Thermal and Convective Loading Methods for Releasing Hydrophobic Therapeutics from Contact Lenses

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Thermal and Convective Loading Methods for Releasing Hydrophobic Therapeutics from Contact Lenses

Ryan Ruben Horne

A thesis submitted to the faculty of Brigham Young University in partial fulfillment of the requirements for the degree of Master of Science

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ABSTRACT

Thermal and Convective Loading Methods for Releasing Hydrophobic Therapeutics from Contact Lenses

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This thesis investigates the feasibility of loading silicone hydrogel (SiHy) contact lenses with two different hydrophobic therapeutics, latanoprost and DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine), for treatment of glaucoma and hyperemia respectively. The two methods of loading were 1) thermal loading in an aqueous medium and 2) convective loading in a solution of n-propanol. Dailies Total1® lenses prepared in this manner were tested for their loading and their release into artificial tears. Continuous release over 1-4 days at therapeutic levels is achievable from thermal loading of DMPC, convective loading of DMPC, and convective loading of latanoprost. The DMPC loading processes can be naturally integrated into standard manufacturing lines for Dailies Total1®. Both DMPC and latanoprost release at rates proportional to the amount loaded into a contact lens. Latanoprost loads into a contact lens strictly proportionally to the loading concentration and the time of loading. The convective loading step represents a significant improvement on both the time of loading (reduced from days to minutes) and the loading capacity of silicone hydrogel contact lenses. This thesis also compares the loading and release of latanoprost in the convective loading procedure using the SiHy contact lenses of Acuvue Advance® (Johnson & Johnson Vision Care, Jacksonville, FL), Air Optix® (Alcon, Copenhagen, Denmark), Biofinity® (CooperVision), PureVision® (Bausch & Lomb), and Dailies Total1® (Alcon), and the polyHEMA lens, SofLens 38® (Bausch & Lomb), finding that silicone hydrogels load an order of magnitude more drug than the polyHEMA lens and release into artificial tears for an order of magnitude longer. Overall, these experiments provide a quantitative understanding of the dynamics of loading and release for both DMPC and latanoprost.

Keywords: drug delivery, controlled release, contact lens, latanoprost, DMPC, thermal loading, convective loading, silicone hydrogel, glaucoma, dry eye
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loading lenses and performing immediate extractions of latanoprost. Error bars represent 95% confidence intervals, n=3.
LIST OF ACRONYMS

ATF – artificial tear fluid (formulation by Alcon Laboratories)
ATS – artificial tear solution (formulation by Pitt Lab)
CI – confidence interval
CL – contact lens
DDH₂O – double distilled water
DMPC – 1,2-dimyristoyl-sn-glycero-3-phosphocholine (agent for treating dry-eye)
DT1 CL – Dailies Total1® contact lens (a type of silicone hydrogel CL made by Alcon)
IOP – intraocular pressure
MPS – multipurpose solution (commercial CL wash and storage solution for CL wearers)
PBS – phosphate buffered saline
PC – phosphatidyl choline
PGA – prostaglandin analogue (class of drugs used to treat glaucoma)
RGCs – retinal ganglion cells
SiHy – silicone hydrogel
INTRODUCTION TO CONTACT LENS DRUG DELIVERY

*Where there is no vision, the people perish.* - Proverbs 29:18

Vision is a foundational sense of human life. Thus deteriorating or problematic vision can wreak havoc on the ability to function in life. Vision problems range from the sight-destroying disease of glaucoma to eye irritation from dry-eye syndrome. The two conditions afflict a projected 2.7 million (National Institutes of Health, 2016) and 5.0 million US citizens respectively (National Eye Institute, 2013). While both conditions respond to eye-drop treatments, the eye-drops come with at least two disadvantages; 1) patients struggle to adhere to eye-drop treatment regimens and 2) eye-drops deliver only a short-lasting drug dose at an initially high concentration that could be potentially problematic (Lavik, Kuehn, & Kwon, 2011). A possible alternative to eye-drops is medicated contact lenses, which have the potential to bypass these issues of adherence and drug release rate.

Researchers have explored using contact lenses to deliver biologically relevant molecules to the eye since 1965 (Sedlacek, 1965). A contact lens is non-invasive, easy to use and replace, and relatively affordable – all distinct advantages for a drug delivery device (Lavik, Kuehn, & Kwon, 2011). Despite these advantages, some agents, particularly those with low solubility in water, do not load well into or release from contact lenses. Two classes of eye-treatment compounds that fall into this category are phospholipids and anti-glaucoma prostaglandin-based drugs.
With the right loading techniques, phospholipids might be able to be released from contact lenses and treat dry-eye. Phospholipids, such as phosphatidyl choline (PC), purportedly aid in alleviating dry eye in the short term when introduced into the tears by stabilizing the tear film (Korb, Greiner, & Glonek, 1996). Conversely, the depletion of PC from the tears would be expected to exacerbate dry-eye. Since contact lenses tend to remove PC from the tears (Svitova & Lin, 2013), contact lenses might introduce or even exacerbate dry-eye in some contact lens wearers. Thus a contact lens that is designed to release PC can offer a net PC gain in the tears and alleviate dry-eye symptoms. Releasable PC could be an attractive option for those with dry-eye, especially for those who already wear contact lenses. However, releasable PC is not yet available in most contact lenses because the customary loading methods do not work for hydrophobic compounds. Traditional contact lens research has focused on ineffective “soak to load” methods, wherein lenses are soaked in an aqueous medium at room temperature to load a contact lens with a dissolved drug. This method requires long loading times, on the order of days, because the drug enters the lens by diffusion. The aqueous loading method also cannot load appreciable amounts of insoluble compounds like PC from the aqueous medium. However, PC does load into silicone hydrogel (SiHy) contact lenses by a novel non-aqueous approach developed in the Pitt lab (Pitt, Jack, Zhao, Nelson, & Pruitt, 2012). The non-aqueous approach leverages the solubility of a hydrophobic compound in an organic solvent to introduce more drug into the contact lens during “soaking”. The term “soaking” is used, but the SiHy lens experiences more than simple diffusion. Rather the lens swells with the organic solvent bringing dissolved drug into the contact lens by convection causing loading to occur on the order of minutes, not days. This loading technique has been demonstrated for a particular type of PC called DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine). Another possible method for loading DMPC
into contact lenses is using the aqueous loading method at elevated temperatures to increase diffusion rates. This “thermal” method is of particular interest to industry because it could be incorporated naturally into an elevated temperature step for contact lens sterilization.

This thesis aims to characterize loading DMPC into contact lenses by elevating the temperature of the aqueous loading solution. Objective 1 of this thesis is to characterize thermal loading DMPC in Dailies Total1® silicone hydrogel contact lenses, which is a lens manufactured by CIBA Vision, a division of Alcon. Objective 2 is to analyze the retention of DMPC through the current Alcon manufacturing process for Dailies Total1® silicone hydrogel contact lenses if the lenses are convectively loaded with DMPC during production. For the convenience of the reader, Chapter 3 provides a summary of the thesis objectives.

The treatment of glaucoma represents another potential area for leveraging the advantages of a contact lens as a drug-delivery device. Glaucoma is defined as the deterioration of the axons of retinal ganglion cells (RGCs) in the eye which leads to vision loss and eventual blindness. This disease is the second highest reason for becoming permanently blind and afflicts an estimated 2.7 million Americans and 60 million people globally (National Institutes of Health, 2016). The major risk factor for worsening glaucoma is elevated intraocular pressure (IOP), or pressure inside the eye (Lavik, Kuehn, & Kwon, 2011). Consequently, most medications that combat glaucoma are designed to reduce IOP – a strategy which generally prevents the progression of glaucoma in 90% of cases when strictly followed (AGIS Investigators T.;, 2000). Despite the therapeutic success of the medications, the current treatment strategy of topical administration struggles from poor patient adherence (Sleath, et al., 2006) and adverse systemic side effects associated with bolus administration of the medication (Lavik, Kuehn, & Kwon, 2011).
The ease of wearing a contact lens and the accompanying gradual release of drug can mitigate the issues with topical bolus administration of anti-glaucoma drugs.

Drugs for treating glaucoma fall into many categories, the most hydrophobic and arguably most effective of them being the prostaglandin analogues. While many methods have been explored for loading contact lenses with prostaglandin analogues, the currently proposed methods either call for inordinately long loading times – anywhere from three hours (Kim, Conway, & Chauhan, 2008) to four days (Peng, Burke, Carbia, Plummer, & Chauhan, 2012) – or would require major restructuring of existing manufacturing processes (Ciolino et al., 2013). Objective 3 of this thesis explores the previously discussed technique of convective loading into Dailies Total1® silicone hydrogel lenses and whether it can be applied to loading a different hydrophobic molecule – one of the commonly used prostaglandin analogues called latanoprost. The motivation for this choice is to investigate the applicability of the convective loading technique to classes of compounds other than DMPC and to develop a contact lens capable of treating glaucoma for multiple days. To identify the best contact lens for treating glaucoma, Objective 4 tests five different types of SiHy contact lenses and one non-SiHy lens for their loading and release of latanoprost.

Overall, these 4 objectives explore techniques for loading SiHy contact lenses with two hydrophobic compounds of interest to ocular systems: DMPC for its role as an anti-dry-eye agent and latanoprost for its role as an anti-glaucoma drug.
2 LITERATURE REVIEW OF CONTACT LENS DRUG DELIVERY

2.1 Literature Review Introduction

This literature review presents the current limits of ocular drug delivery by establishing:

- how convective loading has been shown to work for DMPC so the technique can be best applied to latanoprost
- the limited research performed to-date on thermal loading techniques
- what current researchers are doing to deliver drugs to the eye in treating glaucoma via contact lenses
- the rationale for selection of latanoprost as the anti-glaucoma drug of this study
- what range of delivery rates of latanoprost have the best likelihood for being therapeutic considering physiological factors

Understanding these points provides context for the objectives of this thesis in delivering DMPC and latanoprost to the eye via contact lenses. The research that has previously been established on convective loading of DMPC serves two purposes in this thesis: first, in illuminating how to incorporate the loading techniques into the Alcon manufacturing process, and second, in serving as the framework for investigating the efficacy of loading latanoprost by the same technique. The points pertaining to DMPC will be presented first, followed by those remaining to be discussed for latanoprost.
2.2 Literature on DMPC Loading and Delivery

2.2.1 Convective loading of DMPC into contact lenses via n-propanol

From previous publications originating from the Pitt lab, a daily-use single-wear SiHy contact lens can be loaded with 55 µg of DMPC in about 120 seconds by soaking lenses in a drug solution of n-propanol (Pitt et al., 2014). This is a rapid rate of loading when compared with loading times of three hours (Kim et al., 2008) or 1-4 days (Peng, Burke, Carbia, Plummer, & Chauhan, 2012) seen in other reports. The method is to place a commercially available contact lens into an n-propanol solution of the drug of interest and allow the drug solution to penetrate the contact lens matrix, causing the contact lens to swell to approximately twice its original diameter. Then, the contact lens is returned to an aqueous solution where it returns to its original size. Large hydrophobic molecules like DMPC that have penetrated the contact lens matrix presumably form micro-precipitates within the contact lens as their solubility in the surrounding medium sharply decreases when the lens is transferred from n-propanol to water. Understanding this process of DMPC loading is important to Objective 2, where DMPC convective loading is incorporated into the Alcon manufacturing process for Dailies Total1® contact lenses, and to Objective 3, where latanoprost convective loading is investigated using the techniques employed for DMPC convective loading.

2.2.2 Thermal loading of DMPC into contact lenses

While it seems no journal publication exists on using elevated temperature to accelerate diffusive drug loading in contact lenses, the principle that diffusivity increases with temperature is well established (Bird, Stewart, & Lightfoot, 2007). Zhao et al. originally postulated the idea
of thermal loading of DMPC into contact lenses (Zhao, 2011). Testing this idea constitutes one of the objectives of this thesis.

2.2.3 Release of DMPC from contact lenses into artificial tears

Creating an accurate model of drug release into the eye is challenging. Many researchers use animal models of beagles (Gelatt & MacKay, 2001) or New Zealand white rabbits (Ciolino, et al., 2013), which provide the physiological elements of blink stresses and tear replenishment needed to obtain precise calculations of drug release in contact lenses. Even when not performed in vivo, experiments in vitro show that the presence of corneal epithelial cells can significantly decrease the drug release observed from contact lenses (Mohammadi, Jones, & Gorbet, 2014). Thus, the experiments of this thesis need further refinement in vivo to understand the true dynamics of release. However, in the absence of animal models or corneal cell lines, the dynamics of release are approximated by drug elution from contact lenses into periodically replaced artificial tears with continual gentle stirring (Pitt, et al., 2015). In the case of multi-day lenses, the contact lenses can be cycled through 16 hours of immersion in artificial tears followed by 8 hours in multipurpose solution (MPS) to simulate the 24-hour wear cycle of a daily contact lens wearer (Pitt, et al., 2015).

2.3 Literature pertaining to Latanoprost Loading and Delivery

2.3.1 Anti-glaucoma drugs and their effects

Many types of medications are used for treating glaucoma. Foremost among these in the literature and in clinical practice are the prostaglandin analogues (PGAs). The PGAs combat the degradation of retinal ganglion cells (RGCs) by lowering intraocular pressure (IOP) (Lavik,
Kuehn, & Kwon, 2011). PGAs have fewer side effects and require less doses than other categories of glaucoma drugs (Linden, 2001). Among the PGAs, three major drugs, travoprost (0.004% formulation), bimatoprost (0.03% formulation), and latanoprost (0.005%) hold the majority of the market share (Parrish, Palmberg, & Sheu, 2003). Because of its availability as a radiolabeled drug and potency at lower concentration, latanoprost was selected over the other PGAs for study in this thesis. The anatomical site of therapeutic effect for latanoprost is disputed; however, many researchers argue that the drug leads to collagen dissolution in drainage passageways from the aqueous humor (Russo, Riva, Pizzolante, Noto, & Quaranta, 2008). With less resistance to flow through this drainage pathway, fluid is able to flow more freely from the eye and the intraocular pressure (IOP) is reduced.

While lowering IOP prevents further RGC death in 90% of patients, researchers are also exploring medicines that can protect RGCs directly for those patients whose conditions progresses despite IOP reduction (Lavik, Kuehn, & Kwon, 2011). Some of these drugs include very hydrophobic molecules such as progesterone (Loane & Faden, 2010), memantine (Danesh-Meyer & Levin, 2009), and statins (Loane & Faden, 2010). Because of their hydrophobicity, these drugs might be loadable into contact lenses by convection. However, these drugs have yet to yield successful clinical results, a problem compounded by side effects of the drugs resulting from systemic administration (Danesh-Meyer & Levin, 2009). For the best chance of positive results, the drug must be delivered in a way that mitigates systemic effects, such as continuous release via a contact lens (Lavik, Kuehn, & Kwon, 2011). Though the development and optimization of such a construct is beyond the scope of this thesis, future work could identify which of these three hydrophobic molecules loads the most drug into a contact lens and yields the most advantageous clinical dose.
2.3.2 Loading anti-glaucoma drugs in contact lenses

Despite the lower side effects of PGAs in comparison to other glaucoma drugs, the majority of patients who use the current state-of-the-art PGA eye-drop formulations still report adverse side effects like hyperemia (red eye) (Parrish, Palmberg, & Sheu, 2003). Additionally, the dexterity and consistency required for eye-drop administration prove to be significant barriers to the point that 27-59% of patients fail to adhere to their treatment schedule (Rotchford & Murphy, 1998). Another problem with topical application of the drug is that only 1-7% of the applied dose of drug actually enters the fluid inside the eye (Ghate & Edelhauser, 2008), a costly inefficiency for expensive drugs. Consequently, researchers are investigating drug delivery options that result in better adherence and reduce side effects.

Contact lenses offer many advantages as a platform for eye drug delivery, regardless of if a patient requires vision correction. Glaucoma patients who already use contact lenses must discontinue contact lens use in order to treat their condition with eye-drops (Abelson, 2015). Streamlining vision correction with glaucoma treatment would not only provide better release kinetics, but might reduce patient-adherence issues as well. For those Americans who do not require vision correction, a refractory-neutral contact lens could still deliver drug without altering their vision. With the advent of 30-day-wear contact lenses, a drug-releasing contact lens has become more appealing because of the potential to mitigate some of the patient adherence issues associated with daily treatment routines. If patients only needed to take action in their care once a month instead of once or twice a day as with eye-drops, they might better manage their treatment regimen. If dexterity were an issue, the contact lenses could even be inserted by a family member, friend, or medical professional. Additionally, side effects would be expected to
decrease, because the continuous release of the drug would provide lower maximum concentration compared to the rapid concentration spikes currently produced by eye drops. Adaptation to a new treatment regime in the eye care industry would likely be smooth as 93% of ophthalmic professionals claim they would be interested in a contact lens platform as a treatment option for eye disease if it were available (Karlgard, Jones, & Moresoli, 2004). Contact lens drug delivery thus has the potential to dispense with the issues of poor patient adherence and side effects found in eye-drop drug administration.

However, contact lenses have historically struggled as drug delivery devices. The three biggest challenges are the lower loading capacity of contact lenses (Lavik, Kuehn, & Kwon, 2011), the loss of drug to the packaging medium of contact lenses, and the duration of continuous release not extending past a week (Kim, Conway, & Chauhan, 2008). Thus recent research on contact lens drug delivery has focused on overcoming these three challenges. Other concerns about continuous latanoprost delivery from contact lenses would require further testing once a construct is developed. One of these concerns is that latanoprost could be chemically altered in the contact lens, preventing therapeutic effect. Another concern is that sustained release of latanoprost could cause more discoloration of the iris and skin around the eye than is experienced with eye-drops. Despite these unknowns, continuously released drugs from eye implants have shown decreased or unchanged ocular side effects (Schwartz & Flynn, 2011).

One contact lens drug delivery model employed by researchers at the Massachusetts Institute of Technology has demonstrated constant release from a SiHy contact lens using an entrapped drug layer (Ciolino, et al., 2013). They developed a model that sandwiches a drug-containing layer between two layers of contact-lens polymer to yield controlled release of latanoprost over 30 days at therapeutic levels (Ciolino et al., 2013). Their research demonstrates
proof of concept that a drug-containing matrix with slow internal diffusion of the drug can be a platform for effective treatment in vivo. However, their approach also would require a restructuring of existing contact lens facilities to accommodate the new design if it were to be pursued.

Another decades-old approach to contact lens drug-delivery is the aqueous “soak-to-load” technique; that is, the glaucoma drug of interest is made into an aqueous solution and a contact lens soaks in this medium until a clinically significant amount of drug is loaded. Some models are not able to release into artificial tears past 4 hours, despite 24 hours of loading (Soluri, Hui, & Jones, 2012). Some models release over 2-4 days is achieved in these models after an initial burst, this strategy requires soaking times of 24 hours or more to yield the desired degree of loading for the PGA latanoprost (Peng, Burke, Carbia, Plummer, & Chauhan, 2012). Loading conditions must somehow be different between the loading step and the delivery step if the former is desired to be quick and the latter to be prolonged. The convective loading strategy proposed in this thesis meets this criteria, as loading is done in non-aqueous conditions while release occurs in aqueous conditions.

So far, researchers in the literature have touched only lightly on convective swelling of contact lenses. Ciolino et al. report that both the hydrophobic drug dexamethasone (DX) and the hydrophilic glaucoma drug timolol can be loaded via ethanolic solution and yield zero-order release over 150 days after an initial spike in release (Ciolino et al., 2009). Unfortunately, the release rates are not quantified in their published results. In another study, Kim et al. used a soaking method in ethanol for loading DX and one of its derivatives for 3 hours in 2-2.5 mL to yield steady release of 2 µg/day over 20 days from some of the contact lens polymer mixes that were synthesized in their lab (Kim et al., 2008). The same research group also used the method
of soaking in ethanol to load vitamin E into contact lenses to act as a diffusion barrier (Peng, Burke, Carbia, Plummer, & Chauhan, 2012). However, no research has been published to show just how rapid the loading from an alcoholic medium can be (on the order of minutes, not hours), and no published research has capitalized on the ability to load a hydrophobic anti-glaucoma drug into a contact lens.

### 2.3.3 Qualities of latanoprost well-suited to convective loading

The hydrophobicity of PGAs like latanoprost is analogous to that of DMPC. DMPC loads rapidly and in relatively large amounts (on the order of 100 µg) into contact lenses via the convective loading technique. In this technique, n-propanol permeates the polymer matrix of the lens and convectively carries DMPC into the polymer. DMPC experiences a rapid change of environment when the contact lens is transferred from n-propanol to water. Because DMPC is much less soluble in water than in n-propanol, the DMPC largely associates with itself and the hydrophobic regions of the SiHy contact lens upon introduction of water. Latanoprost also has large differences in its solubility in water and n-propanol, suggesting that it too might possess similar convective loading and entrapment characteristics. One difference between latanoprost and DMPC is their thermal stability; latanoprost degrades over several days at 37°C and much faster at higher temperatures (Johnson TV, 2011), whereas DMPC is relatively stable up to 121°C. Thus this thesis investigates loading latanoprost by convective loading rather than thermal loading.

By the same line of reasoning, other hydrophobic ophthalmic drugs could be good candidates for convective loading and gradual release. The hydrophobic anti-dry-eye drug cyclosporine A (Peng, Burke, Carbia, Plummer, & Chauhan, 2012) could be tested for
convective loading and used if DMPC proves ineffective or problematic. Other ocular issues and their respective hydrophobic drugs include acetazolamide for glaucoma therapy (Lavik, Kuehn, & Kwon, 2011), dexamethasone for treating diabetic macular edema (Kim, Peng, & Chauhan, 2010), fluocinalone acetonide for treatment of diabetic retinopathy (Schwartz & Flynn, 2011) econazole for antifungal properties (Ciolino, et al., 2011), ketotifen for preventing and treating eye inflammation (Soluri, Hui, & Jones, 2012), and the previously mentioned neuroprotective agents of progesterone (Loane & Faden, 2010), memantine (Danesh-Meyer & Levin, 2009), and statins (Loane & Faden, 2010).

2.3.4 Physiologically-based estimates for a continuous latanoprost dose

It is important to determine if a latanoprost-releasing contact lens could deliver the proper latanoprost dose to treat glaucoma. Based on current glaucoma treatments, we can deduce a potential upper limit and lower limit of latanoprost dosage. Thus a contact lens should be “tunable” to provide any latanoprost delivery rate in order to find the optimal continuous release rate in clinical work. What follows is a calculation of that upper and lower therapeutic range.

For the purposes of this thesis, the upper-bound estimate for delivery rate comes from the total latanoprost delivered in a daily latanoprost eye-drop. Current eye-drop formulations of latanoprost deliver approximately 57 µg/mL (Ciolino et al., 2013), which corresponds to a rate of 1.4 µg/day if a 25 µL drop is assumed on a one drop per day treatment schedule. In this upper-limiting case, the rate of latanoprost elution will need to be at most 1 µg per day, or roughly 0.05 µg per hour to have a therapeutic effect. This rate represents an upper-bound, because typically only 1-7% of the eye drop medication reaches the intended target (Ghate & Edelhauser, 2008). While unlikely, this upper-bound release rate might prove too low in practice if the current eye-
drop treatments provide a requisite short-lived latanoprost concentration. However, the more likely scenario is that the contact lens lessens the amount of latanoprost required by reducing some of the inefficiencies of eye-drops. If the contact lens were perfectly efficient, then the lower-bound for release would be about 1% of the upper-bound, or about 0.01 µg per day.

Based on these calculations, a contact lens could foreseeably treat glaucoma if it could continuously release between a rate of 0.01 - 1 µg of latanoprost per day.

2.4 Literature Review Conclusion

A review of the literature indicates that both thermal loading of DMPC and convective loading of anti-glaucoma drugs into contact lenses represent areas where a significant research contribution can be made. The potential to easily incorporate thermal or convective DMPC loading into manufacturing processes to the comfort of contact lens wearers who suffer from dry-eye motivates the research behind loading DMPC into contact lenses. The urgent medical need to offer patients treatment options that are more ergonomically friendly and less regimented motivates the pursuit of drug delivery of anti-glaucoma drugs from contact lenses. Extending existing technology for convective DMPC loading to latanoprost, it might be possible to rapidly load and gradually release latanoprost from contact lenses. Latanoprost release from such a contact lens could possibly treat glaucoma if the release is on the order of 0.01 – 1 µg of latanoprost per day.
3 THESIS OBJECTIVES

There are four general objectives of this master’s thesis:

Objective 1: To investigate the details of thermally loading DMPC at accelerated rates into contact lenses by treating Dailies Total1® silicone hydrogel lenses in an aqueous solution of DMPC in an elevated temperature step via autoclave. Loaded contact lenses will then be tested for whether it is possible to elute DMPC at therapeutically relevant rates into artificial tear solution (ATS) for one week.

Objective 2: To determine whether DMPC can be appreciably retained through the Alcon manufacturing process for Dailies Total1® silicone hydrogel contact lenses if DMPC is loaded via convective swelling in an n-propanol solution. Then, like Objective 1, loaded contact lenses that have gone through the whole process will be tested for elution of DMPC at therapeutically relevant rates into ATF for one week.

Objective 3: To investigate if latanoprost can be loaded into Dailies Total1® silicone hydrogel lenses via convective swelling in an n-propanol solution of latanoprost. If the method proves feasible, contact lenses will then be tested for rates of loading and elution into ATS to see if the construct is one that could foreseeably treat glaucoma.

Objective 4: To compare the convective loading and subsequent ATS release of latanoprost in a variety of contact lenses loaded at the same conditions.
Table 3-1 provides a summary of these objectives and their correlating chapters.

Table 3-1 – The thesis objectives summarized by their respective type of loading, molecule of interest, and contact lens(es) used. DT1 = Dailies Total1® silicone hydrogels. SiHy = silicone hydrogels.

<table>
<thead>
<tr>
<th>Type of Loading</th>
<th>Objective Details</th>
<th>Molecule Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal Loading</td>
<td>Objective 1 (Chapter 5) – DT1 lenses</td>
<td>Latanoprost (not attempted due to latanoprost thermal instability)</td>
</tr>
<tr>
<td>Convective Loading</td>
<td>Objective 2 (Chapter 6) – DT1 lenses</td>
<td>Objective 3 (Chapter 7) – DT1 lenses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Objective 4 (Chapter 8) – Six SiHy lenses (included DT1) and one type of non-SiHy lens</td>
</tr>
</tbody>
</table>
4 EXPERIMENTAL APPROACH

This chapter explains the experimental approach behind the thesis objectives. Many procedures are shared between the various objectives, so these procedures are presented here for convenience. Each objective requires the use of extractions, elutions, and radiolabeled quantification, as well as at least one of the loading procedures. The composition of artificial tear solution (ATS) used in Objective 3 and Objective 4 is also given.

4.1 Thermal Loading

A contact lens is thermally loaded with DMPC by the following procedure:

1) Prepare a liposomal solution of DMPC at a pre-selected concentration by evaporating a pre-determined mass of DMPC suspended in chloroform onto a round-bottom flask, rehydrating with a pre-selected volume of water, and sonicking 3 times for 30 seconds at 25% power. Store in the refrigerator.

2) Prepare unfinished contact lens cores for the final autoclave step using the Alcon proprietary preparation process which involves a series of washes and rinses in aqueous and organic mediums.

3) Pipet 0.35 mL of liposomal DMPC into the hard plastic well of a blister pack, place a contact lens into the solution in the blister pack, and then pipet an additional 0.30 mL of liposomal DMPC to a final volume of 0.65 mL.

4) Ensure the contact lens is immersed in the liposomal DMPC solution.
5) Align the top of the blister pack, a cut, square, metal-foil piece, onto the top of the hard plastic well so as to cover the entire surface of the plastic with room to spare.

6) Press the blister pack for 1 second at about 500 psi of pressure and 435 degrees F to form an air-tight seal.

7) Treat the blister pack containing the liposomal DMPC and contact lens for the pre-selected temperature and length of time in an autoclave.

4.2 Convective Loading of DMPC in the Alcon Process

A set of contact lenses are loaded with DMPC by the following procedure:

1) Prepare a 200 mL loading solution of n-propanol with a 0.15 w/v% DMPC concentration.

2) Remove approximately 0.5 mL of loading solution for making standards for later quantitation.

3) Place contact lens cores in individual water-permeable “snap caps” using plastic tweezers.

4) Keep the desired number of loaded contact lenses in snap caps (9 max per cycle) in DDH$_2$O until the beginning of loading cycle.

5) Perform the Formulation C rinses and subsequent DDH$_2$O step as specified by Alcon (details are proprietary). This step and subsequent steps are done for up to 9 contact lenses at once by placing the contact lenses in snap caps into a light mesh bag and transferring from one solution to the next.

6) Immerse the contact lens into the 200 mL loading solution for 155 seconds while very swirling light mesh bag to prevent the snaps caps from aggregating.
7) Remove the contact lenses from the loading solution precisely on time, and transfer to a 1 minute DDH₂O rinse.

8) Perform the Formulation B rinse and subsequent DDH₂O steps as specified by Alcon (details are proprietary).

9) Remove the contact lenses from their snap caps with plastic tweezers and place them in blister packs with 0.65 mL of Formulation B.

10) Seal the blister packs to an aluminum lid with 1 second of hot pressing (described in detail in Thermal Loading).

11) Autoclave the contact lenses for 45 minutes at 121°C.

12) Remove contact lens for extraction or elution.

4.3 Convective Loading of Latanoprost

A contact lens is loaded with latanoprost by the following procedure:

1) Prepare a 5.5 mL loading solution of n-propanol with a pre-selected concentration of chemical agent (i.e. 3.0 mg of radiolabeled latanoprost per mL of n-propanol) in a 20 mL glass scintillation vial.

2) Remove approximately 0.5 mL of loading solution for making standards for later quantitation.

3) Open a fresh blister pack containing a post-production contact lens.

4) Remove the contact lens from the blister pack with plastic tweezers, suspend the lens over a waste water container, and rinse the lens in a rinse bottle stream of double distilled water (DDH₂O).

5) Blot the lens dry on a KimWipe ®.
6) Immerse the contact lens into the ~ 5.0 mL loading solution for a pre-selected length of time while very gently swirling the vial of the solution to prevent the contact lens from adhering to the glass container.

7) Remove the contact lens from the loading solution precisely on time using rubber-tipped plastic tweezers, and gently running the contact lens across the inner lip of the glass vial in the act of removal to encourage the wetted surface of loading solution to return to the vial.

8) Immediately place the contact lens into a series of three rinses of 5-10 seconds each, with each rinse consisting of gentle swirling of the contact lens in ~15 mL of fresh DDH₂O.

9) Place the contact lens into a small beaker of 5-10 mL of DDH₂O to shrink.

10) Remove the contact lens promptly after 15 minutes of shrinking time to transfer the lens to its new destination (either extractions or elutions).

4.4 Extractions

The amount of a radiolabeled chemical agent in a contact lens can be experimentally determined by the following destructive procedure:

1) Remove the contact lens to be extracted from its solution of origin, rinse thoroughly with DDH₂O to wash away any radiolabeled chemicals found outside the contact lens, and blot dry on a KimWipe ®.

2) Immerse the lens into a 20 mL glass scintillation vial containing a 2.0 mL solution of n-propanol.

3) Cap the glass vial and place in a 35 degree C incubator under gentle shaking (30 rpm) for one hour.
4) After one hour, remove the glass vial from the incubator, suspend the contact lens over the vial with tweezers, and rinse the lens and tweezers with 1.0 mL of n-propanol which is intended to drain into the solution below to make a total volume of 3.0 mL.

5) Repeat steps 2-4 for the number of extractions desired.

6) Discard the lens after the extractions are complete.

7) The amount of radiolabeled chemical from the 3.0 mL extraction medium is quantified by adding 10 mL of scintillation fluid and counting light emissions using a liquid scintillation counter.

4.5 Elutions

The cumulative release of a radiolabeled chemical agent from a contact lens can be experimentally determined by the following procedure:

1) Select the solution of interest for release (i.e. water or artificial tear solution)

2) Prepare a signal background of a 100 µL sample of the solution of interest.

3) Rinse the contact lens to be tested for release with DDH2O and blot dry.

4) Place the contact lens into 3.0 mL of the solution of interest and record the time.

5) Take a 100 µL sample of the solution at the following intervals to model initial release: immediately after start, at 1 hour, 2 hours, and 4 hours. All 100 µL samples should be pipetted into 10 mL of scintillation fluid for later quantitation.

6) Take an additional 100 µL sample sometime between 6 and 18 hours, and record the time elapsed.

7) Take additional 100 µL samples 1-3 times a day as desired. Document all instances of sampling and the time elapsed at each.
8) Eluting solutions may be replenished as desired by removing the contact lens from the old eluting solution and repeating steps 4, 6 and 7. Consistency in sampling schedule between experiments is expected.

9) After the desired timeframe for elution has expired, lenses are then extracted.

### 4.6 Radiolabeled Quantification

For each solution that requires radioactive quantitation, a nonradioactive background solution of the same characteristics is prepared. The background signal in the radioactive sample is then subtracted off, leaving the signal contribution by the radiolabeled compound of interest. A standard signal of known concentration of radiolabeled species is prepared for the purpose of calibrating the radioactive decays per mass of radiolabeled species.

### 4.7 Artificial Tear Solution (ATS) Preparation

While the DMPC experiments use artificial tear fluid (ATF) provided by Alcon, the latanoprost experiments require a volume of artificial tears that exceeds the stock leftover from the Alcon experiments. An artificial tear solution (ATS) is made in house for the latanoprost experiments based off of the formulations of Lorentz et al (Lorentz, et al., 2011) (Lorentz, Rogers, & Jones, The impact of lipid on contact angle wettability, 2007) and the TFOS International Workshop on Contact Lens Discomfort (Craig, et al., 2013).
5 THERMAL LOADING AND RELEASE OF DMPC

Chapter 5 has three sections. Section 1 addresses the process variables involved in the thermal loading of DMPC – that is, how the variables of loading concentration, temperature of the loading step, time of the loading step, and volume of the aqueous DMPC solution affect the loading of DMPC in Dailies Total1® contact lenses (DT1 CLs). Section 2 focuses on the subsequent DMPC release dynamics into artificial tear fluid (ATF). Section 3 addresses the reproducibility of the process when using an optimized DMPC loading process.

By way of overview, the goal of Chapter 5 is to release 1 µg of DMPC per day into ATF (which by happenstance is the desired release rate of latanoprost in later sections) as specified by Alcon. However, Alcon needs to stay under a 100 ppm DMPC concentration in the final storage medium, so the DMPC loading needs to be optimized while meeting that constraint. The goal set by Alcon is to increase the comfort of its Dailies Total1® silicone hydrogel contact lenses by increasing the content of deliverable DMPC. These Dailies Total1® contact lenses already have some DMPC incorporated in them (Alcon, 2015), but adding more would increase contact lens comfort. Little is published about the chemistry of these lenses, but the name of the proprietary silicone hydrogel polymer is Delefilcon A (Alcon, 2015). While the exact chemistry of the lens remains unknown, the following sections show how these lenses take up and release DMPC.
5.1 Section 1: Effect of Loading Conditions on DMPC Thermal Loading

The objective of this section is to study the effects of varying volume, concentration, temperature, and autoclave time on the loading of DMPC. The amount of DMPC loaded from a solution of Formulation A, a proprietary solution that is essentially phosphate-buffered saline (PBS) spiked with DMPC, is summarized in Figure 5-1 through Figure 5-3, with Figure 5-1 showing the effect of time in the autoclave, Figure 5-2 showing the effect of concentration, and Figure 5-3 showing the effect of volume and temperature.

Figure 5-1 - Loading of DMPC as a function of time at a concentration of 80 ppm DMPC, volume of 1 mL, and autoclave temperature of 121°C in Formulation A (essentially phosphate-buffered saline or PBS).

Figure 5-1 shows that increasing the duration of an elevated temperature step increases the loading of DMPC, as would be expected. However, the relationship between loading and time does not appear strictly proportional. One explanation is that the autoclave environment requires time to heat the DMPC solution from room temperature to 121°C. As such, the graphed time of the autoclave step may not properly reflect the thermal history of the DMPC solution.
Figure 5-2 – Loading of DMPC as a function of concentration at a volume of 1 mL, autoclave time of 45 minutes, and autoclave temperature of 121°C in Formulation A (essentially PBS).

Figure 5-2 shows that increasing the concentration of DMPC increases the loading of DMPC. The relationship is proportional. As the concentration gradient between the outside and inside of the lens is increased by a factor of 4, the loading also increases by a factor of 4.

For both the higher temperature and the lower temperature, Figure 5-3 shows that increasing the volume of the DMPC solution increases the DMPC loading. However, increasing the volume by a factor of 9 increased the loading by only a factor of about 3 for both temperatures. Thus DMPC loading is not a strong function of volume. Loading at 121°C was lower than at 129°C for all volumes tested, a finding consistent with diffusivity increasing with temperature.
Overall, the results are encouraging given that at a concentration of 80 ppm DMPC (below the limit of 100 ppm), lower time scales and a temperature of 121°C, target-level amounts of DMPC (approximately 1 µg) are loaded into the lenses. At the higher values of time, concentration, volume, and temperature, the loading of DMPC was many times higher than the target range, with 25 µg being the highest loading achieved. In terms of what the optimal conditions are for DMPC loading, increased loading results from raising variables of time, concentration, volume, and temperature. However, process and legal constraints limit the variables; process autoclave temperatures are designed for 121°C, the DMPC concentration in the blister pack fluid cannot exceed 100 ppm in the final product for legal reasons, and the volume of the blister packs are limited to 0.65 mL. While autoclave times can go beyond 45 minutes, this would require purchasing additional autoclaves or a halving of production to load
additional DMPC. Section 2 will help illuminate if doubling autoclave times would yield a substantial benefit of DMPC loading and release.

5.2 Section 2: Release of DMPC from a Contact Lens into Tears

Section 2 was conducted in blister packs instead of in the glass vials of Section 1. Four different loading conditions were tested for their DMPC release into ATF; two concentrations of 40 ppm and 80 ppm DMPC were paired with two thermal loading times of 45 and 90 minutes. All loading conditions had the same aqueous volume of 0.65 mL and autoclave temperature of 121°C. Figure 5-4 shows a summary of the elution of DMPC from the contact lenses.

At each daily sampling time point, the amount eluted increased in the following order sorted by loading conditions: 45-min-40-ppm < 90-min-40-ppm <45-min-80-ppm <90-min-80-ppm. The 40-ppm samples released slightly more than 2 µg after 7 days. The 80-ppm-45-min samples released about 3.2 µg, and the 80-ppm-90-min samples released about 4.5 µg over 7 days. All of these lenses were releasing more than 1 µg on the first day, which is the day of most importance for a daily-disposable contact lens.

Figure 5-4 - Cumulative elution of DMPC into artificial tears from contact lenses. Confidence intervals of 95% generated the error bars, n=4.
After elution, the lenses were extracted with n-propanol to quantitate the remaining DMPC. This value was combined with the eluted amounts to give the following total of DMPC loaded at each condition, as shown in Table 5-1.

Table 5-1 - Total loading of DMPC into contact lenses (µg/lens)

<table>
<thead>
<tr>
<th></th>
<th>45 minutes autoclave</th>
<th>90 minutes autoclave</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 ppm DMPC in</td>
<td>5.2 ± 0.7 µg/lens</td>
<td>6.8 ± 0.4 µg/lens</td>
</tr>
<tr>
<td>Formulation A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80 ppm DMPC in</td>
<td>8.7 ± 0.7 µg/lens</td>
<td>12.1 ± 0.7 µg/lens</td>
</tr>
<tr>
<td>Formulation A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n=4; DMPC solution volume is 0.65 mL and temperature is 121°C for all samples; mean ± 95% confidence intervals. Formulation A is essentially PBS.

DMPC uptake is a stronger function of DMPC concentration than loading time, because doubling DMPC concentration increased DMPC uptake more than doubling the loading time. In general, about half of the total DMPC eluted in 7 days was released during the first 24 hours. After the first day, the elution rate slowed significantly. The lens loaded for 90 minutes at 121°C with 80 ppm DMPC may have still been releasing DMPC at 7 days, but it appears that the other lenses had finished or nearly finished their release. Upon close examination, it might appear that the loading of Section 2 and Section 1 disagree when comparing similar conditions. However, the thermal history of the two experiments are different, because Section 1 was conducted in glass vials, whereas Section 2 was conducted in polypropylene blister packs. Because the
polypropylene blister packs get up to temperature much faster than the glass vials, DMPC diffuses into the contact lenses in polypropylene blister packs faster initially.

5.3  **Section 3: Reproducibility at Optimal Conditions**

The goal of this section was to investigate in depth the reproducibility of the loading and elution of DMPC from the final selected loading conditions of 0.65 mL of 80 ppm DMPC in Formulation A in a blister pack, and 45 minutes of autoclaving at 121°C. From the 5 lenses that were loaded and extracted, the average loading was 12.8 µg ± 0.3 µg (95% CI). Elution amounts are displayed in Figure 5-5 for daily release and the same data in Figure 5-6 reformatted for cumulative release.

![Figure 5-5](image)

*Figure 5-5- Average amount of DMPC eluted from contact lenses per day in the seven-day simulation of wear. Initial mass loaded was 12.8 µg ± 0.3 µg. Error bars are ± 95 percent confidence intervals; n=5.*

To best simulate contact lens wear, the loaded contact lenses were cycled through ATF for 16 hours (simulating 16 hours of wear) and then in multipurpose solution (MPS) for 8 hours (simulating nightly storage). MPS is the storage and disinfectant solution for nightly contact lens storage. Fresh ATF was used for each cycle to maintain approximate sink conditions.
The release into ATF was much greater than release into MPS, totaling 1.8 µg the first 16 hours in ATF. Daily release decreased dramatically in subsequent daily ATF exposures, following the expected burst and release behavior of typical drug delivery. The 95% confidence intervals are very tight, as indicated by the error bars, indicating the reproducibility of the release into ATF across different contact lenses in the same batch.

After the first few days of rapid release, the release of DMPC into ATF appears to reach a “steady-state” rate of release of about 0.3 µg/16 hours. The difference between the release into ATF and MPS is consistent with the study of Pitt and Zhao (Pitt, Jack, Zhao, Nelson, & Pruitt, 2012). For contact lenses intended for many days of wear, this rate of 0.3 µg of DMPC/16 hours would be expected to be the baseline release rate. The release into MPS over 8 hours was a constant low level. After 7 days of cycling through ATF and MPS, contact lenses lost only about 0.2 µg of the total 12.8 µg of DMPC that had been originally loaded. Similar behavior would be
expected over a month of wear, amounting to at most 0.8 µg of DMPC (or approximately 6\% of the total DMPC loaded into the lens) lost into MPS over a month of nightly storage.

At the end of 7 days, about 1/3 of the DMPC had eluted from the lenses (4.2 of 12.8 µg). However, much of that 4.2 µg is released in the first day. If the lenses were to continue the postulated “steady-state” rate of release of 0.3 µg of DMPC per 16 hour wearing period, then after 30 days, an additional 6.9 µg of DMPC would be released, plus about 0.8 µg that would be lost into MPS from nightly storage. Altogether, the steady-state release plus the first few days of more rapid release plus the loss into MPS would deplete the lens of 11.9 of the 12.8 µg loaded. However, it is doubtful that steady-state release on the order of 0.3 µg per day would be sustained with a near-depleted DMPC reservoir. Thus the true dynamics of release past the 7-day mark would only be known through further experimentation. However, because these particular lenses are intended only for daily use, it is unlikely that the release after 7 days of wear is important. Indeed, if the lenses are worn for only a day as intended, the first day yields 1.8 µg of DMPC, which is very close to the benchmark of 1.0 µg/day. Currently, the FDA has only approved Delefilcon A for single daily wear. Another formulation would need to be develop for 30-day wear lenses.

After 7 days of release into ATF and MPS, the lenses were extracted for their DMPC content. The extracted amount plus the eluted amount summed to only 10.6 µg, so there is some DMPC missing if 12.8 µg of DMPC was initially loaded, as indicated by the lenses that were immediately extracted following loading. The exact cause for the apparent deficiency of DMPC through the elution steps is unknown. It can be speculated that some component of ATF might catalyze DMPC to exhibit an increased degree of affinity for contact lenses, such that when the contact lenses are processed through ATF, less DMPC is extracted than if the ATF treatment had
been skipped. However, further testing would be required to determine if the apparent deficiency of DMPC is actually occurring, and if so, to what extent and for what cause.

These data suggest a potential therapeutic effect from sustained release of DMPC from these lenses. The DMPC release on the first day is 1.8 μg, which is above the daily target of 1.0 μg. If the lenses were replaced daily as they ought to be, then the DMPC content should be more than satisfactory. For cases of longer wear, sustained release into ATF of 0.3 μg /16 hours over an extended time is a distinct possibility, and perhaps over intervals longer than one week. As the formulation currently stands, 0.3 μg of DMPC per day could bring a month of comfort to those contact wearers with dry eye if the same results were seen in a lens intended for longer use.
Chapter 6 addresses the retention of convectively-loaded DMPC throughout a manufacturing process. The manufacturing process in use by Alcon to produce its Dailies Total1® contact lenses is proprietary; Figure 6-1 shows a general overview of the process without the proprietary details.

Figure 6-1 – The latter stages of the Alcon manufacturing process for Dailies Total1® contact lenses without proprietary details.

Without the extra DMPC loading procedure, the standard manufacturing process takes polymerized contact lens “cores” through several rinses of Formulation C (organic solution), a
water rinse, a wash through Formulation B (Stage 1, green solution in Figure 6-1), followed by a series of water rinses (Stage 2, yellow solution in Figure 6-1), and then inserts contact lenses into blister packs with Formulation A for autoclaving (Stage 3, blue blister packs in Figure 6-1). In terms of potential for DMPC loss in the process, Stage 1 of the standard process introduces the contact lens cores to Formulation B (organic solution) where some DMPC might be lost in the organic medium. Stage 2 proceeds with a series of several water rinses, where some DMPC might be lost as the lens shrinks. Stage 3 introduces the contact lenses into blister packs and sterilizes the package in an autoclave, after which the finished contact lenses are ready for shipment in sterilized blister packs. We inserted the DMPC loading step and water rinse (red solutions in Figure 6-1) into the process after polymerization and extraction of non-polymerized monomer and before these final three stages. More specifically, the contact lens cores are soaked all together in 200 mL of the n-propanol/DMPC mixture for 155 s and then rinsed in double-distilled water for 1 minute. In order to facilitate the production of many contact lens cores at once, a large volume of 200 mL of loading solution is required, whereas in thermal loading, only the 0.65 mL volume of an individual blister pack is needed. This chapter studies how much DMPC is retained after each stage of the normal process if the DMPC loading steps are inserted as indicated in Figure 6-1.

Figure 6-2 shows the results of this study. The fraction of DMPC remaining in the lens is plotted immediately after the DMPC loading, after the Stage 2 water rinses, after the Stage 3 packaging steps, and after 7 days of elution into un-replenished artificial tear fluid (ATF). The error bars are the 95% CI.
After the initial loading step of 155 seconds in 0.15 w/v% DMPC in n-propanol, about half of the DMPC is lost over Stage 1 (organic solution) and Stage 2 (several aqueous solutions). No measurements were taken directly after Stage 1 because the water steps are necessary for lens shrinkage. However, Stage 1 is likely where most of the DMPC is being lost because previous studies have shown that DMPC does not elute appreciably into double distilled water (Pitt, Jack, Zhao, Nelson, & Pruitt, 2012). The organic solution of Stage 1 would have ample ability to absorb DMPC from the loaded contact lens. However the amount of retained DMPC does not statistically change past Stage 2 as the lenses are packaged in Formulation A in blister packs and autoclaved. This is likely because the lenses have finished shrinking, and the net amount of DMPC lost to the surrounding aqueous medium of Formulation A is negligible.

To test the packaged lenses for their potential therapeutic effect, the lenses were placed into ATF to simulate wearing. The DMPC release from the completed lens into ATF is another
measure of the potential benefit of the convective-loading process. Figure 6-3 plots the DMPC release from contact lenses that were loaded with DMPC in Stage 1 and processed through Stage 3.

![Graph showing cumulative release of DMPC into 3.0 mL of un-replenished artificial tear fluid.](image)

*Figure 6-3 – The blue circles represent the cumulative release profile of DMPC into 3.0 mL of un-replenished artificial tear fluid and the orange x-mark represents the total DMPC recovered from the lenses after performing extractions (total = cumulative DMPC elution into artificial tear fluid plus DMPC extracted after 168 hours). At 0 hours, 24 µg of DMPC are in the lens. Error bars are 95% confidence intervals, n=3.*

Only a limited supply of the proprietary ATF was available for this experiment, so the experiment proceeded under un-replenished conditions. Over the first 24 hours, the lens released 9% of its DMPC payload into ATF, which is 2 µg of DMPC. This is on target with the goal of 1 µg of DMPC per day set by Alcon. After 24-72 hours, the DMPC cumulative release plateaued, likely because DMPC accumulating in the ATF decreased the driving force for release. If the release rate of the first day were to continue until exhaustion, then the contact lens would be
depleted of DMPC within 11-12 days. However, it is likely that the lens would be able to elute DMPC for a longer period of time, since the release rate of the first day is usually greater than the subsequent days.

The fraction of DMPC release over the first day was 0.09 as compared to the fractional release of 0.14 in Chapter 5 for thermal loading. These are of the same order of magnitude, despite the former being loaded under convective conditions, and the latter being loaded under thermal conditions. The convectively-loaded lens released more slowly over the first day than the thermally-loaded lens. However, because the thermally-loaded lens of Chapter 5 was transferred to fresh artificial tears each 24 hours and the convectively-loaded lens of this chapter was not, no further comparison is possible with this data set. These initial data suggest that the convectively-loaded lens might yield a more gradual release profile than the thermally-loaded lens if the replenishment schedule were enacted at the one day mark for the convectively-loaded lens.

Overall, the results of Chapter 6 reveal that a convective loading step for DMPC can be inserted into the Alcon manufacturing process immediately before the soak in Formulation C. Convective loading in this way yields 50% retention of DMPC at a loading time of 155 seconds and loading concentration of 0.15 w/v % DMPC and release into ATF of 2 µg of DMPC in the first 24 hours. To conserve expensive DMPC, a two-fold lower DMPC concentration of 0.075 w/v % in 200 mL of n-propanol could be used to cut the end ATF release to 1 µg of DMPC in the first 24 hours. Future work could also test the effect of the post-DMPC-loading water-shrink step (the second red solution in Figure 6-1) on the retention of DMPC and the quality of the contact lenses. At only 60 seconds in this study, this water step likely does not allow for full shrinkage of the contact lens. With full shrinkage, the lens might better retain the DMPC going into Stage 1 (green solution in Figure 6-1).
7 CONVECTIVE LOADING AND RELEASE OF LATANOPROST IN DAILIES TOTAL1® LENSES

Beyond the experiments with DMPC in Chapter 5 and Chapter 6, Chapter 7 and Chapter 8 contain results for latanoprost loading and release. Chapter 7 addresses latanoprost loading and release in one type of contact lens exclusively: the Dailies Total1® made by Alcon. Chapter 8 broadens the study to multiple makes of contact lenses, namely Acuvue Advance®, Alcon Air Optix®, CooperVision Biofinity®, Bausch and Lomb PureVision®, and Bausch and Lomb Soflens 38®.

The first section of this chapter covers the effect of loading conditions on latanoprost uptake in Dailies Total1® contact lenses. The second section extends the ideas of the first section by showing how the uptake of latanoprost is indeed by a convective mechanism. The third section of results shows the latanoprost release kinetics from a loaded contact lens into artificial tear solution (ATS). The fourth section examines release from latanoprost-loaded lenses, but into pure water instead of ATS.

7.1 Section 1: Effect of Loading Conditions on Latanoprost Loading

Latanoprost loading into a contact lens varies with the loading conditions of loading time and latanoprost concentration. In the parametric experimental setup, the loading concentration of latanoprost was varied from 1-9 g/L of n-propanol and the loading time was varied from 60-240
seconds. All experiments were conducted at room temperature in a 5.0 mL loading solution into which a contact lens was immersed for the desired loading time. The effect of loading time and latanoprost concentration on latanoprost uptake from convective loading in n-propanol is shown in Figure 7-1 and Figure 7-2 respectively.

![Figure 7-1](image_url)

*Figure 7-1 - Loading of latanoprost into Dailies Total1® contact lenses as a function of loading time. The graph insert shows the same data as the large plot, but with the y-axis representing the mass of loading divided by loading concentration to normalize the effects of loading concentration. Experiments were conducted in three different concentrations of latanoprost in n-propanol, of 1.00 g/L (purple squares), 3.00 g/L (hollow gray circles), and 9.00 g/L (dark blue diamonds). Error bars represent 95% confidence intervals, n=3.*
The data in Figure 7-1 prompt some observations. First, extrapolation to zero time creates a y-intercept indistinguishable from zero and a consistent linear slope, suggesting that latanoprost does not “burst” into the lens, instead entering proportional to loading time. Second, the linearity of time and loading rules out the loading mechanism of diffusion and suggests convection, a topic further explored in later experiments. This strict proportionality is observed for all concentrations tested. Third, longer loading yields less experimental error. The error likely
arises from the different dynamics of lens swelling and consequent crumpling of the lens seen in
the tens of seconds of loading, but which converges to a standard shape when the lens has
become completely swollen by the 240 second mark. Fourth, when the loading concentration is
increased by a factor of 3, the loading also increases by a factor of 3. In fact, loading is
proportional to the concentration of latanoprost in the n-propanol. This phenomenon is
highlighted in the insert, where three statistically indistinguishable lines form when the loading is
divided by the latanoprost concentration. The reason for the normalization is to show that the
loading concentration is strictly proportional to latanoprost loading. This proportionality proves
useful in “tuning” the loading of a lens with the desired quantity of latanoprost.

Rearranging the same data yields Figure 7-2, showing in another way how loading is
proportional to concentration. Naturally, Figure 7-2 prompts similar observations and
conclusions to those reached in the analysis of data in Figure 7-1, but having both figures helps
to illuminate the proportionality in both concentration and time when the data are normalized.
Other factors complicate the loading scenario when varying time; the lens becomes increasingly
swollen, and therefore becomes increasingly fragile the longer it is loaded. However, fragility
faces a trade-off with consistency; the loading is more consistent with longer loading times.

The linearity seen in Figure 7-1 and Figure 7-2 is not observed at times longer than 240
seconds. The linearity in both time and latanoprost concentration up to 240 seconds allows the
regression of a simple equation for prediction of latanoprost loading in DT1 CLs at room
temperature (24 °C) from n-propanol solutions, providing Equation 7-1.
The calculated value for $k$ is $0.21 \pm 0.01 \mu L/sec$ (95% CI), and the variables of $t$ and $c$ represent the variables of loading time and loading concentration respectively. Thus Equation 7-1 can predict the uptake of latanoprost by a contact lens given a latanoprost concentration in n-propanol and time of loading up to 240 seconds.

7.2 Section 2: Effect of n-Propanol Swelling on Latanoprost Loading

As explained in Section 1, the initial experiments suggested a convective loading mechanism for latanoprost loading in n-propanol. The uptake of n-propanol is measured by weighing mass changes of n-propanol soaked lenses, and is itself proportional to time. While a model has not yet been proposed for why this is the case, this relationship has been established experimentally for up to 4 minutes (240 seconds) of loading as shown in Figure 7-3.

![Figure 7-3](image)

*Figure 7-3 – The fractional mass increase in a wet contact lens from n-propanol swelling as a function of time. Linear fit yields $R^2=0.91$, $n=3$, 95% CI.*

Measuring the mass change in contact lenses proved difficult because of the inherent uncertainties in n-propanol measurement such as n-propanol evaporation and the handling of the
lens squeezing out n-propanol. However, Figure 7-3 establishes a general linear trend over the first several minutes of swelling a contact lens in n-propanol. Further experimental refinement can establish a more detailed picture of the swelling profile over time to establish if the linear profile continues to 240 seconds or if the trend should terminate prior to 240 seconds. For the purposes of this thesis, the linearity of the n-propanol uptake will be taken up to 240 seconds. Beyond 240 seconds of swelling in n-propanol, the increase in n-propanol uptake in the contact lenses was not statistically significant.

If both latanoprost loading and n-propanol uptake are proportional to the time of soaking up to 240 seconds, then latanoprost uptake and n-propanol uptake are also proportional to each other. Further analysis supported this observation by comparing the uptake of latanoprost against the uptake of n-propanol, as shown in Figure 7-4.

The creation of Figure 7-4 was possible because the swelling of n-propanol is itself proportional to loading time. It follows that the loading of latanoprost is directly proportional to the mass uptake of n-propanol when concentrations of latanoprost in n-propanol are normalized. Because the rate of n-propanol entry to the contact lens is proportional to the rate of latanoprost entry, this suggests that n-propanol is bringing the dissolved latanoprost into the contact lens. Thus Figure 7-4 further supports the hypothesis for a convection-dominated loading mechanism.

The maximum observed swelling of n-propanol was 1.3 times the mass of the original lens which occurred at 4 minutes of loading. However, when loading times increase beyond 4 minutes to 6 minutes, the linear correlation breaks down between time and n-propanol swelling and latanoprost uptake. In fact, the mass of loaded latanoprost and swelling of n-propanol does not statistically change when loaded for 6 minutes instead of 4 minutes (data not shown). The lens is essentially saturated with n-propanol near the 4-minute mark, so convection into the lens
halts. Further experiments could more precisely reveal the loading characteristics in this “transition” space between the active-swelling stage and the fully-swollen stage and beyond.

![Figure 7-4](image-url)

*Figure 7-4 – Mass of latanoprost loaded into a contact lens as a function of normalized n-propanol uptake (n-propanol mass uptake divided by the mass of a wet contact lens). The graph insert shows the same data as the large plot but with the y-axis representing the mass of loaded latanoprost divided by the concentration of latanoprost in the loading solution. Experiments were conducted in three different concentrations of latanoprost in n-propanol, of 1.00 g/L (purple squares), 3.00 g/L (hollow gray circles), and 9.00 g/L (dark blue diamonds). Error bars represent 95% confidence intervals, n=3.*

However, for times less than 240 seconds, a simple equation can predict the rate of n-propanol uptake into DT1 CLs at room temperature (24 °C), as shown in Equation 7-2.

\[
U = \frac{m_{lat} \cdot \text{y}}{m_{CL}} = U \cdot \left( \frac{m_{lat}}{m_{CL}} \right) \quad \text{Equation 7-2}
\]

In Equation 7-2, \( t \) represents the loading time up to 240 seconds, and the value of \( k \) is \( 0.0065 \pm 0.0008 \) s\(^{-1}\) (95% CI). In calculating \( k \), the average mass measured for a hydrated DT1 CL was 38 mg.
Maximum loading and swelling aside, convection dominates the loading of the first 4 minutes, but it is also of interest to know what percentage of latanoprost stays in the lens during the shrinking step. If a high percentage is retained, then this supports the idea that latanoprost associates favorably with the silicone hydrogel when water diffuses into the swollen lens. If only a low percentage stays, then this supports the idea of latanoprost having lower affinity for the lens than it has for the water. To generate these retention percentages, data from Figure 7-4 was manipulated to give Figure 7-5 which shows the fraction of latanoprost remaining in the lens after shrinking.

![Figure 7-5](image-url)  

*Figure 7-5 – Fraction of latanoprost remaining in the lens after lens shrinking as a function of initial n-propanol mass uptake. The mean values at each mass uptake are not statistically different. Error bars represent 95% confidence intervals, n=3.*

Figure 7-5 assumes that the latanoprost concentration of the loading solution does not change when it is convectively flowing into the contact lens. Under this assumption, the retention of latanoprost is about 78%, and is not a function of loading time or amount. This is relatively high especially when considering that the n-propanol saturated lens loses more than half of the...
system mass during shrinking. The percent retention of latanoprost is consistent across different concentrations of latanoprost and mass uptake of n-propanol, as the data in Figure 7-5 were incorporated from 3 different loading concentrations (1-9 g/L) and 3 different loading times (60-240 seconds).

7.3 Section 3: Release of Latanoprost from a Contact Lens into Tears

With an understanding of the convective loading dynamics and their linearity to loading time and loading concentration, the discussion now transitions to the release of latanoprost from a loaded contact lens.

Glaucoma treatment efficacy varies with the release rate anti-glaucoma drugs, so it is important to understand the dynamics of release for a wide range of latanoprost loadings. Figure 7-6 presents two strengths of latanoprost loading, one of 6.0 µg (loaded at 0.125 g/L and 240 seconds) and the other of 420 µg (loaded at 9.00 g/L and 240 seconds), and the subsequent release into gently-stirred artificial tears held at 35°C.

The experimental procedure behind Figure 7-6 called for regular replacement of the artificial tear solution (ATS) in an effort to maintain near-sink conditions for drug elution. The contact lenses were thus transferred to fresh ATS at the 24, 48, 96 and 144 hour marks. The amounts of latanoprost loaded in these experiments differed by two orders of magnitude, so the cumulative release data were normalized by dividing by the total starting content of latanoprost (6.0 µg and 420 µg) in order to compare the two data sets.
Figure 7-6 – The cumulative fraction of loaded latanoprost released into artificial tears from a loaded contact lens as a function of time. The solid line indicates the maximum cumulative release of 100%. In order to maintain semi-sink conditions, the lenses were transferred to fresh artificial tears at hour 24, 48, 96, and 144 immediately after a sample was taken. Experiments were conducted with low and high loading of latanoprost in the contact lens: 6.0 µg for the low (orange dashes and orange hollow triangles) and 4.2 \times 10^2 µg for the high (dark-blue x-marks and dark-blue diamonds). The dashes and x-marks represent the sample taken immediately before transferring the contact lens to fresh artificial tears. The plot inset presents the same data against the square root of time, revealing a linear trend. Linear regression gives $R^2 = 0.99$ for the first nine points of the 6.0 µg series (short-dashed line) and $R^2 = 0.98$ for the first eleven points of the 4.2 \times 10^2 µg series. Error bars are 95% confidence intervals, n=3.

The shape of the normalized release profiles in Figure 7-6 leads to several observations. First, the contact lenses release essentially all of their loaded latanoprost within 3-4 days, regardless of how much latanoprost was loaded initially. Second, the cumulative release is clearly not linearly proportional to time, suggesting instead that diffusion of latanoprost within the lens is the dominant mechanism because of the proportionality to the square root of time (see inset of Figure 7-6). Third, the two sets of lenses of low and high latanoprost loading have the
same general shape of normalized release profile, which means that release into ATS is proportional to the amount loaded.

This would imply that the release rate into artificial tears can be controlled by controlling the initial loading of latanoprost. A fourth observation is that the lower-loaded contact lens releases about 2 µg in the first day, which is just above the upper-bound therapeutic rate calculated in Chapter 2.

The heavily loaded lens (blue diamonds) yields slightly lower normalized release rates. One possible explanation is that the artificial tear replacement schedule was not frequent enough to maintain perfect sink conditions; the rate of release tapers off up to each point before the lens is transferred to a new container of artificial tears (indicated by the dark-blue x-marks), but increases thereafter. The normalized release rate of the higher-loaded lens was lower because the bulk solution probably approached latanoprost saturation. This assumption is supported by the fact that the latanoprost solubility commercially reported for water is 40 µg/mL (Pfizer Canada Inc., 2014), and the concentration found in the container of artificial tears before the first replenishment was 45 µg/mL ± 6 µg/mL. These concentrations are statistically indistinguishable from each other, which suggests latanoprost could be approaching saturation in the artificial tear solution. This observation comes with uncertainty however, since latanoprost likely is more soluble in artificial tears than pure water because of favorable interactions with hydrophobic proteins in artificial tears. However, as an order of magnitude estimate, latanoprost has a high likelihood of at least approaching saturation conditions in the bulk environment of the heavily loaded lens.

While 30-day release would be optimal, 2-4 day release could still prove useful for a daily replacement treatment schedule. For a daily replacement schedule, one can configure the
precise dose desired in the first 24 hours. Since the rate of latanoprost release into artificial tears is proportional to the amount of latanoprost loaded, this relationship can be written as an equation, namely Equation 7-3,

\[ \text{Initial 24 hour release} = k \times \text{(uptake of latanoprost)} \]

\text{Equation 7-3}

where \( k \) is \( 0.53 \pm 0.05 \text{ day}^{-1} \) (95% CI). The value of \( k \) was calculated using the release rate of the lens loaded with 6 µg of latanoprost in Figure 7-6. While Equation 7-3 will be limited to latanoprost-loading amounts near or below 6 µg, it is precisely this range that was previously calculated in this thesis to be therapeutic. To release at the upper-bound release rate of 1 µg/day, one can use a loading concentration of 0.040 g/L and 4 minutes of loading to load a contact lens with 2 µg of latanoprost, as given by the previously introduced Equation 7-1. This lens will then release about 1 µg/day in the first day of wear. To release at the lower-bound, a loading concentration a hundred-fold less, or 0.0004 g/L can be used.

In order for a one-day lens to be a feasible manufacturing option, a loaded lens would need to release well in artificial tears, but retain latanoprost while in storage solution. The next set of experiments will examine the latanoprost retention in a loaded contact lens when in a storage environment.

### 7.4 Section 4: Release of Latanoprost from a Contact Lens into Water

Contact lenses often must sit for months if not years before they are used. Consequently, a medicated contact lens would need to retain a loaded drug for durations of at least months in order to be commercially viable. To test if latanoprost-loaded contact lenses would retain latanoprost in storage, two sets of contact lenses were loaded with 14 µg (60 seconds of loading and 1.00 g/L) and 35 µg (60 seconds of loading and 3.00 g/L) of latanoprost respectively and
placed in a 3.0 mL unstirred storage medium of double distilled water (DDH₂O). Samples of the storage medium were taken over the course of several weeks to measure latanoprost concentration in the storage medium yielding Figure 7-7.

![Figure 7-7](image_url)

Figure 7-7 - The cumulative fraction of loaded latanoprost that eluted into a double-distilled water storage solution from a contact lens as a function of time. The solid line indicates the maximum cumulative release of 100%. The lenses stayed in the same 3.0 mL of water for the duration of the experiment. Latanoprost was loaded in different amounts into two groups of contact lenses: 14 µg for one group (hollow gray circles) and 35 µg (three-fold more) for the other (purple squares). The plot inset presents the same data against the square root of time, revealing a rough linear trend. Linear regression gives $R^2 = 0.99$ for the 14 µg series (purple line) and $R^2 = 0.95$ for the 35 µg series (gray line). Error bars represent 95% confidence intervals, $n=3$.

Figure 7-7 prompts some key observations. Unfortunately, the primary observation from Figure 7-7 is that all of the latanoprost was lost from a contact lens within 3-4 weeks of sitting in 3.0 mL storage water. It is possible the lens released all of its latanoprost before 3-4 weeks. After
3-4 weeks, there is no statistical difference between the amount loaded into the lenses and the amount that eluted into the storage water. The elution is proportional to how much latanoprost was loaded into the lens, as shown by the similar normalized release profiles for lenses loaded at different amounts. Another observation is that there are large error bars for the later data due to the short loading times of these lenses (as discussed in Section 1). The inset of Figure 7-7 plots the normalized cumulative latanoprost release against the square root of time. However, this inset plot does not indicate a strong case for a square root of time dependency.

The percentage loss of latanoprost over time was statistically indistinguishable for the two different sets of lenses in Figure 7-7 over the first 5 days. For the first week, both lenses exhibited linear release behavior with respect to time, suggesting that the release rate into water is proportional to the initial concentration of latanoprost inside the contact lens. If the linear release of the initial 7 days is extrapolated until depletion, the resultant time to depletion would be 14 days and 17 days for the lenses loaded with 14 µg and 35 µg respectively. While the exact release dynamics at the 2 week time interval are not known, it is possible that all the latanoprost could be eluted within the first 2 weeks. However, it is not necessary to know these dynamics precisely, as further experiments in smaller volumes of storage water might prove more helpful in establishing the best storage method for latanoprost-loaded contact lenses going forward.

The data from Figure 7-7 might present an incomplete picture of complete water elution; if a smaller volume of storage water is used and the lenses are loaded with more latanoprost, the lenses may reach an equilibrium with the bulk storage solution and preserve the amount of latanoprost in the contact lens. Alternatively, the lenses could be stored in a solution already saturated with latanoprost. Future experiments could test these hypotheses.
Overall, Chapter 7 has uncovered some key information about latanoprost loading and release in Dailies Total1® contact lenses:

- Latanoprost loads proportionally with respect to concentration and time up to 4 minutes
- Latanoprost loading is proportional to the amount of n-propanol swelling into the contact lens during the convective loading step
- Latanoprost release into artificial tears is proportional to both the square root of time and the amount of latanoprost loaded.
- Latanoprost release into artificial tears terminates after 2-4 days, with a loaded contact lens delivering essentially all of its original latanoprost payload.
- Latanoprost elutes into 3 mL of double distilled water after 3 weeks

The focus of this paper will now broaden to multiple brands of contact lenses with respect to latanoprost loading and release.
Chapter 8 expands the experiments of latanoprost release into artificial tears to several different silicone hydrogel contact lenses, namely Acuvue Advance®, Air Optix®, Biofinity®, and PureVision®, and the polyHEMA lens, SofLens 38®. Of interest in this comparison is the relative ease of loading at the same conditions and the release dynamics into artificial tear solution (ATS), especially between the different chemistry of the silicone hydrogels and the polyHEMA lenses. Displayed in Figure 8-1 is both the loading level for 240 seconds of loading at 0.125 g/L and the ATS.

Figure 8-1 reveals some intriguing trends. First, the five silicone hydrogels load and release on the same order of magnitude, whereas the Soflens 38® polyHEMA lens (purple dashes) loads one order of magnitude less latanoprost than any of the silicone hydrogel (SiHy) lenses. This supports the hypothesis that latanoprost loads better via convective loading into lenses with more hydrophobic character. The second trend to note is that the normalized release profile has the same general shape for the five silicone hydrogels. Since the normalized release profile of the other four SiHy lenses are all similar to Dailies Total1® (DT1), the observations of Chapter 7 about the normalized release profile of DT1 may also apply to other SiHy lenses; the contact lenses release essentially all their loaded latanoprost; the cumulative release is clearly not linearly proportional to time, but is initially proportional to the square root of time; this
A proportional relationship supports diffusion as a possible mechanism initially for latanoprost transport within the contact lens.

Figure 8-1 – Contact lens cumulative release of latanoprost into artificial tears as a function of time. In order to maintain semi-sink conditions, the lenses were transferred to fresh artificial tears at hour 24, 48, 96, and 144 immediately after a sample was taken. Experiments were conducted with one type of polyHEMA lens, the Bausch & Lomb Soflens 38® (purple dashes and tiny-dots purple line), and five different types of silicone hydrogel contact lenses: Acuvue Advance® (solid blue diamonds and dash-dot line), Alcon Air Optix® (hollow orange triangles and square-dotted line), CooperVision Biofinity® (hollow black circles and long-dashed line), Bausch & Lomb PureVision® (solid yellow squares and thick solid line), and Alcon Dailies Total1® (green x-marks and short-dashed line). All contact lenses were loaded under the same conditions: 240 seconds of loading in an n-propanol solution of 0.125 g/L latanoprost. The horizontal line of each series indicates the amount of latanoprost that was estimated to be loaded into the contact lens. This amount was determined in a separate control experiment by loading lenses and performing immediate extractions of latanoprost. Error bars represent 95% confidence intervals, n=3.

However, some lenses demonstrate more gradual release profiles into artificial tears, the PureVision® lenses being the most pronounced. The data suggest that PureVision® lenses would
be expected to continue latanoprost release for longer durations than the others. The lenses which
demonstrated the quickest and shortest-lived release were the Soflens 38® polyHEMA lenses
followed by the Biofinity® lenses. These would be the least suited lenses to long-term release,
especially considering the payload of the Soflens 38® lens was an order of magnitude less than
the silicone hydrogels.
9 CONCLUSIONS AND RECOMMENDATIONS

The conclusions can be divided into two categories: 1) DMPC and 2) latanoprost.

9.1 DMPC

From the DMPC experiments, we conclude the following:

1) The DMPC experiments proved successful, in that both thermal and convective loading can load DMPC into a Dailies Total1® contact lens.

2) By both thermal and convective loading, a contact lens can be loaded with DMPC and release DMPC at therapeutically conceivable rates of 1.8-2 µg/day into artificial tears.

3) Thermal loading fits naturally into the final autoclave step of the Dailies Total1® contact lens manufacturing process.

4) Convectively loading DMPC can also fit naturally into the normal manufacturing process for retain 50% of the amount loaded.

From the results of these experiments, I have some recommendations. Further experiments should be done to illuminate how DMPC released from a contact lens can provide dry-eye relief in human subjects. On the manufacturing side of the DMPC loading, if DMPC is the limiting economic factor, thermal loading will be the more efficient of the two loading
techniques for the same release rate of 1.8 µg/day of DMPC into artificial tears. This is because in thermal loading, each contact lens only requires 52 µg of DMPC, but in convective loading, each contact lens requires 45,000 µg of DMPC. This large difference in DMPC required per lens arises because both the loading volume and DMPC concentration in the convective loading process (1.2 g/L and 33 mL per contact lens) are much greater than in the thermal loading process (0.08 g/L and 0.65 mL per 1 contact lens).

In terms of loading efficiency, thermal loading puts 25% of the total DMPC in the loading solution into a contact lens, while convective loading puts only puts in 0.1%. While the convective loading solution can be reused, issues of process consistency limit any reuse of DMPC. An exception for the reuse of the DMPC loading solution would be required, because the manufacturing process stipulates that a fresh solution be used for each batch of 6-9 lenses to prevent contamination of the solutions. Even if the loading solution were able to be reused, the depletion of DMPC from this solution each cycle would limit the number of reuses; the loading solution will need to be replaced once the DMPC concentration changes significantly enough to warrant replacement. The allowable loading solution DMPC concentration could perhaps be as low as 1% and as large as 10%, dictating that allowable uncertainty in DMPC release could not vary by more than 1% and 10% respectively. Given this allowable uncertainty, the maximum number of total uses of the loading solution would be 2 and 15 respectively. Allowing reuses then increases the loading efficiency by a factor of 2 and 15 respectively to yield new loading efficiencies of 0.2% and 1.5% respectively. However, even the highest convective DMPC loading efficiency of 1.5% is still much smaller than the 25% loading efficiency observed in thermal loading.
However, if the contact lens must release significantly more than 2 µg per day of DMPC to actually have therapeutic effect on dry-eye, then convective loading would be the only feasible option. Convective loading conditions can be manipulated to release orders of magnitude more DMPC into artificial tears than 2 µg/day (Pitt, et al., 2015), whereas thermal loading already delivers near its maximum capacity at 2 µg/day. Thermal loading already leverages the highest allowable DMPC concentration and volume of fluid in blister pack. The only change in parameters that would yield an increase in DMPC loading would be increased autoclave temperature or increased autoclave duration. However, it is favorable for process synchronization to keep both of these parameters at their current values of 121°C and 45 minutes respectively.

Considering both the expense of DMPC and the achieved target DMPC delivery from thermal loading, I recommend thermal loading as the optimal loading mechanism for DMPC in Dailies Total1® contact lenses. For thermal loading, the optimal process is to load at 80 ppm DMPC in 0.65 mL of Formulation A, at 121°C, for 45 minutes, loading 12.8 µg of DMPC with a loading efficiency of approximately 25%. A contact lens prepared in this way will release 1.8 µg of DMPC into artificial tears over the first day, which is on target with the goal of 1.0 µg of DMPC per day that is expected to provide therapeutic relief of dry-eye.

9.2 Latanoprost

The latanoprost experiments proved successful in that we have come to the following conclusions:
1) Latanoprost can be convectively loaded into silicone hydrogel contact lenses hundreds of times faster than was done in previously reported techniques, and with many times more drug than has been shown in previous techniques.

2) Latanoprost uptake into contact lenses scales proportionally to the time loaded and latanoprost concentration in the loading solution for up to 4 minutes of loading. Because of this relationship, the loading of a contact lens can be adjusted to deliver latanoprost over the full range of what conceivably could be therapeutic for glaucoma. These results can be written as an equation,

\[ \text{Uptake of latanoprost} = ktc \]

where \( k \) is \( 0.21 \pm 0.01 \) \( \mu L/sec \), and \( t \) and \( c \) are the time and concentration of latanoprost loading in n-propanol respectively, where \( t \) must be equal to or less than 240 seconds. This equation can enable any researcher to load any desired amount of latanoprost into a Dailies Total1® contact lens.

3) The amount of latanoprost loaded is proportional to the rate of latanoprost release into artificial tears. This relationship can be written as an equation,

\[ \text{Initial 24 hour release} = k \times (\text{uptake of latanoprost}) \]

where \( k \) is \( 0.53 \pm 0.05 \) \( day^{-1} \) (95% CI).

4) Latanoprost loading correlated with swelling, and about 78% of the latanoprost brought into the lens during the convective loading step remained in the lens.

These results can lead to some promising future work. The proportionality between loading conditions and latanoprost release into artificial tears a contact lens can be loaded to release 1 \( \mu g/day \) of latanoprost in the first day of wear by loading at a concentration of 0.043
g/L in n-propanol and 4 minutes of loading, which is the upper-bound for therapeutic effect. To release at the lower-bound, a loading concentration a hundred-fold less, or 0.0004 g/L and 240 seconds can be used. Thus this contact lens package is ready for further examination *in vivo* to test what loading levels and release rates, if any, yield the best therapeutic outputs for glaucoma when contact lenses are replaced every 1-4 days.

However, a number of technical points of uncertainty remain:

1) The latanoprost release rates from Dailies Total1® contact lenses reported in this thesis are merely an *in vitro* model that would require successful testing in glaucomatous animal models to establish treatment efficacy.

2) Additional experimental refinement is needed to show how latanoprost can remain in a contact lens in storage, perhaps by reducing the volume of the storage solution down from 3 mL to industry standards of 0.8 to 1.0 mL.

3) The contact lens model used here is only intended for daily use in Dailies Total1® lenses. More detailed loading and elution data are needed in an extended-wear silicone hydrogel to properly establish a model for an extended-wear latanoprost-eluting contact lens. The research of this thesis suggests that any of the silicone hydrogels could be a good candidate, with PureVision® contact lenses yielding the best gradual release profile.

4) More work is needed to slow the release of latanoprost into artificial tears if release on the order of weeks is desired. To slow drug release from a contact lens, one study reported loading Vitamin E into contact lenses before loading drug into the lens (Kim, Peng, & Chauhan, 2010). With this “co-loading” of Vitamin E, the release duration increased as much as 6.5 fold (Kim, Peng, & Chauhan, 2010). Building on this
technique of co-loading, release duration of latanoprost could also increase if loaded
with a second molecule that increases the affinity of latanoprost for the environment
of the lens or decreases the diffusivity of latanoprost in the lens. That second
molecule could be DMPC. The co-precipitation of DMPC and latanoprost could yield
delayed release of latanoprost if DMPC and latanoprost have affinity for one another,
or if DMPC could provide significant diffusional resistance to latanoprost.

Beyond the technical drug-delivery considerations, a number of other general points of
uncertainty remain:

1) The FDA would need to approve a latanoprost-eluting contact lens if it were to be
   commercially used. As a combination-device, the approval process might be lengthy.

2) The latanoprost-loaded lens would need to prove manufacturable.

3) The shelf-life of a latanoprost-eluting lens would need to be examined, because the
drug is thermally unstable drug

4) Enough glaucoma patients would need to actually prefer a medicated contact lens to
eye drops to make this product viable. The best market for this technology would
likely be glaucoma patients who are already accustomed to wearing contact lenses
and replacing them daily. A secondary market would be those glaucoma patients who
need vision correction, but who use vision correction methods other than contact
lenses. This second group likely includes many who would struggle with the dexterity
required in a daily routine of contact lens insertion and removal. They would be the
ones who would most benefit from an extended-wear latanoprost-eluting contact lens,
because they could have assistance from others if needed on a weekly or monthly
basis for contact lens removal and replacement.
5) To meet its end purposes, the contact lens would need to prove more effective at managing glaucoma than eye-drops, especially with regards to patient adherence.

6) Some ophthalmologists are still wary of extended-wear lenses because of the increased risk of infection and corneal damage over 30-day wear times. Thus a latanoprost-releasing contact lens might actually prove better overall for the ocular health of glaucoma patients if replaced daily or weekly. Additionally, researchers should closely monitor all aspects of eye health if there is an initial clinical trial for extended-wear latanoprost-eluting contact lenses.

Because rapid convective loading and gradual release proved effective for latanoprost in silicone hydrogel contact lenses, rapid convective loading and release could be possible for many other kinds of hydrophobic ophthalmic drugs in silicone hydrogel contact lenses. Some of these drugs are even more hydrophobic than latanoprost. Consequently, these drugs might exhibit better affinity for a silicone hydrogel contact lens and consequently more extended release profiles into artificial tears on account of their hydrophobicity. Some of these other molecules include acetazolamide for glaucoma therapy (Lavik, Kuehn, & Kwon, 2011), dexamethasone for treating diabetic macular edema (Kim, Peng, & Chauhan, 2010), fluocinalone acetonide for treatment of diabetic retinopathy (Schwartz & Flynn, 2011), econazole for its antifungal properties (Ciolino, et al., 2011), ketotifen for preventing and treating eye inflammation (Soluri, Hui, & Jones, 2012), and the neuroprotective agents of progesterone (Loane & Faden, 2010), memantine (Danesh-Meyer & Levin, 2009), and statins (Loane & Faden, 2010). Of these, ketotifen seems like it would have the widest audience, as many contact lens users experience seasonal or allergic eye inflammation.
Future work and other considerations aside, latanoprost released from a contact lens could effectively treat glaucoma on a 1-4 day replacement schedule for the many glaucoma patients who struggle with daily eye-drops, but who find a contact lens is a better fit.
REFERENCES


