainage}} (C_{\text{aq}} \phi_{\text{aq}} + C_{\text{oil}} \phi_{\text{oil}}) \, dt}{M_0}$$  \hspace{1cm} (5-10)$$

where \(M_0\) is the initial amount of drug in the formulation. By solving the equations in MATLAB we obtain a value of 2.8 % for the bioavailability of cyclosporine A delivered through Restasis®, which amounts to delivery of 0.7 μg/day to cornea and conjunctiva.

5.5.2 Pharmacokinetics of Cyclosporine A Delivery via Punctal Plugs

To develop the pharmacokinetic model for drug released from a plug, we perform a mass balance on the drug released into the ocular tear film. A human eye has a tear volume \(V\) of about 10 μl, and this volume is maintained fixed through a balance between tear secretion from the lacrimal glands and conjunctiva and tear elimination through drainage through the canaliculi into the nasal cavity and also through evaporation. The drug released from the plug into the tear film can either diffuse into the ocular tissue through the cornea and the conjunctiva, or exit the tear volume with tears that drain into the nasal cavity. Thus, a mass balance on the drug released from the insert gives the following equation,

$$V \frac{\partial C}{\partial t} = F - q_{\text{Drainage}} C - k_A C$$  \hspace{1cm} (5-11)$$

where \(V\) is total volume of fluid on ocular surface, \(F\) is the drug release rate by plug (~1.5 μg/day), \(k_A\) is the sum of the product of the area and the permeability for the cornea and the conjunctiva, and \(C\) is the dynamic drug concentration.
Since the time scale for release from the inserts is a few days, which is much longer than the time scale for concentrations in the eyes to reach a steady state, one can assume that the drug release from the inserts could be considered to be at a pseudo-steady state, i.e.,

\[ F = q_{\text{drainage}} C + kAC \]  \hspace{1cm} (5-12)

Based on the definition of bioavailability (F) described above, the value of F for drug released from the plug can be computed as

\[ \text{Bioavailability} = \frac{kAC}{F} = \frac{kA}{kA + q_{\text{drainage}}} \] \hspace{1cm} (5-13)

The presence of a punctal plugs in one of the canaliculus reduces the drainage rate to roughly about half of the normal value. Based on Equation 5-4, the drainage rate under normal physiological conditions from both canaliculi is 1.4µl/min. This value is in reasonable agreement with reported values of 1-2µl/min for tear drainage [95]. Using half of this value in Equation 5-13 (since one of the puncta is blocked by punctal plug) gives a value of 64% for bioavailability of cyclosporine A released from a plug. Based on the model for pharmacokinetics of Restasis® developed above, the therapeutic requirement of cyclosporine A is about 0.7 µg/day, and thus a release of 1.5 µg/day of cyclosporine A from the plug with a bioavailability of 64% may be adequate.

5.6 Conclusion

Dry eyes are a major health care problem in the United States and worldwide. These are frequently treated either by instillation of eye drops, drugs such as Restasis®, and/or by blocking tear drainage through insertion of punctal plugs. Here we propose to combine the two approaches by delivering cyclosporine A through punctal plugs that also block tear drainage. In addition to serving as a dual-approach for
treating dry eyes, drug delivery through punctal plugs will likely increase compliance and reduce side-effects due to systemic uptake of ophthalmic drugs.

The ocular requirements of cyclosporine A are estimated by developing a pharmacokinetic model for Restasis®, which contains 0.05% drug, and two 25 μl drops of this formulation are delivered each day. A drop of Restasis® contains about 12.5 μg and the bioavailability of the drug delivered through Restasis® is determined to be about 2.8 % suggesting that the therapeutic requirements of ocular tissue is about 0.7 μg/day.

Punctal plugs with dimensions similar to commercial plugs and with 40% drug loading can release drug at ~ 1.5 μg/day for more than 2 months. Plugs developed here do not have any initial burst effect, thereby minimizing the chances of having any toxic effects due to sudden release of a large quantity of drug. Also, drug release profiles are approximately linear for more than 40 days, which is an added asset for a drug delivery system. A model has been proposed to predict the linear release profile or zero order release at short times.

The degree of convection plays a prominent role in controlling the release rates particularly at short times. The extent of mixing is not yet established in eyes; therefore in vivo experiments would have to be done to determine the drug release profiles from these drugs under physiological mixing. Also, the results presented in this paper are for cyclosporine A but the concept can be applied to fabricate drug loaded punctal plugs for other eye diseases. Although the devices proposed here are prepared with biocompatible materials, the degree of comfort and biocompatibility is needed to be explored for these devices through testing in animal models.
Figure 5-1. X-SEM images of punctal plugs with 20% drug loading.

Figure 5-2. Effect of crosslinking on cumulative drug release profiles from the punctal plugs with 20% drug loading (131 μg). Data represented as mean ± SD (n=3). The solid line is the prediction of the profile for the 20%, 0X system based on the model described below.
Figure 5-3. Effect of increased drug loading on cumulative drug release profiles from the punctal plugs with 100X crosslinking. Data represented as mean ± SD (n=3). The solid line is the prediction for the 40%, 100X system based on the model described below.

Figure 5-4. Effect of increased convection on cumulative drug release profiles from the punctal plugs with crosslinking of 100X and drug loading of (A) 20% (131 μg) and (B) 40% (225 μg). Data represented as mean ± SD (n=3).
Figure 5-5. Drug release profile of plugs totally covered with silicon (only ends are exposed, sides are covered) Total dia=1.96mm, EGDMA shell dia=1.47mm, core dia=1.02mm.
Table 5-1. Physiological parameters used for the pharmacokinetic model

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<td>R_0</td>
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<tr>
<td>σ</td>
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</tr>
<tr>
<td>p_0</td>
<td>400Pa</td>
</tr>
<tr>
<td>p_sac</td>
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CHAPTER 6
CONCLUSION

Millions of people in the United States are suffering from severe eye problems; therefore effective ways of delivering drugs is an area of current research. Bioavailability of drugs delivered via eye drops has been reported to be low (< 5%) due to their rapid elimination from eyes through tear drainage. Therefore our work has focused on understanding the transport of drugs in front surface of the eyes specifically in cornea, which could be of immense help in designing better ophthalmic formulations. Also, we have developed drug delivery devices such as conjunctival inserts and punctal plugs which would not only improve bioavailability of drugs but would also greatly enhance patient compliance.

Conventional pharmacokinetic models that depict cornea as compartments are unsuitable to determine bioavailability of topical drugs. The mechanistic model developed in this thesis accurately characterizes the transient transport behavior of drugs across cornea. Chapter 2 in the thesis presents the mechanism and model development of transport of hydrophobic molecules while chapter 3 deals with presenting the phenomenon for hydrophilic drugs. Lipophilic drugs exhibit slow accumulation in cellular layers presumably due to their slow transport from the membrane bilayers to intracellular hydrophobic sites. While, for hydrophilic drugs transport across the transcellular layer plays a rate determining step in transport across the endothelial layer of cornea. Sensitivity analysis of the parameters obtained from the model clearly indicates that the parameters are sensitive to the model and are well identified.
Drug delivery devices such as cylindrical conjunctival HEMA inserts (chapter 4) and punctal plugs (chapter 5) were prepared and presented as an alternate solution for the treatment of dry eyes. The devices were fabricated via thermal polymerization in presence of cyclosporine A drug at high loadings to create a system containing particles of drug dispersed in the matrix. The drug release rates were measured to explore the effect of length, drug loading, crosslinking, and mixing in the release medium. The inserts release the drug for a period of about a month while punctal plugs release drug for more than two months at therapeutic rates. The rates of drug release are zero-order and independent of drug loading and crosslinking for certain period of time. These effects were shown to arise due to a mass-transfer boundary layer in the fluid and a mathematical model was developed by coupling mass-transfer in the devices with that in the boundary layer in the surrounding fluid. The model with diffusivity in the polymer matrix and boundary layer thickness as parameters fits the experimental data and explains all trends in release kinetics. The fitted diffusivity is about twice that obtained by direct measurements, which agreed well with the value obtained by using the Brinkman’s equation but only after accounting for drug binding to the polymer. Pharmacokinetic models are also developed for Restasis®, commercially existing ophthalmic emulsion, to obtain the bioavailability of the drugs delivered through these eye drops. These models suggest that the amount delivered by our devices is therapeutically sufficient and similar to the commercially existing ophthalmic solutions.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Chhavi Gupta completed his undergraduate studies from Indian Institute of Technology, Guwahati (IITG), India in May 2006. He joined Department of Chemical Engineering at the University of Florida in a Ph.D. program in Fall 2006. He joined Dr. Anuj Chauhan’s lab in Spring 2007 and since then began working on his thesis titled “Devices and mechanisms for ophthalmic drug delivery”.

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