# Contact Lenses for Ciprofloxacin Drug Delivery

by

Alex Hui

A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Doctor of Philosophy
in
Vision Science

Waterloo, Ontario, Canada, 2014

# AUTHOR'S DECLARATION

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

# STATEMENT OF CONTRIBUTIONS

I would like to acknowledge the names of my co-authors who contributed to this thesis:

- Dr. Lyndon Jones, PhD
- Dr. Adrienne Boone, PhD
- Dr. Heather Sheardown, PhD
- Dr. Mark Willcox, PhD

# ABSTRACT


## PURPOSE

The purpose of this thesis was to evaluate the potential of contact lenses as ciprofloxacin drug delivery devices.

## METHODS

Investigations into ciprofloxacin uptake and release characteristics, and the possibility of their eventual clinical applications were elucidated through three broad experiments:

- In the first experiment (Chapter 3), the uptake and release characteristics of commercially available contact lenses, both hydrogels and silicone hydrogels, were examined *in vitro.*
- In the second experiment (Chapter 4), novel contact lens materials were manufactured using a molecular imprinting strategy to modify *in vitro* ciprofloxacin release characteristics.
- In the final experiment (Chapter 5), utilizing the results gleaned from the first two experiments, contact lenses were manufactured using the molecular imprinting strategy. The material properties of the novel lenses were evaluated. The antibacterial activity of these lenses were evaluated both *in vitro* and in an *in vivo* rabbit model of microbial keratitis.

## RESULTS

Examination of commercial contact lens materials for their ciprofloxacin delivery potential demonstrated a measurable difference between the different lens types, with the hydrogel lenses taking up more ciprofloxacin and releasing more over time. Silicone hydrogels

as a group did not release as much antibiotic as the hydrogels, but neither group of lenses were able to release the antibiotic for any extended periods of time.

Novel materials created using a molecular imprinting strategy demonstrated substantial improvements to release times measured *in vitro*. Some of the materials were able to demonstrate sustained release within the vials for up to two weeks. The molecular imprinting strategy was subsequently applied to contact lenses manufactured in-house, which were shown to have similar contact lens properties to lenses already on the market. Testing of the lenses *in vitro* and *in vivo* demonstrated a superior profile in eradicating pathogenic bacteria in models of microbial keratitis.

## CONCLUSIONS

The results from this thesis detail the potential for novel, custom-made contact lenses with extended ciprofloxacin releasing characteristics. These novel lenses may influence or be a part of future treatment paradigms for ocular infections.

# ACKNOWLEDGEMENTS

I would like to extend my most sincere thanks to Dr. Lyndon Jones for his support, mentorship and guidance these past 8 years. He has given me ample opportunities to carve out success, and for that I will always be grateful. He has truly been my ideal supervisor, and I cannot thank him enough for that.

I would like to thank the members of my committee, Drs. Heather Sheardown, David McCanna, Maud Gorbet and Vivian Choh for your insights and support. Your supervision has been most appreciated, and I will take the lessons I've learned from each and every one of you to heart in my future career. I would also like to thank Dr. Barry Weissman for agreeing to travel from California to be my external examiner.

Thanks are also extended to Drs. Mark Willcox, Ajay Vijay and Ms. Denise Lawler at the University of New South Wales for agreeing to host my visiting stint in Sydney, New South Wales, Australia. Their support and aid has had a significant impact on the experimental completion of this thesis, and I thank them for that.

A special thanks is also given to research associate Miriam Heynen. I have appreciated all of our discussions, and your insight and support has proved invaluable. I also wish to thank Drs. Lakshman Subbaraman, Sruthi Srinivasan, Doerte Luensmann and Holly Lorentz, all of whom have served as mentors and role models for me as a graduate student and researcher within the laboratory.

In my time as a graduate student at the University of Waterloo, I have been proud and fortunate to have been part of two extended families. The Centre for Contact Lens Research (CCLR) has been an ideal workplace and environment. Its strength lies within its people, and

these people continue to make the CCLR a fantastic place to work in every day. The Graduates in Vision Science (GIVS) have been incredibly supportive of each other as we all pursue our studies. The camaraderie I experienced with each of you has been an incredible source of strength and motivation. I could not have asked for more from my dear friends.

Finally, thanks to Drs. David Guttman, Pauline Wang, John Stavrinides and Wenbo Ma. Professor Guttman and all the members of his laboratory were kind enough to welcome me into their laboratory at the University of Toronto while I was a mere undergraduate student, and it was working with those fantastic people that planted a seed of interest in research and academia that continues to grow to this day.

This thesis was made possible through support from the Natural Sciences and Engineering Research Council (NSERC) of Canada Alexander Graham Bell Doctoral Scholarship, the NSERC 20/20 Network for the Development of Advanced Ophthalmic Materials, the Canadian Optometric Education Trust Fund (COETF), the Endeavour Research Fellowship administered by the Australian Government through the Australia Awards Initiative, and the American Optometric Foundation (AOF)'s Ezell Fellowship and Vistakon Research Grant programs.

# DEDICATION

To my family. My mother, my father, my brother, my sister, and Lyn. I could not have done this without you.

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

# LIST OF SYMBOLS

| | |
|---|---|
| % | percentage |
| ° | degree |
| °C | degrees centigrate |
| D | diopters |
| g | gram |
| L | liter |
| M | molar |
| mg | milligram |
| mL | milliliter |
| mM | millimolar |
| mm | millimeter |
| nm | nanometer |
| pH | negative logarithm hydronium ion concentration |
| pKa | logarithmic acid dissociation constant |
| µg | microgram |
| µL | microliter |
| µm | micrometer |

# LIST OF ABBREVIATIONS

| | |
|---|---|
| ANOVA | analysis of variance |
| AOF | American Optometric Foundation |
| AUC | area underneath the curve |
| BAB | blood aqueous barrier |
| BRB | blood retinal barrier |
| C&S | culture and sensitivity testing |
| CCLR | Centre for Contact Lens Research |
| CFU | colony forming unit |
| CL | contact lens |
| CLSI | clinical and laboratory standards institute |
| CMV | cytomegalovirus |
| DMAA | *N,N*-dimethylacrylamide |
| EGDMA | ethylene glycol dimethacrylate |
| EUCAST | European committee on antimicrobial susceptibility testing |
| EVA | ethylene vinyl acetate |
| FDA | United States Food and Drug Administration |
| FQ | fluoroquinolone |
| FM0411M | α-Methacryloyloxyethyl iminocarboxyethyloxypropyl-poly(dimethylsiloxy)-butyldimethylsilane |
| HEMA | hydroxylethyl methacrylate |
| HPLC | high performance liquid chromatography |
| HIV | human immunodeficiency virus |
| HOB | 2-Hydroxybutyl methacrylate |
| IBM | Isobornyl methacrylate |
| IOL | intraocular lens |
| IOP | intraocular pressure |
| M3U | α ω-Bis(methacryloyloxyethyl iminocarboxy ethyloxypropyl)-poly(dimethylsiloxane)-poly(trifluoropropylmethylsiloxane)- |

| | |
|---|---|
| | poly(ω–methoxy-poly(ethyleneglycol)propyl methylsiloxane) |
| MAA | methylacrylic acid |
| MBC | minimum bacteriocidal concentration |
| MIC | minimum inhibitory concentration |
| $MIC_{90}$ | minimum inhibitory concentration for 90% of bacterial isolates |
| NVA | (*N*-vinyl amino acid) |
| NVP | (*N*-vinyl pyrrolidone) |
| MEHQ | 4-methoxyphenol |
| MK | microbial keratitis |
| mPDMS | monofunctional polydimethylsiloxane) |
| NaCl | sodium chloride |
| NSAID | non-steroidal anti inflammatory drug |
| NSERC | Natural Science and Engineering Research Council of Canada |
| PABA | p-amino benzoic acid |
| PBS | phosphate buffered saline |
| PBVC | poly[dimethysiloxy] di [silylbutanol] bis[vinyl carbamate] |
| PC | phosphorylcholine |
| PMMA | poly(methyl methacrylate) |
| PLGA | poly(lacto-co-glycolide) |
| pHEMA | poly(hydroxylethyl methacrylate) |
| PRK | photorefractive keratectomy |
| PVA | poly vinyl alcohol |
| PVP | poly vinyl pyrrolidone |
| TAIC | 1,3,5-Triallyl-1,3,5-triazine-2,4,6(*1H,3H,5H*)-trione |
| TEGDMA | tetraethyleneglycol dimethacrylate |
| TPVC | tris-(trimethylsiloxysilyl) propylvinyl carbamate |
| TRIS | Methacryloxy propyl tris (trimethylsiloxy)silane |
| VMA | N-Vinyl-N-methylacetamide |

# CHAPTER 1 - INTRODUCTION

## 1.1 BIOMATERIALS

Mankind has been using materials to aid in improving the human condition since ancient times. Early examples of the use of gold in dental fillings, and of using wood for prosthetics for severed limbs are evident from anthropological history. [1, 2] However, it took until the turn of the 20th century for advancements in materials and chemical engineering, as well as our understanding of relationships between materials and their effect on the body and vice versa to develop sufficiently to allow for the field of "biomaterials" to truly develop. Biomaterials are commonly defined as any nonviable material used in a medical device, but recent examples of non-medical applications of biomaterials such as DNA microarrays and cell culture platforms suggest that the common definition may be a bit too narrow. [1, 3] Regardless, success of biomaterials used in humans span diverse applications from scaffolding for the growth of new bones, stents to keep narrow blood vessels open and dental implants to replace lost teeth. Reaching modern day levels of success did not come overnight. Unfavorable host-material interactions initially prevented successful use of materials in medical applications. [3] Use of organic material such as wood as medical devices inevitably led to undesirable immunologic responses by the body and device failure. [1] The success of modern biomaterials has seen insights into the design, synthesis and application of materials to have favorable host-material interactions.

### 1.1.1 CHARACTERISTICS OF SUCCESSFUL BIOMATERIALS

The use of intraocular lenses (IOLs) after cataract extraction surgery, where an artificial lens is implanted into an eye in place of the removed, cloudy, natural lens, provides a useful example of the properties needed for successful biomaterials. Success of a biomaterial depends

on two distinct, broad properties. First, it must be somehow accepted by the body. The material cannot cause an immunologic reaction that results in persistent inflammation or a host immune response or the material will eventually be rejected. The material also cannot be toxic to the area of the body where it is to be used. Second, the biomaterial must in some way fulfill a function. [3] In the majority of cases, the function of the biomaterial will be defined by the disease that is being treated. [4] These two defining features of biomaterials are described in the broadest terms available to illustrate the fact that what is needed will necessarily be disease and location specific. In the design of IOLs, for example, the choice of material such as acylates, hydrogels and silicone were chosen not only because they had favorable host-material interactions with the eye, but also because they could be manufactured with the desired optical properties to correct vision. [5] Properties that facilitate success within blood vessels, where desirable properties may include prevention of blood clot formation, and stability in enlarging the blood vessels, may not be applicable for biomaterials used in the mouth for example, where the toughness of the material against wear and corrosion are more important. [1] Consideration must also be made for the timeframe of use of the biomaterial, as materials that are intended to be used indefinitely within the body may have required properties different than those that will be removed at a later date. Materials used indefinitely within the body have to be carefully studied for any long term adverse effects, and maintenance of desired function. For example, while one may think hip replacement surgery to be an indefinite operation, the lifespan of the materials in this very mobile joint is only 10-15 years. [6]

Initially, the aim of biomaterials was biological inertness in conjunction with their intended function. Materials were chosen that would not interact biologically with the host organism and behave effectively invisible to the immunological process. The majority of these

early materials served a more mechanical support type of role. Titanium and titanium alloys for orthopedic applications are a prime example of an early biomaterial whose main biological aim was inertness. [4] Titanium alloys benefitted from having strength and structural rigidity to aid in the healing of broken bones, with the added benefit of being corrosion resistant and invisible to the immune system for years after implantation. [4] In contrast, as the field of biomaterials has continued to evolve, newer materials are being engineered to positively interact and affect the course and management of diseases in addition to mechanical support. An example of these newer materials have been introduction to the market of heart stents which release compounds to prevent vessel re-stenosis, and thus improve recovery time after a cardiac ischemic event. [1] The materials still have a mechanical function in keeping the blood vessel open, but now they also can release factors to encourage the body to behave in a therapeutically beneficial way.

## 1.2 CONTACT LENSES

Arguably, contact lenses (CLs) are the most commercially successful biomaterial ever released, with estimates of between 125-140 million wearers as of 2010. [7] CLs are small biomaterials specifically engineered to correct for refractive error while being worn on the ocular surface. [8] CLs have become so common in modern society that it is often easy to overlook the challenges in their development before they reached commercial success. As CLs are biomaterials, the challenge to the successful commercialization of CLs was the same as the challenges to all biomaterials - the material needed to be designed to perform an intended function, while also being positively received by the body. The function of CLs is the correction of refractive error. They needed to be formed of a material that had the appropriate optical characteristics in terms of light transmission and ability to refract light rays. Second, the lens needed to be compatible with the ocular surface with which it would interact. [9] The need for CL

biocompatibility is what prevented successful use of CLs until midway into the 20th century. Mankind already had a long history of using glass for optical applications such as glasses, telescopes and microscopes before the invention of CLs, and was thus the material used for the very first CLs. [9] Glass ultimately failed as a possible material for CLs because it has poor biocompatibility. Early examples of large CLs made from glass, even when lathed with as much precision as possible so that they fit into the contours of the cornea and the rest of the ocular surface, were so uncomfortable that wearers could not wear them for long periods of time even with the aid of anesthetics. [9] Glass also does not transmit any oxygen to the cornea, hampering normal corneal physiology.

### 1.2.1 History and Development of Contact Lenses

Poly(methyl methacrylate) (PMMA) was used as early as the 1930s as the first successful CL material. The material, an acrylate polymer, was used because it was found to have the desired optical properties while also being relatively well tolerated by the ocular surface during wear and for many years was the basis for successful rigid CL wear. [10] Indeed, the biocompatibility of PMMA in the eye was further elucidated by events in World War II. A British ophthalmologist, Harold Ridley, made the observation that shards of PMMA embedded into the eyes of fighter pilots from canopies blown out by machine gun fire did not induce large inflammatory reactions as they healed, and thus did not need to be removed. [3] Ridley eventually used the material in his design of an IOL. It would take until the 1960s for Otto Wichterle, a Czech chemist, to fashion an efficient and simple means to form poly(hydroxylethyl methacrylate) (pHEMA) lenses through spin casting, which would serve as the basis of future soft CLs. [9] pHEMA was biocompatible with the ocular surface, and importantly, because of the ability of the material to absorb and retain water, it had significantly better comfort when worn

on the ocular surface compared to rigid lenses made from PMMA. [11] Further developments in spin casting allowed for the reproducible large scale industrial production of lenses to be a reality and allow for successful commercialization of this means of refractive error correction. pHEMA and variants of pHEMA dominated the CL market for many years since their commercial introduction, but they did suffer from some drawbacks, chief among them being reduced oxygen flow to the ocular surface. [11] The primary sources of oxygen to the ocular surface are through the tears which are in contact with the outside air, and the circle of blood vessels which encircle the cornea in the area known as the limbus. By introducing another barrier between the ocular surface and the eye, the cornea was relatively oxygen starved whenever CLs were worn. [10] Prolonged wear of CLs based on pHEMA material eventually led to symptoms associated with hypoxia, as metabolic functions of the cornea are hampered by the scarcity of oxygen. In the short term, the ability of the endothelium, a layer of cells in the cornea whose primary function is to regulate the hydration of the cornea, is affected and led to clinical manifestations of edema within the cornea, affecting vision and comfort. [12] Long term, chronic starvation of oxygen by the cornea induced the growth of new blood vessels into the normally avascular cornea, leading inevitably to an inflammatory response and decreased CL tolerance. [12] There was also some speculation by practitioners within the CL field that the relative hypoxia experienced by the cornea during CL wear led to decrease ability of the eye to ward off pathogens and thus explained the increased risk of developing sight threatening microbial keratitis when wearing CLs. [13]

### 1.2.2 Development of Silicone Hydrogels

The solution from the CL industry to the problems associated with pHEMA-based materials was to search for novel ways to increase the amount of oxygen that was able to flow

through the lens and be delivered to the cornea. Within pHEMA lenses, oxygen transport is dictated by the water content and thickness of the lens. [10] The water content in pHEMA based lenses thus put a limitation on the amount of oxygen that could theoretically be delivered. High water content CLs also suffered from protein deposition and poor lens reproducibility. For many years, it had been known that silicone had excellent oxygen transport capabilities. Indeed, to combat the effects of CL induced hypoxia in children who required CLs after pediatric cataract surgery, they would be fitted with CLs made solely of silicone rubber. [14-18] The large downside to these silicone lenses was their extreme hydrophobicity, preventing appropriate lens wetting in contact with the tear film, and leading to increased deposition of the hydrophobic components of the tear film such as lipids, eventually fouling the lenses. [17] For commercial applications for the simple correction of refractive error, the issues of discomfort would be enough to prevent silicone rubber lenses becoming commercially viable, and the use of silicone elastomers today is restricted to pediatric cases. [10]

The introduction of silicone hydrogels the late 1990s revolutionized the CL market by providing the high oxygen properties of silicone, while retaining the desirable handling and comfort characteristics of hydrogels by incorporating hydrophilic monomers with silicone containing materials. [19] The silicone within these new materials would provide the desired oxygen transmission, and the hydrophilic monomers would provide the water and ion permeability that are critical for lens comfort. [20] The hydrophobicity of the silicone within the lenses was combated by modifying the surface. Bausch & Lomb's lens, the Purevision (balafilcon A) went through a plasma oxidation process by putting the material in a plasma chamber, which partially transformed the surface silicone into wettable silicate. [19] As the entire surface was not converted into silicate, the surface was described as being composed of silicate

"islands", which when worn gave the effect of a continuous wettable surface. CIBA Vision's Focus NIGHT AND DAY (lotrafilcon A) material used a plasma coating process to put down an ultrathin wettable coating onto the lens surface. [19] The initial aim of these materials were overnight, continuous 30 day and night's wear. [11] The thinking was that with improved oxygen transport, complications with chronic hypoxia would be eliminated, and by eliminating the need and use of solutions, complication from preservatives would also be eliminated. Practitioner prescribing habits ultimately derailed attempts at eliminating solutions, as overnight, extended wear was not widely prescribed due to the increased risk of developing sight threatening infections with this type of wear modality. Other generations of silicone hydrogels followed. The Acuvue silicone hydrogel product range from VISTAKON, Acuvue OASYS (senofilcon A) and Acuvue Advance (galyfilcon A) utilized incorporation of an internal wetting agent, polyvinylpyrollidone, to improve the wettability of the surface, without modifying the surface in any way. [20] CooperVision's Biofinity line (comfilcon A) represents the third generation of silicone hydrogel technology. The comfilcon A material does not utilize any wetting agent or surface modification - rather the design of the silicone polymers incorporates a highly wettable moiety to improve comfort. [11] The aim of all of these developments were to improve patient comfort and acceptance. It was disappointing to clinicians that with the introduction of silicone hydrogels, and the significant improvement in the amount and severity of hypoxic complications, that the rate of CL drop-out did not significantly change. [21] Clearly, hypoxia was but one piece of the puzzle to CL discomfort. Contemporary materials will likely continue to utilize silicone for the superior oxygen transmission profiles, the challenge to the manufacturers will continue to be to improve the notion of CL "comfort" to prevent patient drop out.

### 1.2.3 Demographics of Contact Lens Wear

International surveys paint an interesting picture of CL wear and CL prescribing within the contemporary eye care market, with the results from the most recent worldwide surveys being detailed in publications headed by Morgan and Efron as part of the International Contact Lens Prescribing Consortium. The results of surveys being sent to practitioners worldwide for many consecutive years yield data in terms of wear modality, refractive errors being corrected and lens replacement frequency, and the general results are summarized in Table 1-1. It was found that there is a continual decrease in the prescribing of rigid CLs over soft lenses, with rigid gas permeable lenses accounting for only about 10% of all lens fits. [22] When prescribed, rigid lens patients tended to be older, male, and more likely to be fit with multifocal or bifocal lens designs. The worldwide distribution of rigid CL prescribing also varies greatly, from a high of 37% of all fits in Malaysia to only 0.2% of all fits in Lithuania. [22] Regardless of the worldwide distribution, the authors noted that the prevalence of rigid CLs has decreased over time, but there still exists a population of practitioners and patients who prefer this type of lens material.

The refractive condition corrected by CLs has also expanded greatly. Initial designs for CLs were merely for the correction of spherical refractive error, hyperopia and myopia, but designs to correct astigmatism and presbyopia are now also available and commonly prescribed. [23] The demographics for presbyopic CL wearers skews to significantly more females. The majority of presbyopic patients were not corrected with some form of presbyopic correction, as multifocals or monovision was only prescribed 37% of the time in presbyopic patients. [23] When presbyopic corrections were prescribed, three times more patients were fit with multifocal CLs versus monovision, highlighting the greater acceptance by practitioners and patients of multifocal contact lens designs. There was again spread of presbyopic prescribing seen

throughout the world, with a high of 79% of presbyopes fit with presbyopic CLs in Portugal, to a complete lack of any presbyopic CL prescriptions for patients in Singapore. [23] Correction of patients with astigmatism, in contrast to patients with presbyopia, has seen a gradual increase in the number of toric astigmatic CLs being prescribed. On the whole, patients being fit with toric lenses tended to be older and of male gender. [24] There was significantly less patients fit in daily disposable wear modalities for patients being fit with toric lenses. Overall, 25% of patients were fit with toric lenses. This figure still falls short of the presumed 45% of the population who are estimated to have more than 0.75D of astigmatism, but has been rising over the years that the survey has been performed. [24] Again, regional differences were found, with a high of 48% of patients in Portugal being fit with toric lenses, and a low of only 6% being fit in Russia. [24]

The wear modality and replacement frequency demographics that have been gleaned from these surveys also illustrate the efforts of the industry to shape the CL wearing experience. Extended wear, once thought to be the wear modality of the future, was only prescribed 7.8% of the time. [25] The trend for extended wear appears to be downward, with decreasing prevalence throughout the years measured by the survey after reaching a peak in 2006. [25] Patients who were prescribed extended wear tended to be male, older and were fit with silicone hydrogels. [25] The authors of this study suggest that it is likely that, given the established risk that extended wear has on the development of sight threatening microbial keratitis, patient and practitioners do not see the benefits outweighing the potential complications of extended wear. [25] Malaysia had the lowest rate of extended wear being prescribed, with only 0.6% of all fits using this modality, compared to the high of 27% extended wear seen in Norway. [25] Daily disposable CL wear, where a lens is worn once straight out of the packaging before being replaced the next day with a new lens, is another example of the ways in which the CL industry has attempted to regulate the wear

modality of the CL population. Daily disposable lens prescribing has been increasing over time, accounting for 24% of all fits by the end of the survey. [26] Daily disposable CL wearers tended to be older and of the male gender. Interestingly, there is a positive correlation between the proportion of CL fits being daily disposable and the country's gross domestic product, suggesting that the initial lens cost may be a factor in the ability of the lenses to be dispensed. [26] Nepal saw the lowest prescriptions for daily disposable lenses (0.6%), while 66% of patients in Qatar were fit with daily disposable lenses. [26]

Finally, even though silicone hydrogels are not being used extensively for extended wear applications, they have seen a continued increase in popularity for fits on a daily wear basis. The proportion of fits with silicone hydrogels have increased from 3-4% of all fits the year after they were released on to the market to approximately 36% today. [27] Silicone hydrogel prescribing is not significantly different between the two genders, or between the different age groups. Highlighting the perceived utility of silicone hydrogels in combating complications, a large proportion of silicone hydrogels were refits rather than initial fittings. Regionally, Australia had the highest penetration of silicone hydrogels at 65%, while delayed introduction into the Japanese market let to a relative low market penetration in that country (20%). [27]

| Studied Factor | % Proportion of All Fits | Demographics of contact lens population | Most often prescribed country (%) | Least often prescribed country (%) | Trend over time |
|---|---|---|---|---|---|
| Rigid Lenses | 10 % | - Older<br>- Male | Malaysia (37%) | Lithuania (0.2%) | Decreasing |
| Presbyopic Correction (multifocal or monovision) | 37 % (of all presbyopic patients) | - Female | Portugal (79%) | Singapore (0%) | Increasing |
| Toric Lenses | 25 % | - Older<br>- Male | Portugal (48%) | Russia (6%) | Not reported |
| Extended Wear | 7.8 % | - Older<br>- Male | Norway (27%) | Malaysia (0.6%) | Decreasing after peaking in 2006 |
| Daily Disposable | 24 % | - Older<br>- Male<br>- Affluent Country | Qatar (66%) | Nepal (0.6%) | Increasing |
| Silicone Hydrogel | 36% | - Refits | Australia (65%) | Japan (20%) | Increasing |

**Table 1-1 Demographics and Trends in Contact Lens Prescribing**

There has been considerable change in availability and options to prescribers of CL in terms of material (eg soft, rigid, silicone hydrogel), wear modality (eg non-replacement, monthly wear, daily wear, daily disposal, extended wear), and refractive conditions correctable by CLs (astigmatism, presbyopia) in the past 15 years. The interplay between the industry, eye care practitioners and patients will continue to dictate what types of lenses will have success and market share. [22-27]

### 1.2.4 CONTACT LENS MANUFACTURING

The materials used for CLs throughout history have centered around two man-made materials which have excellent light transmittance characteristics: Glass and polymers. Glass, which had a much longer history than polymers, was initially used in attempts to make CLs but proved to be very uncomfortable. [28] The work of Dallos in making eye impressions improved the comfort somewhat by providing a "negative" mould to which the lens could be formed, and continues to be useful for creating larger custom designed scleral lenses. [28] The first true revolution in CLs was in the introduction of polymers in the 1930s and 40s, and the techniques used in attempts to mould the material to the patient's eye shape.

Initial techniques in the forming of polymer CLs involved lathing, where a cylindrical or round piece of preformed material was mounted on an apparatus to grind and cut away the front and back surface to give a desired shape/refractive power. [29] In this application of lathe cutting, the manufacturers at the time were using knowledge gained from the manufacture of glass lenses for spectacle applications. The downside of this technique were that it was time consuming, and that, at least at the beginning, was dependent on the skill of the technician operating the lathe. Understandably, reproducibility of the lenses were an issue. [28] It also took some time to develop the proper skill to be able to lathe both the front and back surface. Lathe cut CLs still exist today, with the major change being the use of computer controlled lathes to greatly improve the precision, reproducibility and time needed to produce the lenses. These lenses are mainly used in specialty applications. [28]

The introduction of soft CL materials expanded the possibilities of manufacture. Spin casting was the initial method proposed by Wichterle in producing CLs from liquid solutions. [30] In spin casting, the liquid mixture to be polymerized is placed into a mould which forms the front

surface of the lens. The lens is forced to assume the contours of the mould by centripetal force as the mould is spun, and the speed of rotation is controlled to produce the desired posterior surface shape. [31] Cast CL materials are also possible with liquid polymerization mixtures. In cast moulding, the polymerization mixture is injected between two moulds which provide the anterior and posterior shape of the lens as the lens is polymerized. [28]

Regardless of spin or cast moulding, the manufacture of soft CLs is classified as a polymerization reaction. In polymerization reactions, long, repeating molecular chains are produced. When exposed to certain wavelengths of light, or raised to certain temperatures, special molecules within the polymerization mixture known as "initiators" will produce energetic unpaired electrons called free radicals. [32] These free radicals provide energy to facilitate the joining of molecules, and the process repeats and continues until the reaction mixture is exhausted of raw materials or free radical generation stops. During this time, the individual molecules are attached to the ever growing and elongating molecule, much like adding links on a chain. The end result of a polymerization reaction is the conversion of a reaction mixture of simple monomers into a long polymer chain. [32] In CLs, the main monomer used in the polymerization reaction is hydroxyl ethyl methacrylate (HEMA) (Figure 1-1a), which when elongated is termed poly(HEMA) or pHEMA (Figure 1-1b). To successfully form pHEMA, the elongated chains also require a crosslinker, a molecule to join these long repeating chains together so that a meshwork can be formed that has the ability to absorb water. The molecule most often used is EGDMA (ethylene glycol dimethacrylate) (Figure 1-1c). As can be seen by its structure, EGDMA contains two double bonds on either side, each of which can participate in the polymerization reaction and thus can serve to attach chains together. Modern silicone hydrogel

materials also incorporate into the mixture silicone containing monomers such as tris(trimethylsiloxy)silane.

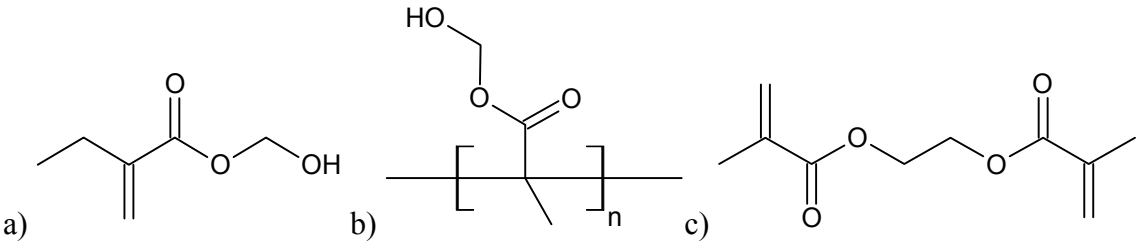



**Figure 1-1 Chemical Structure of Contact Lens Components**

Chemical structure of components commonly used in CLs. a) Hydroxyl ethyl methacrylate (HEMA), the common base material b) Structure of polymerized HEMA, pHEMA c) Ethylene Glycol Dimethacrylate (EGDMA), variants of which are commonly used as crosslinkers to join extending pHEMA chains together to form the meshwork of the CL.

Today, initiation of the free radical reaction is through UV light initiated photopolymerization. The initiators of reaction are photoinitiators which absorb particular wavelengths of light to produce the free radicals needed for the polymerization reaction. [32] The advantage of photoinitiated polymerization reactions are that large volumes of individual lenses can be polymerized at once, the reaction tends to occur very quickly, and the process can be started and stopped quickly by modifying how long the light is on. In comparison, heat initiated polymerization suffers from lower degrees of polymerization control, and longer rates of reaction, with the advantage of lower temperatures being reached in the reaction and longer polymer chains eventually being produced. [31]

## 1.3 PHARMACOLOGY

Pharmacology is the study of the interactions between drugs and the body. [33] Drugs can be man-made, endogenous or naturally occurring, but can be broadly defined as substances

which have some physiological effect on the body and are used to treat or diagnose a disease. [34] Much of the advances in modern medicine rely on the advances in our understanding of molecules and their effects on the body, which are elucidated by those in the pharmacological field. [34] The study of pharmacology is necessarily an interdisciplinary study, as elements of biology, medicine, molecular biology, organic chemistry, physics, physiology and biochemistry are all necessary to understand the role that drugs have on the body and disease.

### 1.3.1 PHARMACOKINETICS AND PHARMACODYNAMICS

Key to our understanding of pharmaceuticals and their effects when administered is the study of two fundamental concepts - pharmacokinetics and pharmacodynamics. [34] Pharmacokinetics studies how an administered agent is handled by the body, while pharmacodynamics is the study of the effect of the drug on the body. [35] Pharmacokinetics are generally studied in four main stages, which are commonly expressed through the acronym ADME - which represent the pharmacokinetic stages of Absorption, Distribution, Metabolism and Excretion/Elimination. [36] The study of pharmacokinetics is fundamentally the study of the passage of a drug through the body, and for the majority of drugs it is concerned with the concentration of a drug in the plasma or circulatory system. [34] Study of Absorption is intractably linked to the route of administration, or the method to which the drug is given to the body. There are many different ways in which a drug can be administered to the body, but most commonly, they include oral administration and intravenous injection. Other methods include sublingual administration, by inhalation, intramuscular and subcutaneous injections, rectal suppositories and topical applications. [37] Regardless of the route of administration, the goal is to deliver the drug to the body through the most efficient way possible to treat the specific disease. In general, a drug is expected to be absorbed faster if administered to areas with greater blood flow and with

greater surface areas. [35] The second step in the study of pharmacokinetics is the study of Distribution. An administered drug has potential to be distributed throughout the entire body through either the blood or lymphatic system. Even seemingly locally administered drugs, such as direct intramuscular injection or transdermal patches, are transported through the body. [37] Depending on the solubility characteristics of the drug, a drug may distribute preferentially to certain sites in the body, such as within the fatty tissue, or within the blood system. Metabolism occurs when the drug begins to be changed or modified by the body. The key player in the metabolism of drugs is the liver and the various cytochrome oxidase enzymes (also known as the cytochrome P450 series of enzymes) produced by the liver. [36] The goal of metabolism from the body's standpoint is to modify a foreign substance to allow for easier excretion from the body. The liver cytochrome enzymes primarily work in oxidative reactions to increase the polarity of molecules to allow easier excretion by the kidneys in the urine. Other reactions by the liver conjugate the drug with other molecules to create more stable, insoluble complexes, which can eventually be mixed with bile acids and excreted in the stool. [37]

The primary activity of the liver in metabolizing drugs coupled with the preferential passage of the blood from the gastrointestinal tract to the liver through the hepatic portal vein can pose a significant problem for drug administered orally. [35] As blood from the small intestine is passed through the liver before being circulated through the rest of the body, an orally administered drug that is metabolized by the liver may see a significant proportion inactivated before it can reach the systemic circulation and its site of action. This phenomenon is known as the hepatic first pass effect. [35] This limitation to oral bioavailability, or the amount of drug administered orally that reaches its target site of action unchanged, leads to investigations into alternative routes of administration for certain diseases. For example, in the treatment of angina

pectoris, a painful sensation in the chest and heart area due to cardiac ischemia, treatment involves dilation of the coronary blood vessels using nitroglycerin, which becomes nitric oxide, a potent vasodilator. Nitroglycerin is given underneath the tongue (sublingually) in part because the hepatic first pass effect would inactivate the majority of nitroglycerin administered orally. [33, 36] The final stage in the study of pharmacokinetics is Elimination/Excretion. Through the actions of metabolism, the administered drug is made to be excretable through the urine or the stool and is removed from the body. Though less common, drugs can also be excreted through other body secretions such as sweat or saliva, or through the lungs by exhalation. [33] Ultimately, phamacokinetics is the study of the effect that the body has on an administered drug. The impact of this knowledge guides the selection of a particular drug for a particular disease situation, and critically, the dosage and the dosing frequency needed to efficiently combat the disease of interest. [37]

In contrast to pharmacokinetics, the study of pharmacodynamics concerns the effect that a drug has on the body. The purpose of an administered drug is to have some sort of change in biological function, to affect some component of a disease process. [34] Central to our modern understanding of the molecular mechanism of drugs and their effects is the study of cell receptors and cells signaling. [34] The ability of a drug to lower blood pressure, to kill cancerous tumour cells or aid in sexual dysfunction ultimately come down to the ability of the drug or the by-products of the drug's metabolism to interact with cell receptors, whether they be on the surface of the cell or within, and initiate a cascade of cell signaling to affect a desired clinical outcome. Thus, for a drug to be effective, cells at the site of action require receptors that can interact with that drug, and interaction of that drug with those receptors must then initiate the desired outcome. [36] In the absence of those specific receptors in that part of the body, the drug

will have no effect. Consideration also has to be given to the distribution of these receptors in other places within the body, and the effect that the drug may have on those cells and those signaling processes, as these can lead to undesirable side effects. [35] For example, in the chemical treatment of cancer, chemotherapy drugs affect cancerous cells by selectively killing rapidly growing and dividing cells. Unfortunately, there are many cells within the body that are also rapidly growing and dividing through normal actions which are affected by chemotherapy treatment. This leads to the typical side effects of hair loss and nausea during cancer treatment, as the rapidly dividing hair follicle cells and epithelial cells of the intestine are destroyed as "collateral damage" to the destruction of cancerous cells. [36] It is also important to acknowledge the set of tools that administered drugs must work with. The ability of drugs to affect change within the body is limited by the abilities of the cells themselves – a drug cannot signal for cells to perform actions that they do not have the capacity to already perform. The search for new drugs in the treatment of disease thus becomes intertwined with our ability to understand the body's systems and cell processes.

## 1.3.2 OCULAR PHARMACOLOGY

### 1.3.2.1 OCULAR ANATOMY AND PHARMACOLOGY

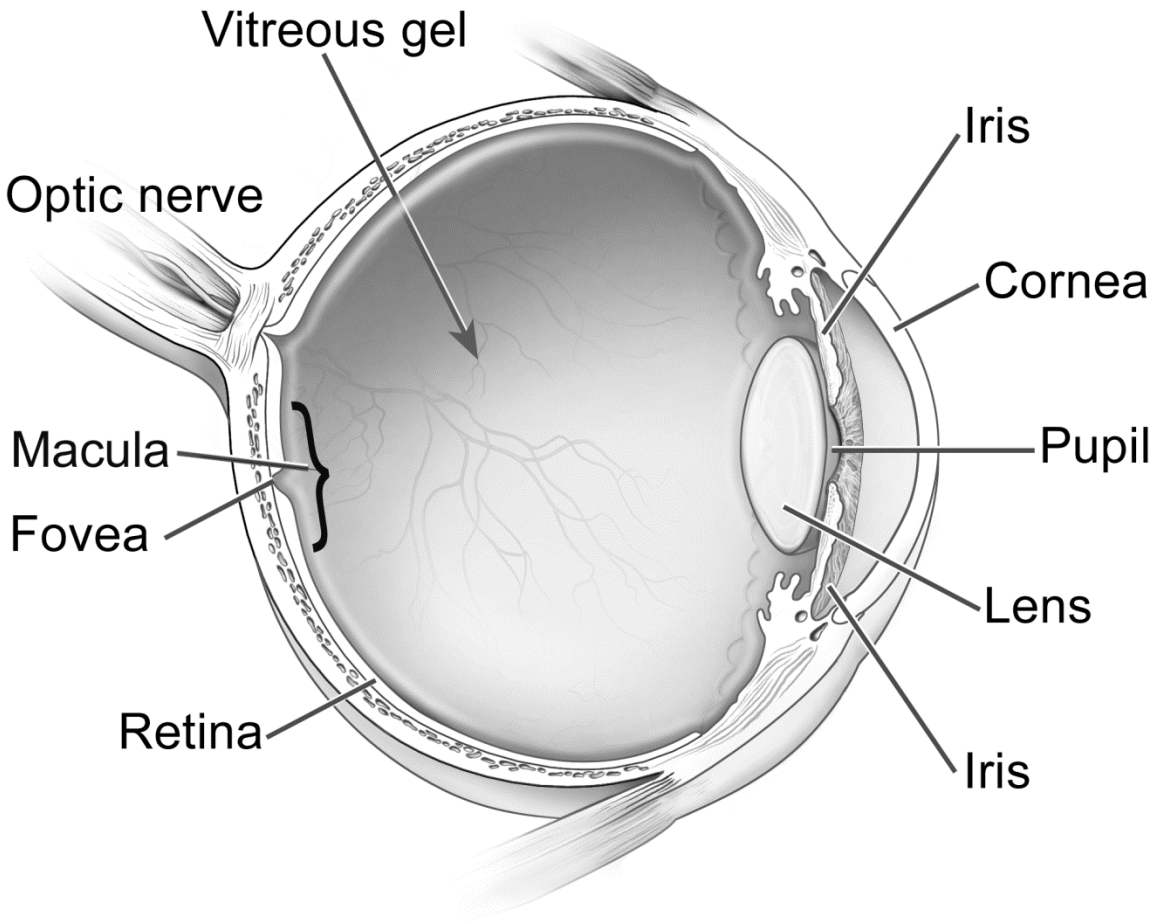



**Figure 1-2 Anatomical Structures of the Eye**

Figure courtesy of the National Eye Institute, National Institute of Health, Ref #NEA09

The key structures in the anatomy of the eye is detailed in Figure 1-2. The anatomy of the eye, from a pharmacological perspective, can be divided into the anterior and posterior segment, which is roughly defined as the areas anterior and posterior to the physiological lens. [38] The

reason for this segmentation is that it corresponds to the tissues that can reasonably be reached through topical application on the ocular surface (anterior segment), and what tissues require more invasive local treatment or systemic treatment (posterior segment). [39] The anterior segment of the eye is surrounded by the ocular orbit and ocular adnexa, consisting of the eyelids and eyelashes which cover the ocular surface when the eyes are closed. The most anterior structure of the eye proper is the clear cornea. The cornea consists mainly of an organized, avascular, hydrated collagen network bordered on either side by epithelium termed the stroma, which consists of 90% of the corneal thickness. [39, 40] On the anterior surface, the corneal epithelium is several layers thick and are held together with tight junctions which seal the surface as much as possible from the influx and efflux of water and other aqueous soluble substances. [39-41] On the posterior side, the cornea is covered with a single layer of specialized epithelium called the endothelium, whose primary function is to control the corneal hydration and thus transparency. [42, 43] The three section structure of the cornea has implications for topical drug delivery, as the two epithelial layers primarily serve as a barrier to hydrophilic molecules while allowing lipophilic molecules through, while the very hydrated stroma behaves exactly the opposite. [39, 44] The design of a drug to be delivered through the cornea thus requires both hydrophilic and hydrophobic characteristics, or have some sort of vehicle or strategy to penetrate these barriers. [45] Surrounding the cornea is the highly vascularised area known as the limbus, which is the site of the corneal epithelial stem cells, and the membranous, vascularised conjunctiva, which serves to aid in the lubrication of the eye through secretion of mucous. [46] Due to a higher surface area and blood supply, uptake of instilled molecules can be an order of magnitude larger for the conjunctiva when compared to the cornea. [40, 45] Uptake into the conjunctiva is not considered useful to the eye as absorbed molecules are quickly moved to the systemic circulations by the

blood vessels and lymphatics. [39] The sclera is a continuation of the cornea beyond the anterior portion of the eye. [46] In comparison to the clear cornea, because the collagen is not precisely organized, the sclera is completely white and opaque rather than being clear, but is extremely tough. [46] The entire anterior surface of the eye, cornea, conjunctiva and sclera are lubricated through the production of tears secreted by the lacrimal glands. The tears serve a host of diverse functions - lubrication, transport of nutrients, removal of waste, refraction of light and defense against pathogens. [47, 48]

Posterior to the cornea lies an area known as the anterior chamber. The anterior chamber is usually filled with a liquid termed the aqueous humour, a low protein fluid produced by the ciliary body. The aqueous humour serves several functions, including a mechanical function supporting the shape of the eye through its effect on intraocular pressure, providing nutrition and removal of waste for several energy intensive structures such as the endothelium and trabecular meshwork, and finally, as an optical component to aid in the proper focus of light rays as they traverse through the eye. [44, 48] The production of aqueous from the blood is highly controlled, involving active secretion of select components by the ciliary epithelium. This energy dependent, specific generation of aqueous from the blood differentiates aqueous from a mere blood filtrate. The separation from the aqueous and the blood is termed the "blood-aqueous" barrier (BAB). [38, 39] The highly vascular iris separates the anterior chamber from the posterior chamber. The iris primarily serves to control the amount of light that enters the eye by controlling the size of the pupil. [49] Posterior to the iris is the posterior chamber, which is also filled with aqueous humour and also contains the lens. The lens is one of the main refractive elements of the eye, and in conjunction with the ciliary body, is responsible for the ability of the eye to change its focal length and allow for the eye to focus from optical infinity and closer. [48, 50] The lens serves as the

de facto separation between the anterior and posterior segment of the eye pharmacologically because it is realistically the most posterior structure that treatments applied to the ocular surface (eye drops, ointments, etc) can possibly reach and have an effect. [39] All of the structures mentioned anterior to the lens can be targeted to some degree with topical therapy, with structures beyond the lens minimally affected by agents applied on the surface. [41, 46]

The space between the lens and retina is filled with the vitreous. The composition of the vitreous is similar to the cornea in that it is avascular, and composed mainly of water with a small amount of dissolved collagen. It also contains very few cells. [44] The vitreous has recently become an important target for the treatment of posterior segment disorders because agents can be safely injected into the vitreous to eventually spread to other posterior structures such as the retina which cannot be targeted directly without a high degree of risk. [51] The retina is nervous tissue adapted to the capture and processing of light. It consists of specialized cells to capture light (the rods and the cones) and networks of neural tissue (the bipolar, ganglion and amacrine cells) to provide rudimentary processing of light information before being sent from the eye to the central nervous system through the optic nerve. [44] The retina is a highly metabolic area of the body, and is fed by a highly vascularised area posterior to the retina known as the choroid, which is in the same anatomical layer in the eye as the ciliary body and iris, which all together form the uveal tract. [42, 46]

Like the central nervous system, the retina also has preferential and privileged blood flow. [52] Control of blood components transported to the retina is tightly controlled to protect these vital and sensitive tissues, and this preferential transport of blood components is termed the "blood retinal barrier" (BRB). [38, 39, 46] The BRB often prevents successful systemic treatment from affecting the retina, necessitating surgical intervention and its associated risks. [52]

Considering the anatomy of the eye, all of its barriers and mechanisms to remove unwanted molecules, the design and engineering of successful ophthalmic pharmaceuticals can be quite complex. As always, the ideal characteristics of a drug will be defined by the disease being treated, the location in which it is occurring, how the drug will be administered and the health status of the patient. All things being considered, the ideal drug would be able eradicate the disease in question quickly in a minimum number of doses and a minimum amount of side effects. For the vast number of treatments these ideals are not reached, and so a cost benefit analysis must be undertaken - weighing the cost of treatment in terms of economics and side effects against the potential benefit of eradicating the disease, or decreasing recovery time. [53] The eye is readily accessible to treatment, and thus the preference is local therapy to the eye only, to prevent the side effects associated with systemic therapy. [38, 52] The eye also has a very specific function in conveying visual information. Treatments should preserve this function as much as possible if they are to be successfully adopted by practitioners and patients alike. [43, 53] The frequency of dosing needs to also be considered. It is well known that patient compliance with treatment decreases as the number of drugs and the frequency that those drugs need to be taken increases, and thus a formulation which maximizes the therapeutic effect of treatment with a minimum number of instillations and drugs is preferred. [40, 45]

For the eye, the majority of the drug that has an effect is absorbed through the cornea. If a topical treatment is to penetrate past the ocular barriers, the molecule would be best served if it could traverse both hydrophobic and hydrophilic environments, as this would allow easy passage through the relatively hydrophobic corneal epithelial layers, while also passing through the hydrophilic stroma and final passage into the aqueous humour. [44, 52] Varied factors, from the size

of the molecule, the charge, shape and degree of ionization all have a potential effect on the ability of the molecule to pass through the cornea. [40, 43] Many molecules do not possess the ideal properties for corneal passage, and thus the drug concentration and dosing frequency are often modified to overcome the molecular shortcomings. Tear flow and tear drainage also serve as a significant barrier to efficient anterior drug transport. [54] The sudden increase in volume on the ocular surface by an instilled drop causes reflex tearing and blinking, which dilute and flush away the drug. Estimates of ocular residence time of an eye drop can be as short as only 3-5 minutes. [45] The immunopriviledged status of the eye through the incorporation of the two blood barriers (the BRB and BAB) limit the effectiveness of systemically applied therapy. [43, 52] Treatments intended for the eye which are given systemically through the oral or intravenous route face significant hurdles in trying to pass the BRB and BAB, as unless the molecule partitions preferentially into the nervous tissue, a very large dose would be needed to be administered systemically for the drug to force its way past these barriers. [55] For this reason, systemic treatment for strictly ocular disorders have uneven effectiveness, and are fraught with the potential to cause significant side effects due to the large doses often required. [38]

#### 1.3.2.3 Advantages and Disadvantages of Eye Drops and Ointments

The advantages of using eye drops and ointments for topical treatment of ocular disorders over other forms of treatment centre around the location and accessibility of the eye. [45] The anterior segment of the eye is readily accessible externally, and thus treatment with eye drops and ointments can directly target the eye and surrounding structures. [52] Frequent dosing can allow for relatively high concentrations of the drug to be reached within the ocular tissues fairly easily, as long as the patient is compliant. [40] By applying the treatment directly to the ocular tissues, the hepatic first pass effect can be bypassed compared to orally administered agents. [45]

The chief challenge to effective eye drop therapy are issues with patient compliance. Often, the dosing frequency may be too difficult to manage for patients with acute conditions, and during treatment of chronic conditions even once a day dosing can be very difficult to adhere to 100% of the time. [56, 57] Eye drops have also been shown to be a relatively inefficient means of delivering drugs to the eye. [49] Computer modeling and measurements estimate that only between 1-7% of the active ingredient within an eye drop is able to overcome all of the ocular barriers and exert a therapeutic effect within the eye. [45, 52, 58] This is clearly economically and therapeutically disadvantageous, as drugs that do not reach the site of action are wasted and unnecessary. [59] Current eye drops thus achieve appropriate concentration profiles by utilizing highly concentrated solutions instilled frequently. [59] The volume being instilled from a commercial eye drop bottle is also significantly larger, at 35-56 μL, than what can be reasonably accommodated by the ocular surface which has a theoretical maximum capacity of 30 μL, and routinely only has 7 μL of tears on it. [39, 45, 59] The excess fluid overflows out of the eye and spreads across the patient's cheek. Approximately 75% of the instilled drop which is retained by the ocular surface is removed by the nasolacrimal duct, with the remaining 25% being lost to the conjunctival vasculature. [46] The surface area of the conjunctiva is approximately 17 times larger than that of the cornea, limiting direct diffusion and absorption of the drop by the cornea. [45] The pharmacokinetics of eye drops are also not ideal. Eye drop pharmacokinetic profiles are characterized by pulsatile delivery, with only a short period of time within the therapeutic window, surrounded by periods of either overdose or underdose. [60] Finally, as mentioned previously, treatment of the eye through the anterior segment is also unable to affect the posterior segment. [38, 39]

Ophthalmic ointments partially alleviate some of the disadvantages of eye drops by significantly improving ocular residence time. [45] The ointment vehicle is often a petroleum or jelly-like substance with low water solubility, and thus when placed on the ocular surface is much more difficult to be flushed away through actions of the tears or blinking. [38] The major disadvantage of ophthalmic ointments is their unfortunate side effect of impairing vision. [45] The thick ointment causes a significant blockage of light transmission, such that they are generally only useful for overnight use when the visual function of the patient is not a primary concern. [59]

## 1.4 OPHTHALMIC DRUG DELIVERY SYSTEMS

The use of drug impregnated reservoirs for the treatment of ocular disorders has a long and ancient history. It has been recorded that the ancient Romans utilized honey soaked bandages as ophthalmic dressings, and there are some recordings of the Egyptians also utilizing drug soaked bandages for treatment of ocular infections. [59] In modern times, the interest and development of drug releasing devices for the eye continues in attempts to improve patient compliance and decrease treatment side effects.

### 1.4.1 ANTERIOR SEGMENT DRUG DELIVERY SYSTEMS

An overview of commercially available anterior segment drug delivery systems is summarized in Table 1-2. To date, the most widely known commercially available anterior segment ocular drug delivery system has been the Ocusert® pilocarpine insert, introduced to the USA market in 1974 by Alza. [61] The system consisted of a small disc containing two insoluble semipermeable membranes made of ethylene vinyl acetate (EVA) encasing the pilocarpine active ingredient. The system was designed to control intraocular pressure by precisely releasing pilocarpine over the course of 7 days. [40] By modulating the release of pilocarpine from the device in an almost linear fashion, rather than pulsatile delivery as is often seen in eye drops,

most of the significant side effects of pilocarpine treatment such as induced myopia and pupillary miosis were significantly reduced. [61] The system suffered from several significant disadvantages unfortunately, chief among them being device awareness leading to a foreign body sensation and patient discomfort. [61] The device was also difficult to insert and remove from the eye, and in some cases would also spontaneously eject. [46, 62] Even though the system was designed for 7 days, the recommendation was that these devices should not be worn for more than 12 hours, and as such required the patient to remove it themselves rather than an eye care provider as had been envisioned. [61] The system is no longer available, likely due to the evolution of glaucoma treatment away from pilocapine as a first, second or even third line agent in the treatment of that disease, in addition to the noted device shortcomings.

Other attempts through the 1990s to develop anterior segment devices had only minimal success. Collagen shields, thin dissolvable films formed from porcine sclera, are often used as a bandage for a damaged or scratched eye. Suggestions have been made that they could be used to concurrently deliver drugs, with the significant advantage that they eventually are completely dissolved and cleared from the eye in a relatively short amount of time without any additional intervention. [63] Investigations of these shields to release anti-glaucoma agents, antibiotics, anti-inflammatories and some combination drugs have been performed, but no commercially available product has yet to be released. [64-69] Rod shaped devices, designed to be improved versions of the discs used for the Ocusert® device have been investigated as they potentially have better retention properties within the conjunctival fornix. The Lacrisert® system by Merck uses such a rod, which is placed in the fornix to slowly release a moisturizing agent (hydroxypropyl cellulose) in the treatment of dry eye over the course of a single day. [41] Use of Lacrisert® in trials was shown to be preferred by patients when compared to four times a day

dosing with artificial tears. [41] The Minidisc Ophthalmic Therapeutic System by Bausch & Lomb was designed to mimic a CL, but to be fitted to the shape of the superior or inferior sclera, and in trials was investigated to release the antibiotics gentamicin and sulfisoxazole for over 100 hours, and was reported to be easier to insert and had better comfort than the fornix-located Lacrisert® device. Unfortunately, the device was never released to the market. [61] The failure of inserts as a whole likely stems from the lack of any real commercial traction seen by Ocusert®.

Advances to the design of eye drops have focused on the development of gels or mucoadhesive drops which increase residence time, or the application of penetration enhancers such as preservatives to aid in drug penetration, although these preparations are often limited by their blurring effects on vision or poor ocular tolerability. [55, 58] Durasite®, a propriety vehicle from InSite vision in California, is a polycarbophil vehicle, designed to specifically hydrogen bond with the ocular mucus and ocular epithelium. This greatly improves the residence time of the instilled drop. AzaSite, from Inspire Pharmaceuticals in North Carolina, was the first eye drop formulated with Durasite® to be released into the market. [46] With this formulation, the dosing schedule for the antibiotic is significantly reduced for cases of bacterial conjunctivitis, from the usual four times a day for most antibiotics, to only twice a day on the first two days, then only a single drop for a further five days. [70] The vehicle also appears to be well tolerated in patients. [46]

Given that such a large proportion of an instilled drop is drained through the nasolacrimal duct, thoughts on utilizing a punctal plug as a drug delivery system has been explored. Some evidence exists that in the treatment of glaucoma with eye drops, if the puncta is occluded it may lead to further decreases in the observed IOP. [71, 72] The next step has been to design the punctal plug to be the reservoir of the drug, which is slowly eroded by the tears, releasing the drug into

the tear film and onto the ocular surface. Phase II trials have shown that such devices releasing glaucoma drugs such as latanoprost and brimatoprost have good retention within the puncta, but their effectiveness at lowering IOP were not ideal. [46]A punctal plug to release antihistamines in the treatment of allergic conjunctivitis is also being examined, as well as one to release the antibiotic moxifloxacin. [46, 73] Punctal plugs have to be used with caution, as they may cause epiphora (excess tearing) if used in patients with normal tear production and blocked drainage, and may be difficult to remove if the patient is experiencing adverse effects from the drug.

A sustained drug delivery system for the anti-inflammatory dexamethasone has recently been investigated for use in the anterior chamber. Termed Surodex™ from Allergan in California, the implant is used after cataract surgery to control postoperative inflammation. The device is bioerodable, formed from poly lacto-co-glycolide (PLGA) and is designed to release dexamethasone for 7-10 days. Current clinical trials however have demonstrated that the device may have no better clinical outcome compared to topical anti-inflammatory therapy, so its usefulness has come into question. [42]

| Device Name | Manufacturer | Composition | Active Agent(s) | Time Frame | Indications | Design |
|---|---|---|---|---|---|---|
| Ocusert® | Alza Corp | Ethylene vinyl acetate | Pilocarpine | Up to 7 days, but recommendation is only 12 hours | Primary Open Angle Glaucoma | Small disc placed in inferior cul-de-sac |
| Lacrisert® | Merck | Hydroxypropyl cellulose | Hydroxylpropyl cellulose | 1 day | Dry Eye | Rod place in upper or lower fornix, biodegradable |
| Minidisc Ocular Therapeutic System | Bausch & Lomb | Polyhydroxymethyl methacrylate, proprietary monomers, hydroxypropyl cellulose | gentamicin and sulfisoxazole | 100-300 hours | Prophylaxis against bacterial infections | Miniature contact lens - designed to fit on sclera |
| AzaSite® | Inspire Pharmaceuticals | Durasite®, a proprietary polycarbophil vehicle | Azithromycin | 1 day | Bacterial Conjunctivitis | Mucoadhesive Eye Drop |
| Surodex™ | Allergan | Poly-lacto-co-glycolide (PLGA) | Dexamethasone | 7-10 Days | Postoperative Inflammation | Anterior Chamber Injection, biodegradable |

**Table 1-2 Summary of Commercially Developed Anterior Segment Drug Delivery Devices**

Anterior segment commercially developed drug delivery systems. Ocusert® is no longer commercially available, and the Minidisc Ocular Therapeutic System never reached the market. [40-42, 46, 61, 70]

### 1.4.2 POSTERIOR SEGMENT DRUG DELIVERY SYSTEMS

Commercially developed posterior segment sustained drug delivery devices are summarized in Table 1-3. In contrast to the anterior segment, the posterior segment of the eye has seen a greater interest in the development of sustained drug delivery devices. This interest likely stems from the inaccessibility of the posterior segment of the eye without some sort of surgical intervention, and the associated risks with repeated surgeries and injections. [53] In the modern treatment of posterior segment diseases, the trend has been toward multiple treatments/injection of agents into the back of the eye to treat diseases of an aging population such as age related macular degeneration, diabetic macular edema and chronic inflammation. Development of a sustained released device would have clear advantages, particularly with regards to decreased surgical complications and increased patient compliance by reducing the number of necessary visits, and several devices have been developed sufficiently to reach commercialization.[74]

The first commercially released posterior segment sustained drug release device was the Vitrasert® device designed for the sustained released of ganciclovir, an antiviral agent, in 1992. [53] Cytomegalovirus (CMV) infection of the retina is visually devastating and common in those who are immunocompromised, mainly due to the human immunodeficiency virus (HIV). A large and consistent dose of an antiviral is necessary to prevent infectious sight loss. The Vitrasert® device is a non-degrading implant that will release ganciclovir for more than 80 days, and in the Food and Drug Administration (FDA) approval studies demonstrated good control of CMV retinitis. [53] The device is relatively large and requires a sclerotomy to be implanted and the device sutured into place. [43] Since it is not degradable, a second surgery is needed to remove the device after it has been drained and to implant a second device if necessary. [75] The device

consists of the drug, and coats of EVA, which is a relatively impermeable barrier to aqueous fluids, and polyvinyl alcohol (PVA), which allows ganciclovir flow. [76] The device suffered from a relatively high (12%) complication rate from the implantation procedure, commonly causing undesirable outcomes such as cataracts, endophthalmitis or retinal detachment, which impacted visual acuity and visual function. [77] It is expected that earlier detection and treatment of CMV retinitis in at-risk HIV patients will decrease the prevalence of the disease and thus the need for the Vitrasert® device.

The Retisert® ophthalmic device is based on the Vitrasert® device and was developed by Bausch & Lomb in Rochester, New York. The device is designed to release the corticosteroid fluocinolone acetonide for approximately 1000 days. [78] It is composed of a silicone elastomer cup containing an orifice to allow drug diffusion. The orifice is covered by PVA to serve as an additional barrier, and the entire device is anchored through a suture tab to the sutured hole made during implantation. [79] Clinical trials for the device were initiated for the treatment of recurrent posterior uveitis and recurrent diabetic edema, and although benefit from the device was seen for both of these conditions, the rate of complications from the device were severe enough to limit application to recurrent uveitis only. [78] In a 34 week trial, the device reduced uveitis recurrence from 51% in the treated eyes to 6%, while the fellow, untreated eyes had recurrence jump from 20% to 42%. At this time, 50% of the patients required anti-ocular hypertensive medications, with 6% requiring more serious glaucoma filtering surgery. At 34 weeks, 10% of the patients required cataract surgery. [78] These trends continued as more time passed. 1 year post implantation saw a uveitis recurrence rate in treated eyes of only 5.4%, compared to 46% in the control eye. [38] 3 years post treatment there was a near universal need for cataract surgery. [80]

The Iluvien® (formerly Medidur®) device from Alimera Sciences, Atlanta, Georgia, attempts to alleviate some of the glaucoma complication rates seen with the Retisert® device. While also a fluocinolone releasing device, the size of the device is significantly smaller and rod shaped, allowing for significantly simpler implantation through injection with a proprietary 25 gauge needle, allowing for self-sealing of the wound without the need for stitches. [76] Unlike the previous devices, the device is freely floating within the vitreous after implantation. [81] The device implant location and steroid release profile is suspected to be contributory to the decreased rate of ocular hypertension and glaucoma seen with this device, as the need for IOP lowering intervention is seen with only 38% and 47% of patients with the low and high doses of the implant, compared to over 70% for the Retisert® device. [81] Accelerated cataract formation was still observed, with peaks at 6 to 18 months. The company is attempting to gain approval for the device application for treatment of recurrent diabetic macular edema, as well as other trials evaluating its efficacy in treating wet age-related macular degeneration, geographic atrophy and macular edema secondary to retinal vein occlusion. [82]

Ozurdex® is a degradable intravitreal implant designed for the release of dexamethasone. Composed of PLGA, the device is targeted for the treatment of persistent macular edema. [76] The device is administered through a specially designed injector directly into the vitreous cavity. [83] In a six month trial, two different dosing formulations of the device were able to improve patient's vision faster, to a greater degree and with less likelihood of significant vision loss. [84] 18% of treated patients achieved a greater than 15 letter improvement, deemed to be a significant visual improvement, versus only 6% of the sham treated controls. [84] The device did cause a significant IOP spike, but returned to normal and was no different to the sham treatment by the 180th day

post implantation. [84] The device is also approved for use in non-infectious uveitis, and is being investigated for its use in diabetic macular edema.

| Device Name | Manufacturer | Composition | Active Agent(s) | Time Frame | Indications | Design |
|---|---|---|---|---|---|---|
| Vitrasert® | Bausch & Lomb | Ethylene Vinyl Acetate | Ganciclovir | 80 Days | Cytomegalovirus Retinitis | Large tab, surgically implanted and sutured into place |
| Retisert® | Bausch & Lomb | Silicone Elastomer | Fluocinolone | 1000 Days | Recurrent Posterior Uveitis | Disc shaped implant surgically implanted and sutured into place |
| Iluvien® (formerly Medidur®) | Alimera Sciences | Polyvinyl Alcohol, Silicone | Fluocinolone | 18-30 Months | Recurrent Macular Edema | Small rod injected with proprietary needle |
| Ozurdex® (formerly Posurdex®) | Allergan | Poly lacto-co-glycolide | Dexamethasone | 180 Days | Macular edema secondary to retinal vein occlusion, recurrent uveitis | Small biodegradable rod injected with proprietary needle |

**Table 1-3 Summary of Commercially Developed Posterior Segment Drug Delivery Devices**

Posterior segment commercially developed posterior segment drug delivery devices. The Iluvien® system is currently in trials for treatment of various forms of age related macular degeneration, while Ozurdex® is currently seeking approval for treatment of diabetic macular edema. [38, 41, 54, 76, 78, 81, 84]

## 1.4.3 CONTACT LENS DRUG DELIVERY SYSTEMS

### 1.4.3.1 ADVANTAGES OF CONTACT LENS DRUG DELIVERY SYSTEMS

Use of CLs to serve as a drug reservoir for drug delivery to the anterior segment of the eye is not a new idea. Sedlacek first proposed the idea in 1965, and this was followed by further postulations in the 1970s by Gasset, Kaufman and Waltman. [85-88] The theory was that the CL could serve as a depot for the therapeutic agent, decreasing the number and frequency of administrations, and potentially increasing the drug's effectiveness by improving drug penetration or improved drug residence time. [89, 90] There are also several commercially available CLs (balafilcon A, lotrafilcon A, etafilcon A) which have a special designation by the FDA for "therapeutic use", that is, they can be employed in situations primarily to aid in the management of diseases or disease process within an individual, rather than for the correction of ametropia. The FDA categorizes "therapeutic use" of CLs into applications for pain relief, the promotion of wound healing (such as after a corneal abrasion), providing mechanical support (such as post traumatic injury), maintenance of corneal hydration (in severe dry eye cases) and drug delivery. [90] The use of lenses as a drug delivery device is underutilized, while the first four applications are routinely seen and used in clinical practice when indicated.

The main goals in investigating CLs as a drug delivery platform are to improve ocular bioavailability, improve patient compliance, decrease drug wastage and prevent undesirable side effects. [60] In this application, the CL can be loaded with the drug by soaking within a drug solution before lens insertion, or combined with eye drop instillation over the top of an already worn lens. There exist some evidence that simply by wearing a CL on the ocular surface during drop instillation increases drug penetration, with one study suggesting that diffusion into the cornea may be up to five times higher. [90] Indeed, mathematical modeling suggests that up to 50%

bioavailability from a drug releasing CL, versus the 1-7% seen with eye drops alone. [91, 92] CLs would thus provide an ideal platform for treatments which require a large amount of drug to enter into the anterior segment. Investigations into the concurrent use of CLs and antibacterials, anti-inflammatories, an anti-allergy eye drops found that they offered increase corneal and aqueous humour concentrations when compared to the use of CLs alone. [93-96] The increase in drug penetration and bioavailability would have a direct effect on the efficiency and rates of adverse reactions systemically, as more of the active agents reach the intended tissues. [91] The concentration of the solution used to load the lenses could also be decreased due to better bioavailability. [29] The effect on patient compliance is unknown, but is speculated to have a positive effect due to the dual nature of CLs in drug delivery applications. By coupling the delivery of needed medication to the eye, which can be easily ignored for many diseases which have little or no symptoms until the latter stages of the disease (such as glaucoma), to the correction of refractive error, which provides a real, immediate and tangible benefit to the patient, it is expected that patient compliance can readily be improved. [91] Simplification of drug treatment regimens have also been shown to improve patient compliance, which is particularly important if one wishes to prevent the development of bacterial resistance in the use of antibiotics. [60] CL manufacturing is also well established on a large, industrial scale, which would drive the unit cost of each one of these lenses down. [91] Finally, the biocompatibility and complications of CL wear are well known, and there is comfort in both prescribing eye care practitioners and patients alike in the use of CL technology. [89] Insertion and removal of CLs is relatively easy, ensuring that they will both be used appropriately, and can be withdrawn quickly if they are causing any complications. When surveyed, eye care practitioners have indicated that they would use a CL drug delivery device, should one be available. [97]

Ultimately, the overarching challenge of a CL drug delivery system will be demonstration that the system is able to improve patient outcomes, whether through improved drug delivery and bioavailability, improved recovery time, improved patient compliance, decreased adverse events or decreased economic burden. [91] Success will depend on correct identification of a disease or disease process which could conceivably be treated with a CL. As discussed previously, there is minimal impact within the posterior segment of the eye through topical treatment, and thus treatments of posterior segment diseases will unlikely to be targeted with such a device. The course of treatment needed for the disease must also be considered. For prolonged treatments, the drug delivery system must be sufficient to deliver an appropriate amount of the drug and for the appropriate duration of time, all in the absence of causing significant adverse reactions. [98] For example, if the system is designed for the chronic, indefinite treatment of glaucoma through the release of IOP lowering drugs, then consideration has to be made to the wear modality of the lens (daily wear or extended, overnight wear), the drug release kinetics needed to maintain target intraocular pressures, the total amount of drug needed to be released over the course of treatment, and whether the chosen lens material can be worn safely for the treatment period [98] Each of these factors is a significant engineering and development challenge that needs to be addressed through the course of development of the devices .

The focus of the majority of the research into CL drug delivery devices has understandably been on the drug release kinetics, as this poses the largest engineering challenge. However, addition of drug delivery properties, and all of the modifications that they entail, cannot adversely affect the significant and useful ocular properties that are necessary for successful CL wear. [60] The optical transmission, water content, ion transmission, oxygen

transport, lipid and protein deposition profile need to all be tightly maintained and regulated within a small range of acceptable values if the lenses are to be successfully worn by patients. [91] Establishment of the device's CL material properties are arguably as important as establishment of its drug delivery properties, as it is such a crucial element in the overall success of such a combination device that if any elements are missing it cannot go forward. Consideration has also to be made to the eventual manufacturing methods that the device will go through. If the process needed to manufacture these CL-drug delivery devices involve a significant change in method, raw materials or equipment then the economic feasibility of the device will be put into question by the CL manufacturers. [91] The active agent being investigated must also be compatible with CL manufacturing. Much of the polymerization of CLs begin with irradiation using light in the ultraviolet range. If the pharmaceutical being delivered is required to be part of the polymerization process then exposure to the UV light source, or any other procedure in the lens manufacturing, sterilization and storage, should not affect its pharmaceutical activity. [99]

Finally, the most critical component for these devices if they are to reach the market is consideration of patient and prescribing practitioner acceptance. The superiority, or at the very least, non-inferiority, of the CL drug delivery device can be demonstrated in the laboratory, in animal subjects and in clinical trials, but if the proposal is too radical or too far from contemporary clinical practice then the system will likely be met with resistance.

#### 1.4.3.3 Drug Delivery from Unmodified Commercial Contact Lens Materials

Initial studies on CL drug delivery focused on the use of unmodified commercial CL materials. The strategy with commercial materials would be to soak the lenses within a drug loading solution, allowing for drug uptake. Once worn, the lens would be able to serve as a depot of the drug as either a primary means of treatment or as an augment or supplement to eye drops.

This methodology would appeal to those in clinical practice, as the materials, namely CL samples, and the eye drop pharmaceuticals are often available in office separately, and thus could be quickly combined on an as-needed basis. There are significant advantages to using lenses which are already commercially available. They can be obtained easily on a large scale with consistent quality control checks built in by multinational corporations that need to comply with federal regulators, ensuring quality and consistency. The biocompatibility of the materials and the treatment drops would presumably also be a non-issue, as each individual component has already been tested for use in the eye, and thus testing for biocompatibility can focus on the safety of the combination rather than each component itself.

The first studies into drug soaked CLs mainly focused on the treatment of glaucoma or the release of prophylactic amounts of antibiotics from CLs in the 1970s. [100-105] In some of the early papers by Hillman, it was demonstrated that by soaking a CL in only 1% pilocarpine before application to the eye that IOP control was equivalent to that of "intensive" control using 4% pilocarpine drops for the treatment of glaucoma. [100] Experiments with volunteers wearing CLs soaked in the antibiotic gentamicin were shown to maintain antibacterial concentrations three days after lens insertion, with no toxic or adverse effects. [102]

More recently, the work on commercial lenses has moved from the clinic using human participants to the laboratory and *in vitro* models of drug release kinetics from the lenses. Lenses soaked in the pharmaceutical of interest would have their uptake and release elucidated through various laboratory based assays. Antibiotics (ciprofloxacin hydrochloride), anti-inflammatories (dexamethasone phosphate, ketorolac tromethamine), anti-allergy agents (ketotifen fumarate, cromolyn sodium) and surface rewetting agents (polyvinyl alcohol) have all been investigated from unmodified, off the shelf CLs. [106-110] From these studies, a few common conclusions can be

reached about release from commercial materials. First, the absolute amount of drug released from different commercial materials can vary substantially. On the whole, silicone hydrogels tend to release a lower amount of drug than hydrogel materials. [109] The water content of the lenses appears to have an effect, with the higher the water content the more drug possibly being released. [107] The total amount of drug loaded is however limited, as is the drug release times. [98] The vast majority of lenses tested *in vitro* do not demonstrate any sustained release characteristics, as the monitored drug concentrations very quickly reach their maximum and plateau for the rest of the experimental time. [107-109] The majority of lenses reach release plateaus within one to two hours. For the development of CL drug delivery devices, these unfavorable release kinetics is a major impediment for the applicability of commercial lenses as therapeutic, sustained drug releasing devices, and so alternative strategies were sought to improve the drug kinetics

#### 1.4.3.4 Drug Delivery from Vitamin E Coated Contact Lenses

Considering the poor release characteristics of commercially available lenses, but their advantages regarding availability and biocompatibility, researchers have looked into methodologies to modify commercial materials post-manufacture. The use of Vitamin E, an antioxidant, has been the most extensively studied for its ability to retard drug diffusivity and thus extend drug release times. By coating commercially available silicone hydrogels in Vitamin E, a transport barrier is formed that forces loaded drug molecules to travel a long and tortuous path to be released into solutions or the ocular surface. [111] The authors of this group have used this technique to demonstrate extended release *in vitro* of lenses loaded with antifungals (fluconazole), anti-glaucoma agents (timolol), anti-inflammatories (dexamethasone), anaesthetics (lidocaine) and immunomodulators (cyclosporine A). This approach has exhibited various levels

of success, with some of the modified lens and drug combinations showing *in vitro* release times of over 400 times higher than from unmodified lenses. [111-116] Addition of Vitamin E to the lenses decreased UVA and UVB light transmittance, and slightly decreased the oxygen permeability, but not to the extent that the authors were concerned with the possibility of the development of hypoxic complications. [111]

*In vivo* testing has been performed with lenses that have been modified using these protocols for the delivery of the IOP lowering drug timolol maleate. [116] In a head to head trial, the ability of timolol releasing CLs to control IOP was compared to the IOP using topical timolol drops. The experiment was performed in a species of beagle dogs who spontaneously develop glaucoma due to their consistently high IOPs. [116] Treatment with the extended release lenses were equal in their ability to reduce IOPs when compared to eye drops. The concentration of drug needed to be loaded into the lenses could also be reduced with little effect on the response, suggesting a greater bioavailability of the drug when given as a CL. [116]

#### 1.4.3.5 Drug Delivery from PLGA Drug Impregnated Films within contact Lenses

One of the drawbacks to regular CLs as drug delivery is the limited loading capacity of the lenses. The total amount of drug loaded into a lens is of less importance if the disease treated requires only a minimum amount of drug to be effective, or if the lens can be replaced with a high frequency. These two factors do not appear to be applicable when one considers the use of a lens to treat an active infection. In that case, not only would a drug delivery device require a large amount of drug to be released to combat fast replicating microorganisms, but also that this dose be sustained over long periods such as overnight to combat the infection as the patient sleeps. To solve these two problems simultaneously, researchers have devised a radical change in CL design. Rather than utilizing the CL material as a drug reservoir, a drug reservoir was formed

using PLGA, a polymer formed between lactic and glycolic acid, and previously discussed for its use in the posterior segment drug delivery devices such as Ozurdex®. [117] This PLGA film was loaded with a large amount of the antibiotic ciprofloxacin, and the polymerization of the pHEMA CL material is done in two steps. [117] The first step creates the bottom portion of the lens before the drug impregnated PLGA film is placed on to the surface, before more of the liquid mixture is placed overtop, and the polymerization mixture initiated again to seal the film in between the two pHEMA halves. Release studies *in vitro* were able to demonstrate continual release for 25 days, and the ciprofloxacin released retained antibacterial properties even after going through the lens polymerization process. [117] The authors have also demonstrated similar release kinetics and antimicrobial activity with a lens designed for the release of the antifungal agent econazole. [118] The limitation to the design of these lenses lie in the CL properties. The PLGA films do not transmit light in the visible spectrum, being opaque, white substances when loaded with the pharmaceuticals. Thus, if the film covers the entire area of the CL then vision would be impossible. The authors combat this problem by cutting a small, 3 mm pupil into the centre of the lens that would be used for viewing, but whether this would be sufficient to allow for both patient and prescribing practitioner acceptance is doubtful. The modifications also increase the size and thickness of the lens (450 μm thickness compared to regular CLs of 80-100 μm thickness), [111] which would decrease the oxygen transmission, adversely affecting the biocompatibility of the lens, and thus limit the device's commercial viability.

#### 1.4.3.6 Drug Delivery from Molecular Imprinted Contact Lenses

Molecular imprinting is a polymerization based strategy to increase the affinity of a molecule of interest to a polymer to slow down diffusion. [119] Originally, molecular imprinting was used in the field of chromatography to aid in the selective removal of particular molecules

from solutions. [119] This was accomplished by modifying the polymerization mixture. Prior to the initiation of polymerization, a template molecule of interest is also dissolved within the reaction mixture, as well as other small molecules which have been termed "functional monomers". The purpose of the functional monomers is to form non-covalent interactions with the template molecule within the polymerization solution so that after polymerization, shape specific and functional group specific areas are created within the material for the template. The interactions can be in the form of hydrogen bonding, ion pairing and dipole-dipole interactions. [119] These areas of recognition for the template have alternatively been termed as "biomimetic", "cavities" or "molecular memory". [120] Regardless, the effect of the molecular imprinting modification to the standard polymerization reaction is to increase the affinity of the template molecule to the material, and thus slow down the diffusion of the template from the material. [121] This would clearly be advantageous in terms of drug delivery applications, as this could prove to be useful in modifying or extending release times from polymerized molecules or membranes such as CLs.

Molecular imprinting is the most widely utilized strategy by different groups investigating CL drug delivery systems in attempts to modify release times.[121] Investigators have produced materials capable of releasing antibiotics (norfloxacin), anti-inflammatory (dexamethasone, prednisolone, diclofenac), anti-allergy (ketotifen fumarate), and anti-glaucoma (timolol, dorzolamide) treatments. [122-131] Through this body of work, several general conclusions about effective molecular imprinting CLs have emerged.

The first conclusion is that the nature of the polymers and functional monomer selection were crucial to sustained drug release characteristics. [132] Certain combinations of template (drug) molecules, CL monomers such as pHEMA or *N,N*-dimethylacrylamide (DMAA) and functional monomers such as methacrylic acid (MAA) are better at loading larger amounts of drug, or

releasing for longer periods of time. In one study, a threefold difference in measured release time was seen between the two extremes of the releasing lenses formed using different monomer compositions. [132]

The second critical insight was in the concentration of the crosslinker. The greater the amount of the crosslinker added to the hydrogel mixture, the greater the stiffness of the material and thus selectivity and specificity of the formed "molecular memory". [123] There exists, however, an upper limit to the amount of crosslinker that can be present if the material is to be formed into a comfortable CL, as well as a lower limit if the imprinting process is to be formed efficiently and effectively. [123] Through this work, there was an established minimal crosslinker concentration of 80 mM within the polymerization mixture to allow for efficient imprinting to occur. Above this, there was no significant change in the release coefficients of the hydrogels produced. [123]

The third key finding was the relationship between the amount of the functional monomer to the amount of the template within the polymerization mixture, [124] termed the Monomer to Template ratio (M:T). For most monomer-template combinations used for molecular imprinting, there exists an ideal M:T ratio that allows for the slowest diffusion times, and ratios above or below this ratio are less effective. The theory behind this phenomenon is that when there is a low M:T ratio, there exists very little monomer relative to the template to form complexes and the cavities needed. At M:T ratios that are too high, the fraction of monomers which are randomly distributed are high compared to those that are interacting with the template and thus molecular imprinting efficiency decreases. In the ideal ratio, the functional monomer surround the template molecules efficiently and this creates the molecular memory within the final polymerized product. Through a series of experiments with the antibiotic norfloxacin,

Alvarez-Lorenzo and colleagues were able to demonstrate that the ideal ratio of acrylic acid functional monomer to norfloxacin template was approximately 4:1. [124] Utilizing the ideal M:T ratio increased the loading capacity of the hydrogels and extended release times to 5 days, which was significantly improved over the less optimally imprinted materials, which differed little from non-imprinted controls. Differences between the lenses was also most readily seen when the lenses were loaded through soaking in the least concentrated solution, so that the bulk of the release characteristics could be dominated by the molecular imprinted cavities versus simply the bulk polymer or water content of the lens. [124] It should be noted however that the ideal M:T ratio will vary with both the choice of the monomer and the choice of the template.

In recent years, as other research groups have continued to investigate and expand our understanding of the process of molecular imprinting, other factors have been shown to have some effect on the ability to control drug release rates. Some groups have demonstrated that more than one functional monomer can be used simultaneously, and a combination of functional monomers can have a greater effect on extending the release than one functional monomer alone. [125] Sophisticated release experiments have also begun to evolve *in vitro*. To better mimic the tear production, flow and drainage that is actually seen on the eye, a sophisticated "microfluidic" device was engineered by the authors of one study. [126] With such a device, a loaded CL can be placed under "physiological" flow rates of saline or artificial tear fluid, and the amount of drug being released over time can be monitored in a closer approximation of what occurs on the eye. Use of such a device with molecular imprinted lenses showed that the modification allows for almost zero order/concentration independent release from the lenses for periods of several days, which offer significant improvements over the monitored controls. [126]

Several studies have also investigated the use of molecular imprinted lenses *in vivo*. A molecular imprinted lens for the delivery of ketotifen fumarate, a mast cell stabilizer and anti-histamine, was compared head to head with the instillation of topical drops. [133] The use of the CL led to a sustained therapeutic concentration on the ocular surface for more than 24 hours, while the eye drop concentration dropped off within a single hour, and a non-imprinted lens maintained drug residence on the ocular surface for only 3 hours. [133] Other studies have investigated imprinted CLs for the delivery of timolol, a beta blocker which is used in the treatment of glaucoma. Again, these lenses were applied to rabbits, and the concentration within the tear fluid of the drug was monitored over time. [134] Use of imprinted lenses improved the length of time that measurable concentrations could be found within the tear film 2 and 3 fold times more than non-imprinted lenses and drops respectively. [134]

The advantage of using a molecular imprinting technique to sustain drug delivery from a CL is that the factors that contribute to drug release are well known. Modification of the polymer compositions, functional monomer selection and concentration, concentration of the crosslinker and template can all be optimized to design the desired release characteristics. [123, 132, 134, 135] The downside to using molecular imprinting is that a new material is being created, and thus all of the relevant testing of appropriateness of the material to serve as a CL are also required. There are also limitations to the amount of drug that can be loaded onto the lens through this process, and as mentioned previously, the effect of the imprinting is seen more strongly when the materials are loaded with low concentrations of the drug. The drug molecules used as a template within the polymerization process may also have to be removed. If the drug is light or heat sensitive and does not survive the polymerization process intact, then it needs to be removed before being

reloaded. [136] It may also be desirable to remove the template so that a precise amount of drug can be loaded into the material to prevent overdose or toxicity.

## 1.5 BACTERIOLOGY

### 1.5.1 BACTERIAL ORGANISMS

Phylogenetically, a significant division in the diversity of organisms occurs according to cell structure and organization. Eukaryotic organisms, which include humans, animals, fungi, algae and protozoans, all consist of cells which contain membrane bound organelles as well as a nucleus which houses the organism's genetic code. [137] By enclosing organelles within membranes, specialized areas within the cell can perform specific functions such as energy production, DNA replication and transcription, or protein synthesis without interfering or affecting activities elsewhere within the cell. In contrast, prokaryotic organisms lack membrane bound organelles and more importantly, do not have a membrane bound nucleus. The genetic material of the cell is localized within a specialized area of the cell known as the nucleoid, but is not separated or protected from the activities occurring within the cell. [138] Prokaryotes consist of bacteria and archaea. They are generally smaller than eukaryotic cells, are invisible to the naked eye, and of less complexity due to their lack of organelles. However, although each individual bacterial cell may be small, they have the ability to replicate extremely quickly and are found in every possible environment on Earth. [139] Indeed, the combined biomass of all prokaryotes is estimated to significantly outweigh that of all plants and animals. [137] Bacteria have significant effect on the life cycle of living organisms on Earth. They can serve as a food source for other organisms and have an effect on agriculture and human food production. Symbiosis with some plant foods, such as legumes with bacteria, allow for nitrogen fixation, reducing the need for fertilizer. They are a significant part of the decomposition of matter, converting carbon, nitrogen,

phosphorous and other raw materials from deceased organisms into useful forms that can be absorbed by living organisms, and thus part of the life cycle and nutrient cycle of ecosystems, biomes and the planet as a whole. [137]

The study of bacteria is known as bacteriology. The key development in the study of bacterial organisms was the invention of the light microscope, which allowed for the first time small microscopic organisms to be seen by early pioneers such as Robert Hooke and Antoni van Leeuwenhoek. [140] The later seminal work of Louis Pasteur and Robert Koch served to reinforce the ubiquity of bacteria in our environment and establishment of the "Germ Theory" of disease based on Koch's postulates - namely that a pathogen can be isolated in pure culture from a diseased organism, that when introduced into a second, healthy, susceptible organism, the pure culture will again induce the disease, and that the organism can be re-isolated from the second organism again in pure culture. [141] Later, identification of a key feature in the classification of the bacteria, the presence or absence of a second phospholipid bilayer outside of the bacterial cell wall, led to classification of Gram positive or Gram negative based on the results of the procedure to produce the Gram stain. Gram positive organisms lack a second external phospholipid envelope and have a rather thick cell wall. Gram negative organisms have a significantly smaller cell wall and a second membrane surrounding the cell wall externally. [138]

Modern understanding of bacteriology has had a significant impact on medicine and the treatment of diseased individuals. Improvements in culturing has allowed for rapid isolation and identification of disease causing organisms, and thus an associated increase in the speed of identifying useful treatments. Knowledge of the ubiquity of microorganisms has changed surgical techniques to prevent post-operative infections. DNA sequencing and generation of bacterial phylogeny has identified the evolutionary history of organisms and thus given insight

into their host and pathogen relationship, and the evolutionary pressures that each organism has exerted on each other. [138]

### 1.5.2 ANTIBIOTICS AND ANTIBIOTIC THEORY

It could be argued that the most prevalent diseases of modern society - cancer, diabetes, cardiovascular disease - are all a by-product of the effect of the introduction of antibiotics. Prior to the discovery of antibiotics to combat infections, the majority of deaths by human beings were due to infectious disease. [142] With the significant decrease in infectious disease in the population, life expectancy has increased and allowed for the diseases of old age to begin to present within the population. Antibiotics are produced from one microorganism to prevent growth of another microorganism. [137] Key for the use of antibiotics in humans and other animals is their relative lack of significant side effects or impact on the diseased host. This lack of significant side effects is one of the main differentiations between antibiotics and other agents used to kill microorganisms, such as antiseptics or disinfectants, which often have detrimental effects on the host cells, in addition to bacterial cells. [137] The selectivity of antibiotics stems from the subtle or significant differences between bacterial and host cells. [141] Antibiotics target bacteria-specific areas such as the bacterial cell wall, the bacterial cell membrane, the bacterial protein synthesis pathway and the bacterial nutrient synthesis pathway.[138] The effectiveness of antibiotics also may depend on the form of the bacteria, planktonic, or free floating, and bacteria found as part of a bacterial community adhered to a surface in an extracellular matrix known as a biofilm.[143] The planktonic form of the bacteria are typically much more susceptible to antibiotics than bacteria found within biofilms. [143]

The majority of modern antibiotic agents are semi-synthetic derivatives of molecules found in nature, formed by a microorganism to combat the growth of another microorganism.

For example, Penicillin, which was first widely isolated and used in World War II to treat wounds and prevent sepsis, was discovered by Alexander Fleming in 1929, when one of his Petri dishes containing growing *Staphylococci* was contaminated by a spore of the fungi *Penicillium*, which create a zone of inhibition surrounding it. [144] This molecule was eventually isolated, purified and mass produced as penicillin, and derivates of the molecule continue to play a role today in the management of infections.

The discovery of new classes of antibiotics went through a significant period of growth following the discovery of penicillin, but in recent decades the rate of discovery has slowed considerably. This is concerning, as bacteria have begun to develop resistance to common antibacterial agents. Due to their very short generation time and vast numbers, mutations and recombinations within the genome of bacteria can easily be introduced, and these changes can be selected for by natural selection if they confer a survival advantage against such things as antibiotics. [145] Use of an antibiotic confers a significant selection pressure for these mutants, spurring selection for survival of a population of resistant mutants, if antibiotics are not given appropriately. [145] This fact, coupled with the unnecessary overprescribing of antibiotics to the population at large, has led to a decrease in effectiveness of common antibiotics, and worryingly, selection of strains of common bacteria resistant to multiple antibiotics at once. The problem is not only limited to the administration of antibiotics to humans, as the vast majority of antibiotics are now being administered to agricultural animals, which eventually work their way up the food chain. [146] Bacteria are also able to share genetic information through horizontal gene transfer, likely spreading the resistance throughout the bacterial community. [145] Older, less effective or agents with more side effects are increasingly being used to control these resistant infections, and with the lack of any clear cut new antibiotics being discovered leads to worrisome trends in

future medicine if infection control cannot be achieved. There were already penicillin resistant strains of bacteria identified before the commercial release of the antibiotic. [146] More than 1000 different genetic mutations have been discovered in bacteria which confer penicillin resistance, exemplifying the numerous avenues that bacteria can become resistant to antibiotics. [146] The worry is that medicine may be entering into the "post-antibiotic" era, where a clear lack of effective antibiotic agents to prevent and treat infections may have a massive impact on the way modern medicine is practiced. [146]

Antibiotic agents can be classified based on their effects on bacterial cells. Bacteriostatic agents do not kill the bacteria outright. Rather, they prevent adequate or efficient cell replication, allowing for the host immune system to clean up the infection. Bacteriocidal agents directly kill the cell by interfering with some type of critical cellular process. Finally, bacteriolytic agents are also bacteriocidal, and kill bacteria by inducing cell lysis. [137]

### 1.5.3 Ocular Antibiotics and Mechanism of Action

A summary of commonly used ocular antibiotics is listed in Table 1-4.

#### 1.5.3.1 Beta Lactams and Cephalosporins

The beta lactams were the first antibiotic agents to gain widespread use, and are named based on the presence of a characteristics chemical structure containing a "beta lactam" ring. Beta lactams include the penicillins and the cephalosporins. Their mechanism of action is to serve as an ineffective building block of bacterial cell wall synthesis and repair. [147] The bacterial cell wall is used to provide structural rigidity against osmotic stress. The cell wall is constantly undergoing remodeling and repair. The structure of the beta lactams is such that it can be incorporated into a growing bacterial cell wall, but lacks the correct moieties to allow for crosslinking of cell wall chains, destabilizing the structure of the cell wall and eventually leading

to cellular lysis. [147] Common examples include penicillin G, amoxicillin, cefazolin, and cefalexin. Their spectrum of activity depends on the generation being used. Early generations were generally effective against Gram positive organisms, while later iterations had extended activity against a greater number of Gram negative organisms, leading to the classification of "extended" spectrum of activity. [144] Clinically, what has been shown for the beta lactams is that the shape of the concentration-time curve has the most effect on the ability of the drug to kill bacteria. For the beta lactams, what is most important in their ability to kill microorganisms is the amount of time that the drug is maintained at concentrations above the minimum inhibitory concentration (MIC). Increasing the concentration to levels beyond the MIC has only marginal effects. [148] The main methods of resistance to the beta lactams is through the production of beta lactamases, enzymes which cleave the beta lactam ring and prevent incorporation of the molecules into the growing bacterial cell wall.

The ocular use of the beta lactams is mainly during severe infections. Fortified cephazolin, a cephalosporin antibiotic, is often used in the mix for the topical treatment of microbial keratitis. Oral amoxicillin can be used for the treatment of eyelid infections such as internal hordeolums, and injections of penicillin can be used for cases of endopthalmitis. [149]

#### 1.5.3.2 AMINOGLYCOSIDES

The aminoglycosides are one of the most frequently used ophthalmic antibiotics and were considered to be the drug class of choice for ocular infections before the introduction of the fluoroquinolones. They are generally not used systemically because they have a high rate of both nephrotoxicity and ototoxicity. [144] They are bacteriocidal, and their mechanism of action is blockage of bacterial protein synthesis through binding of the bacterial 30S ribosome subunit. [147] The rate of bacterial killing is concentration dependent. [148] They are derived from the

*Streptomyces* species of bacteria. They are generally useful against Gram negative organisms, but are considered to be broad spectrum because of their moderate effectiveness against Gram positive organisms. [144, 150] Examples of aminoglycosides include streptomycin, gentamicin, neomycin and tobramycin. Mechanisms of resistance to the aminoglycosides include mutations in the 30S ribosomal subunit, and production of enzymes which destroy the drugs. [144]

The ocular indications of aminoglycosides vary from mild conjunctivitis to sight threatening microbial keratitis. [151] Often, in the treatment of microbial keratitis, fortified and compounded tobramycin will be administered alongside the beta lactam cefazolin, to ensure the broadest Gram negative and Gram positive coverage. [152, 153] Some ocular preparations of aminoglycosides suffer from poor patient tolerance. Neomycin has a particularly high rate of hypersensitivity reaction (15-30%) when given for long periods, limiting their usefulness. [153] Tobramycin is commonly used as a combination drop with the steroid dexamethasone.

#### 1.5.3.3 MACROLIDES

The macrolides are a series of antibiotics which were also discovered from various members of the *Streptomyces* family. Their mechanism of bacterial inhibition is through inhibition of protein synthesis through binding of the 50S ribosomal subunit. [144] When used clinically, it is considered to have a bacteriostatic effect. [150] They generally are effective against Gram positive organisms. Examples of macrolides include erythromycin, clarithromycin and azithromycin. Resistance to macrolides is through methylation of the ribosomal subunit, which prevents macrolide binding, and unfortunately, resistance to one macrolide confers resistance against the entire class. [144]

Ophthalmically, erythromycin ointments are commonly used in neonatal conjunctivitis because of its favorable pediatric safety profile. More recently, new formulations of

azithromycin in proprietary vehicles has been shown to be an effective treatment for bacterial conjunctivitis, with a significantly simplified dosing schedule due to improved ocular retention and bioavailability conferred by the mucoadhsive vehicle. [154] The clinical effect of the macrolides is not necessarily concentration dependent. They exhibit a property known as a "post antibiotic effect", in that exposed bacteria to the antibiotic which haven't been killed are much slower to grow after the antibiotic has been completely removed than non-treated bacteria. This likely stems to the injury that the antibiotic has incurred on the bacteria. [148]

#### 1.5.3.4 SULPHONAMIDES

The sulphonamides are a group of molecules which are competitive inhibitors of the molecule p-aminobenzoic acid (PABA). PABA is used in the folic acid synthesis pathway that is critical in the creation of nucleic acids for DNA replication and synthesis in bacterial cells. [144] The sulphonamides compete with PABA to bind with the bacterial enzymes and thus prevent bacterial growth. A related molecule, trimethoprim, inhibits a second enzyme further downstream in the folic acid synthesis pathway to the sulphonamides, leading to synergistic activity when both agents are administered simultaneously.[144] The sulphonamides and trimethoprim are considered to have a bacteriostatic effect individually on bacteria, but when combined they have a bacteriocidal effect. [150] The sulphonamides were the first antibiotics discovered by chemists working on investigating dyes that could inhibit bacterial growth. [144] They are limited in their use through the development of allergy in patients. Resistance to the sulpha drugs is due to overproduction of PABA, changes to the binding affinity for the enzymes in folic acid synthesis and changes in drug transport. [144]

In the eye, trimethoprim is often combined with the basic polypeptide detergent Polymyxin B. The combination of the two is termed "Polytrim" and is effective against Gram positive (trimethoprim) and Gram negative (Polymyxin B) organisms. [150]

#### 1.5.3.5 TETRACYCLINES

The tetracyclines are bacteriostatic agents derived from the soil bacterium *Streptomyces aureofaciens*.[155] They specifically work by inhibiting protein synthesis through binding of the 30S segment of the bacterial ribosome. [156] They were one of the first antibiotics to be labeled as truly "broad spectrum" as they were able to affect the growth of a both gram positive and gram negative organisms. Examples include tetracycline, doxycycline and minocycline. Resistance is conferred through changes in the drug transport into the bacterial cell. [144] There is some concerns about their use in pediatrics, as the drug will deposit in growing bones and teeth, leading to discoloration. [147]

Ophthalmic use of tetracyclines has previously centered on the treatment of the ocular manifestations of Chlamydia in both neonates and adults, but recently the discovery of the large host of anti-inflammatory activity of these molecules has led to renewed interest in using them for such diseases as blepharitis, meibomian gland dysfunction and acne rosacea. [155]

#### 1.5.3.6 FLUOROQUINOLONES

The fluoroquinolones (FQ) are bacteriocidal and are the newest generation of antibiotics, and were released only in the 1980s. They are unique in that they are completely synthetic and not derived from a microorganism source, as they were discovered as a by-product of industrial chloroquine synthesis. [157] Their mechanism of action is inhibition of bacterial DNA replication, specifically through inhibition of the enzyme DNA Gyrase, as well as, in later iterations, Topoisomerase IV, enzymes which are involved in cutting and unwinding of bacterial DNA

strands to allow for efficient replication. [158] Common examples include ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin, gatifloxacin and besifloxacin. The newest generation of agents show broad spectrum activity, but earlier examples were mainly effective against Gram negative organisms. [157] Their rate of bacterial killing is concentration dependent. The more times concentrations are reached above the minimum inhibitory concentration, the faster the bacteria will be eradicated. [148] The main method of bacterial resistance against the FQs is mutation of either of DNA Gyrase or Topoisomerase IV enzymes, as well as the generation of active efflux pumps which remove the molecules from within the cells into the external environment. [157]

The FQs are some of the most frequently used antibiotics in ophthalmology, because of their broad spectrum of use and their availability as ophthalmic preparations. Some FQs (such as gatifloxacin) are only available for ophthalmic use due to systemic side effects. [157] They are indicated for use most commonly to treat bacterial conjunctivitis, while a few (such as ciprofloxacin and ofloxacin) are also approved for the treatment of bacterial keratitis. [159] While they do not have the indication to treat bacterial keratitis, many of the newer agents are used to treat microbial keratitis off-label. [160] The newer generation of FQs such as moxifloxacin and gatifloxacin have an enhanced spectrum of activity compared to older products such as ciprofloxacin, and are thought to suffer less likelihood of resistance development due to their dual targeting activities. Mutations to both enzymes are required simultaneously to prevent effectiveness of the agents. [159, 161]

| Antibiotic Class | Mechanism of Action | Isolated from | Effect on Bacteria | Spectrum of Activity | Common Uses | Ophthalmic Example |
|---|---|---|---|---|---|---|
| Beta Lactams and Cephalosporins | Inhibition of cell wall synthesis | Fungi - *Pennicilium* and *Acremonium* | Bacteriolytic | Gram positive or Extended Spectrum | Septicemia | Oral amoxicillin for internal hordeolums |
| Aminoglycosides | Protein Synthesis (30S Ribosome Binding) | Bacteria - *Stremtomyces* | Bacteriocidal | Mainly Gram Negative | Endocarditis | Fortified gentamicin for treatment of corneal ulcers |
| Macrolides | Protein Synthesis (50S Ribosome binding) | Bacteria - *Streptomyces* | Bacteriostatic | Gram Positive | Respiratory Tract Infection | Oral azithromycin for Chlamydia conjunctivitis |
| Sulphonamides/ Trimethoprim | Inhibition of Folic Acid Metabolism | Synthetic Dyes | Bacteriostatic (individually) Bacteriocidal (combination) | Mainly Gram Positive | Seborrheic Dermatitis | Polymyxin B/ Trimethroprim solution for pediatric conjunctivitis |
| Tetracyclines | Protein Synthesis (50S Ribosome Binding) | Bacteria - *Streptomyces* | Bacteriostatic | Broad Spectrum | Treatment of Lyme disease | Acne Roasacea Blepharitis |
| Fluoroquinolones | Inhibition of DNA replication (DNA Gyrase and Topoisomerase binding) | Synthetic - by-product of chloroquine production | Bacteriocidal | Early examples Gram positive, Later examples Broad Spectrum | Urinary tract infections | Moxifloxacin drops for surgical prophylaxis |

**Table 1-4 Characteristics of Ophthalmic Antibiotics**
The antibiotics listed all have some use in the treatment of ocular infections. [144, 150, 155-157, 159, 162, 163]

### 1.5.4 MINIMUM INHIBITORY CONCENTRATIONS (MIC)

The translation between the laboratory testing of antibiotic agents to the bedside when the antibiotics are used clinically is difficult. [164] In a controlled setting such as within a laboratory, where pure cultures of bacteria can be grown under ideal conditions with plentiful food and nutrients, the overall effect of an antibiotic on the growth of the bacteria can be clearly demonstrated. Unfortunately, the conditions within the laboratory do not mimic the conditions seen when an infection is raging within a living organism, and thus results from testing within the laboratory have to be scrutinized carefully. [165] Still, results from the laboratory testing of antibiotics can be useful in the setting of antibiotic concentration goals within the body during treatment. [165, 166]

A central tenet in testing of antibiotics within the laboratory is the concept of the Minimum Inhibitory Concentration (MIC). The MIC is the lowest concentration of the antibiotic within solution that will prevent the growth of bacteria, and is used to determine the potential effectiveness of an antibiotic in treating the infection caused by the organism. [165, 167, 168] A related concept is that of the Minimum Bacteriocidal Concentration (MBC), which is the minimum concentration of a bacteriocidal antibiotic to completely kill the bacteria. In general, the MBC will be at a higher concentration than the MIC, but the MIC is the measure that is generally discussed clinically. MIC testing can be performed in a variety of ways, but the two most common laboratory tests are the agar gradient diffusion and microbroth dilution methods. [167] In the agar disk diffusion method, also known as the E-test method, a strip which contains a continuous gradient of an antibiotic is placed on an agar plate which has been seeded with a lawn of bacteria and incubated at an appropriate temperature. Over time, the bacteria grow over the

entire surface of the plate except in areas surrounding the test strip that have high enough antibiotic concentrations to inhibit their growth. [165] The pattern created is an ellipse of no bacterial growth surrounded by complete bacterial growth, and the MIC is determined at the intersection of the bacteria growth and the test strip. [168] The broth microdilution method utilizes a 96 well microplate. Each well contains Mueller Hinton Broth growth medium and $5x10^4$ colony forming units (CFU) of bacteria. The antibiotic is added to each well in two fold dilution steps, and chosen to cover the range of antibiotic concentrations typically found within the plasma or serum when given to a patient using a typical dosage. [167]After incubation for 18-24 hours, the wells are checked for turbidity and the MIC determined to be the lowest concentration of antibiotic that prevents bacterial replication. [167] The antibiotic dilutions and broth preparation can be done manually in house, or there are a number of commercially available kits which contain dried antibiotic and growth media, and simply require addition of the bacterial solution. [168]

Ultimately, the goal of MIC testing in a clinical setting is to determine the causative organism's susceptibility to a panel of antibiotics, and recommendation of which is the best antibiotic to be used in treatment. Translation of the MIC results obtained from the laboratory to clinical recommendations is not clear cut or as simple as it may appear on first glance. Initial attempts in the 1970s tried to set interpretive "breakpoints" found within MIC testing. The hope was to differentiate between "susceptible" and "resistant" isolates of the bacteria to the antibiotic, through the basis of what was the numerical value of the MIC. [166] The usefulness of these categories was debatable, as they did not take into account patient and bacterial variables that are inherent in all treatments. There is a variation in the susceptibility of the bacteria within the wild type population to antibiotics. There is variation in the blood serum concentration of the

antibiotic when given to a healthy volunteer versus to an infected individual based on differences in the pharmacokinetics and pharmacodynamics within the population and within the health status of the patient. [164] There is also variation in the effectiveness of different antibiotics on the growth of bacterium based on certain parameters. For example, the effectiveness of penicillins within the body at eradicating bacteria is a function of the size of the area underneath the curve (AUC) of a time versus plasma penicillin concentration plot. [148, 164] Thus, it is not necessarily the concentration of penicillin that is achieved within the bloodstream that is important, but also the length of time that the penicillin is administered and found within the bloodstream. Other antibiotic effectiveness is measured in how the peak concentration within the plasma compare to the MIC, and others, how much time are MIC concentration levels reached and maintained. [164] Clearly, there is a significant amount of variation to be found in 1) the concentrations of the antibiotic that can kill a bacterium within the laboratory, 2) the peak concentration of the antibiotic found within the blood stream and 3) the time that the antibiotic is found within the bloodstream, and recommendations of treatment for infections clinically need to take all of these variations into account. [164]

There are two major panels which provide these recommendations, the Clinical and Laboratory Standards Institute (CLSI) in the USA, and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). [169] EUCAST has a publically available website where breakpoints, both laboratory and clinical, are presented and available for consideration when interpreting MIC results from routine laboratory testing. [164, 169]

## 1.6 MICROBIAL KERATITIS

### 1.6.1 OVERVIEW – CLINICAL DIAGNOSIS, SIGNS AND SYMPTOMS

A microbial keratitis (MK) is an infection of the cornea by replicating microorganisms. The cornea can be infected by a variety of different organisms, such as fungi or protozoans, but for the vast majority the infections are caused by bacterial organisms and so they will be the focus of the discussion with respect to MK. A patient is diagnosed with presumed MK if they present with a break in the corneal epithelium/ulceration overlying a corneal infiltrate. [170] As MK is an ocular emergency, diagnosis of presumed MK is sufficient to begin initiating treatment before culture and susceptibility (C&S) testing is completed, as any delay in the administration of antibiotic agents will have a negative impact on the overall outcome. [171, 172] Other clinical signs are discharge, hyperemia and an anterior chamber reaction. A patient with MK will present with complaints of severe pain and discomfort, light sensitivity, discharge and variable decrease in vision (from 20/20 to no light perception). [172, 173] Without culturing, the ability of eye care practitioners to correctly distinguish between the different types of causative organisms in MK based on clinical presentation alone is limited. [174]

### 1.6.2 DEMOGRAPHICS AND RISK FACTORS

The risk factors for MK can vary by geography and by climate. [173, 175] Contemporary epidemiological studies of the risk factors of MK have identified CL wear as a significant risk factor. In certain studies, the proportion of patients who present to tertiary referral centres for MK associated with some form of CL wear approaches 50%, with other risk factors such as ocular trauma or history of keratoplasty significantly less frequent. [173] Several CL behaviors have been identified as increasing the risk of MK, including overnight wear of CLs, reusing CL

solutions, poor hygiene associated with CL wear, swimming with CLs, internet supply of CLs and poor compliance with lens replacement schedules. [171, 176, 177] The annualized incidence of MK in daily CL wear is between 2.7 and 6.4/10 000, based on several independent studies in Australia, Scotland, the USA, Holland and Hong Kong, with the risk of those in extended wear increasing their risk broadly 10 times, with an annualized incidence rate of 21/10 000. [178, 179] Given the prominence of CL wear as a modifiable risk factor for MK, several studies have been developed to identify the wear modalities with the greatest risk. Overnight wear of soft CLs, regardless of the type of lens being worn, continues to be a significant risk factor, increasing the relative risk over planned replacement lenses to 5.4 times higher. [180] Unfortunately, "modern" CL wear modalities, namely high oxygen transmitting soft silicone hydrogel materials and daily disposable wear modalities did not significantly decrease the risk of developing MK in certain studies. [180] In other studies, daily disposable CL wear was associated with the lowest incidence of MK in soft CL wearers, while silicone hydrogel wear was associated with higher incidences of MK when compared to other daily wear CL wear modalities. [179]

Non CL related risk factors include male gender, younger age, smoking status, ocular surface disease, history of ocular trauma or ocular inflammation and depressed immune system. [171, 177, 181-187] The epidemiology of MK is extremely different in the developing world. The rates of MK in the developing world are estimated to be 30-70 times more frequent than in the developed world. [172] In the developing world, the major risk factor is ocular trauma. The poor socioeconomic status of much of the developing world also puts a large proportion of patients there at risk. [172] There has also been an identification of a bimodal age distribution in cases of MK, with a cluster in the younger age groups who tend to wear CLs (who are also at a greater

risk for ocular trauma), and a cluster in the older age group, who have the significant risk factor of ocular surface disease. [173]

### 1.6.3 Causative Organisms in Microbial Keratitis

Identification of causative organisms during the course of MK often depends on the timing through which a corneal scrape or sample was taken, and the susceptibility of the causative organism to the initial empirical therapy. If the organism is susceptible to initial therapy, then delayed corneal scraping may yield no organisms. [174] The strategy when bacterial MK is suspected is to use three to four different media (chocolate agar, blood agar, thioglycolate broth and brain heart infusion broth) to allow identification of the organism. In general, it is expected that the rate of capturing a positive culture is approximately only 50%. [170]

Discerning between Gram positive and Gram negative organisms for cases of MK have shown some mixed data. In tertiary referral centres, where the MK can be due to any number of risk factors including trauma and CL wear, the data shows that the vast majority of the infections are caused by Gram positive organisms, of which they are mainly identified as coagulase negative *Staphylococcus*. [173, 175] When only the CL wearing population with MK is examined, the causative organisms tend to be Gram negative, and mainly identified as being *Pseudomonas aeruginosa*. [171, 173]

### 1.6.4 Treatment, Management and Prognosis

It is recommended that all cases of MK be sent for C&S testing, should initial empirical therapy fail. This requires a corneal scrape to be sent to the laboratory for analysis. The recommended treatment for cases of presumed MK before results of C&S testing are returned, is frequent dosing with a topical antibiotic. [188] The choice of which topical antibiotic often depends

on the risk factors of the patient, but for most practitioners preference is shown towards fortified antibiotics compounded by a hospital pharmacy, including tobramycin, gentamicin, vancomycin and cephazolin in some combination to provide broad spectrum gram positive and gram negative coverage. [173] Commercially, only the fluoroquinolones ciprofloxacin, ofloxacin and levofloxacin have been approved by the United States Food and Drug Administration for treatment of MK as commercially available monotherapy. [189] Newer and more advanced antibiotics have been approved for bacterial conjunctivitis only, and usage of those for treatment of MK is off-label. The treatment of MK with fluoroquinolone monotherapy is controversial. [190, 191] The fluoroquinolones have been shown in trials to have a broad spectrum of activity effective at eradicating microorganisms implicated in MK, and lack the majority of the corneal toxicity seen with prolonged fortified antibiotic therapy. However, concerns about developing resistance to the fluoroquinolones give pause to practitioners considering monotherapy. Trials have demonstrated the equivalence between fluoroquinolone monotherapy and fortified antibiotic therapy. [192, 193]

Recommended dosage of the antibiotic is quite frequent. Upon diagnosis, dosages given every 15-30 minutes are common in attempts to quickly saturate the cornea to high levels of the antibiotic, and this frequency of administration may be continued for 36 hours or more in severe cases. [172, 175] Continued monitoring of the patient is necessary to chart improvement and progress, with acknowledgement that the eye may not appear to be improving due to the large number of drops being instilled, as well as manipulations related to taking corneal scrapings. Modification of the dosing or type of agent used occurs when there is an improvement in the clinical signs and symptoms, or results from the C&S testing return with indicated susceptibilities. Improvement of the MK is seen as a decrease in the size of the infiltrate, healing

and reepithelialisation of the corneal surface, reduction in the anterior chamber reaction and symptomatic improvement in the level of pain experienced. Hospitalization to manage these conditions occur frequently, with one study reporting an average stay in hospital of 9 days. [173, 188] Hospitalization is frequently necessary because of the frequency of the dosing schedule and the likely need to take the antibiotics around the clock, which can prove to be difficult, if not impossible, on an outpatient basis. Hospitalization is strongly recommended in all MK cases involving pediatric, monocular or non-compliant patients.[173, 188]

The long term outcome of the disease depends on the underlying pathogen and the condition of the cornea. In one study, while the majority of the cases were cured by treatment, only a slight majority (60%) of the patients had improvements to their visual acuity compared to their admittance baseline acuity. One patient in twenty also had an extremely poor visual outcome. Several patients in that study had severe complications, including endophthalmitis, posterior synechiae, and ocular hypertony. As a result, some patients required penetrating keratoplasty and for some enucleation was necessary. [173]

### 1.6.5 Pathogenesis and Contact Lenses and Microbial Keratitis

Issues at hand for the CL industry and the fear of developing a MK are compliance, wear modality, hypoxia and bacterial organism. Exposure to microorganisms is not sufficient to cause an infection, as the ocular surface without CL wear is constantly being exposed to bacteria and yet it rarely becomes infected. [194] Compliance is not a magic bullet, as even the best care system used for CLs does not completely disinfect the lenses themselves. The efforts to improve compliance may also be in vain. [194] Further understanding is needed of the link between extended wear and MK. The understanding of the pathogenesis of MK in overnight wear centres

on a change in virulence of the causative organisms, purportedly due to development of biofilms on the posterior surface of the CL, in combination with a cornea under stress and less able to up regulate defence mechanisms. [195] Hypoxia is an interesting topic because of the introduction of silicone hydrogels. As previously mentioned, while the rates of hypoxic related complications decreased with the introduction of these lenses, the rates of MK stayed the same. Some data suggest that the severity of the disease in these patients with MK is decreased compared to other wear modalities, but the rate of disease being similar highlights our continued lack of a complete understanding of the link between hypoxia and MK, if any. [196] Finally, questions abound as to why *Pseudomonas aeruginosa*, a common commensal bacteria found on the skin and in water, is the most commonly identified organism in MK cases involving CL wear. [197] Current thinking is that *P. aeruginosa* is able to exploit the CL wearing patient due to a large genome encoding virulence and survival factors. Coupled with the decrease in frequency of the blink response in patients wearing CLs provides for longer residence and contact time between the microorganism and the surface it wishes to invade. [194] The organism has also been shown to have significantly better lens adherence properties than other bacteria. [198]

### 1.6.6 Animal Models of Microbial Keratitis

The main animals that have been studied as models for MK are rabbits, mice and guinea pigs. The use of rabbits as a model for MK has had a long history because of desirable ocular characteristics, chiefly the similarity in size to the human eye (13 mm rabbit corneal diameter versus 11 mm corneal diameter in humans). [199] They are also much easier to handle with ophthalmic devices designed for human eyes when compared to a mouse or a guinea pig, and can be fitted with commercially available CLs. [200] Commonly, New Zealand White rabbits, a non-

pigmented rabbit strain is used, but pigmented strains of rabbit such as Dutch Belted rabbits have also been used. [199] *Pseudomonas aeruginosa* and *Staphylococcus aureus* are commonly the two organisms used to infect the corneas. Reproducible models of *S. aureus* MK typically involve intrastromal injections of the bacterial solution. [201, 202] *P. aeruginosa* MK models are, in contrast, much more amenable to alternative methods of infection. Classically, infection of the cornea was induced through passing of a silk thread that had been soaking in a *P. aeruginosa* solution into the corneal stroma. [199] Intrastromal injection models are still very viable, but infections have also been shown to be possible through topical application of bacteria after a corneal scratch or epithelial debridement, or application of a bacterial contaminated CL. [203-208] Rabbit models of MK are used to investigate the efficacy of new or developing treatments and therapeutic agents, as well as to elucidate different mechanisms of disease. Rabbits can be used as models for CL induced MK, but this often requires the aforementioned extreme experimental measures to ensure infection. The rate of development of MK with animal models under various established CL risk factors, such as overnight, extended wear, or poorly maintained CLs, remain surprisingly understudied. The drawbacks to using rabbits as *in vivo* models of MK are their relatively higher cost when compared to mice, and the corresponding increased operator time due to their larger size. [200]

Mice, in comparison to rabbits, have significantly smaller eyes and are more difficult to assess using clinical tools designed for human sized eyes. Their advantage lies in the wealth of resources behind mice-based animal research. Mutated or inbred strains containing certain genes, or knockout mice with certain genes removed are readily available and thus can be used to study contributory factors to MK pathogenesis. [199] In general, the mouse model of infection typically

involves a corneal scratch before introducing the bacteria onto the ocular surface. [200] Mice are also typically used to investigate the mechanisms of MK pathogenesis, by investigating both host and microorganism related factors in diseased versus non-diseased eyes. [199]

Guinea pigs have been used as a model of MK since 1975, with the guinea pigs being inoculated through intrastromal injection for use in antibiotic quantification studies. [209] There have also been some studies that have used specially designed CLs to fit the guinea pig eye in modeling CL related MK or inflammation. [210] Guinea pigs were seen as an advantageous alternative to using rabbits because they are smaller, easier to handle, eat less and tend to not bite or scratch. [209] The model of MK in guinea pigs has also been deemed to be highly reproducible. [199]

There are several disadvantages to the use of animal models for studies of MK, and mainly these stem from differences in anatomy, behaviour and genetic diversity. The majority of laboratory animals are inbred, and so results from those studies may have limitations in their applicability on the human population as a whole. [199] As previously mentioned, the size of the eye in a rabbit is quite close to that of a human, but it is still slightly larger, and the cornea of the mouse and guinea pig are significantly smaller. [199] Rabbits also have the presence of a "third" eyelid, the nictitating membrane which moves horizontally across the eye, that is absent in humans. [199] Finally, the tear film composition is markedly different between the three animals, with different levels and amounts of antibacterial proteins such as lysozyme. [211] The blink interval also varies widely, from a short 2-6 seconds in humans to over 30 seconds for rabbits. [199] For CL MK research, the rates of MK development with extended wear of CLs are not well established. Regardless, even with all of these limitations, the advancements gleaned from the

results of experiments of animals in the study of MK have been substantial in advancing our understanding of the disease, and in improving prevention, diagnosis, treatment and prognosis.

# CHAPTER 2 - OBJECTIVES AND RATIONALE

There has been a significant growth in the ocular pharmaceutical market over the past 10 years. As the population demographics of North America shift towards the older ages, age-related diseases of the eye are expected to increase in prevalence. [1] While there are many different avenues and modalities of treatment for ocular disease, there exists substantial room for improving drug delivery for both acute and chronic diseases. [2] The mainstay treatments for the anterior segment of the eye (eye drops and ointments), have several limitations that make them inefficient and ineffective vehicles of pharmaceuticals to the eye for treatment. [3] The majority of the active agents within an eye drop are drained away quickly from the eye, leading to wastage and potential systemic side effects. [4] To be effective, eye drops thus require both an increase in concentration and dosing frequency, which are hampered by cost and patient compliance. Even with optimal dosing, the reality is that the pharmacological effect of using eye drops provides only narrow windows of time wherein the drug is at therapeutic concentrations, interspaced between times of over and under dosing. [2] Investigations into alternative means to deliver drugs to the eye are thus warranted in attempts to combat these economical, efficiency and therapeutic challenges.

The eye is ideally suited to investigate the potential of long term drug delivery devices, because its relative accessibility and immune privileged status allow for relatively isolated, targeted therapy. [5] To date, there have been several commercially available sustained drug delivery systems for the eye, but their clinical impact has been minimal as they have not been widely used. [6] Given the success of contact lenses, both in terms of commercialization as well as biocompatibility, the potential for utilizing them as a platform for sustained drug delivery to the

anterior segment of the eye holds great promise. There have been some experiments which have demonstrated that simply soaking an off-the-shelf lens within a drug solution before application to the eye can have some benefit in ocular residence time, but systematic investigations of contact lenses as drug delivery devices are lacking. [7-10]

Microbial Keratitis (MK) represents a true ocular emergency, representing an infection of the cornea by replicating microorganisms, and requires frequent dosing of an antibiotic to the surface of the eye if remaining normal sight is to be protected. [11, 12] The frequency of drops during episodes of MK can be so frequent as to require hospitalization to ensure adherence to therapy. [13] Patients often require treatment at all times, even while sleeping. Thus, if a suitable sustained release system for an antibiotic could be found, potentially patient compliance and clinical outcomes could be vastly improved in the management of MK. The improvement in patient compliance is particularly significant in this age of developing antibiotic resistance, as any improvements to patient compliance can stem the growth of resistant microorganisms.

The purpose of this thesis was to investigate the potential for contact lenses as a means of delivery of the antibiotic ciprofloxacin. With this goal in mind, Chapter 3 begins with investigations into the potential of several commercially available contact lens materials for their ability to uptake and release the antibiotic ciprofloxacin *in vitro*. Nine different contact lenses were surveyed, and the concentration of ciprofloxacin within the solutions investigated through fluorescence spectrophotometry.

Chapter 4 details the first attempts to manufacture model contact lens materials for the express purpose of sustained release of ciprofloxacin. The model materials were generated using a molecular imprinting strategy, in the hopes of modifying their release characteristics. The

effect of different functional monomers, concentrations of functional monomers, and ratios of the functional monomer to the template ciprofloxacin were investigated for their ability to affect ciprofloxacin release and release times *in vitro*. Material properties such as the dry weight, water content and centre thickness of these materials were also evaluated.

The final project (Chapter 5) utilized the results from projects detailed in Chapters 3 and 4 to design actual contact lenses which can sustain the release of ciprofloxacin. The methods needed to be used to create sustained ciprofloxacin releasing contact lenses, as well as their material properties such as water content, surface wettability and light transmission were elucidated. Given the success at extending the release times of these materials, they were tested for their ability to control bacterial growth *in vitro*, as well as within an *in vivo* rabbit model of MK.

In the next chapter, the ability of commercially available CLs to uptake and release the antibiotic ciprofloxacin will be measured over time using fluorescence spectrophotometry, and comparisons between the different lens types will be made.

# CHAPTER *3* – UPTAKE AND RELEASE OF CIPROFLOXACIN-HCL FROM CONVENTIONAL AND SILICONE HYDROGEL CONTACT LENS MATERIALS

Alex Hui[1], Adrienne Boone[1] PhD, Lyndon Jones[1] PhD

[1]Centre for Contact Lens Research, School of Optometry and Vision Science, University of Waterloo, Waterloo, Ontario, Canada

This chapter has been published as follows:

Hui A, Boone A, Jones L. Uptake and release of ciprofloxacin-HCl from conventional and silicone hydrogel contact lens materials. *Eye & Contact Lens* 2008;34:266.



| Author | Concept/Design | Acquisition of Data | Analysis | Write Up/ Publication |
|---|---|---|---|---|
| Hui | Y | Y | Y | Y |
| Boone | Y | | | Y |
| Jones | Y | | | Y |

## 3.1 OVERVIEW

### 3.1.1 OBJECTIVES

To investigate the uptake and release characteristics of the antibiotic Ciprofloxacin-HCl in conventional and silicone hydrogel lenses, and evaluate their potential as therapeutic drug delivery devices.

### 3.1.2 METHODS

Nine differing soft contact lens materials were soaked in a 0.3% Ciprofloxacin-HCl solution at 34º. The uptake of the drug into the lenses was measured by the change in concentration over 24 hours using fluorescence spectrophotometry. The lenses were then placed in a buffered saline solution, and the release of the drug from the lenses was also measured using spectrophotometry.

### 3.1.3 RESULTS

The release of drug varied from 0.016 ± 0.004 mg/lens for lotrafilcon A lenses to 0.42 ± 0.03 mg/lens for etafilcon A lenses, with an average of 0.133 mg/lens. The three conventional lenses used in the study released a statistically significantly different amount of drug when compared to the silicone hydrogels. The release of drug was very rapid, with drug release reaching a plateau after no more than ten minutes for the majority of the lenses. The majority of the lenses were able to release enough drug to achieve $MIC_{90}$ for most resistant ocular pathogens. Ciprofloxacin was found to heavily precipitate on the etafilcon A lenses during the release phase at physiological pH.

### 3.1.4 CONCLUSIONS

Whilst balafilcon A released the most drug from the SH materials, all materials released the drug too quickly to be effective as drug delivery devices.

## 3.2 INTRODUCTION

Patients who develop a severe ocular infection of the outer surface of the eye require frequent dosing of an antibiotic to resolve this potentially sight-threatening complication. The dosing regimens for the antibiotics can be very frequent. As an example, for a microbial ulcer, a dosing regimen of a drop of antibiotic every fifteen minutes is common, at least in the early stages, and thus many patients may require hospitalization to comply with the dosing regimen. [1] Therefore, development of a slow-release ocular drug delivery device could potentially ease the amount of labor required during treatment of ocular infections and may provide benefits for both the patient and the clinician in charge of the medical management. The use of contact lenses as drug delivery devices was proposed as early as 1965, [2] but complications with long-term, overnight wear of older, conventional contact lenses reduces the desirability of these lenses as drug delivery devices for a number of reasons. Most contact lenses based on polyHEMA do not transmit sufficient oxygen to the cornea to allow for normal metabolic activity during sleep, [3] resulting in edema and inflammatory reactions of the cornea, [4] decreasing both the healing process and patient comfort. The introduction of highly oxygen permeable soft contact lenses (also known as "silicone hydrogels", "siloxane hydrogels" or "high Dk soft lenses) [5, 6] has revolutionized the way in which clinicians can use contact lenses as drug delivery devices, as these lenses transmit substantially more oxygen that conventional, older materials. [7-9]

These new silicone hydrogel lenses would thus be ideal candidates for use as extended wear, drug delivery devices. However, the kinetics of delivery of drugs using contact lenses is still only poorly understood and few publications have addressed this issue with modern lens

materials. [10, 11] While some information is available on the uptake and release of certain agents, [10-12] newer, second-generation silicone hydrogel lens materials have yet to be evaluated.

Ciprofloxacin is a second generation fluoroquinolone antibiotic with a wide range of activity against gram negative and gram positive bacteria, [13, 14] and is very commonly applied topically as an eye drop after corneal abrasions or in the treatment of microbial keratitis, conjunctivitis or endophthalmitis. [15, 16] It preferentially inhibits bacterial DNA Gyrase [17] and has low solubility at physiological pH due to the presence of aromatic ring structures, and has increased solubility in acidic or basic mediums due to the presence of ionizable functional groups. [18] A notable side effect of treatment with ciprofloxacin treatment is the formation of white corneal precipitates after prolonged use, which may delay epithelial healing. [19]

The current study examined the time course for the uptake and release of Ciprofloxacin-HCl in six commercially available silicone hydrogel contact lenses - balafilcon A (PureVision, Bausch & Lomb, Rochester, NY), comfilcon A (Biofinity, CooperVision, Scottsville, NY), galyfilcon A (Acuvue Advance, Johnson & Johnson, Jacksonville, FL), lotrafilcon A (Night and Day, CIBA Vision, Duluth, GA), lotrafilcon B ($O_2$Optix, CIBA Vision, Duluth, GA), and senofilcon A (Acuvue OASYS, Johnson & Johnson, Jacksonville, FL) and three "old generation", conventional soft contact lenses - alphafilcon A (SofLens 66, Bausch & Lomb, Rochester, NY), etafilcon A (Acuvue2, Johnson & Johnson, Jacksonville, FL) and polymacon (SofLens 38, Bausch & Lomb, Rochester, NY), to evaluate their usefulness as drug delivery devices.

## 3.3 MATERIALS AND METHODS

### 3.3.1 PREPARATION OF DRUG SOLUTIONS

Ophthalmic ciprofloxacin solutions typically have a concentration of 0.3% (w/v). A 0.3% (w/v) Ciprofloxacin-HCl (LKT Laboratories Inc, St.Paul, Minnesota) solution was prepared in Unisol®4 saline solution (Alcon, Fort-Worth, Texas) and the pH was adjusted with HCl to pH 4.0. A pH 4.0 solution was chosen as it allowed for complete solubility of the drug into solution at 0.3% (3 mg/mL). [18] At physiological pH, ciprofloxacin only has a solubility of 0.09 mg/mL. [18] The drug is light sensitive, and thus experiments and storage were performed in light minimizing amber glassware.

### 3.3.2 SPECTROGRAPHIC ANALYSIS OF CIPROFLOXACIN-HCL

The absorbance and emission spectra of the Ciprofloxacin-HCl solution was determined using a Hitachi F-4500 Fluorescence Spectrophotometer (Hitachi Ltd, Tokyo, Japan). Ciprofloxacin-HCl was determined to have a maximum excitation wavelength of 274 nm, resulting in a maximum emission at 419 nm and thus these were the conditions chosen for study.

### 3.3.3 DETERMINATION OF CIPROFLOXACIN-HCL CONCENTRATION – PREPARATION OF THE STANDARD CURVE

Samples of the 0.3% Ciprofloxacin-HCl solution were diluted to a range of concentrations from 0.0001 mg/mL to 0.0014 mg/mL, to create a linear standard curve to be used as a reference to correlate absorbance readings to Ciprofloxacin-HCl concentrations in solution. The generated curves typically had a correlation coefficient above 99.5%.

### 3.3.4 LENSES USED

Six silicone hydrogel lenses (balafilcon A, comfilcon A, galyfilcon A, lotrafilcon A, lotrafilcon B, senofilcon A) and three conventional lenses (alphafilcon A, etafilcon A and polymacon) were used. All lenses (except for comfilcon A) were -3.00 D prescription, obtained from the manufacturer in their original packaging solution. The comfilcon A lenses were of -2.50 D prescription, due to limited power availability at the time of the study. Table 3-1 and Table 3-2 contain detailed lens properties.

| | Night & Day | $O_2$OPTIX | PureVision | Acuvue OASYS | Acuvue Advance | Biofinity |
|---|---|---|---|---|---|---|
| United States adopted name | lotrafilcon A | lotrafilcon B | balafilcon A | senofilcon A | galyfilcon A | comfilcon A |
| Manufacturer | CIBA Vision | CIBA Vision | Bausch & Lomb | Johnson & Johnson | Johnson & Johnson | CooperVision |
| Center thickness (@ –3.00 D) mm | 0.08 | 0.08 | 0.09 | 0.07 | 0.07 | 0.08 |
| Water content (%) | 24 | 33 | 36 | 38 | 47 | 48 |
| Oxygen permeability ($\times 10^{-11}$) | 140 | 110 | 91 | 103 | 60 | 128 |
| Oxygen transmissibility ($\times 10^{-9}$) | 175 | 138 | 101 | 147 | 86 | 160 |
| Surface treatment | 25 nm plasma coating with high refractive index | 25 nm plasma coating with high refractive index | Plasma oxidation process | No surface treatment. Internal wetting agent (PVP) throughout the matrix that also coats the surface | No surface treatment. Internal wetting agent (PVP) throughout the matrix that also coats the surface | None |
| FDA group | I | I | III | I | I | I |
| Principal monomers | DMA + TRIS + siloxane macromer | DMA + TRIS + siloxane macromer | NVP + TPVC + NVA + PBVC | mPDMS + DMA + HEMA + siloxane macromer + TEGDMA + PVP | mPDMS + DMA + EGDMA + HEMA + siloxane macromer + PVP | FM0411M + HOB + IBM + M3U + NVP + TAIC + VMA |

**Table 3-1 Types and Properties of Silicone Hydrogels Used in Study** [34,35]

DMA (*N,N*-dimethylacrylamide); EGDMA (ethyleneglycol dimethacrylate); FM0411M (α-Methacryloyloxyethyl iminocarboxyethyloxypropyl-poly(dimethylsiloxy)-butyldimethylsilane); HEMA (poly-2-hydroxyethyl methacrylate); HOB (2-Hydroxybutyl methacrylate); IBM (Isobornyl methacrylate); MA (methacrylic acid); mPDMS (monofunctional polydimethylsiloxane); NVP (*N*-vinyl pyrrolidone); TEGDMA (tetraethyleneglycol dimethacrylate); TPVC (tris-(trimethylsiloxysilyl) propylvinyl carbamate); TRIS (trimethylsiloxy silane); M3U (α ω-Bis(methacryloyloxyethyl iminocarboxy ethyloxypropyl)-poly(dimethylsiloxane)-poly(trifluoropropylmethylsiloxane)-poly(ω−methoxy-poly(ethyleneglycol)propyl methylsiloxane)); NVA (*N*-vinyl amino acid); PBVC (poly[dimethysiloxy] di [silylbutanol] bis[vinyl carbamate]); PC (phosphorylcholine); PVP (polyvinyl pyrrolidone); TAIC (1,3,5-Triallyl-1,3,5-triazine-2,4,6(*1H,3H,5H*)-trione); VMA (N-Vinyl-N-methylacetamide).

| | Proprietary Name | | |
|---|---|---|---|
| | SofLens 38 (formerly Optima FW) | SofLens 66 | Acuvue2 |
| USAN | polymacon | alphafilcon A | etafilcon A |
| Manufacturer | Bausch & Lomb | Bausch & Lomb | Johnson & Johnson |
| Water Content (%) | 38 | 66 | 58 |
| Dk | 10 | 30 | 22 |
| FDA Group | I | II | IV |
| Surface Treatment | None | None | None |
| Principal monomers | pHEMA | HEMA + NVP | HEMA + MA |

**Table 3-2 Types and Properties of Conventional Lenses Used in Study [34]**

HEMA (poly[2-hydroxyethyl methacrylate), MA (methacrylic acid), NVP (N-vinyl pyrrolidone)

### 3.3.5 Uptake and Release Studies – Uptake

Three lenses of a given type were removed from their packaging and placed into 5 mL of Unisol®4 in 15 mL, round bottom polypropylene tubes (VWR International, Mississauga, Ontario) and gently shaken for 30 minutes to remove any packaging solution. The lenses were removed and partially dried on lens paper, before being transferred into an amber vial (Wheaton, Millville, NJ) containing 2 mL of 0.3% Ciprofloxacin-HCl solution. The vial was incubated in a shaking water bath at 34 °C, to simulate eye conditions. [20] Uptake evaluations were undertaken at various times over a 24 hour period. During the first 30 minutes, readings were taken every 5 minutes. For the next 1.5 hours readings were taken every 15 minutes. Thereafter, readings were taken every hour, out to 24 hours. At each time point under investigation, 5 μL of solution was removed, and diluted 4000 fold, to obtain a reading within the linear range of the standard curve.

### 3.3.6 Uptake and Release Studies – Release

After the 24$^{th}$ hour uptake reading was taken, the lenses were removed from the Ciprofloxacin-HCl solution and briefly dipped into Unisol®4 to remove any residual drug solution not absorbed or adsorbed. The lenses were then partially dried on lens paper, and placed into a fresh amber vial containing 2 mL of Unisol®4 (at a measured pH of 7.4). The vial was incubated in a shaking water bath at 34 °C to simulate eye conditions. At time points identical to that used during the uptake phase of the study, either 5 μL or 10 μL of release solution (depending on lens type) was removed and diluted to 1 mL (1:200 or 1:100 dilution) with Unisol®4.

### 3.3.7 Photographs

Photographs of the lenses were undertaken using a slit lamp mounted lens holder, and taken with a Panasonic 3CCD digital camera, using diffuse and direct lighting with 5X, 8X or

12X magnification, as indicated. This was conducted to visualize the optical impact of the uptake of the ciprofloxacin into the various lens materials.

### 3.3.8 Statistics and Calculations

Statistical analysis was performed using Statistica Ver7.1 (StatSoft Inc., Tulsa, OK, USA). All the data are reported as mean ± standard deviation, unless otherwise indicated. In all cases, calculations took into account the volume change due to sampling, through comparison of the concentrations actually measured and theoretical concentrations (if no lens was present). A repeated measures Analysis of Variance (ANOVA) was performed to determine differences across various time points within the same lens type. An ANOVA was performed to determine differences between lens types. Post-hoc Tukey HSD tests were used as needed. Statistical significance was considered when p values of less than 0.05 were obtained.

## 3.4 Results

Typical uptake curves over a 24 hour period for four of the lenses (etafilcon A; senofilcon A; lotrafilcon A; galyfilcon A) are illustrated in Figure 3-1, and summarized for all lens types in Table 3-3. Inspection of Figure 3-1 shows that up to 60 minutes post exposure to the ciprofloxacin solution that all lens materials showed an uptake of the drug ($p<0.05$), and that the differences between lens types was minimal (p=NS).

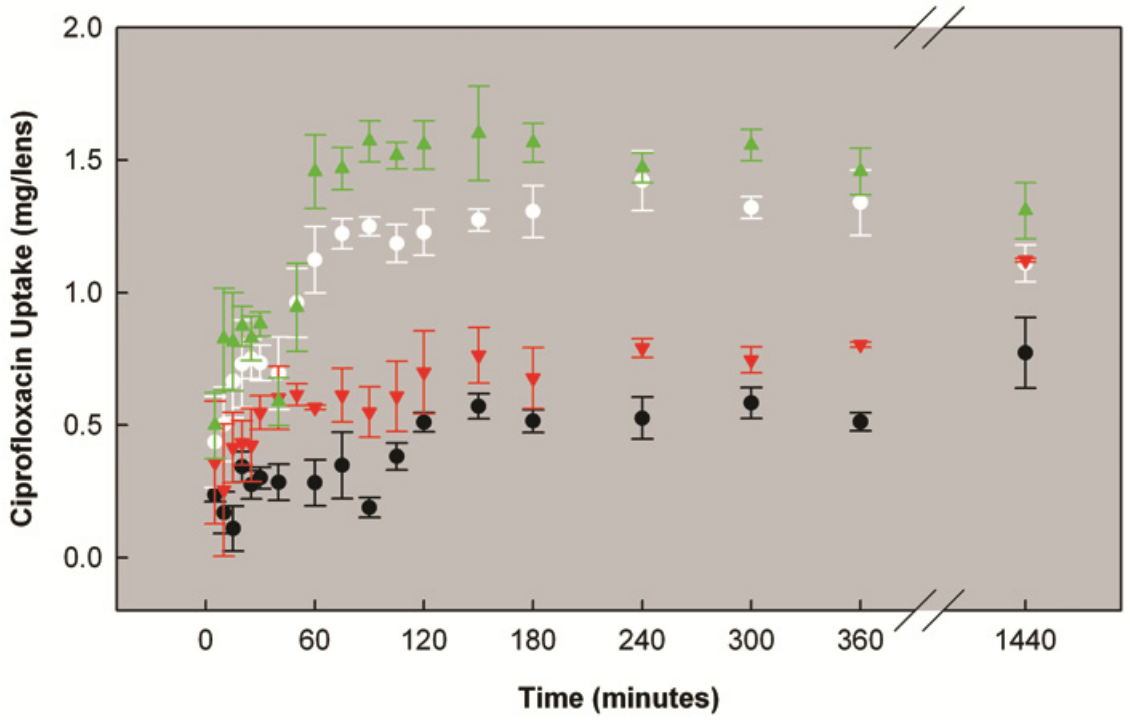



**Figure 3-1 Ciprofloxacin Uptake (mg/lens)**

Ciprofloxacin uptake (mg/lens) for etafilcon A (▲),senofilcon A (○), lotrafilcon A (▼) and galyfilcon A (●),over time determined by spectrophotometry. Values plotted are the mean ± standard deviation. Of the silicone hydrogel lenses, the senofilcon A lenses took up the most drug (1.29 ± 0.13 mg/lens; range 0.39 ± 0.36 to 1.29 ± 0.13 mg/lens), although these differences were not statistically significant (p=NS). Senofilcon A was only second in its uptake of ciprofloxacin to etafilcon A lenses (1.51 ± 0.14 mg/lens).

| Lens | mg of ciprofloxacin taken up / lens | | | |
|---|---|---|---|---|
| | 60 mins | 180 mins | 360 mins | 1440 mins |
| Alphafilcon A | 0.15 ± 0.12 | 0.37 ± 0.18 | 0.18 ± 0.08 | 0.68 ± 0.18 |
| Balafilcon A | 0.39 ± 0.21 | 0.18 ± 0.04 | 0.16 ± 0.15 | 0.53 ± 0.28 |
| Comfilcon A | 0.80 ± 0.10 | 0.85 ± 0.05 | 0.89 ± 0.04 | 0.93 ± 0.04 |
| Etafilcon A | 1.46 ± 0.14 | 1.56 ± 0.07 | 1.46 ± 0.09 | 1.31 ± 0.11 |
| Galyfilcon A | 0.28 ± 0.09 | 0.51 ± 0.04 | 0.51 ± 0.03 | 0.77 ± 0.13 |
| Lotrafilcon A | 0.57 ± 0.01 | 0.68 ± 0.12 | 0.80 ± 0.01 | 1.12 ± 0.01 |
| Lotrafilcon B | 0.62 ± 0.06 | 0.64 ± 0.13 | 0.76 ± 0.10 | 1.01 ± 0.07 |
| Polymacon | 0.49 ± 0.08 | 0.59 ± 0.04 | 0.87 ± 0.05 | 0.71 ± 0.03 |
| Senofilcon A | 1.12 ± 0.13 | 1.31 ± 0.10 | 1.34 ± 0.12 | 1.11 ± 0.07 |

**Table 3-3 Summary of Uptake of Ciprofloxacin-HCl into Different Lens Types**

Results are expressed as the mean ± standard deviation of 3 lenses.

Typical release curves over a 24 hour period for five of the lens types (etafilcon A; alphafilcon A; polymacon; balafilcon A; comfilcon A) are illustrated in Figure 3-2 and summarized for all lens types in Table 3-4. All lenses released a statistically significant amount of drug when compared to the initial time point ($p<0.001$). The majority of the lenses released their drug within the first 10-15 minutes, as evidenced by the lack of statistically significant difference (p=NS) between sequentially measured values after the initial fifteen minutes. Of all the lenses, only etafilcon A and polymacon lenses showed statistically significant ($p<0.05$) changes over time beyond the first few measurements; etafilcon A released drug significantly for the first 25 minutes before reaching a plateau (changing from $0.10 \pm 0.03$ mg/lens to $0.37 \pm 0.01$ mg/lens), while polymacon continued to show significant changes for 20 minutes before reaching a plateau (changing from $0.01 \pm 0.007$ mg/lens to $0.19 \pm 0.005$ mg/lens). With respect to lens type, the conventional lens types (etafilcon A, polymacon and alphafilcon A) all had statistically significantly higher ($p<0.001$) release of drug over all time points, when compared to all other lens types. Release from etafilcon A lenses were statistically different ($p<0.001$) from alphafilcon A and polymacon lenses after 10 minutes had elapsed; statistical difference between the remaining two conventional lenses is seen after 30 minutes had elapsed ($p<0.001$).

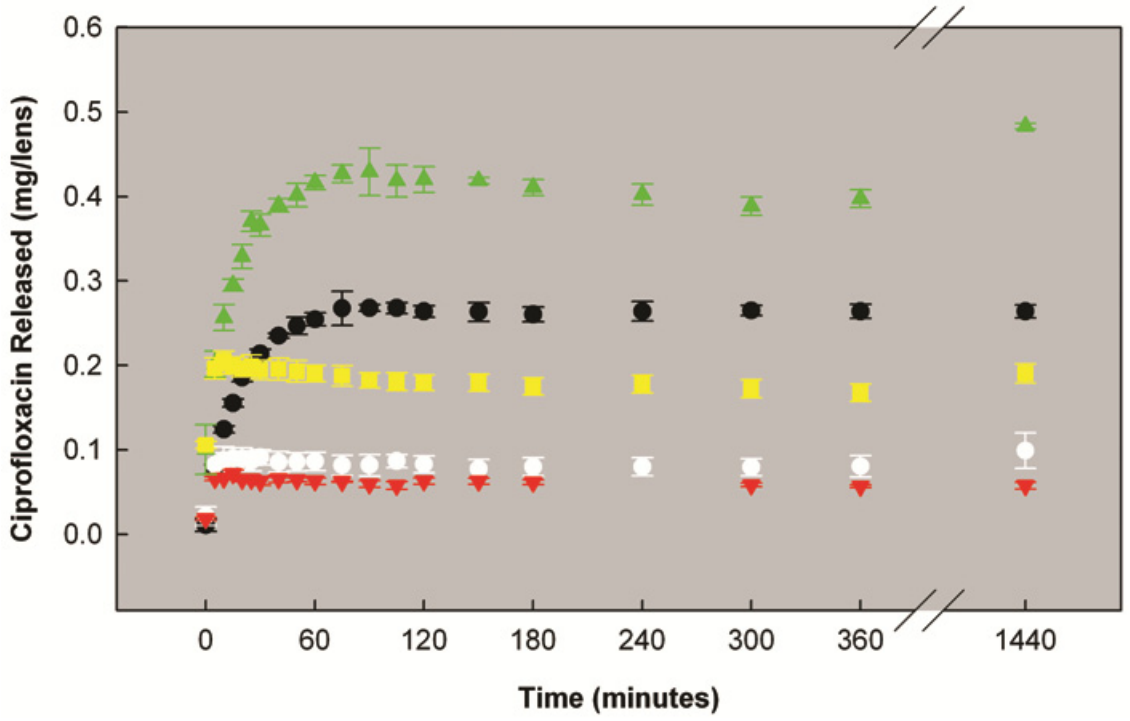



**Figure 3-2 Ciprofloxacin Release (mg/lens)**

Ciprofloxacin release (mg/lens) from etafilcon A (▲), polymacon (●), alphafilcon A (■), balafilcon A (○) and comfilcon A (▼) over time determined by spectrophotometry. Values plotted are the mean ± standard deviation. The etafilcon A lenses released the most ciprofloxacin, with a maximum release of 0.42 ±0.03 mg/lens., the polymacon lens released 0.27 ± 0.01 mg/lens, the alphafilcon A lens released 0.18 ± 0.01while the silicone hydrogels balafilcon A and comfilcon A released only 0.078 ± 0.01 and 0.06 ± 0.004 mg/lens respectively. Release from the conventional lenses (alphafilcon A, etafilcon A and polymacon) was statistically different from each other, as well as all other lenses ($p<0.001$). All lenses except for lotrafilcon A lenses showed statistically significant release when compared to the first time point ($p<0.001$), but only etafilcon A and polymacon showed a statistical significant change over time; etafilcon A release was statistically significant for the first 25 minutes ($p<0.001$) while release from polymacon lenses was statistically significant for the first 20 minutes ($p<0.001$). There is was no significant difference between balafilcon A and comfilcon A lenses over time.

| Lens | mg of ciprofloxacin released / lens | | | |
|---|---|---|---|---|
| | 60 mins | 180 mins | 360 mins | 1440 mins |
| Alphafilcon A | 0.19 ± 0.01 * | 0.18 ± 0.01 * | 0.17 ± 0.01 * | 0.19 ± 0.01 * |
| Balafilcon A | 0.086 ± 0.01 | 0.080 ± 0.01 | 0.08 ± 0.01 | 0.099 ± 0.02 |
| Comfilcon A | 0.063 ± 0.004 | 0.062 ± 0.003 | 0.057 ± 0.001 | 0.058 ± 0.004 |
| Etafilcon A | 0.42 ± 0.009 * | 0.41 ± 0.009 * | 0.40 ± 0.011 * | 0.48 ± 0.003 * |
| Galyfilcon A | 0.077 ± 0.004 | 0.075 ± 0.003 | 0.071 ± 0.003 | 0.063 ± 0.002 |
| Lotrafilcon A | 0.016 ± 0.003 | 0.014 ± 0.001 * | 0.013 ± 0.001 * | 0.022 ± 0.005 * |
| Lotrafilcon B | 0.05 ± 0.005 | 0.046 ± 0.002 | 0.044 ± 0.003 | 0.057 ± 0.01 |
| Polymacon | 0.25 ± 0.008 * | 0.26 ± 0.009 * | 0.26 ± 0.008 * | 0.26 ± 0.008 * |
| Senofilcon A | 0.047 ± 0.002 | 0.059 ± 0.009 | 0.060 ± 0.007 | 0.056 ± 0.002 |

**Table 3-4 Summary of Release of Ciprofloxacin-HCl from Different Lens Types**

Results are expressed as the mean ± standard deviation of 3 lenses. * Represents values significantly different ($p<0.05$) from all other lens types at that time point.

Visually, most of the lenses remained clear throughout both the uptake and release phases of the experiment. The only lens with a marked change was etafilcon A, which showed a dramatic precipitation of the drug. The drug precipitated on the lens during the uptake phase (at pH 4.0), and became completely white and opaque once placed within the release solution at pH 7.4 (Figure 3-3a and Figure 3-3b). There was also some evidence of precipitation on the alphafilcon A (Figure 3-3c) and polymacon lenses during the release phase of the experiment. This was in contrast to the lack of any noticeable precipitate on all of the other lenses (Figure 3-3d).

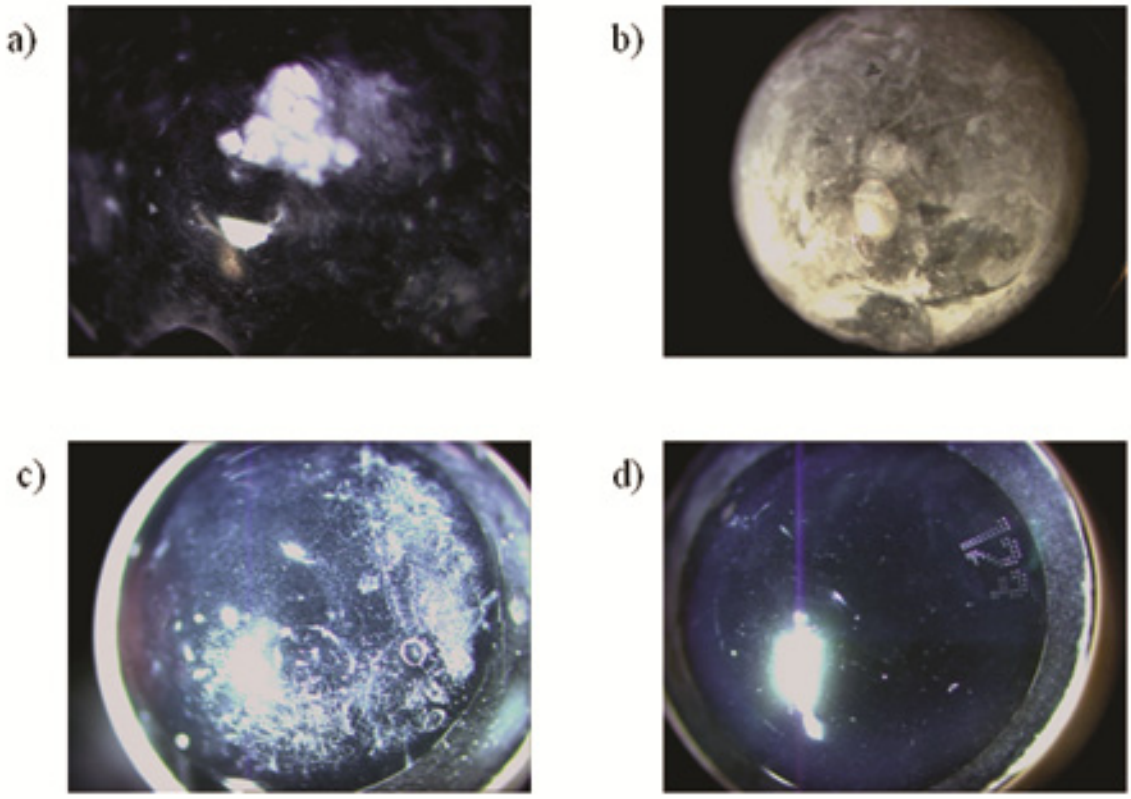



**Figure 3-3 Ciprofloxacin Precipitates**

Ciprofloxacin precipitates on etafilcon A during (a) Uptake (12X magnification) and (b) Release (5X magnification) and on (c) alphafilcon A (8X magnification) during release. Absence of precipitate is seen on (d) galyfilcon A (8X magnification) during release. Etafilcon A lenses show the most uptake and release of ciprofloxacin but the presence of white precipitates on the lens limit their use as a drug delivery device. Alphafilcon A lenses also show a high amount of ciprofloxacin release but also presence of precipitates. In contrast, the silicone hydrogel lenses (such as the representative galyfilcon A lens pictured) do not show any precipitate, but also release much less drug.

## 3.5 DISCUSSION

Uptake profiles (Table 3-5) indicate that the highest uptake is with etafilcon A, followed by senofilcon A. Release profiles (Table 3-5) indicate that the highest release is with etafilcon A, followed by polymacon and alphafilcon A, with lotrafilcon A having the lowest release. Table 3-5 also shows the percentage of ciprofloxacin that is released, as a proportion of that taken up. These values show that the three conventional lens materials (etafilcon A, polymacon and alphafilcon A) release the highest percentage of the drug that is taken up, and lotrafilcon A releases the least. These values suggest that conventional materials typically are able to release the drug more easily than silicone hydrogels, which appear to bind the drug relatively firmly to the lens material. This is obviously a disadvantage for a drug delivery device, although could be an advantage, if the delivery was sustained at a low level for a long period of time.

| Lens | Mass (mg) of ciprofloxacin per lens | | Percentage of Drug Released | Max ciprofloxacin released (mg/lens) x Dk |
|---|---|---|---|---|
| | Uptake | Release | | |
| Alphafilcon A | 0.32 ± 0.24 | 0.18 ± 0.008 * | 56.2 | 5.4 |
| Balafilcon A | 0.39 ± 0.36 | 0.078 ± 0.01 | 20 | 7.1 |
| Comfilcon A | 0.89 ± 0.06 * | 0.06 ± 0.004 | 6.7 | 7.7 |
| Etafilcon A | 1.51 ± 0.14 * | 0.42 ± 0.03 * | 27.8 | 9.2 |
| Galyfilcon A | 0.57 ± 0.12 | 0.071 ± 0.005 | 12.5 | 4.3 |
| Lotrafilcon A | 0.79 ± 0.19 | 0.016 ± 0.004 * | 2.0 | 2.2 |
| Lotrafilcon B | 0.75 ± 0.16 | 0.047 ± 0.006 | 6.3 | 5.2 |
| Polymacon | 0.72 ± 0.13 | 0.27 ± 0.008 * | 37.5 | 2.7 |
| Senofilcon A | 1.29 ± 0.13 | 0.057 ± 0.002 | 4.4 | 5.9 |
| | | | Average | 5.5 |

**Table 3-5 Average Ciprofloxacin Uptake and Release after Plateau from 120 Minutes to 1440 Minutes**

Results are expressed as the mean ± standard deviation of 3 lenses. * Represents values significantly different ($p<0.05$) from all other lens types.

Based on ocular studies, the range of minimum inhibitory concentrations for 90% of bacterial isolates ($MIC_{90}$) range from 0.00025 to 0.032 mg/mL[21] for ciprofloxacin against common susceptible and resistant ocular pathogens. To achieve this concentration within the 2 mL volume of the test cuvette, a lens must release a minimum of 0.0005 mg/lens for susceptible isolates, or up to 0.064 mg/lens for more resistant isolates. All lenses released enough drug to meet the $MIC_{90}$ concentrations for the more susceptible isolates, but lotrafilcon A, lotrafilcon B, senofilcon A and comfilcon A do not release enough drug to meet the concentrations needed for more resistant organisms. As evidenced by Figure 3-1 and Figure 3-2, for the majority of lenses, the time course for the uptake and release of the antibiotic occurred very quickly. For uptake, typically by 60 minutes the uptake of drug into the lens had essentially plateaued, with minor increases thereafter. For release, within the first 20 minutes the majority of lenses had released their maximum amount of drug into solution.

Compared to a previous study by Karlgard et al., [10] the results from the current study appear to indicate a lower amount of drug being taken up by the lenses, as well as that being released. The Karlgard study [10] established a maximum uptake for all lenses of approximately 1.8 mg/lens, compared to the current study, which shows an uptake varying from 0.47 mg/lens to 1.5 mg/lens (mean 0.96 mg/lens). The amount of drug released also differs considerably, with the previously reported release ranging from 0.065 mg/lens to 0.217 mg/lens (mean: 0.140 mg/lens), while the current study varies from 0.021 mg/lens to 0.48 mg/lens (mean 0.145 mg/lens). The reasons for the discrepancy between these two studies may be attributable to the doping solution used. The current study utilized a pH-adjusted solution, which allowed for a fully dissolved, uniform, concentrated 0.3% ophthalmic solution to be used, compared to a 0.15% partial suspension in unmodified Unisol®4 in the previous study. [10]

Although etafilcon A lenses took up and released the most ciprofloxacin when compared to all the other lens types, its use as a drug delivery device for the delivery of ciprofloxacin should be discouraged, as the lens is rendered completely opaque by drug precipitates at physiological pH, as seen in Figure 3-3b. While the dosage of the drug delivered is exceptional, the decrease in visual performance and comfort provided by the lens would limit its use in a practical sense. The precipitates seen on polymacon lenses would also discourage their use, as this precipitation would likely decrease visual performance as well as comfort.

Previous studies have shown that drug - lens interactions are multi-faceted and are influenced by several factors, including water content, ionicity, porosity, surface treatment, surface morphology of the lens material under test and also the organization of water in and around the material. [10, 22-25] The results from this study show that among the silicone hydrogel lens materials, balafilcon A had the highest percentage of drug released (Table 3-5). Balafilcon A is a low water content lens material with a negative charge due to the presence of *N*- vinyl amino acid (Table 3-1), and is thus classified as a United States Food & Drug Administration group III material. The balafilcon A material undergoes a surface modification process which results in a mosaic-like surface with glassy discontinuous silicate coatings, at a thickness of approximately 10-25nm. [26] In addition, balafilcon A is relatively more porous when compared to other SH lens materials. [26] Ciprofloxacin has an amino group (pKa of 8) and a carboxylic acid group (pKa of 6). [18] At the doping pH of 4, Ciprofloxacin will carry a net positive charge as the amino group is protonated and the carboxylic acid will be non-ionized, resulting in Ciprofloxacin being adsorbed into the negatively charged balafilcon lens material. Whereas, at the release pH of 7.2, Ciprofloxacin will be neutral, as the carboxylic acid will carry a negative charge and the amino group will carry a positive charge, resulting in the drug being easily released from the lens

material. Thus, the combination of a discontinuous surface treatment, open pore-like network and the charge on the drug may result in an increased percentage of ciprofloxacin being released from the balafilcon A material.

The ideal "bandage lens drug delivery device" would be able to deliver a high amount of ciprofloxacin over an extended period of time, while also transmitting a high amount of oxygen to the cornea, as determined by its quoted oxygen permeability (Dk) or transmissibility (Dk/t). An index relating these two important parameters could be determined by multiplying the maximum ciprofloxacin release by the material Dk, and this "index" is shown in Table 3-5. These values show that etafilcon A has the highest index, due to its high release, but the aforementioned precipitates preclude its usefulness. Of the silicone hydrogels, it should be noted that balafilcon A and comfilcon A are above average, while lotrafilcon A and galyfilcon A perform below average, when compared to the rest of the lenses.

In conclusion, nine different soft contact lenses were tested for their uptake and release characteristics when soaked in the ocular antibiotic Ciprofloxacin-HCl. The majority of the lenses were able to release levels of the antibiotic into solution which would be clinically relevant, but released them too quickly under experimental parameters to be clinically useful as drug delivery devices. These results suggest that etafilcon A lenses should not be used as a drug delivery device or as a bandage lens concurrent with the delivery of ciprofloxacin-HCl eyedrops, as drug precipitation renders it ineffective for adequate visual performance. If a practitioner is looking for a balance of high oxygen transmissibility with "high" delivery levels of ciprofloxacin, then balafilcon A lenses appear to provide an encouraging mixture of these two factors. Balafilcon A lenses are FDA approved for use as therapeutic lenses and previous studies have shown that they work well in this format. [7 27 28] It is worth noting that the other two silicone

hydrogel lens materials approved for therapeutic use (lotrafilcon A and senofilcon A) both released lower amounts of ciprofloxacin than balafilcon A. To date, no studies have been published on the use of senofilcon A as a bandage lens, but studies looking at lotrafilcon A have also shown this material to be an excellent therapeutic lens. [8 27 29 30 31 32 33] However, these studies did not investigate its performance when used with concurrent drug delivery.

## 3.6 ACKNOWLEDGEMENTS

The authors would like to thank Sruthi Srinivasan for photography and help with statistical analysis and Miriam Heynen and Elizabeth Heikkila for laboratory and technical aid. We also acknowledge financial assistance from Beta Sigma Kappa and Natural Sciences and Engineering Research Council (NSERC) Canada.

In the next chapter, a molecular imprinting strategy is employed in an attempt to modify the ciprofloxacin release kinetics *in vitro*. The effect of different ratios of functional monomer to template ratio within the polymerization mix, the concentration of the functional monomer and the concentration of the loading solution were all examined for their effects on ciprofloxacin release kinetics. The structure of the chapter is dictated by the journal into which it was published, *Materials* by MDPI.

# CHAPTER 4 - ACETIC AND ACRYLIC ACID MOLECULAR IMPRINTED MODEL SILICONE HYDROGEL MATERIALS FOR CIPROFLOXACIN-HCL DELIVERY

Alex Hui OD [1], Heather Sheardown PhD [2], Lyndon Jones PhD [1,2]

[1]Centre for Contact Lens Research, School of Optometry, University of Waterloo, 200 University Avenue West, Waterloo, Ontario N2L 3G1, Canada
[2]Department of Chemical Engineering, McMaster University, 1280 Main St. W. Hamilton, Ontario L8S 4L7, Canada

This chapter has been published as follows:

Hui A, Sheardown H, Jones L. Acetic and Acrylic Acid Molecular Imprinted Model Silicone Hydrogel Materials for Ciprofloxacin-HCl Delivery. *Materials* 2012;5:85-107



| Author | Concept/Design | Acquisition of Data | Analysis | Write Up/ Publication |
|---|---|---|---|---|
| Hui | Y | Y | Y | Y |
| Sheardown | Y | | | Y |
| Jones | Y | | | Y |

## 4.1 OVERVIEW

Contact lenses, as an alternative drug delivery vehicle for the eye compared to eye drops, are desirable due to potential advantages in dosing regimen, bioavailability and patient tolerance/compliance. The challenge has been to engineer and develop these materials to sustain drug delivery to the eye for a long period of time. In this study, model silicone hydrogel materials were created using a molecular imprinting strategy to deliver the antibiotic ciprofloxacin. Acetic and acrylic acid were used as the functional monomers, to interact with the ciprofloxacin template to efficiently create recognition cavities within the final polymerized material. Synthesized materials were loaded with 9.06 mM, 0.10 mM and 0.025 mM solutions of ciprofloxacin, and the release of ciprofloxacin into an artificial tear solution was monitored over time. The materials were shown to release for periods varying from 3 to 14 days, dependent on the loading solution, functional monomer concentration and functional monomer:template ratio, with materials with greater monomer:template ratio (8:1 and 16:1 imprinted) tending to release for longer periods of time. Materials with a lower monomer:template ratio (4:1 imprinted) tended to release comparatively greater amounts of ciprofloxacin into solution, but the release was somewhat shorter. The total amount of drug released from the imprinted materials was sufficient to reach levels relevant to inhibit the growth of common ocular isolates of bacteria. This work is one of the first to demonstrate the feasibility of molecular imprinting in model silicone hydrogel-type materials.

**Keywords:** molecular imprinting; ciprofloxacin; antibiotic; contact lens materials; silicone hydrogel; drug delivery; combination devices

## 4.2 INTRODUCTION

As the contact lens industry continues to grow and develop, novel uses and applications of contact lenses are constantly being contemplated and investigated. Contact lens materials as a vehicle for sustained ophthalmic drug delivery to the eye has had a renewal of interest in the past decade, mainly due to the advent of silicone hydrogel materials, which provide sufficient oxygen delivery to the eye to permit hypoxia-free wear during overnight use [1]. Indeed, in the original patents and designs of soft contact lens materials, the concept of using contact lenses as a reservoir for drugs delivered to the eye was noted, although little work investigating this application has been conducted for over thirty years [2]. Recently, there has been an explosion in the number of studies and groups who have demonstrated an interest in the development of contact lens drug delivery materials. The rationales for the use of contact lenses as drug delivery devices are numerous. First, contact lenses are arguably the most successful biomaterial currently available, with estimates of over 140 million wearers worldwide [3], and are thus firmly embraced by patients and, more importantly, practitioners. Second, contact lenses have already been demonstrated to successfully correct refractive errors in patients. The addition of drug delivery to this correction of refractive error can potentially increase the quality of life in patients by decreasing dosing frequency, while also potentially increasing compliance rates in acute or chronic ophthalmic treatment. Third, there is some evidence that concurrent contact lens and topical ophthalmic treatment is more effective than topical treatment alone. Use of contact lenses has been demonstrated to increase the residence time and/or increase ocular penetration of topically administered agents [4, 5]. Use of contact lenses may thus decrease the amount of drug needed to successfully treat ocular disease in patients. Finally, there are many situations or locales around the world where access to pharmacological therapy is inconsistent at best, necessitating the use of treatments that can be administered at a single time and have a long lasting effect.

Development of these devices to combat these medical challenges is thus warranted and potentially useful.

There are several clinical scenarios in which a contact lens is already used medically to aid the healing of a patient, with topically prescribed agents being used concurrently with contact lenses. For example, following photorefractive keratectomy (PRK), an ocular laser surgical method used for the correction of refractive error, a bandage contact lens is used for several days post-surgery, due to the absence of the corneal epithelium, which is removed during the course of the procedure [6]. Antibiotic drops are used on top of the lens prophylactically to prevent any post-surgical infection. In patients who present with a traumatic corneal abrasion, a bandage contact lens is often used to increase the rate of healing, while also providing symptomatic pain relief. These patients are often prescribed an antibiotic agent, either prophylactically or to treat any current infection sustained during the trauma. It is evident that if the bandage lens was concurrently providing the symptomatic relief as well as the release of the prophylactic antibiotic agent, then the patient could be permitted to rest and recuperate rather than worrying about drug dosing schedules.

The extended release of drugs from soft contact lens materials (hydrogels) is unfortunately not that simple. Previous studies have demonstrated that commercially available lenses soaked in ophthalmic pharmaceuticals are capable of releasing clinically relevant amounts of drugs, but the release times from these materials is in the order of only minutes to hours [7-10]. Furthermore, these materials are not designed for extended wear, so even if long term release was achieved, the hypoxia of the cornea that would occur with extended wear would necessitate their removal. Thus, strategies to optimize release times to be more on the order of days or even weeks are needed, if these devices are to be used and marketed effectively.

Numerous strategies have been investigated to slow and/or control the release of pharmaceuticals from contact lenses. Some investigators have found the addition of a diffusion barrier could impede the movement of the drug out of the lens, thus slowing the release. In recent studies investigating this concept for the delivery of dexamethasone and timolol [11, 12], vitamin E was used as a diffusion barrier and the authors were able to demonstrate sustained release from these materials for days to weeks, with the time for release being controlled by the amount of vitamin E used. This technique may prove particularly beneficial as it can be used with commercially available materials, thus shortening the regulatory approval processes. Other authors have proposed the use of a drug-impregnated coating on the surface of the lens, using cyclodextrins, nanoparticles or liposomes [13-15]. This strategy may be particularly useful for drugs with poor solubility in aqueous environments, as the microenvironment of the coating can be different from the rest of the lens.

One of the more successful strategies in generating extended release times from contact lens materials has been molecular imprinting. Molecular imprinting is a polymerization strategy in which a molecule of interest is present within the pre-polymerization solution of a polymer. The addition of other molecules known as functional monomers, which serve to interact with the functional groups of the template molecule, create “cavities” or “molecular memory” within the material after polymerization is complete [16]. These “cavities” specifically interact with the template molecules, slowing the diffusion of the templates out of the material into solution, and thus extending release times [17]. This technique was originally designed for highly crosslinked, hard plastics for the specific removal of components out of solutions [18]. The challenge has been to adapt this technique for contact lenses, in which a highly crosslinked, rigid type material would not be useful. Despite these challenges, several recent papers have shown this technique to

be applicable to the creation of contact lens materials to deliver anti-glaucoma, antibiotic, antihistamine, non-steroidal anti-inflammatory agents (NSAIDs) and wetting agents [17, 19-22]. The gains in delivery time for materials created using this concept have been substantial; whereas non-modified materials may release for only a few hours at most, delivery from imprinted materials in the order of several days have been achieved [22]. Several key insights have been gleaned from previous authors. First, the choice of the template and functional monomer is crucial. There has to be an appropriate interaction between the template and functional monomer to efficiently create the cavities to be fixed during the polymerization process [22]. Second, the amount of functional monomer relative to the template in the polymerization mix is also important. A low functional monomer:template will yield an insufficient number of cavities being created around the template; a too high functional monomer:template ratio will lead to inefficient creation of cavities, as much of the functional monomer will not have the opportunity to interact with the template [20]. Much of the work to-date on imprinted molecules have involved "conventional" higher water content hydrogel materials based on poly-hydroxyethyl methacrylate (pHEMA) [21, 23], but more recent work has been performed on the more oxygen permeable siloxane-based hydrogels [24].

Ciprofloxacin-HCl is a second generation fluoroquinolone antibiotic. It interferes with bacterial DNA gyrase, preventing bacterial DNA replication [25]. It is a broad spectrum antibiotic, with activity against both gram-negative and gram positive bacteria [26, 27]. It is used ophthalmically as either an eye drop or as an ointment. It is commonly used as a treatment for bacterial conjunctivitis, and is one of only a few drugs that have United States Food and Drug Administration (FDA) indications for the treatment of bacterial ulcers/microbial keratitis [28, 29]. Ciprofloxacin exhibits poor aqueous solubility at physiological pH due to its overall neutral

charge as a zwitterion at this pH, and the presence of its dual aromatic rings [30]. Its solubility in aqueous media is greatly enhanced in acidic or basic solutions, leading to commercially available ophthalmic preparations having a pH of approximately 4.0, which may cause some stinging or irritation upon instillation [29, 30]. When dissolved in high concentrations, ciprofloxacin solutions have a yellowish colour. During a severe infection, the dosing of ciprofloxacin can be as frequent as two drops every fifteen minutes. This high dose and long term use, coupled with poor solubility of the drug at physiological pH, can lead to the development of white, crystalline precipitates in the cornea or inferior conjunctival sac, although this does not necessarily indicate the need to discontinue treatment [31].

In this current study, molecular imprinting techniques were used to create model silicone hydrogel materials for the delivery of the antibiotic ciprofloxacin-HCl. Acetic and acrylic acid were used as functional monomers, and the effect of functional monomer:template ratio, overall functional monomer concentration and drug loading concentration were all investigated and explored. This study is one of the few studies investigating the use of silicone hydrogel-type materials for the delivery of pharmaceuticals using a molecular imprinting strategy.

## 4.3 Results and Discussion

### 4.3.1 Pilot Study: Ciprofloxacin pHEMA-Methacryloxypropyltris (Trimethylsiloxy) Silane (TRIS) Materials with Acetic Acid Functional Monomers

The water content and dry weight of the different acetic acid imprinted model materials is detailed in Table 4-1. Model lenses created would all be classified as being of low water content, and would require some increase in water content if they were to be used as actual contact lenses on the eye. There was no statistically significant difference between the pHEMA-TRIS-Acetic

Acid controls and the pHEMA-TRIS-Acetic Acid Ciprofloxacin imprinted materials, based on a one way analysis of variance (ANOVA) ($p > 0.05$).

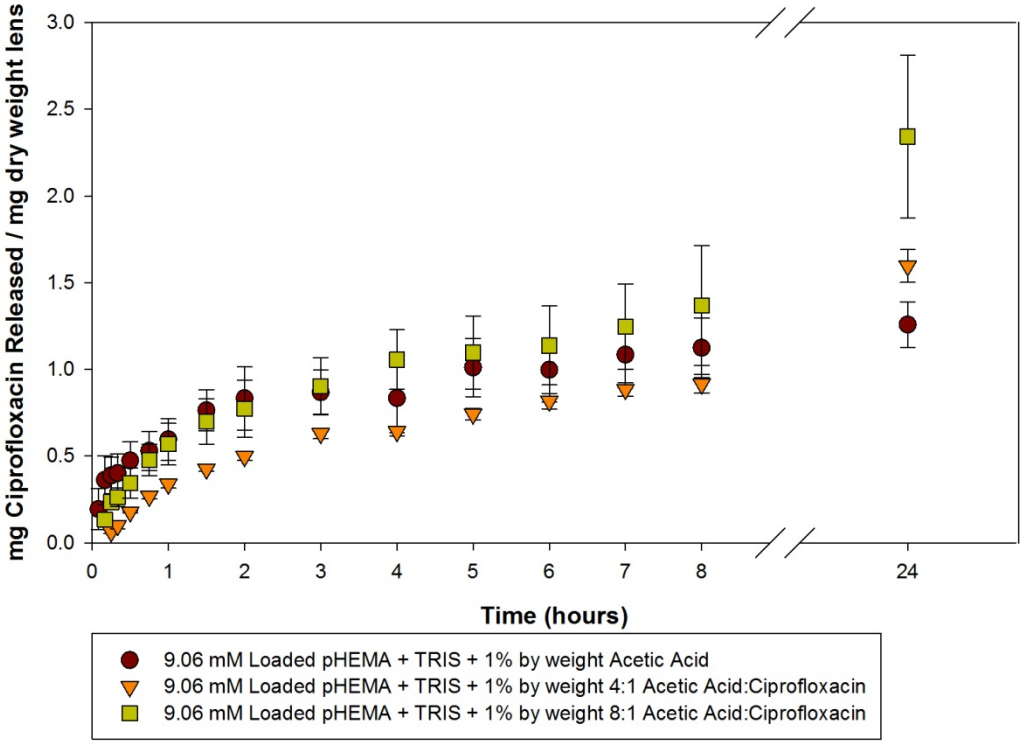
**Table 4-1** Dry Weight and Water Content of Acetic Acid Imprinted pHEMA-TRIS Materials.

| Model Lens Type | Dry Weight (g) (Average ± Standard Deviation) | Water Content (%) (Average ± Standard Deviation) | Centre Thickness (mm) (Average ± Standard Deviation) | Volume ($mm^3$) (Average ± Standard Deviation) |
|---|---|---|---|---|
| pHEMA + TRIS + 1% by weight Acetic Acid Control | 0.0457 ± 0.0089 | 15.5 ± 2.7 | 0.87 ± 0.12 | 68.1 ± 9.3 |
| pHEMA + TRIS + 1% by weight 4:1 Acetic Acid:Ciprofloxacin | 0.0428 ± 0.0078 | 14.8 ± 2.5 | 0.93 ± 0.16 | 73.2 ± 12.9 |
| pHEMA + TRIS + 1% by weight 8:1 Acetic Acid:Ciprofloxacin | 0.0396 ± 0.0059 | 16.7 ± 2.1 | 0.99 ± 0.14 | 77.3 ± 10.8 |

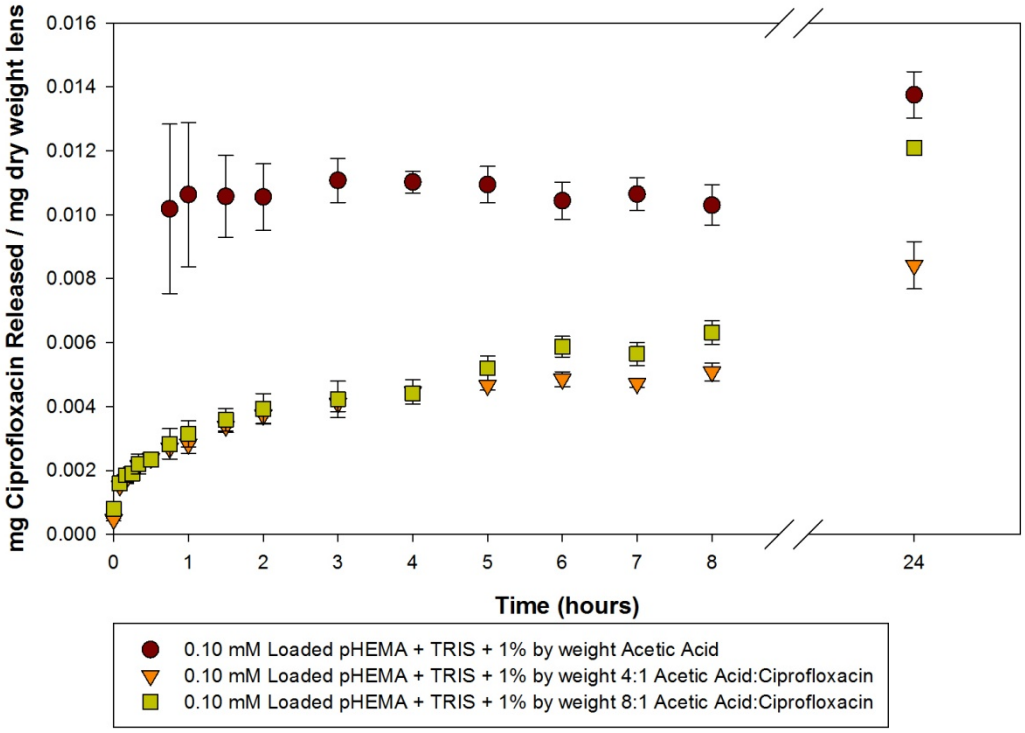
The release curves from these materials loaded with 9.06 mM, 0.10 mM and 0.025 mM ciprofloxacin over the first 24 hours are seen in Figure 4-1(a–c). There was no statistically significant difference seen between the imprinted and control model lenses loaded with 9.06 mM, over the course of the 24 h ($p > 0.05$). The initial release from the 0.10 mM and 0.025 mM model lenses are of interest. For 0.10 mM loaded model lenses, the control exhibited a very fast release and almost immediate plateau, at a level higher than the two imprinted materials. For the 0.025 mM loaded model lens, the control model lens again almost immediately reached its final plateau level, but in this situation it was at a level that was below that of the two imprinted materials. Whether this was caused by some residual loading solution on the 0.10 mM loaded discs is unknown. For the imprinted materials, for both the 0.10 mM and 0.025 mM loaded model lenses, there was a slow release of ciprofloxacin into solution over the course of the 24 hours, but there was no statistical significance between the 4:1 and 8:1 imprinted materials.

**Figure 4-1 (a–c)** **Release Curves from Acetic Acid Imprinted Materials over 24 hours**

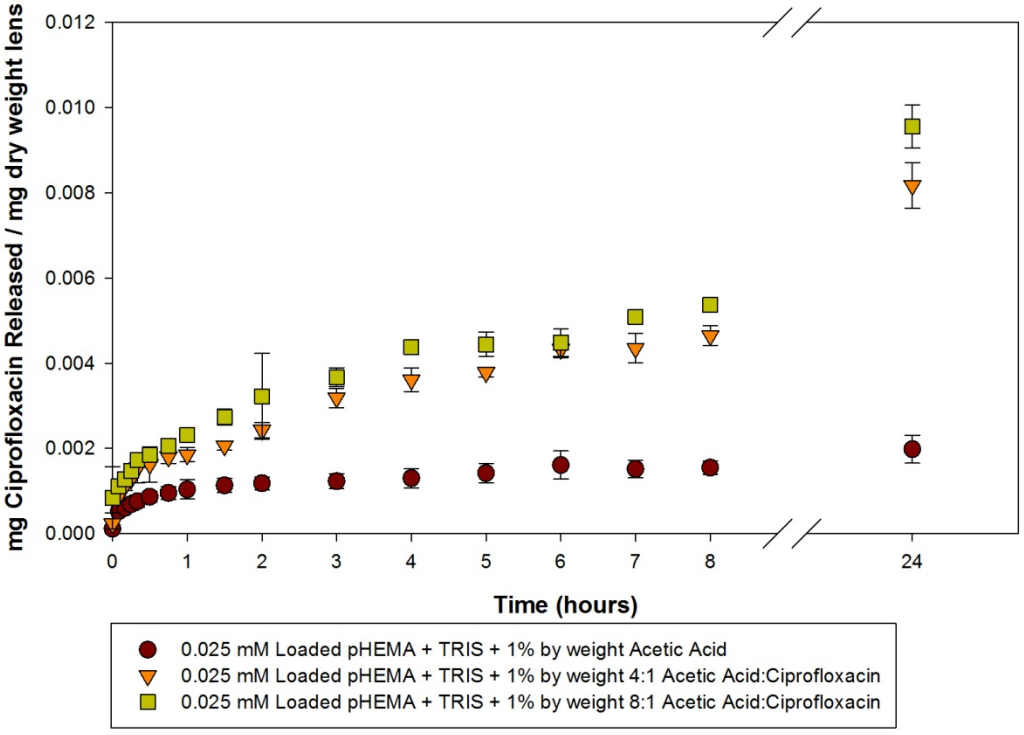
Release curves from acetic acid imprinted materials loaded with (**a**) 9.06 mM ciprofloxacin; (**b**) 0.10 mM ciprofloxacin and (**c**) 0.025 mM ciprofloxacin over 24 h. Values plotted are means ± standard deviations.




(**a**)


mg Ciprofloxacin Released / mg dry weight lens
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
0
1
2
3
4
5
6
7
8
24
Time (hours)
0.10 mM Loaded pHEMA + TRIS + 1% by weight Acetic Acid
0.10 mM Loaded pHEMA + TRIS + 1% by weight 4:1 Acetic Acid:Ciprofloxacin
0.10 mM Loaded pHEMA + TRIS + 1% by weight 8:1 Acetic Acid:Ciprofloxacin

(**b**)

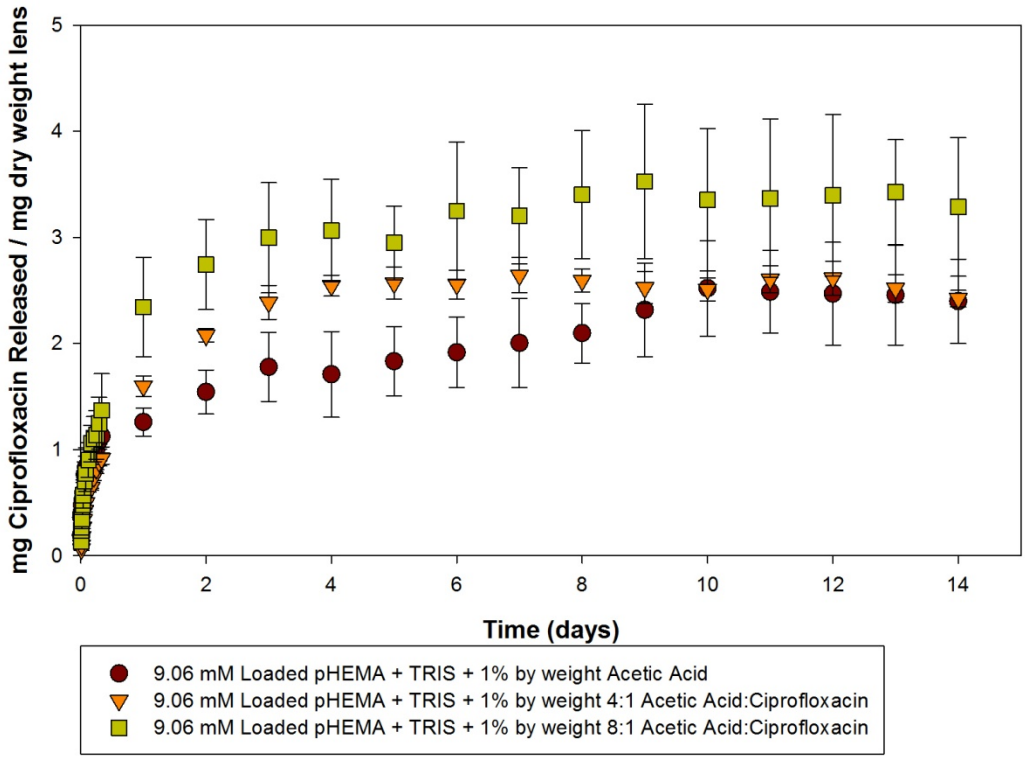

mg Ciprofloxacin Released / mg dry weight lens
0.012
0.010
0.008
0.006
0.004
0.002
0.000
0
1
2
3
4
5
6
7
8
24
Time (hours)
0.025 mM Loaded pHEMA + TRIS + 1% by weight Acetic Acid
0.025 mM Loaded pHEMA + TRIS + 1% by weight 4:1 Acetic Acid:Ciprofloxacin
0.025 mM Loaded pHEMA + TRIS + 1% by weight 8:1 Acetic Acid:Ciprofloxacin

(**c**)

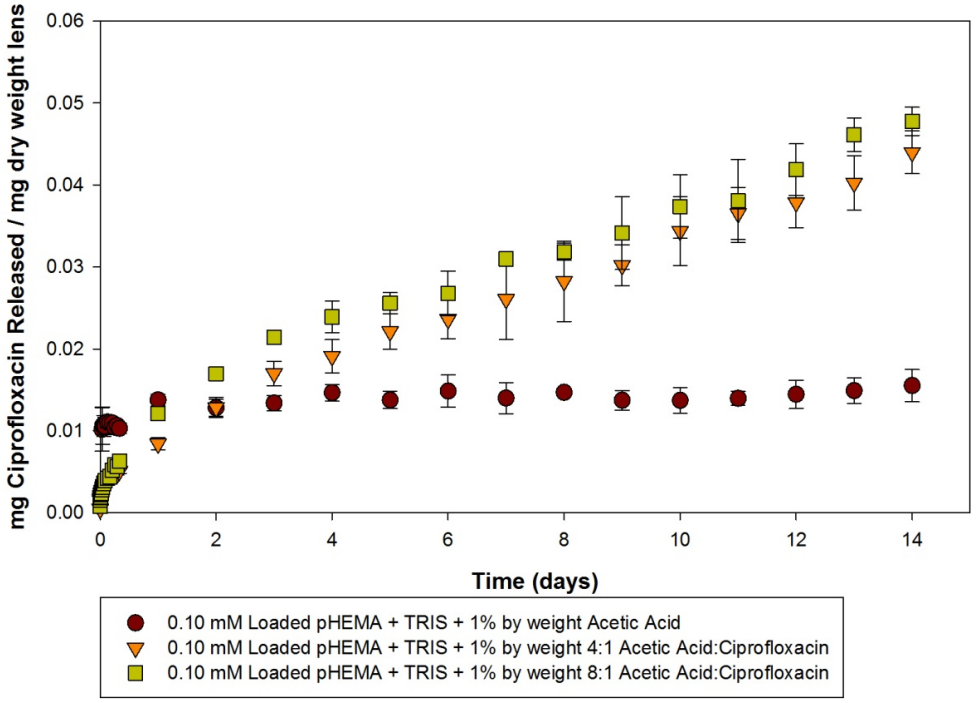
The release curves of the acetic acid imprinted materials and controls after 14 days of release is seen in Figure 4-2 (a–c). For model lenses loaded with 9.06 mM of ciprofloxacin, there was an overall statistically significantly greater amount of drug released by the imprinted materials compared to the control ($p < 0.05$), but there was no significant difference between the two imprinted materials ($p > 0.05$). The time to reach the plateau was also different; interestingly, the imprinted materials appeared to reach their plateaus within 4 or 5 days, while the statistics suggest that the control was releasing for up to 8 days. Unfortunately, there is a greater amount of variation in the determination of the concentration of ciprofloxacin within the solution when loading with such a high concentration, as dilutions are necessary to reach concentrations relevant to the linear portion of the standard curve, potentially confounding results. The effect of imprinting in comparison with the non-imprinted controls is most evident again when the materials are loaded with the lower concentration solutions (0.10 mM and 0.025 mM), as seen in Figure 4-2b and Figure 4-2c. Here, the imprinting demonstrates two key advantages over the non-imprinted control, with a longer release time and a greater amount of ciprofloxacin being released. For the 0.10 mM loaded materials, analysis suggests that a plateau level is reached in as little as 45 minutes for controls. In contrast, the 4:1 imprinted and 8:1 imprinted materials demonstrate continued significant release compared to earlier time points out to 10 days. Similar results are seen in model lenses loaded with 0.025 mM solutions. The control released so little that there was statistically no difference over the course of the 14 days compared to the initial time point, whereas the imprinted materials were releasing for up to 8 days. As can be clearly seen from the release curves, there was no statistically significant difference between the two ratios of acetic acid to ciprofloxacin in terms of the plateau amount of ciprofloxacin released, or the time to reach a plateau.

**Figure 4-2 (a-c)** **Release Curves from Acetic Acid Imprinted Materials over 14 Days**

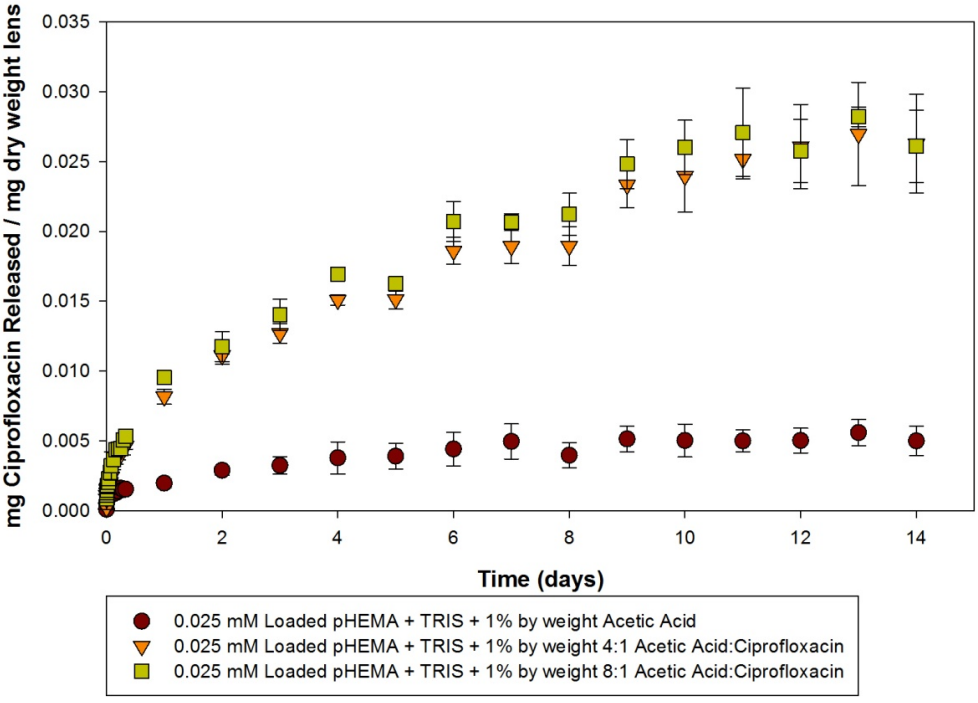
Release curves from acetic acid imprinted materials loaded with (a) 9.06 mM ciprofloxacin; (b) 0.10 mM ciprofloxacin and (c) 0.025 mM ciprofloxacin over 14 days. Values plotted are means ± standard deviations.




**(a)**


mg Ciprofloxacin Released / mg dry weight lens
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0
2
4
6
8
10
12
14
Time (days)
0.10 mM Loaded pHEMA + TRIS + 1% by weight Acetic Acid
0.10 mM Loaded pHEMA + TRIS + 1% by weight 4:1 Acetic Acid:Ciprofloxacin
0.10 mM Loaded pHEMA + TRIS + 1% by weight 8:1 Acetic Acid:Ciprofloxacin

(**b**)


mg Ciprofloxacin Released / mg dry weight lens
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
2
4
6
8
10
12
14
Time (days)
0.025 mM Loaded pHEMA + TRIS + 1% by weight Acetic Acid
0.025 mM Loaded pHEMA + TRIS + 1% by weight 4:1 Acetic Acid:Ciprofloxacin
0.025 mM Loaded pHEMA + TRIS + 1% by weight 8:1 Acetic Acid:Ciprofloxacin

(**c**)

The results from these initial attempts to create imprinted silicone hydrogel materials were very encouraging in that they achieved two separate goals. First, the effect of the imprinting was demonstrated when the model lenses were loaded with lower concentrations of the drug, as there was a clear difference between the imprinted and non-imprinted materials in their ability to deliver drugs for an extended period of time, as evidenced by drug release occurring for a period of 8 to 10 days (depending on the loading concentration). Second, we were able to confirm the delivery of relevant amounts of the antibiotic. When loaded with the clinical concentration of ciprofloxacin (9.06 mM), concentrations were achieved in the 2 mL reaction vial that were clinically relevant in achieving the minimum inhibitory concentration ($MIC_{90}$) of common ocular isolates [32]. Not surprisingly, when the loading concentration was decreased by approximately 100 times, the amount of drug released was less, and the $MIC_{90}$ only reached concentrations relevant to more susceptible bacteria. Finally, the pilot study failed to demonstrate any differences between the ratio of acetic acid to ciprofloxacin used to create the imprinting that has been demonstrated previously [2, 17, 18, 20, 22]. This was possibly due to the lack of precision in choosing to add the imprinting mixture on the basis of percentage weight rather than by molar concentration of the functional monomer, in relation to the number of moles of the other components of the polymerization as a whole.

### 4.3.2 Ciprofloxacin pHEMA-TRIS Materials with Acrylic Acid Functional Monomers

To further explore the effect of imprinting on the model silicone hydrogel materials, a second, larger study was conducted with a few key modifications to the imprinting process. The overall functional monomer concentration within the polymerization mix was varied between two concentrations (100 mM and 200 mM), and the functional monomer was changed to a related molecule, acrylic acid, which has had some success in the literature in terms of efficiently

creating imprinted cavities [20]. The same three loading concentrations were used, and three separate imprinted ratios of acrylic acid to ciprofloxacin were used: 4:1, 8:1 and 16:1. The dry weight (g) and the water content (%) of the created materials are listed in Table 4-2. Similar to the model materials, the majority of the model materials were of low water content, and some degree of modification would be necessary to increase the water content if these materials were to be used on the human eye. A one way ANOVA revealed a significant difference between the dry weights and water contents of the materials ($p < 0.05$). Post Hoc Tukey tests revealed that this difference was mainly confined to two model—the pHEMA+TRIS+ 200 mM Acrylic Acid, 8:1 ratio to ciprofloxacin and the pHEMA + TRIS + 200 mM Acrylic Acid, 4:1 ratio to ciprofloxacin were found to be statistically different than the other model lens materials ($p < 0.05$).

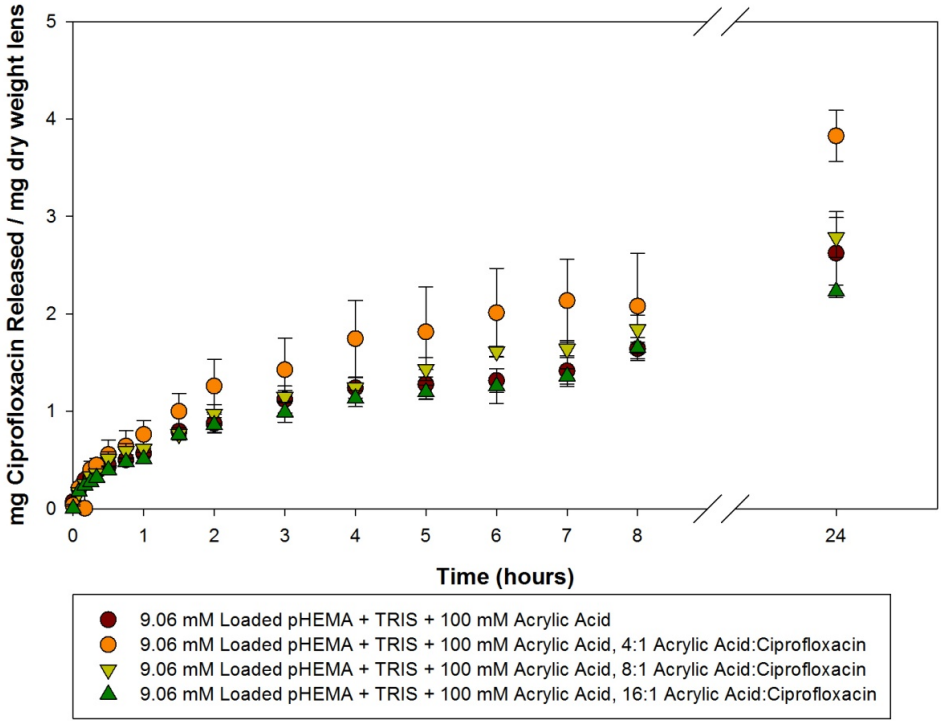
**Table 4-2** Dry Weight and Water Content of Acrylic Acid Imprinted pHEMA+TRIS Materials.

| Model Lens Type | Dry Weight (g) (Average ± Standard Deviation) | Water Content (%) (Average ± Standard Deviation) | Centre Thickness (mm) (Average ± Standard Deviation) | Volume ($mm^3$) (Average ± Standard Deviation) |
|---|---|---|---|---|
| pHEMA + TRIS + 100 mM Acrylic Acid Control | 0.0417 ± 0.0058 | 16.8 ± 4.1 | 0.96 ± 0.07 | 75.5 ± 6.0 |
| pHEMA + TRIS + 200 mM Acrylic Acid Control | 0.0454 ± 0.0064 | 15.1 ± 1.8 | 1.05 ± 0.16 | 82.2 ± 12.6 |
| pHEMA + TRIS + 100 mM Acrylic Acid, 4:1 ratio to ciprofloxacin | 0.035 ± 0.0076 | 16.2 ± 3.6 | 0.78 ± 0.16 | 60.87 ± 12.3 |
| pHEMA + TRIS + 200 mM Acrylic Acid, 4:1 ratio to ciprofloxacin | 0.0576 ± 0.011 | 12.6 ± 2.2 | 1.13 ± 0.3 | 88.3 ± 23.6 |
| pHEMA + TRIS + 100 mM Acrylic Acid, 8:1 ratio to ciprofloxacin | 0.0428 ± 0.0054 | 14.5 ± 2.1 | 1.01 ± 0.12 | 79.6 ± 9.3 |
| pHEMA + TRIS + 200 mM Acrylic Acid, 8:1 ratio to ciprofloxacin | 0.0397 ± 0.010 | 17.7 ± 3.6 | 0.97 ± 0.22 | 75.8 ± 17.4 |
| pHEMA + TRIS + 100 mM Acrylic Acid, 16:1 ratio to ciprofloxacin | 0.0497 ± 0.0053 | 14.3± 2.2 | 1.13 ± 0.13 | 88.6 ± 9.9 |
| pHEMA + TRIS + 200 mM Acrylic Acid, 16:1 ratio to ciprofloxacin | 0.0523 ± 0.0062 | 13.6 ± 1.4 | 1.20 ± 0.14 | 94.6 ± 10.9 |

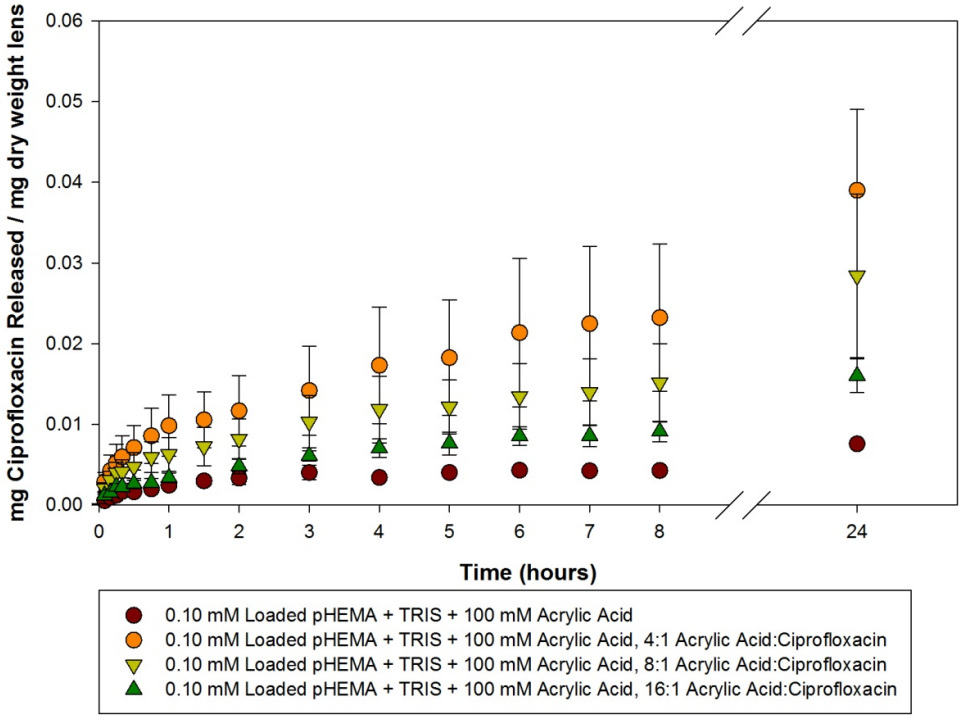
Ciprofloxacin release curves from 100 mM acrylic acid materials loaded with 9.06, 0.10 and 0.025 mM of ciprofloxacin within the first 24 h are detailed in Figure 4-3 (a–c). A similar trend to that seen with the acetic acid imprinted materials is seen, as there are little differences in the amount or the rate at which ciprofloxacin was released from the 9.06 mM loaded model lenses, but when the materials were loaded with progressively lower amounts of ciprofloxacin the difference between the imprinted and the non-imprinted control became more apparent, with the imprinted materials releasing relatively more and at a greater rate. The release curves from these materials over the course of two weeks are detailed in Figure 4-4(a–c). Analysis of the model lenses loaded with 9.06 mM ciprofloxacin (Figure 4-4a) showed that the control model lens was only releasing for a maximum of 3 days before reaching a plateau, while the imprinted materials were releasing for periods up to 7 days. At plateau, the materials with 4:1 imprinting were found to be statistically significantly higher than the other model lens types ($p < 0.05$). The other model lens types (including the control) tended to cluster together. Analysis of the 0.10 mM loaded materials showed no significant release compared to the initial time point for the control, and significant release from the imprinted materials for up to 14 days in the case of the 8:1 imprinted material. The 16:1 imprinted material was found to be different from the other two imprinted materials ($p < 0.05$), while releasing for 11 days. The 4:1 imprinted materials released the most drug, but for the shortest period of time, at only 5 days. For materials loaded with 0.025 mM ciprofloxacin, the results were similar but with more extended release times. The 4:1 and 8:1 model lenses tended to cluster together and release the most amount of drug, while the 16:1 was statistically significantly lower, but still higher than the control ($p < 0.05$). All of the imprinted materials in this case took 10 days to reach a plateau level.

**Figure 4-3 (a-c)** **Release Curves from 100 mM Acrylic Acid Imprinted Materials over 24 Hours**

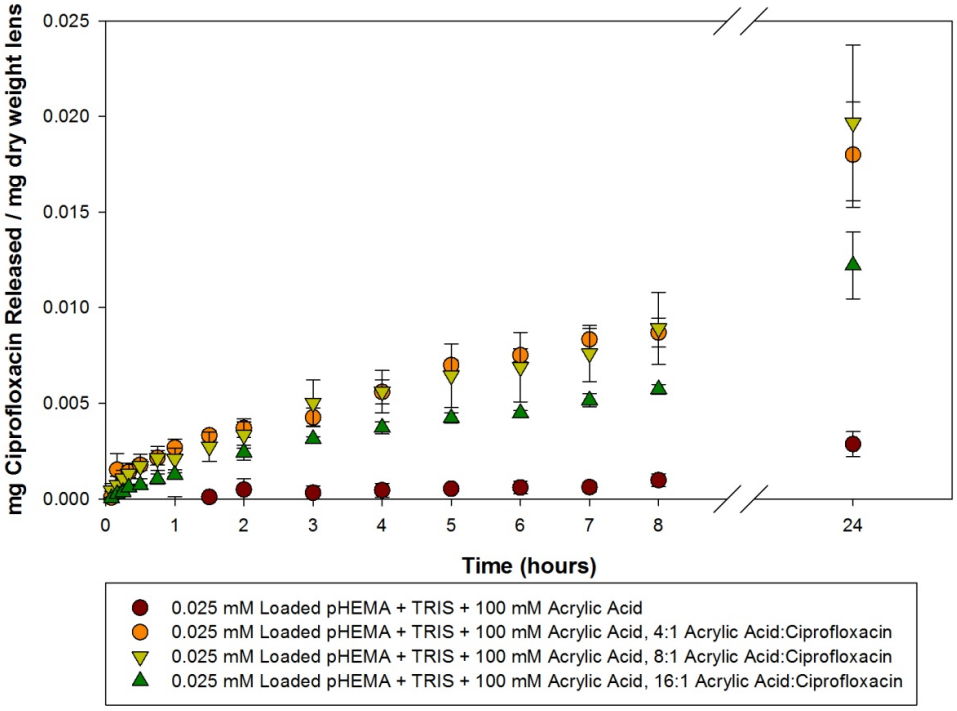
Release curves from 100 mM acrylic acid imprinted materials loaded with (a) 9.06 mM ciprofloxacin; (b) 0.10 mM ciprofloxacin and (c) 0.025 mM ciprofloxacin over 24 hours. Values plotted are means ± standard deviations.




(**a**)


mg Ciprofloxacin Released / mg dry weight lens
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0
1
2
3
4
5
6
7
8
24
Time (hours)
0.10 mM Loaded pHEMA + TRIS + 100 mM Acrylic Acid
0.10 mM Loaded pHEMA + TRIS + 100 mM Acrylic Acid, 4:1 Acrylic Acid:Ciprofloxacin
0.10 mM Loaded pHEMA + TRIS + 100 mM Acrylic Acid, 8:1 Acrylic Acid:Ciprofloxacin
0.10 mM Loaded pHEMA + TRIS + 100 mM Acrylic Acid, 16:1 Acrylic Acid:Ciprofloxacin

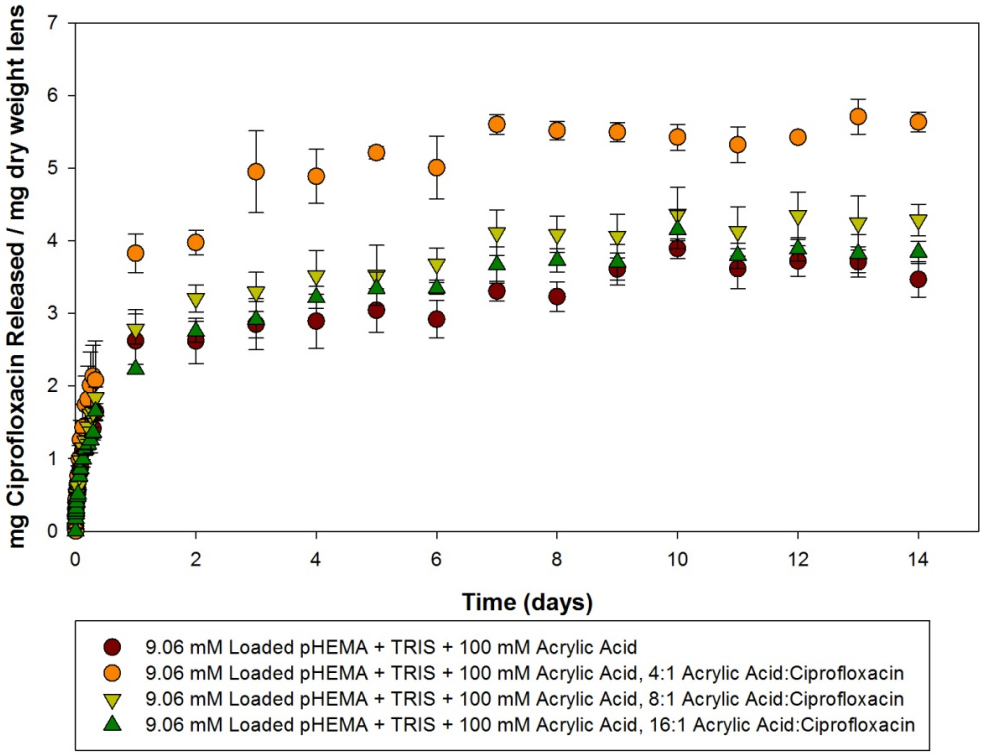
(**b**)


mg Ciprofloxacin Released / mg dry weight lens
0.025
0.020
0.015
0.010
0.005
0.000
0
1
2
3
4
5
6
7
8
24
Time (hours)
0.025 mM Loaded pHEMA + TRIS + 100 mM Acrylic Acid
0.025 mM Loaded pHEMA + TRIS + 100 mM Acrylic Acid, 4:1 Acrylic Acid:Ciprofloxacin
0.025 mM Loaded pHEMA + TRIS + 100 mM Acrylic Acid, 8:1 Acrylic Acid:Ciprofloxacin
0.025 mM Loaded pHEMA + TRIS + 100 mM Acrylic Acid, 16:1 Acrylic Acid:Ciprofloxacin

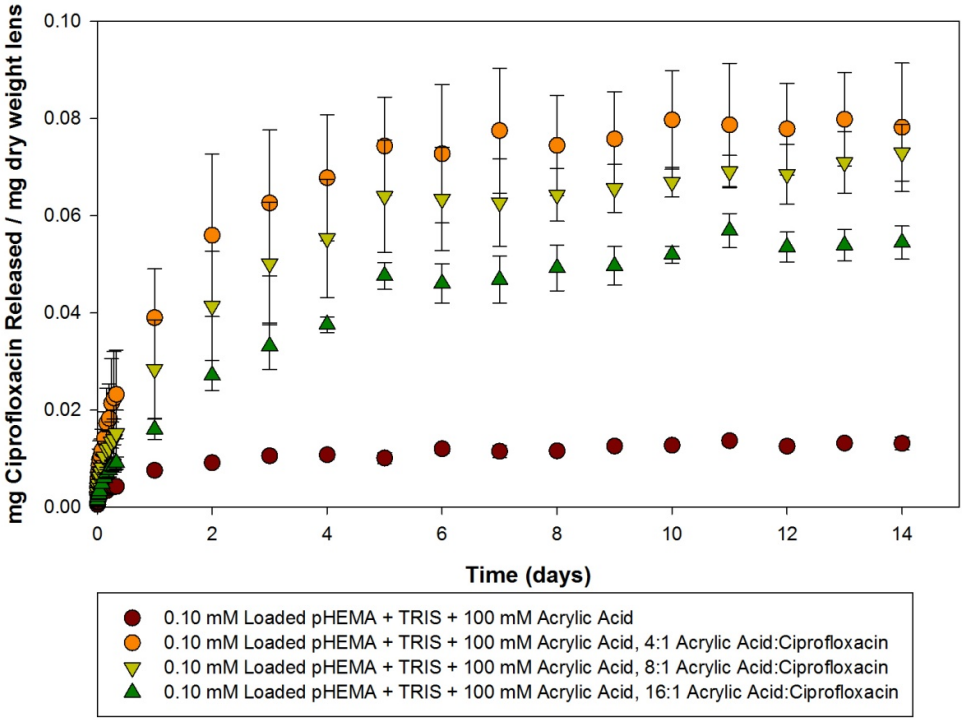
(**c**)

**Figure 4-4 (a-c)** **Release Curves from 100 mM Acrylic Acid Imprinted Materials over 14 Days**

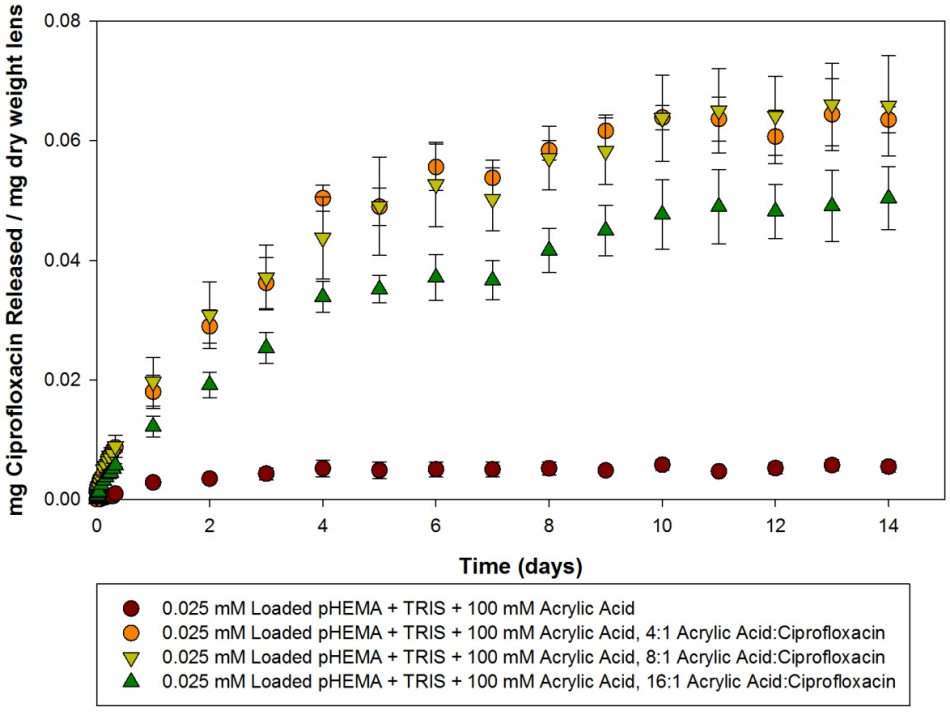
Release curves from 100 mM acrylic acid imprinted materials loaded with (**a**) 9.06 mM ciprofloxacin; (**b**) 0.10 mM ciprofloxacin and (**c**) 0.025 mM ciprofloxacin over 14 days. Values plotted are means ± standard deviations.




(**a**)


mg Ciprofloxacin Released / mg dry weight lens
0.10
0.08
0.06
0.04
0.02
0.00
0
2
4
6
8
10
12
14
Time (days)
0.10 mM Loaded pHEMA + TRIS + 100 mM Acrylic Acid
0.10 mM Loaded pHEMA + TRIS + 100 mM Acrylic Acid, 4:1 Acrylic Acid:Ciprofloxacin
0.10 mM Loaded pHEMA + TRIS + 100 mM Acrylic Acid, 8:1 Acrylic Acid:Ciprofloxacin
0.10 mM Loaded pHEMA + TRIS + 100 mM Acrylic Acid, 16:1 Acrylic Acid:Ciprofloxacin

(**b**)

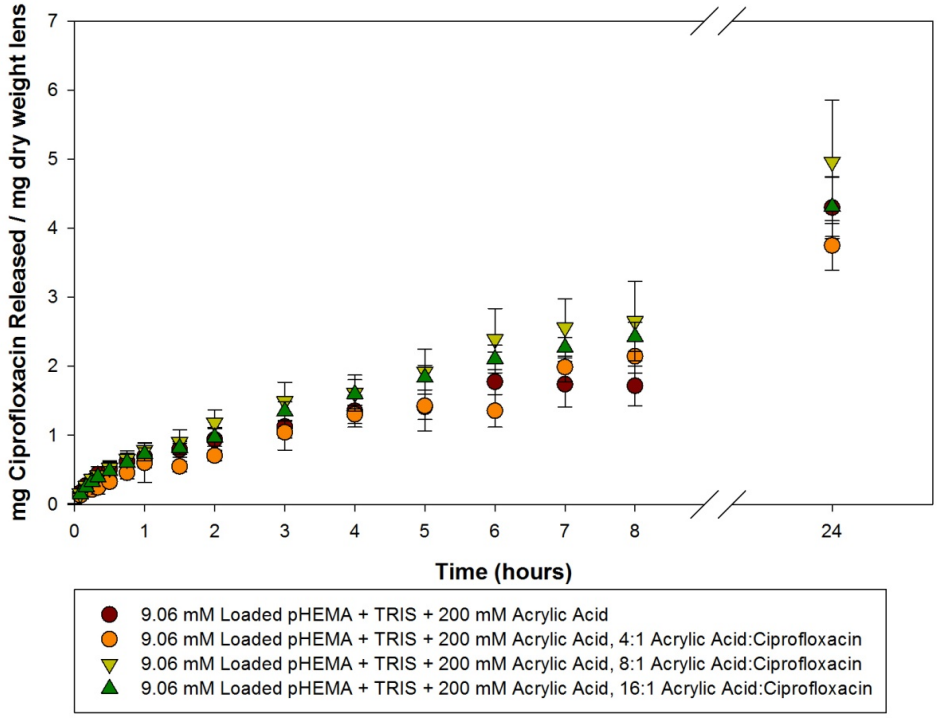

mg Ciprofloxacin Released / mg dry weight lens
0.08
0.06
0.04
0.02
0.00
0
2
4
6
8
10
12
14
Time (days)
0.025 mM Loaded pHEMA + TRIS + 100 mM Acrylic Acid
0.025 mM Loaded pHEMA + TRIS + 100 mM Acrylic Acid, 4:1 Acrylic Acid:Ciprofloxacin
0.025 mM Loaded pHEMA + TRIS + 100 mM Acrylic Acid, 8:1 Acrylic Acid:Ciprofloxacin
0.025 mM Loaded pHEMA + TRIS + 100 mM Acrylic Acid, 16:1 Acrylic Acid:Ciprofloxacin

(**c**)

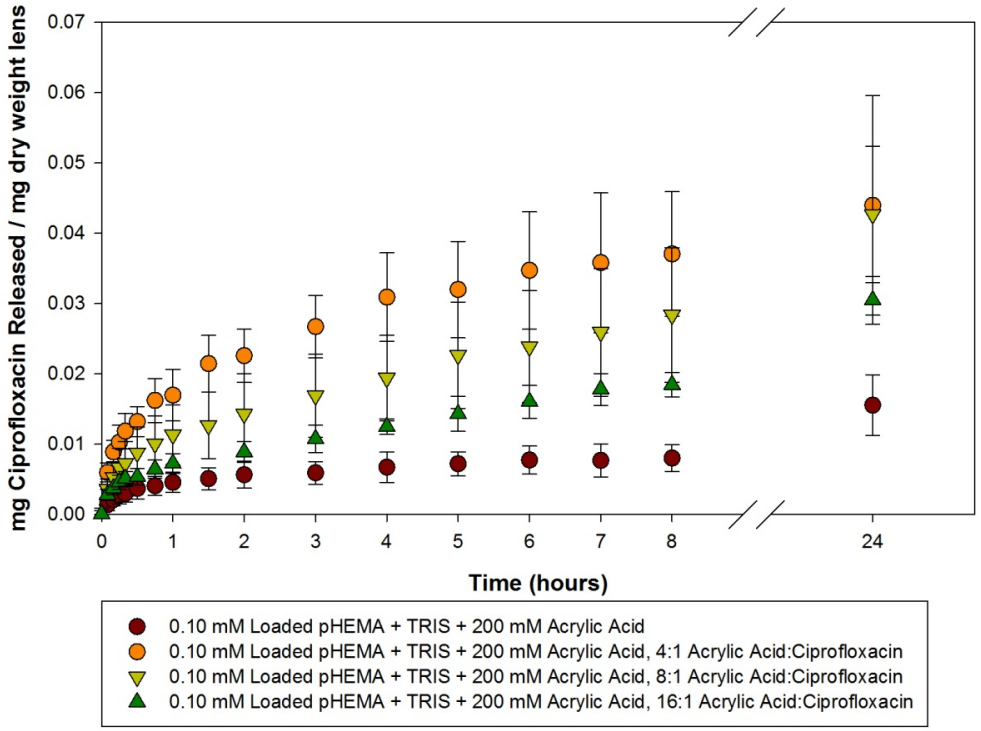
Ciprofloxacin release curves from 200 mM acrylic acid imprinted materials loaded with 9.06 mM, 0.10 mM and 0.025 mM ciprofloxacin solutions for the first 24 h is presented in Figure 4-5(a–c). The loading of the high concentration (9.06 mM) led to all materials releasing a significant amount of drug, but there was no difference between the imprinted materials and the control ($p > 0.05$) over the first 24 h. For the model lenses loaded with 0.10 mM and 0.025 mM, the imprinted materials released a larger amount and at a faster rate compared to the control ($p < 0.05$), but there was no difference between the imprinted materials, although it appeared that the 4:1 loaded materials released more than the 8:1, and the 16:1 imprinted material released the lowest amount.

**Figure 4-5 (a-c)** **Release Curves from 200 mM Acrylic Acid Imprinted Materials over 24 Hours**

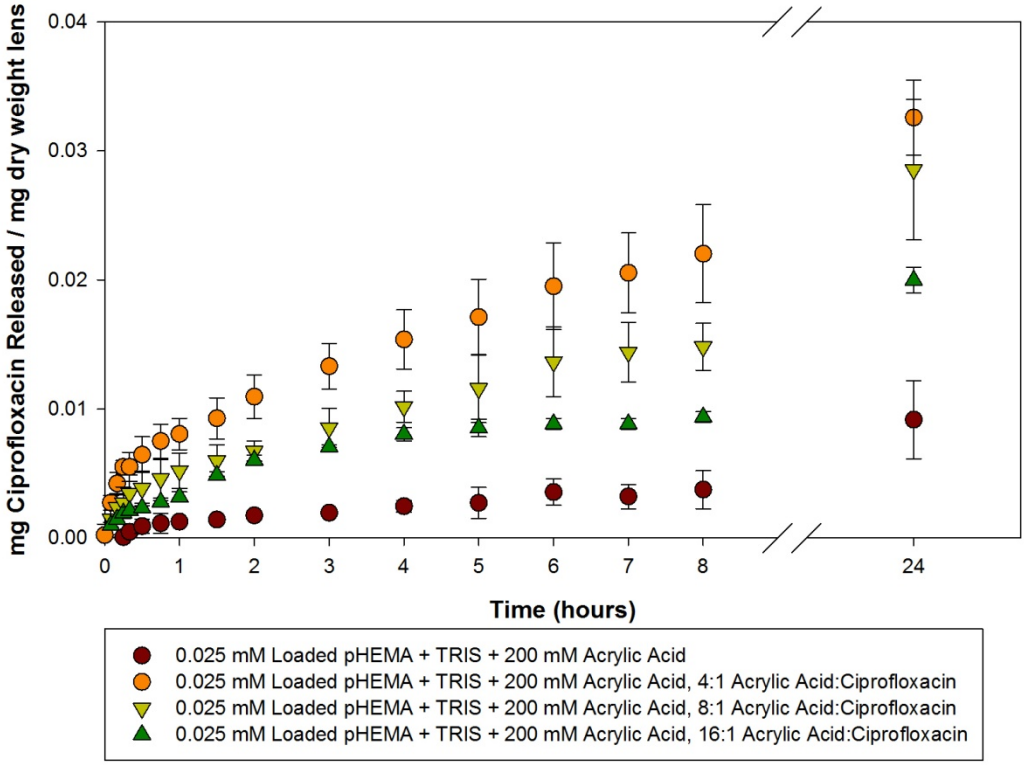
Release curves from 200 mM acrylic acid imprinted materials loaded with (a) 9.06 mM ciprofloxacin; (b) 0.10 mM ciprofloxacin and (c) 0.025 mM ciprofloxacin over 24 hours. Values plotted are means ± standard deviations.




(**a**)


mg Ciprofloxacin Released / mg dry weight lens
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0
1
2
3
4
5
6
7
8
24
Time (hours)
0.10 mM Loaded pHEMA + TRIS + 200 mM Acrylic Acid
0.10 mM Loaded pHEMA + TRIS + 200 mM Acrylic Acid, 4:1 Acrylic Acid:Ciprofloxacin
0.10 mM Loaded pHEMA + TRIS + 200 mM Acrylic Acid, 8:1 Acrylic Acid:Ciprofloxacin
0.10 mM Loaded pHEMA + TRIS + 200 mM Acrylic Acid, 16:1 Acrylic Acid:Ciprofloxacin

(**b**)

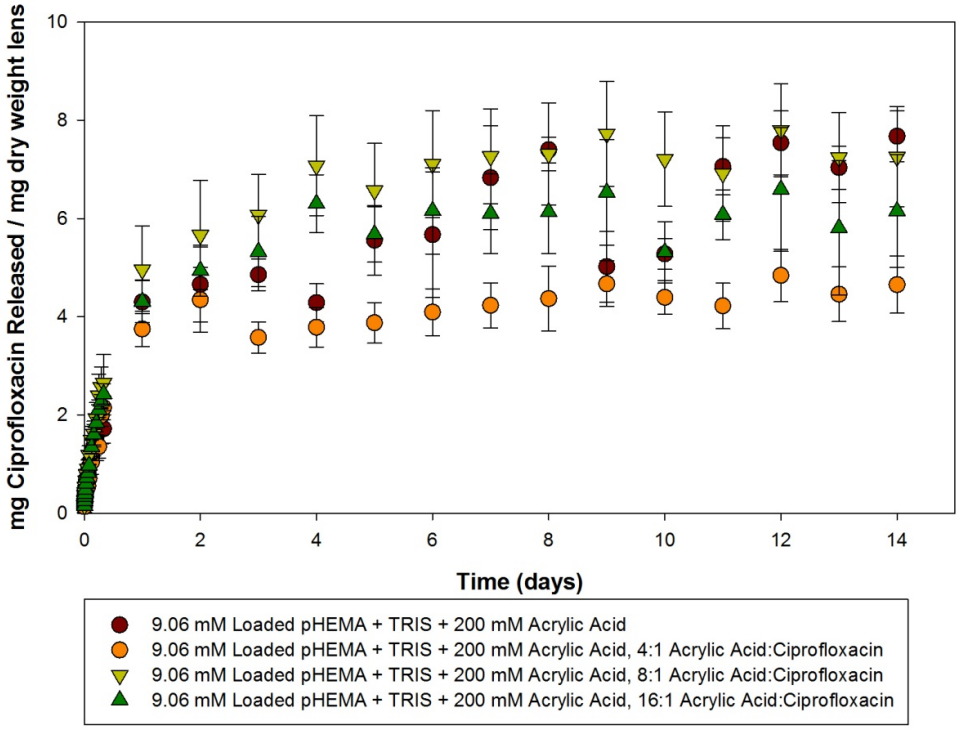

mg Ciprofloxacin Released / mg dry weight lens
0.04
0.03
0.02
0.01
0.00
0
1
2
3
4
5
6
7
8
24
Time (hours)
0.025 mM Loaded pHEMA + TRIS + 200 mM Acrylic Acid
0.025 mM Loaded pHEMA + TRIS + 200 mM Acrylic Acid, 4:1 Acrylic Acid:Ciprofloxacin
0.025 mM Loaded pHEMA + TRIS + 200 mM Acrylic Acid, 8:1 Acrylic Acid:Ciprofloxacin
0.025 mM Loaded pHEMA + TRIS + 200 mM Acrylic Acid, 16:1 Acrylic Acid:Ciprofloxacin

(**c**)

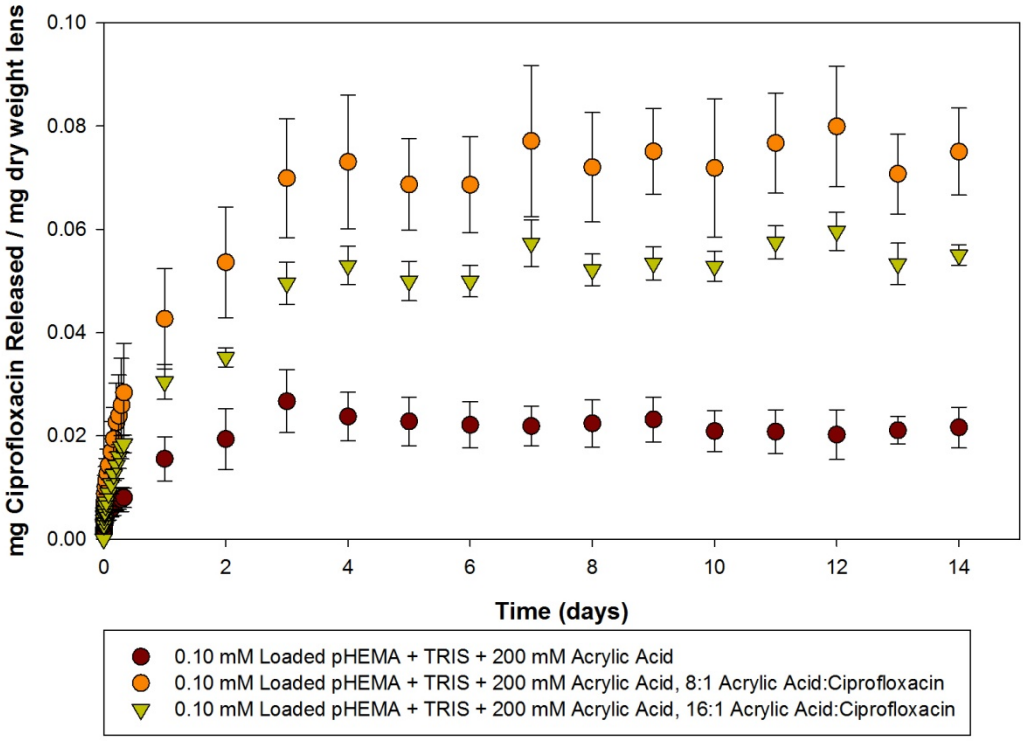
Ciprofloxacin release curves from 200 mM acrylic acid imprinted materials loaded with 9.06 mM, 0.10 mM and 0.025 mM ciprofloxacin solutions over the course of 14 days is presented in Figure 4-6 (a–c). The 9.06 mM loaded materials again showed a large amount of variation, and there was not statistically significant difference between the various imprinted materials versus the controls. The materials did release more than the required amount of antibiotic to be clinically relevant against common ocular pathogens. In the course of measurement over the two weeks, there was one anomalous group of readings. The 0.10 mm loaded, 4:1 imprinted materials began to show a declining concentration of ciprofloxacin within solution over time. Whether this was due to contamination, or drug degradation is unknown, regardless, the data is not presented here. Examination of the other 0.10 mM loaded materials shows that the imprinted materials released for up to 4 days, significantly different than the control ($p < 0.05$). The 0.025 mM loaded model lenses demonstrated significant differences between the 4:1 loaded and the other imprinted materials and the control, although the release time was relatively short at only 2 days. The 8:1 and 16:1 imprinted materials released comparatively less ciprofloxacin, but released it for longer periods of 13 and 14 days respectively. The control material loaded with 0.025 mM in comparison released relatively little ciprofloxacin over the course of 4 days, before no further changes were measured.

**Figure 4-6 (a–c)** **Release Curves from 200 mM Acrylic Acid Imprinted Materials over 14 Days**

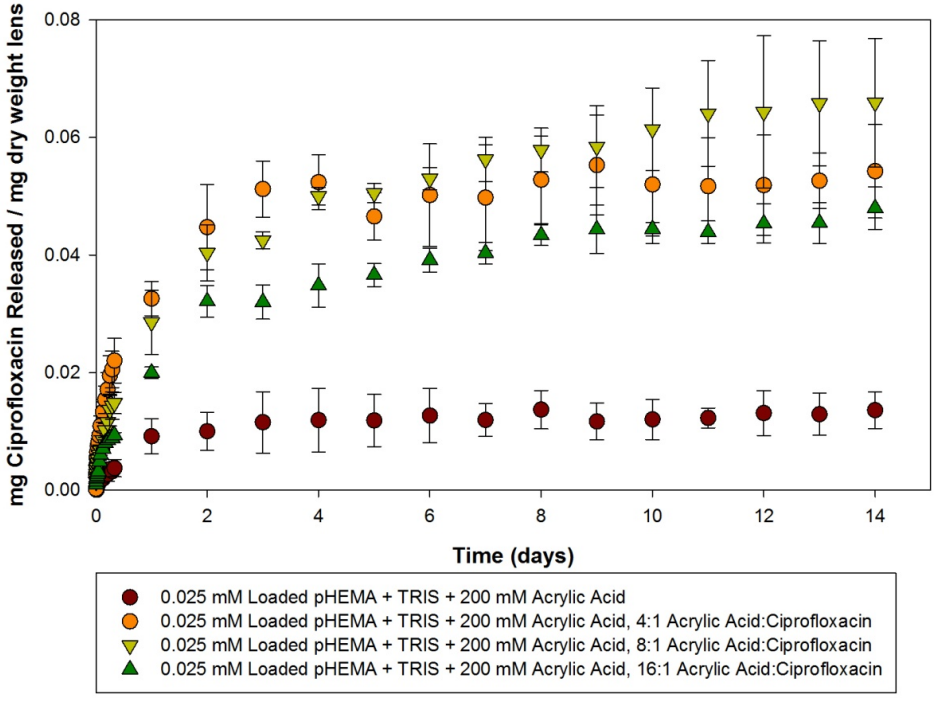
Release curves from 200 mM acrylic acid imprinted materials loaded with (**a**) 9.06 mM ciprofloxacin; (**b**) 0.10 mM ciprofloxacin and (**c**) 0.025 mM ciprofloxacin over 14 Days. Values plotted are means ± standard deviations.




(**a**)


mg Ciprofloxacin Released / mg dry weight lens
0.10
0.08
0.06
0.04
0.02
0.00
0
2
4
6
8
10
12
14
Time (days)
0.10 mM Loaded pHEMA + TRIS + 200 mM Acrylic Acid
0.10 mM Loaded pHEMA + TRIS + 200 mM Acrylic Acid, 8:1 Acrylic Acid:Ciprofloxacin
0.10 mM Loaded pHEMA + TRIS + 200 mM Acrylic Acid, 16:1 Acrylic Acid:Ciprofloxacin

(**b**)


mg Ciprofloxacin Released / mg dry weight lens
0.08
0.06
0.04
0.02
0.00
0
2
4
6
8
10
12
14
Time (days)
0.025 mM Loaded pHEMA + TRIS + 200 mM Acrylic Acid
0.025 mM Loaded pHEMA + TRIS + 200 mM Acrylic Acid, 4:1 Acrylic Acid:Ciprofloxacin
0.025 mM Loaded pHEMA + TRIS + 200 mM Acrylic Acid, 8:1 Acrylic Acid:Ciprofloxacin
0.025 mM Loaded pHEMA + TRIS + 200 mM Acrylic Acid, 16:1 Acrylic Acid:Ciprofloxacin

(**c**)

Thorough examination of the acrylic acid imprinted materials leads to several conclusions. The loading concentration of ciprofloxacin has a large role on the ability to detect the effect of the molecular imprinting. When the model lenses are loaded with a large concentration (9.06 mM), which is equivalent to the concentration of ciprofloxacin in commercially available 0.3% eye drops, there is little to no difference in the various imprinted materials and the controls. In this situation, it is likely that the majority of the ciprofloxacin was loaded into the material through non-specific concentration gradients, and the release from all the materials reflected that. One cannot discern the effect of the need for dilution to generate readings in the range of the linear standard curve as this could potentially affect the sensitivity to detect subtle changes in concentration within the solution, and may have contributed to the variability. However, this effect would be minimal.

When loaded with lower concentrations of ciprofloxacin, a different picture emerges from the data, in that the effect of imprinting these materials with template and the functional monomer become apparent. The imprinted materials release a larger amount compared to similarly loaded control materials, and for a significantly longer time. Release times for up to 14 days were seen in some cases, such as the 0.025 mM loaded, 200 mM acrylic acid 8:1 imprinted material, while control materials were confined to minimal release amounts for periods of only a few days. Interestingly, there was little to no difference between materials created with the two different concentrations of acrylic acid in terms of the amount or rate of ciprofloxacin being released. There has been some evidence in the literature that not only is the functional monomer:template ratio important, but so is the functional monomer:cross linker ratio [33]. In this experiment, there was no variation in the amount of crosslinker chosen, which was ethylene glycol dimethacrylate (EGDMA), so it would be interesting to see if the drug release rate

dependence on functional monomer to crosslinker ratio would prove to be important in this model silicone hydrogel-type system.

In comparison with the pilot study, the functional monomer was changed to acrylic acid, and the precision to which the imprinting process was performed was more carefully controlled. In doing so, greater differences in the imprinted materials were demonstrated, with materials imprinted with the 4:1 ratio in general releasing the greatest amount of drug, with decreasing release from 8:1 and 16:1 imprinted materials respectively. This is similar to the results that were seen in a previous paper imprinting norfloxacin, another fluoroquinolone antibiotic [20].

The majority of the model lenses released enough antibiotic to reach concentrations that were clinically relevant for common bacterial isolates, especially with model lenses loaded with the clinical concentration of ciprofloxacin [32]. The difficulty is that sustained release over time was really only observed when loading with much lower concentrations, which can pose a problem with antibiotic therapy in preventing the development of bacterial resistance. To combat this, future studies should use newer and more potent antibiotics, whose minimum inhibitory concentrations are much lower than ciprofloxacin, such as the fourth generation fluoroquinolones moxifloxacin and gatifloxacin [34]. The challenge for these contact lens combination devices, especially antibiotic ones, beyond the demonstrated ability to sustain drug release, is acceptance into clinical practice. Considering the perception of the role of contact lenses in the etiology of severe ocular infections, use of a contact lens in such a situation faces an uphill climb in acceptance, and it will be the challenge to researchers and companies marketing such products to demonstrate advantages of such a device over traditional therapy.

The results from this study were generated using what is commonly known as the "infinite sink" technique, in which the release of drug is into the same static solution over time.

This clearly does not necessarily mimic the ocular surface, in which tear production, evaporation and drainage can play a significant part in drug residence time and ultimately bioavailability to the cornea. The use of a static solution can also have a significant effect on release times for a drug such as ciprofloxacin, which is poorly soluble at physiological pH, potentially limiting release times due to the drug reaching a maximum soluble concentration within the solution. Several authors have proposed different solutions to this infinite sink problem. The simplest is to transfer the lenses to fresh solutions free of any drug at various time points, and sum up the release from all these release solutions [35]. A more sophisticated solution involves creation of an ocular tear flow device, in which the flow into, and drainage out of a tear solution as it interacts with the drug delivery device is controlled to mimic ocular tear flow. When such a system is used, authors have found that release rates are much slower than in infinite sink conditions, which is probably due to significantly smaller volumes of solution available to the device at any one given time. The release was also shown to follow zero order kinetics [23], and it would be interesting to test the materials created in this study under such conditions to observe any changes in release kinetics.

## 4.4 EXPERIMENTAL SECTION

### 4.4.1 MATERIALS

2-Hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), acrylic acid, acetic acid, and ciprofloxacin-HCl were purchased from Sigma-Aldrich (Oakville, ON, Canada). Methacryloxy propyl tris (trimethylsiloxy) silane (TRIS) was purchased from Gelest (Morrisville, PA, USA). IRGACURE was purchased from CIBA (Mississauga, ON, Canada). The HEMA and TRIS monomers were purified of the polymerizer inhibitor 4-

methoxyphenol (MEHQ) by passing through an Aldrich inhibitor removers (Sigma-Aldrich). All other materials were not modified and used as obtained.

### 4.4.2 Model Silicone Hydrogels

Model silicone hydrogel materials were created using a UV induced polymerization process. 3.6 g of HEMA was mixed with 0.4 g of TRIS. 0.2 g of EGDMA was subsequently added, allowed to mix, and finally 0.02 g of the photoinitiator IRGACURE was added. The mixture was poured into aluminum foil molds, and cured in a UV chamber (CureZone 2 Control-cure) for 20 minutes at 340 nm. The surfaces were then placed in a 50 °C oven overnight to ensure completion of polymerization. Samples were then placed in Milli-Q water for a minimum of two days to rehydrate, with the water being changed daily to remove any unreacted monomers [36].

### 4.4.3 Molecular Imprinted Materials—Acetic Acid Functional Monomer

Acetic Acid imprinted materials were created using a similar process to the model silicone hydrogels. To each polymerization mix before the addition of the IRGACURE initiator, acetic acid solution with various amounts of ciprofloxacin dissolved within it were added to the reaction mixture, creating an approximate 0.01 M acetic acid concentration in the final polymerization mixture. Control materials had a solution of acetic acid added without any ciprofloxacin.

### 4.4.4 Molecular Imprinted Materials—Acrylic Acid Functional Monomer

The imprinting of acrylic acid materials was more carefully controlled to determine the effect of the imprinting on the drug release characteristics of the technique. To that end, materials were

created using similar procedures to the model silicone hydrogels. Before the addition of the IRGACURE initiator, acrylic acid was added to a final concentration of either 100 mM or 200 mM. Ciprofloxacin powder was subsequently added to the mixture, in molar ratios to the acrylic acid varying from 1:4 to 1:16, and the polymerization of the materials was initiated as previous.

### 4.4.5 Molecular Imprinted Materials—Washout

Materials imprinted with ciprofloxacin were rehydrated in Milli-Q water in glass jars, with the water being changed daily. The water used in the washout period was measured for ciprofloxacin concentration, and materials were only used after ciprofloxacin concentrations within the water were at minimal/non-existent levels.

### 4.4.6 Drug Solutions

A 0.3% (w/v) (9.06 mM) stock solution of ciprofloxacin-HCl was created in a phosphate buffered saline. The pH of the solution was adjusted to 4.0 to ensure the complete solubilization of the ciprofloxacin at this high concentration. Using this stock solution, samples were diluted approximately 4,000 times to be read by a Hitachi F-4500 fluorescence spectrophotometer (Hitachi Ltd., Tokyo, Japan), with an excitation wavelength of 274 nm and an emission peak at 419 nm to create a linear standard curve. This standard curve was used to correlate emission amounts with the concentration of ciprofloxacin within the solution.

### 4.4.7 Water Content, Centre Thickness, Volume and Dry Weight Determination

After soaking in Milli-Q water for a minimum of two days, discs of the materials were punched out using a #4 cork borer with a diameter of 5 mm. The water content of these discs was determined using the gravimetric method, using the Sartorius MV 100 (Sartorius Mechatronics Canada, Mississauga, ON, Canada). The dry weight of the disc was also determined. The centre

thickness was determined using a dial lens gauge for rigid contact lenses (Vigor Optical, Carlstadt, NJ, USA), and the volume was calculated from thickness and diameter data, assuming a cylindrical shape.

### 4.4.8 Drug Loading into Materials

After determination of the water content, discs were placed in a ciprofloxacin drug loading solution. Three separate concentrations were used—the stock 9.06 mM, and two diluted loading concentrations, 0.10 mM and 0.025 mM. 2 mL of the loading solution was used, and this was undertaken in amber vials, as ciprofloxacin is light sensitive. Loading discs were left at room temperature for one week.

### 4.4.9 Drug Release Kinetics

Loaded discs were removed from the loading solution amber vials using plastic tweezers. The surface was partially dried on lens paper to remove any excess loading solution, and the disc placed into another amber vial containing 2 mL of an artificial tear solution (NaCl 90 mM, KCl 16 mM, $Na_2CO_3$ 12 mM, $KHCO_3$ 3 mM, $CaCl_2$ 0.5 mM, $Na_3$Citrate 1.5 mM, Glucose 0.2 mM, Urea 1.2 mM, $Na_2HPO_4$ 24 mM, HCl 26 mM, pH 7.4) [37]. The vials were then placed in a shaking water bath at 34 °C. At various time points, the concentration of ciprofloxacin in the solution was determined using spectrophotometry. For model lenses loaded with 9.06 mM ciprofloxacin solution, samples were removed and diluted 100X to get into the range of the standard curve. For the other two loading conditions, 1 mL of the release solution was removed from the vial, read in the spectrophotometer, and returned to the vial. Readings were taken every 5 minutes for the first 20 minutes, then after 30, 45, 60 and 90 min. Readings were then taken hourly until 8 hours had passed, then daily until 14 days had passed.

### 4.4.10 Statistical Analysis

Statistical analysis was performed using Statistica version 8 (StatSoft Inc, Tulsa, OK) using a repeated measures ANOVA, and post hoc Tukey tests as indicated. A p value of less than 0.05 was considered statistically significant.

## 4.5 Conclusions

In this study, model silicone hydrogels for the delivery of the antibiotic ciprofloxacin were developed using a molecular imprinting strategy. Synthesized materials had water contents in the mid to low teens, and when loaded with various solutions of ciprofloxacin they demonstrated different release kinetics. Loading with high concentrations of ciprofloxacin led to very few differences in the various imprinted materials and the control. When loaded with lower concentrations, the effect of the imprinting was more clearly seen, with model lenses created using a 4:1 ratio of acrylic acid to ciprofloxacin template consistently releasing the greatest amount of drug, and certain model lenses continuing to release the drug for up to 14 days. As the use of these contact lens combination devices will likely involve some element of overnight or extended wear, the results from this study using model silicone hydrogel materials has provided some insight into how these materials behave as drug delivery devices when formed using molecular imprinting.

## 4.6 Acknowledgments

The authors would like to thank Lakshman Subbaraman for his help in making the materials and manuscript editing. AH is supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada, the Canadian Optometric Education Trust Fund (COETF) and a Vistakon® Research Grant and Ezell Fellowship, both administered by the American Optometric

Foundation (AOF). This study is also supported by the NSERC 20/20 Network for the Development of Advanced Ophthalmic Materials.

In this next chapter, the results from the previous two chapters were combined to generate contact lenses using the molecular imprinting strategy to increase the release times of ciprofloxacin. These materials were evaluated for their contact lens properties, *in vitro* ciprofloxacin release rates, *in vitro* antimicrobial activity, and *in vivo* antimicrobial activity in a rabbit model of microbial keratitis.

# CHAPTER 5 – *IN VITRO* AND *IN VIVO* EVALUATION OF NOVEL CIPROFLOXACIN RELEASING SILICONE HYDROGEL CONTACT LENSES.

Alex Hui, OD[1], Mark Willcox, PhD[2], Lyndon Jones, PhD[1]

[1]Centre for Contact Lens Research, School of Optometry & Vision Science, University of Waterloo, Waterloo, Ontario, Canada

[2]School of Optometry and Vision Science, University of New South Wales, Sydney, New South Wales, Australia

This chapter has been accepted for publication in *Investigative Ophthalmology and Vision Science* as of June 27, 2014

| Author | Concept/Design | Acquisition of Data | Analysis | Write Up/ Publication |
|---|---|---|---|---|
| Hui | Y | Y | Y | Y |
| Willcox | Y | | | Y |
| Jones | Y | | | Y |

## 5.1 OVERVIEW

### 5.1.1 PURPOSE

The purpose of this study was to evaluate molecular imprinted ciprofloxacin releasing silicone hydrogel contact lens materials *in vitro* and *in vivo* for the treatment of microbial keratitis.

### 5.1.2 METHODS

Model silicone hydrogel contact lens materials were manufactured using a molecular imprinting technique to modify the release kinetics of the antibiotic ciprofloxacin. The light transmission, wet and dry weight, centre thickness, water content and surface wettability were determined, and the *in vitro* ciprofloxacin release kinetics elucidated using fluorescence spectrophotometry. The materials were then evaluated for their ability to inhibit *P. aeruginosa* strain 6294 growth *in vitro* and in an *in vivo* rabbit model of microbial keratitis.

### 5.1.3 RESULTS

The novel contact lenses synthesized had similar material properties to commercial contact lens materials. There was a decrease in light transmission in the shorter wavelengths due to incorporation of the antibiotic, but over 80% light transmission between 400 and 700 nm. The modified materials released a statistically significantly larger amount of antibiotic and for a longer period of time when evaluated *in vitro* compared to unmodified controls ($p<0.05$), with the modified materials releasing for more than eight hours compared to only two hours for the control. When tested *in vivo*, there was no statistically significant difference between the number of colony forming units (CFU) recovered from corneas treated with eye drops and those treated with one of two modified contact lenses ($p>0.05$), which is significantly less than corneas treated with unmodified control lenses or those which received no treatment at all ($p<0.05$).

### 5.1.4 CONCLUSIONS

These novel, molecular imprinted contact lens materials developed for the extended release of ciprofloxacin may be beneficial to supplement or augment future treatments of sight threatening microbial keratitis.

Keywords: Contact Lens, Microbial Keratitis, Drug Delivery, Molecular Imprinting, Ciprofloxacin

## 5.2 INTRODUCTION

Microbial keratitis (MK), an infection of the cornea by pathogenic microorganisms, represents a true ocular emergency. Unless immediate treatment is initiated with appropriate antimicrobial agents, the probability of retaining normal vision is unlikely. [1] Epidemiological studies have identified certain risk factors for the development of MK, including male gender, younger age, overnight wear of contact lenses, smoking, poor hygiene and internet supply of lenses. [2-4] Unfortunately, even with all of the advances in our understanding of MK and implementation of solutions to lower modifiable risk, the incidence of the disease has remained largely unchanged. [5] Contemporary treatment of patients with MK involves the frequent use of topical antibiotic agents, often fortified by a compounding pharmacy. [6] In the early stages of treatment, drop instillation as frequently as every 15 minutes is common to quickly saturate the cornea to therapeutic antibiotic levels. Even with these frequent dosing schedules, practical considerations on the pharmacokinetics of eye drops suggests that the therapeutic windows are only reached for relatively short periods of time, interspersed between times of therapeutic overdose and underdose. [7] Indeed, measurements and modeling suggest that at most only between 5 and 10% of an instilled eye drop ultimately exerts therapeutic action, with the remainder flushed away and absorbed systemically. [8] This is disadvantageous both economically

and therapeutically, as useful molecules are lost without exerting a therapeutic effect, and systemically absorbed agents have the potential to cause side effects. Adherence to strict and frequent treatment regimens for the management of MK is understandably difficult for outpatients, thus management often requires hospitalization, costing both the heath care system and the individual significant amounts of time and money. [9] Economic analysis of the MK costs in Australia suggest that each case costs upwards of AUD $10,000. [10] Given these therapeutic, practical and economic challenges, development of alternative MK therapies are warranted and may prove to be beneficial.

Even though contact lenses (CLs) are risk factors for developing MK, the use of CLs as vehicles to deliver therapeutics to the eye has been suggested and is not a recent idea. Utilizing hydrogels such as CLs as a reservoir for a drug during the treatment of anterior segment disease was proposed as early as 1965 by Sedlacek, and has received renewed research interest of late. [11, 12] The appeals of a CL drug delivery device are numerous. The materials have a proven track record of biocompatibility and patient and practitioner acceptance. [13] Modern manufacturing methods have driven the unit cost of each lens to affordable levels. If CLs are used in a drug delivery application, they can also simultaneously correct for refractive error, allowing for continued clarity of vision by the patient undergoing treatment. The oxygen permeability of the lenses have also increased significantly with the introduction of silicone hydrogel materials in the late 1990s, allowing for potentially extended or overnight treatments with CLs without fear of hypoxic complications. [14] There also exists evidence that combination CL and drug delivery devices would be well accepted by eye care practitioners. In surveys of practicing optometrists and ophthalmologists in North America, a large proportion of practitioners surveyed utilized CLs as bandages when indicated, and crucially, concurrently also prescribed topical medications such

as antibiotics and anti-inflammatories, and would be willing to accept a lens that did both simultaneously. [15]

The contemporary challenge in the development of a successful drug delivery contact lens has been the drug release kinetics. Not surprisingly, off the shelf commercial contact lens materials show less than ideal drug release characteristics. The majority of lenses examined showed very rapid release kinetics when tested *in vitro*. Antibiotics (ciprofloxacin), [16] anti-inflammatories (ketorolac, dexamethasone) [17, 18] and anti-allergy agents (ketotifen fumarate) [19] have all been tested, and while differences in the absolute amount of the drug being released between commercial lens types are seen, the release time is typically limited to one or two hours. Given this restriction, the focus of research has centred on modifying, extending and controlling release times. Numerous techniques have been investigated. For example, a group has investigated modification of commercial materials through the incorporation of a Vitamin E coating, to serve as an additional diffusion barrier for drug migration. This technique allowed for extension of release times from several minutes to several hours *in vitro*, and has been used to investigate release of timolol, an anti-glaucoma treatment. [20] The authors were able to demonstrate improved intraocular pressure control using the experimental contact lens system in comparison with eye drops in a glaucomatous dog model. [21] A novel design involving a drug impregnated film sandwiched between two hydrogel pieces has also been investigated for delivery of antibiotics and antifungals. [22, 23] Use of such a system showed a significant increase in the amount of drug released and favorable release kinetics *in vitro*. Unfortunately, the design was limited by the optical properties of the lens, as the film used as the drug reservoir was opaque, necessitating that the lens require a small, 3mm pupil cut in the middle of the film to be used for vision, a design that is unlikely to resonate with eye care practitioners or patients.

Molecular imprinting is a strategy that has been derived from work in chromatography. Originally, polymers created by this technique were used to preferentially remove certain components from solutions. [24] In this technique, the molecule of interest to ultimately be released is dissolved in the pre polymerization mixture. [25] Inclusion of a separate small molecule, denoted as the functional monomer, to specifically interact with the molecule of interest through non covalent interactions such as hydrogen bonding creates shape specific and functional group specific complexes deemed "cavities" or "molecular memory" within the final polymerized product. [26] This "molecular memory" can significantly slow the movement of the drug of interest from the material, thus extending drug release times. [12] Previous work has demonstrated that selection of the appropriate functional monomer and the ratio of the functional monomer to the template, are the most crucial aspects in generating materials with desired extended drug release properties. [27] This technique has been used to successfully increase the drug release times observed *in vitro* for antibiotics, anti-inflammatories, anti-glaucoma and anti-allergy medications. [28-35]

In this current study, novel silicone hydrogel contact lenses were created using a molecular imprinting technique to increase the release times of the fluoroquinolone antibiotic ciprofloxacin. The molecule acrylic acid had previously been shown to be a useful functional monomer to increase fluoroquinolone release times. [28, 34] The materials were tested for their contact lens properties, *in vitro* drug release characteristics, and sustained antibacterial activity in an *in vivo* rabbit model of microbial keratitis.

## 5.3 MATERIALS AND METHODS

### 5.3.1 REAGENTS

2-hydroxylethlymethacrylate (HEMA), Methacryloxy propyl tris (trimethylsiloxy) silane (TRIS), ethylene glycol dimethacrylate (EGDMA), acrylic acid, ciprofloxacin-HCL, Irgacure-1173, polyvinylpyrrolidone (PVP), phosphate buffered saline (PBS) and chloroform were purchased from Sigma-Aldrich (Oakville, ON, Canada). Nutrient Agar was purchased from Sigma-Aldrich PTY Australia. BBL cation adjusted Mueller-Hinton II Broth and Dey/Engley Neutralizing Broth were purchased from BD Australia. Polypropylene contact lens moulds were kindly donated from Alcon Vision Care (formerly CIBA Vision – Fort Worth, Texas). The polymerizer inhibitor 4-methoxyphenol (MEHQ) was removed from the HEMA and TRIS monomers by passing through a column of Aldrich inhibitor removers. All other reagents were used as received.

### 5.3.2 MOLECULAR IMPRINTED CONTACT LENS SYNTHESIS

3.6 g of filtered HEMA was mixed with 0.4 g of filtered TRIS, 0.1 g of EGDMA and 0.3 g of PVP. To this, a 1 mL acrylic acid and ciprofloxacin solution dissolved in chloroform was added so that the final concentration of acrylic acid within the mixture was 100 mM. Control lenses were created by omitting the ciprofloxacin in the acrylic acid solution. Various ratios of acrylic acid to ciprofloxacin solutions were made, ranging from 4:1 moles of acrylic acid:ciprofloxacin, 8:1 moles acrylic acid:ciprofloxacin and 16:1 moles acrylic acid: ciprofloxacin (hereby denoted as lens "4:1 Imprinted", "8:1 Imprinted" and "16:1 Imprinted" respectively). 1 mL of isopropanol was added as a diluent, and 0.04 g of the photoinitiator Irgacure 1173 added and the solution mixed for five minutes at room temperature. 100 μL of the solution was injected into plastic moulds, and cured for five minutes using a UV oven (Dymax

Silver EC Series UV Light Curing Flood Lamp System, Ellsworth Adhesives Canada, Stoney Creek, Ontario). The cured lenses were removed from the moulds, and lenses rinsed daily with acetate buffer (pH 4.0) until no ciprofloxacin could be detected by spectrophotometry. The lenses were then soaked in isopropanol for one day to remove any leftover monomers, before being rinsed and stored in PBS.

### *5.3.3* Determination of Material Properties – Water Content, Wet and Dry Weight, Light Transmission, Centre Thickness, Surface Wettability

The water content and wet and dry weight of lenses was determined using the gravimetric method (Sartorius MA 100, Sartorius Canada Inc, Mississauga, Ontario), where the change in weight as the lens was heated to 105°C over the course of 7 minutes was correlated to the water content of the lens. The centre thickness of a fully hydrated lens was measured using a contact lens thickness gauge (Vigor Contact Lens Thickness Gauge, Vigor Optical, Carlstadt, New Jersey). To determine the light transmission, individual lenses and 1 mL of PBS were placed into wells of a 24 well plate, and a wavelength scan from 300 nm to 750 nm was conducted using a plate reader (Spectramax M5 Microplate reader, Molecular Devices, Sunnyvale, California). The advancing contact angle, a measure of the surface wettability, was determined using the sessile drop method employing the Optical Contact Analyzer (OCA, Dataphysics Instruments GmbH, Filderstadt, Germany). A fully hydrated lens was removed from the PBS soaking solution, and the surface dried on lens paper for 20 seconds before being placed on a custom designed lens holder. 5 μL of High Performance Liquid Chromatography (HPLC) water was dispensed from a syringe, and an image of the contact of the water droplet with the lens surface after settling captured using a high speed camera. [36, 37] The contact angle between the settled drop and the lens surface was analyzed using custom software (SCA 20 software, Version 2.04, Build 4).

### 5.3.4 *IN VITRO* TESTING OF CIPROFLOXACIN RELEASE

Prepared lenses were removed from PBS and placed into 4 mL of a 0.3% (3000 μg/mL) ciprofloxacin solution prepared in acetate buffer (pH 4.0). The lenses were autoclaved, and allowed to take up ciprofloxacin from the solution for one week. After one week, the amount of ciprofloxacin loaded into the lenses was determined using fluorescence spectrophotometry in comparison to previously generated standard curves (excitation wavelength 274 nm, emission 419 nm). The lenses were then removed and the surface briefly dried on Lens Paper (VWR Scientific Products, Westchester, Pennsylvania) before being placed into 2 mL of PBS. 100 μL of PBS was removed at set intervals over the course of 24 hours, and the concentration of ciprofloxacin determined by spectrophotometry. After 24 hours, the lenses were removed, the surface briefly dried on lens paper, and placed into a second vial with 2 mL of fresh PBS, and the time course release was again monitored for another 24 hours. This process was repeated one additional time for a third day to generate release curves.

### 5.3.5 BACTERIAL STRAIN AND GROWTH, MINIMUM INHIBITORY CONCENTRATION DETERMINATION

*Pseudomonas aeruginosa* strain 6294, a bacterial strain previously isolated in the USA from a human case of microbial keratitis, [38] was streaked on nutrient agar plates from -80°C frozen stocks and incubated at 34°C for 18 hours. A single colony was picked and grown overnight in Mueller-Hinton Broth before being centrifuged, rinsed in PBS and re-suspended in PBS to an optical density of 0.1 at 660 nm (approximately $1x10^8$ colony forming units (CFU)/mL). The Minimum Inhibitory Concentration (MIC) of the test organism was determined using the broth microdilution method. [39] $5x10^4$ CFU of *Pseudomonas aeruginosa* strain 6294 was added to each well of a 96 well plate, with each well containing a doubling dilution concentration of ciprofloxacin in Mueller-Hinton Broth. The plate was incubated overnight, and

the turbidity of the solution in individual wells used to determine the minimum concentration of the antibiotic that prevents bacterial growth.

### 5.3.6 *IN VITRO* TESTING OF ANTIMICROBIAL ACTIVITY

Test lenses were removed from the loading solution and briefly dipped in PBS before being added to 2 mL of Mueller-Hinton Broth seeded with $1x10^8$ CFU/mL *P. aeruginosa*. 100 µL was sampled hourly into Neutralizing Broth, and serial dilutions plated on nutrient agar plates. The plates were incubated at 34°C for 18 hours before counting for CFU. The lenses were removed from solution after 24 and 48 hours, briefly dipped in PBS and placed into fresh Mueller-Hinton bacterial solutions, and the procedure repeated.

### 5.3.7 *IN VIVO* TESTING OF ANTIMICROBIAL ACTIVITY – RABBIT SCRATCH MODEL OF MICROBIAL KERATITIS

All animal procedures were approved by the executive of the animal care and ethics committee at the University of New South Wales, and performed in accordance with the ARVO statement for the use of animals in ophthalmic and vision research. 4 kg New Zealand White rabbits were sourced from S&J Hurrell in Sydney, New South Wales, Australia. After acclimatization for one week, the nictitating membrane was surgically removed from both eyes under general anaesthesia. Recovery was allowed for a minimum of one week, at which time two 5 mm central corneal scratches on one eye were induced using a 23 gauge needle under general anesthesia, and 20 µL of the *P. aeruginosa* strain 6294 solution placed on the eye (approximately $2x10^6$ CFU). The eyes were held closed for 2 minutes, after which the rabbit was allowed to recover from the anaesthetic before being returned to the pen. Pain control was achieved through subcutaneous injection of 0.02 mg/kg buprenophine every 12 hours. 16 hours after the scratch and bacterial introduction, the rabbits were randomly assigned to one of three intervention

groups: intervention by hourly instillation of 3000 μg/mL ciprofloxacin drops for 8 hours, intervention by one of three types of contact lenses (control, 4:1 imprinted and 8:1 imprinted) loaded in 30 μg/mL ciprofloxacin solution for 8 hours or no intervention for 8 hours (three rabbits per treatment condition). The animals were euthanized by lethal injection of 1 mL of sodium pentobarbital intravenously 24 hours post scratch, and the cornea excised. The cornea was homogenized in neutralizing broth, and serial dilutions of the homogenate plated on nutrient agar for 18 hours at 34°C before CFU were counted.

### *5.3.8* STATISTICS

All statistics were performed using STATISTICA Version 7 (Tulsa, OK). Analysis of *in vitro* release curves and bacterial growth curves was done using a repeated measures ANOVA, with lens type as a categorical factor, time as a within effects factor and μg/g dry weight ciprofloxacin released or CFU as a dependent factor. Comparison of bacteria recovered from rabbit corneas or material properties was done using a one way ANOVA, with lens type as a categorical factor, and the measured property as a dependent factor. Post hoc Tukey tests were used as necessary. A p-value of less than 0.05 was deemed to be statistically significant.

## 5.4 RESULTS

### 5.4.1 MATERIAL PROPERTIES

The water content, wet and dry weight, centre thickness, water content and advancing contact angle are summarized in Table 5-1.

| Lens Type | Wet Weight (g) | Dry Weight (g) | Centre Thickness (µm) | Water Content (%) | Advancing Contact Angle (°) |
|---|---|---|---|---|---|
| Control | 0.027 (0.004) | 0.016 (0.003) | 63 (14) | 42.3 (4.5) | 94.6 (1.5) |
| 4:1 Imprinted | 0.029 (0.004) | 0.019 (0.003) | 64 (15) | 36.2 (3.4) | 77.4 (2.0) |
| 8:1 Imprinted | 0.028 (0.006) | 0.017 (0.004) | 62 (19) | 43.3 (3.0) | 81.5 (1.3) |
| 16:1 Imprinted | 0.028 (0.005) | 0.016 (0.002) | 61 (19) | 41.7 (3.0) | 89.2 (2.0) |

**Table 5-1 Material Properties of Experimental Lenses.**

All values are presented as averages (standard deviation) (n = 6). The imprinted lenses are denoted by the ratio of the moles of the functional monomer acrylic acid to the moles of the template ciprofloxacin.

There was no statistically significant difference in the wet weight and centre thicknesses of the lenses. The 4:1 imprinted lenses were found to have dry weights statistically different than the control and 8:1 imprinted lens ($p<0.05$). The 4:1 imprinted lens was statistically significantly different than all the other lenses in terms of water content ($p<0.05$). All the lenses were statistically different when compared to each other with respect to contact angle ($p<0.001$).

The light transmission of the four different lens types tested is shown in Figure 5-1. Increasing the amount of ciprofloxacin into the lens material lead to increased yellow coloration of the lens, and thus greater loss of light transmission in the shorter wavelengths.

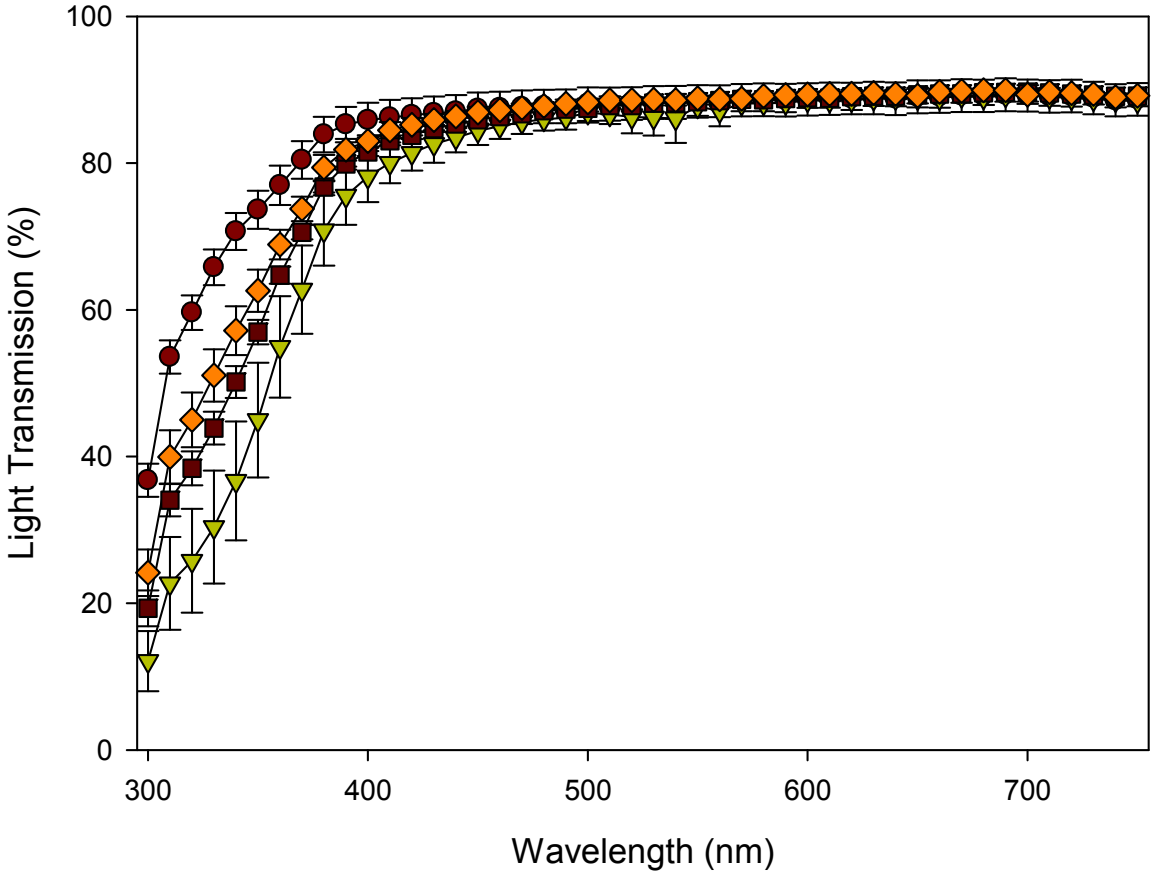



**Figure 5-1 Percentage Light Transmission Curves**

Control (●), 4:1 imprinted (▼), 8:1 Imprinted (■) and 16:1 imprinted lenses (◆). Increased incorporation of ciprofloxacin into the material leads to increased yellow coloration of the lens, and a decrease in transmission in the shorter wavelengths. Symbols represent averages ± standard deviation (n=3).

### 5.4.2 *IN VITRO* TESTING OF CIPROFLOXACIN RELEASE

After autoclaving and allowing to uptake of ciprofloxacin from the loading solution for one week, the amount of ciprofloxacin taken into each lens type is presented in Table 5-2. There was no statistically significant difference between the amounts taken up by the different lenses.

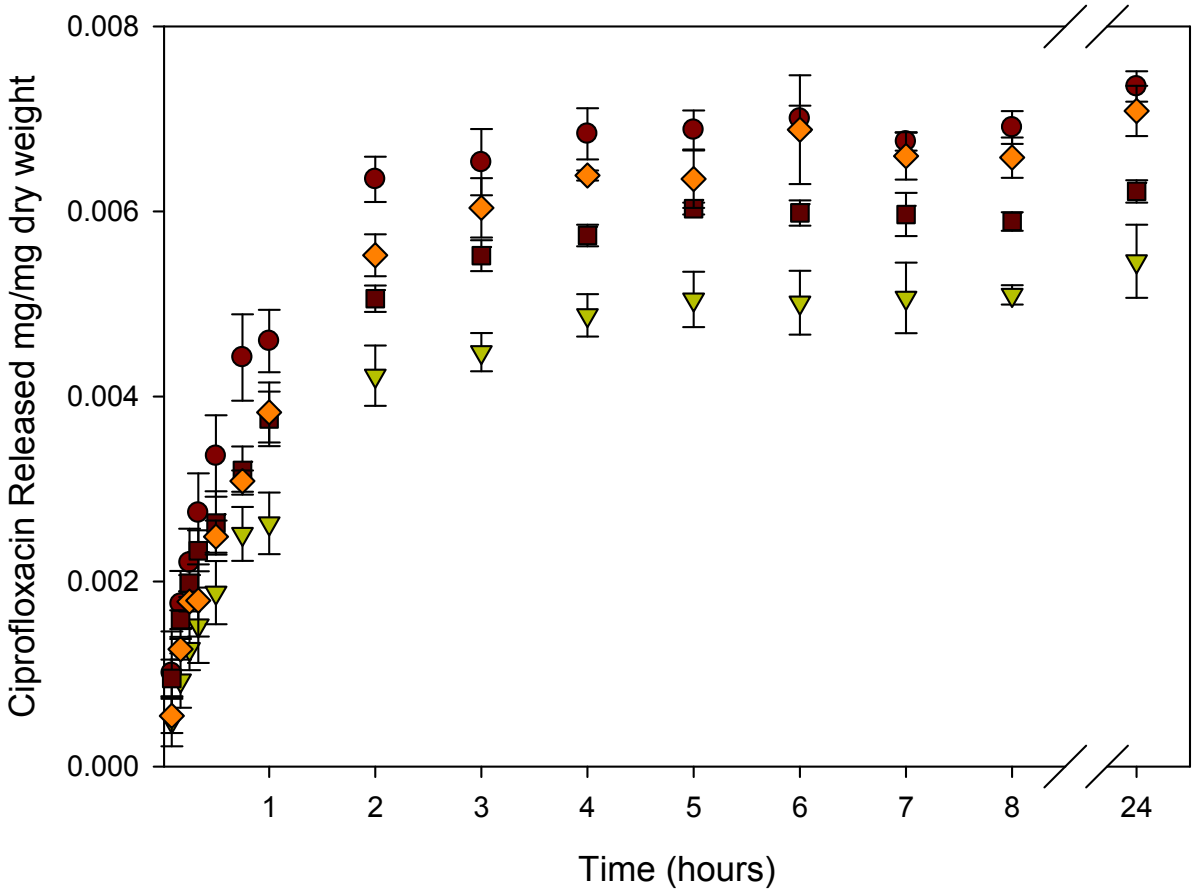
| Lens Type | Ciprofloxacin Loaded (µg/lens) |
|---|---|
| Control | 1383 (144) |
| 4:1 Imprinted | 1509 (291) |
| 8:1 Imprinted | 1133 (264) |
| 16:1 Imprinted | 1234 (295) |

**Table 5-2 Uptake of Ciprofloxacin into Each of the Four Tested Lenses.**
All values are presented as averages (standard deviations) (n=3)

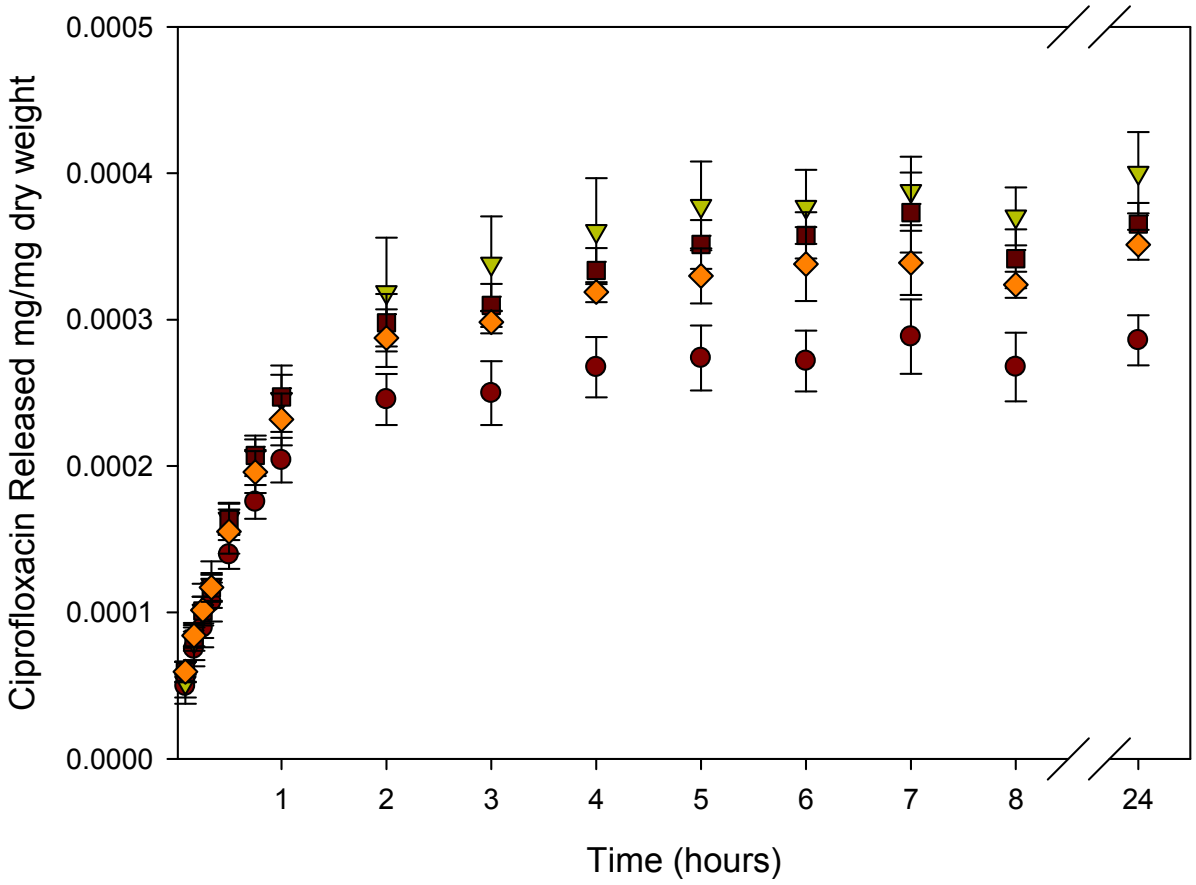
The *in vitro* release curves over the course of three days and three releasing solutions are presented as Figure 5-2 a-c. On the first release day, the control material reached a plateau concentration after three hours, while the imprinted materials released ciprofloxacin for five hours or more. The plateau concentration of the control material was higher than the imprinted materials, although this difference was not statistically significant ($p>0.05$). On the second release day in the second release solution, the control and imprinted materials reached a plateau concentration after four hours and there were no statistically significant differences between them. In the third release medium on the third day, the control material reached a plateau concentration after a mere two hours, while the 4:1 and 8:1 imprinted materials continued to

release for over eight hours. The plateau concentration reached by the 4:1 and 8:1 imprinted materials were also statistically different than the concentration reached by the control ($p<0.05$). The plateau concentration reached by the least imprinted material, the 16:1 lens, was not statistically different than the control.

a)



b)


Ciprofloxacin Released mg/mg dry weight
0.0005
0.0004
0.0003
0.0002
0.0001
0.0000
1
2
3
4
5
6
7
8
24
Time (hours)

c)

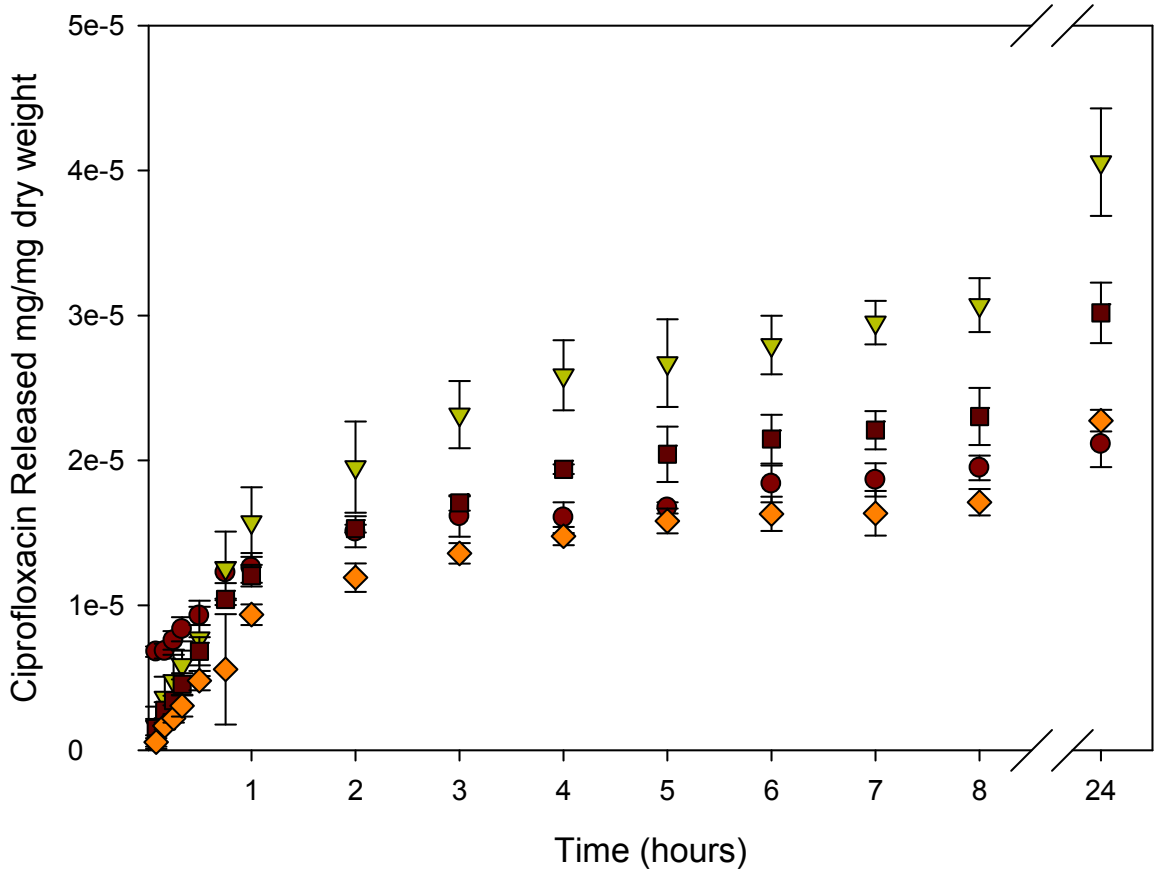



**Figure 5-2** ***In vitro*** **Ciprofloxacin Release**

Control (●), 4:1 imprinted (▼), 8:1 Imprinted (■) and 16:1 imprinted lenses (◆) on Day 1 (a), Day 2 (b) and Day 3 (c) after loading in a 0.3% ciprofloxacin solution for one week. Symbols represent averages ± standard deviation (n=4).

### 5.4.3 *IN VITRO* ANTIBACTERIAL ASSAYS

The as tested MIC of the *P. aeruginosa* strain 6294 was 0.4 μg/mL. All lenses loaded with 0.3% ciprofloxacin were able to completely inhibit the growth of bacteria for the first two days, suggesting that inhibitory amounts of the antibiotic were being released from the lenses. The ability of the lenses to inhibit the growth of *P. aeruginosa* strain 6294 in Mueller Hinton Broth on the third day is presented as Figure 5-3. There was an initial decrease in concentration of bacteria as the final reserves of ciprofloxacin were released from the lenses. The rate at which the number of viable bacteria were decreasing is indicative of concentration of antibiotic in solution, suggesting that the control lens initially reaches a higher concentration than the two imprinted lenses, which correlates to the released data seen in Figure 5-2c. As complete inhibitory concentrations were not reached by any of the lenses, by the 8 hour time point the bacteria population began to rebound. There was a statistically significant decrease in the number of bacteria from the beginning to the end of the monitoring period for all three lenses tested ($p<0.05$). The differences between the lenses however was not statistically significant ($p>0.05$).

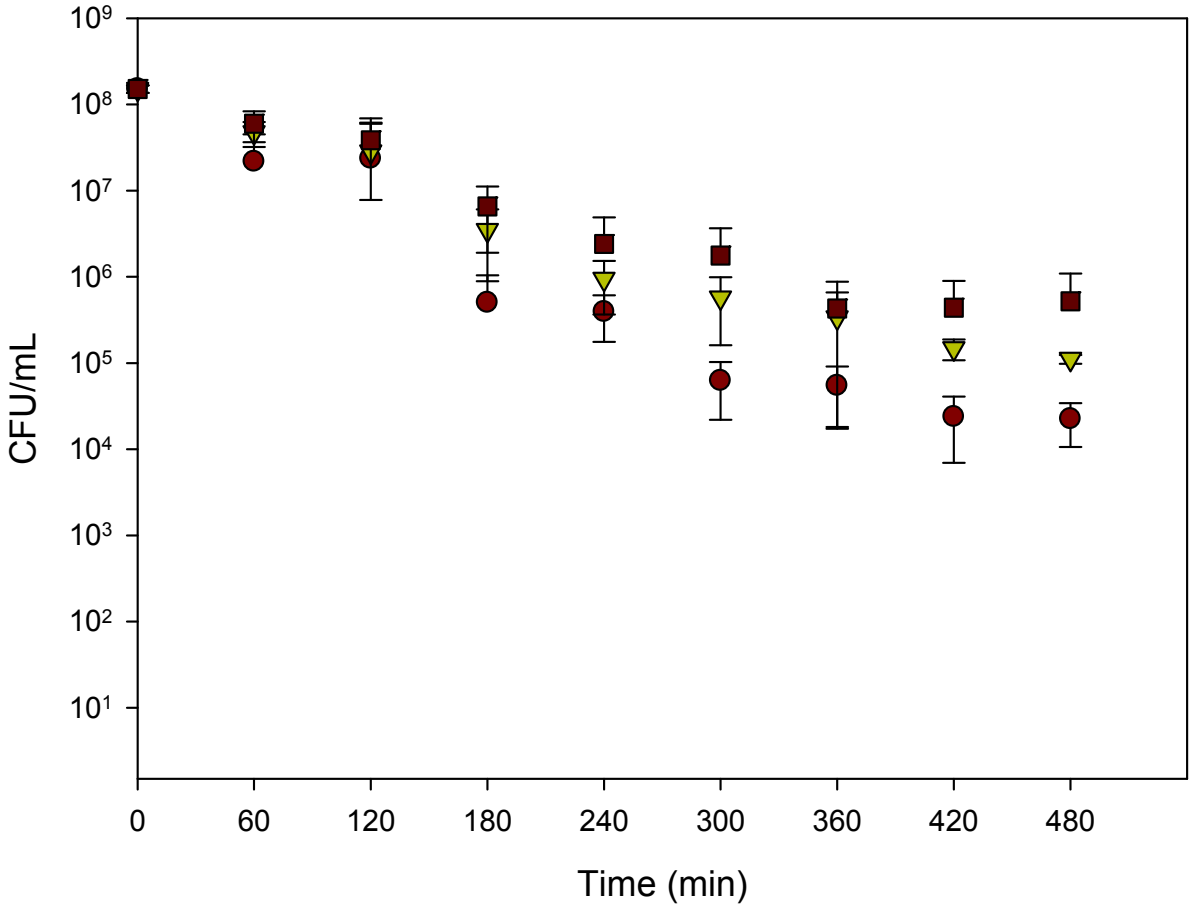
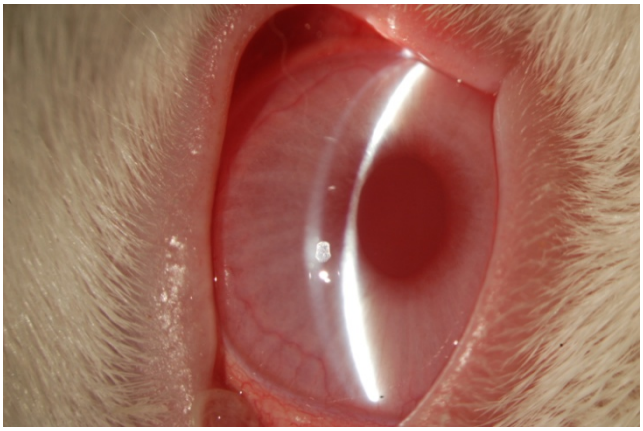


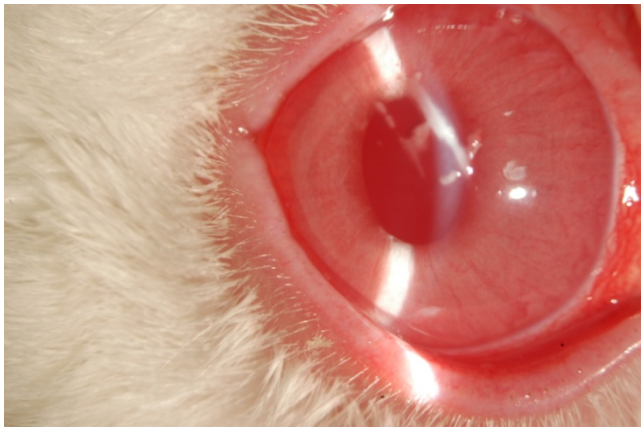

**Figure 5-3** ***Pseudomonas aeruginosa*** **strain 6294 Growth Curves in Presence of Ciprofloxacin Releasing Contact Lenses.**

No viable bacteria were recovered from the first two growth medias on the first two days, as sufficient antibiotic concentrations were reached in solution. The presented curves are from the third bacterial solution on the third day, after a significant amount of antibiotic was already released from the lenses. As inhibitory concentrations were not reached, by the 8 hour time point the bacteria numbers are beginning to recover and growth is beginning to increase. Control (●), 4:1 imprinted (▼), 8:1 imprinted (■). Note exponential scale. (n=3)

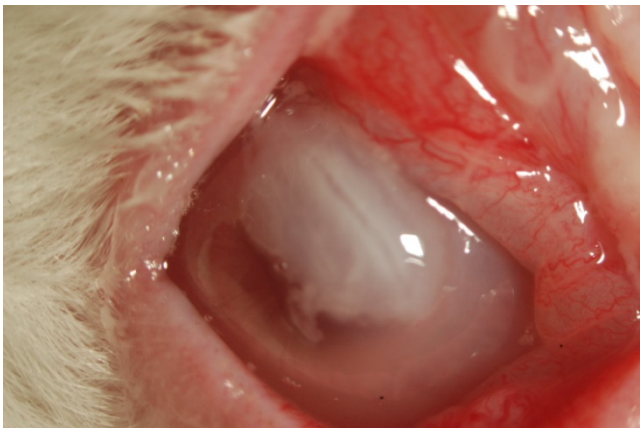
### 5.4.4 *IN VIVO* MODEL OF MICROBIAL KERATITIS

Corneas scratched and exposed to *P. aeruginosa* strain 6294 began to show an infection response after 16 hours, characterized by development of infiltrates, discharge and redness as shown in Figure 5-4b. Left untreated, the severity of the infection increased dramatically over the next 8 hours before euthanasia of the rabbit (Figure 5-4c). Treatment intervention with a modified contact lens at the 16 hour point partially resolved the infiltrate or discharge by the 24 hour point (Figure 5-4d).

a) 

b) 

c)



d)

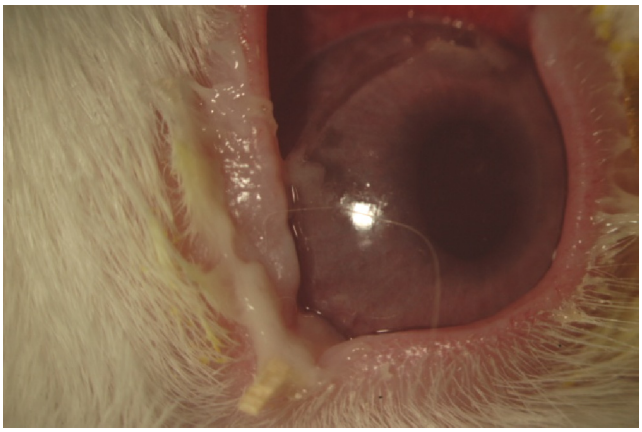


**Figure 5-4 Rabbit Model of Microbial Keratitis**

a) Cornea appearance prior to corneal scratching and bacteria introduction b) Cornea appearance 16 hours post scratch, showing infiltrate, redness and discharge c) Cornea appearance 24 hours post bacteria introduction without treatment showing a large increase in size and severity of the microbial keratitis and d) Cornea treated with experimental contact lens for 8 hours 16 hours after bacteria introduction.

The number of CFU recovered from excised and homogenized infected corneas are presented in Figure 5-5. Left untreated, approximately $10^6$ CFU per cornea were recovered, while treatment with hourly instillation of ciprofloxacin eye drops lead to complete sterilization and lack of any recoverable bacteria from the cornea after only 8 hours. Treatment with lenses soaked in only 30 μg/mL ciprofloxacin solutions (100 times less than the clinical drops) lead to differences in bacterial recovery. The number of bacteria recovered from corneas treated with the control (i.e. no molecular imprinting) lenses that had been soaked in ciprofloxacin was not significantly different than that of the non treated control lenses ($p>0.05$). However, there is a significant reduction in the number of recoverable bacteria from the corneas treated with the slow release, molecularly imprinted lenses ($p<0.05$ when compared to untreated control or untreated corneas). Many of the corneas treated with the imprinted lenses were rendered sterile through treatment, and overall no statistically significant difference was found in the number of bacteria recovered from those corneas and corneas treated with antibiotic eye drops ($p>0.05$).

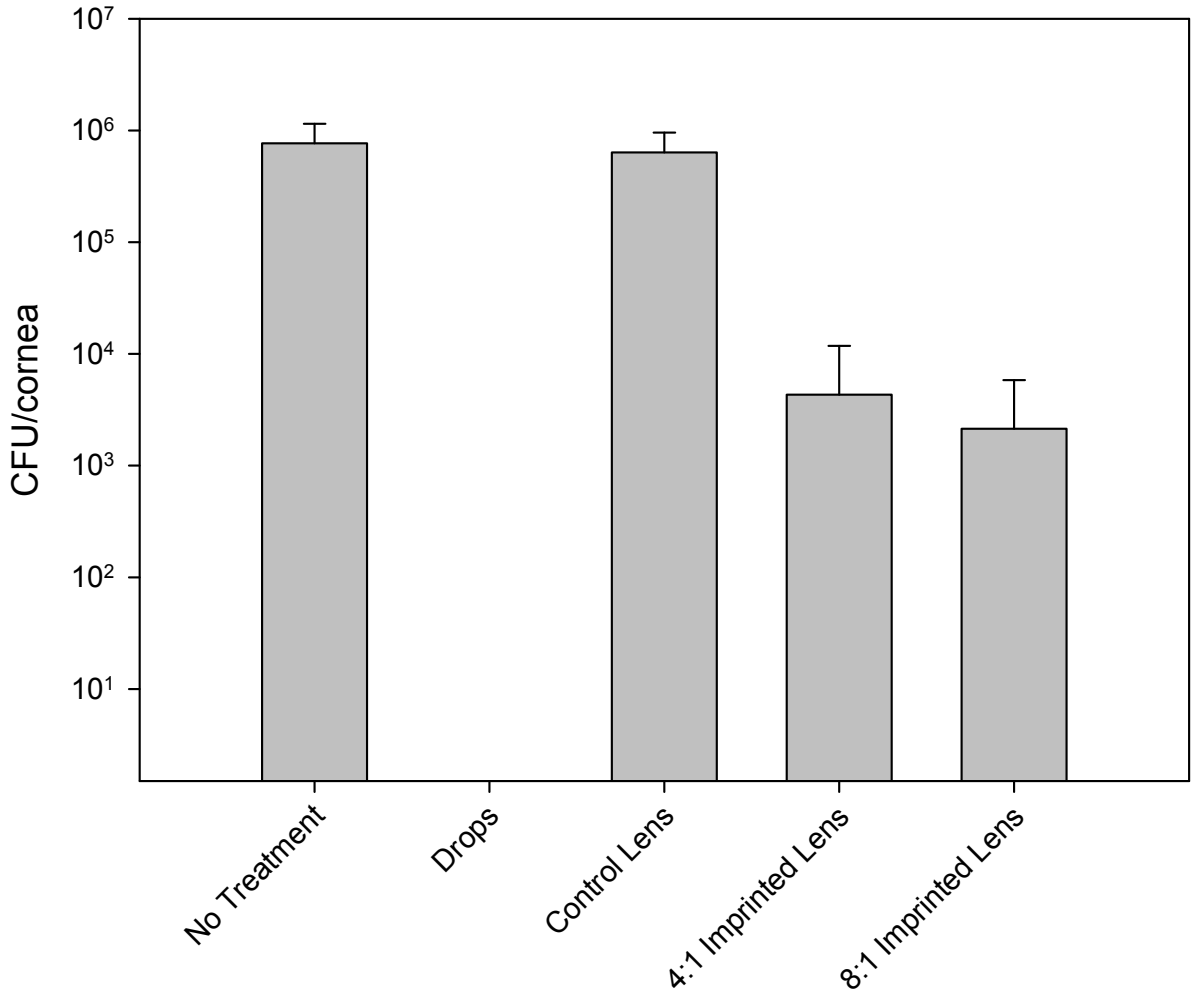



**Figure 5-5 Bacteria Recovered from Excised, Homogenized Corneas**

The bacteria were recovered 24 hours post corneal scratch and introduction of *P. aeruginosa* strain 6294, and 8 hours of different treatment conditions. Corneas treated with drops had 10 µl of a 3000 µg/mL ciprofloxacin solution instilled hourly. Lenses used in the treatment were presoaked and autoclaved in a 30 µg/mL solution of ciprofloxacin. The number of bacteria recovered from the no treatment (range $10^{5.68}$ - $10^{6.08}$ cfu/cornea) and control lens (range $10^{5.51}$ - $10^{5.98}$ cfu/cornea) treatments were significantly different than those treated with ciprofloxacin eye drops (range 0 - 0 cfu/cornea) or 4:1 (range 0 - $10^{4.11}$ cfu/cornea) or 8:1 (range 0 - $10^{3.80}$ cfu/cornea) imprinted lenses ($p<0.05$). n = 3 for each treatment group. Note exponential scale.

## 5.5 DISCUSSION

The challenge in the development of contact lens drug delivery devices remains the relevant drug release kinetics. Previous investigations into drug release from commercially available materials demonstrated less than clinically useful drug release times, [40] [41] prompting the need for custom design lenses to be developed. As seen from the drug release curves presented in Figure 5-2, by using a molecular imprinting technique the ciprofloxacin release profiles from contact lens materials were significantly altered. By incorporating acrylic acid as a functional monomer within the pre-polymerization mixture in various ratios to ciprofloxacin, materials were modified to release the antibiotic at various rates, with a ratio of 4:1 functional monomer to template molecule showing the greatest extension of release times. The influence of the ratio of functional monomer to the template on the efficiency of molecular imprinting has been presented in the literature. [42] Away from the optimum monomer to template ratio, cavities created within the polymerization structure will be inadequately or inefficiently created, and thus shift the equilibrium toward disassociation and release of the template, leading to faster release times when release studies are performed *in vitro*. [42] That the 4:1 ratio was shown to be the most effective in slowing the release of ciprofloxacin is not surprising, as the ratio had previously been demonstrated as most effective in experiments with molecular imprinting and norfloxacin, a first generation fluoroquinolone. [28] Through use of isothermal titration calorimetry, a saturation in the binding of norfloxacin within the hydrogels at a functional monomer to template ratio of 4:1 was observed, and thus would be the ratio predicted to most perfectly create the imprinted cavities and most prolong release times. [28] This prediction was demonstrated by the norfloxacin release data, as ratios above or below 4:1 did not as effectively control norfloxacin release. [28] This experiment was an improvement to previous *in vitro* experiments [34] in that the releasing medium was changed on a daily basis to better simulate the changing concentration gradients that are

likely to be seen if these materials were placed on the eye. On the initial day, the unmodified control lenses released a very high concentration of drug, while the modified materials released for longer periods but reached lower final concentrations. As the release medium solutions were changed, the advantage of the molecular imprinted materials began be more apparent. The control material continued to release extremely rapidly, and reached lower plateaus than the modified materials. This was best exemplified by the data from the third releasing medium on the third day, when the control material reached a fast plateau of ciprofloxacin concentration within 2 hours, while the 4:1 material continued to release for more than 8 hours, and reached a significantly higher concentration in solution.

*In vitro* testing of the antibacterial activity of the test materials served as a complement to the release of ciprofloxacin in solution. Here, the differences in the recovered bacteria were seen as a surrogate of the amount of ciprofloxacin released. The test organism, *P. aeruginosa* strain 6294, is ciprofloxacin sensitive, with a MIC of 0.4 μg/mL. The growth of the bacteria within the media is a function of several factors - not only the concentration of ciprofloxacin within the solution, but also the initial seeding concentration of bacteria, the growth phase of the bacteria and the availability of resources, including nutrients and oxygen. [43-45] The plotted growth curves in Figure 5-3 reflect all of these factors simultaneously. There are several conclusions that can be reached by considering the growth of bacteria in the presence of these lenses. First, each of the lenses were initially inhibiting the growth of the bacteria, presumably due to release of the antibiotic. Second, inhibition of growth by these lenses waned over time. By the 8 hour mark, the bacterial population stabilized or started to grow as the limited amount of ciprofloxacin released from the lenses was exhausted or insufficient to prevent multiplication, leaving bacterial growth limited only by available resources. Third, while the differences between the different lenses

were not statistically significant, examination of the different growth curves can be suggestive of the effect of imprinting. The rate at which the population growth was reduced by the lenses is reflective of the concentration of the antibiotic in the solution. The control lens, as previously demonstrated, released the majority of the available ciprofloxacin very quickly, and reached higher concentrations of antibiotic in solution faster than the two slow release materials (Figure 5-2c). Thus, the decrease in bacterial concentration from systems treated with the control material are expected to be faster than when treated with the two slow release lenses, which is what is seen (Figure 5-3). Thus, within a closed, fixed *in vitro* solution, the control lens released ciprofloxacin which reached a high concentration quickly can be considered to be a superior lens used to control bacterial growth.

*In vitro* testing of antibacterial activity is unfortunately an inadequate model for *in vivo* applications. In the controlled, closed system of a test tube or vial, the bacteria were exposed to all of the antibiotic released from the experimental materials, which would kill the bacteria cells. This was regardless of the rate at which the antibiotic was being released. This is in contrast to what occurs when antibiotic drops or lenses are placed on the ocular surface, where pharmacokinetic factors including tear production and drainage, epithelial/corneal penetration and drug metabolism play significant factors in the amount of drug exerting an effect. If the eye was a closed system, then the fast burst release from a control lens could be advantageous and quickly raise drug concentrations to effective levels. Unfortunately, because of tear drainage and corneal cellular barriers, it is likely that much of the antibiotic released in such a burst fashion will very quickly be cleared from the ocular surface, limiting its usefulness, which is why frequent dosing with eye drops is necessary. In contrast, with a sustained release contact lens supplying the antibiotic, a continual replenishment of the antibiotic is possible. A fast rise to a

high concentration is less likely, but over time there is greater potential for therapeutic concentrations to be reached, and more importantly, for them to be sustained over a longer period of time. This is exemplified by the *in vivo* results seen in Figure 5-5. Even with the superior performance of the control lens (i.e. normal lenses soaked in ciprofloxacin) against the bacteria *in vitro*, this superiority did not translate in the *in vivo* rabbit model. The control lens did not appreciably impact the number of recoverable bacteria compared to no treatment, presumably because all of the antibiotic was released at once, and any of the antibiotic not absorbed was quickly drained away. In contrast, the two imprinted lenses performed significantly better in reducing the number of recoverable bacteria, as the reserves of ciprofloxacin were released slowly over time and could replenish lost drug that was drained away from the surface.

If the field of contact lens drug delivery is to continue and be eventually accepted by both practitioners and patients alike, the wearer experience must be similar to regular contact lenses on the market. The optical transmission in the visible range needs to be acceptable for wear in day to day life, the water content and wettability have to remain with a certain narrow set of parameters to ensure acceptable comfort during wear, and the amount of oxygen being transmitted must be adequate to prevent complications. As shown from the results of our experimental lenses, while not surface treated, they had acceptable wettability measures in line with other non-surface treated silicone hydrogels that incorporate an internal wetting agent such as polyvinylpyrrolidone. [46] The light transmission in the visible range is acceptable, other than slight tinting of the lenses due to ciprofloxacin drug incorporation causing a mild yellow coloration. The centre thickness and water contents were also in line with commercial contact lenses. The incorporation of silicone monomers into the material will allow for superior oxygen transmission properties. Between the lenses, there were some significant differences in water

content and advancing contact angle. The water content of the lenses which incorporated the greatest amount of ciprofloxacin in the imprinting process, the 4:1 imprinted lenses, also had the lowest water content, with the 16:1 and control lenses having increasing water content comparatively. The one anomaly to this trend was the 8:1 imprinted lenses, which measured the highest water content. There were also significant differences in the advancing contact angle, with a trend toward lower contact angles as more ciprofloxacin was used. Considering that the only difference in the synthesis of all of the lenses is the amount of ciprofloxacin added, it can be surmised that this difference in water content and advancing contact angle is likely due to irreversible binding of some ciprofloxacin within the materials during synthesis. The continued yellow coloration of the contact lenses which had ciprofloxacin incorporation during the molecular imprinting process would lead credence to this theory. The permanently bound ciprofloxacin is not expected to have had a significant effect on the ciprofloxacin release characteristics from these lenses.

In this study, the ultimate test of the effectiveness of the modified contact lens drug delivery device was performance in an *in vivo* model of microbial keratitis in New Zealand White rabbits. The use of rabbits as a model for microbial keratitis is well known, as they have an adequate eye size to allow for contact lens wear. [47] The methods and selection of bacteria for infection are also critical. Classically, to achieve infections of the cornea, animal models of keratitis have required either passing of a silk suture soaked in a bacterial solution into the corneal stroma or direct injection into the corneal stroma of a bacterial solution to get a consistent and repeatable keratitis response. [47] The method chosen in this study involved the creation of a superficial scratch through the epithelium of the rabbit cornea before exposure to a bacterial solution. This method mimicked to some extent the contact lens rabbit model of Hume

et al., but without the need to add spermidine. [48] Usage of a highly virulent strain of bacteria, *P. aeruginosa* strain 6294 allowed for consistent keratitis responses to be seen under these experimental conditions. The timing of the treatment was also carefully chosen for two separate reasons. Sixteen hours were allowed to pass so that the MK response could be seen. It was also chosen to mimic a more real world situation, where a patient may be reluctant to seek treatment after the initial insult, and rather chooses to delay medical attention until the condition and symptoms had significantly worsened. We were limited ethically to an experiment of no more than 24 hours to prevent significant pain, suffering and distress to the experimental animals. As is evident by the data (Figure 5-5), corneas treated with ciprofloxacin eye drops were rendered sterile after only the short 8 hour treatment time frame. Indeed, this was also seen in treatment trials with the molecularly imprinted contact lenses, as 2 out of the 3 corneas in both of the modified lens trials were also rendered sterile. However, the clinical picture at this time does not reflect the sterility of the cornea as all eyes at the 24 hour time point regardless of treatment type continued to show significant infiltrates, redness, edema and discharge, although the severity varied between the different treatment conditions. If the study could have continued for longer, an alternative, more clinically relevant outcome measure to recoverable bacteria could have been used such as time to resolution of the infiltrate and/or re-epithelialisation of the corneal surface. The sterility of the corneas is also in contrast to what is often seen in the experimental trials of novel antibiotic drops. For example, in a recent trial testing the efficacy of a new fluoroquinolone antibiotic drop, treatment with the new antibiotic (and other commercially available antibiotics) did not completely sterilize the cornea, rather it merely significantly impacted the number of bacteria recovered compared to non treatment controls. [49] The difference observed in this trial likely stems from the method of infection used. In antibiotic drop efficacy

studies, corneal infection are generally achieved using an intrastromal injection of the offending organism. In contrast, in the current study infection was preceded by a corneal scratch and break in the corneal epithelium. This break in the epithelium can provide an avenue for the antibiotic to reach the microorganisms, while in intrastromal injection models, the antibiotic must traverse through the significant intact epithelial barrier. The performance of the unmodified control lenses in comparison to the treatment with eye drops is illustrative of the dosing needed to eradicate the bacterial organisms. Eye drop therapy was able to sterilize the corneas, but only after repeated instillations over time to ensure that an adequate amount of the antibiotic reaches the ocular structures. Based on its *in vitro* release kinetics, the control lenses release ciprofloxacin as a very quick initial burst, and any of the drug that is not absorbed is presumably lost. In this manner, the dosing provided by application of a ciprofloxacin loaded, unmodified control lens behaves much like a single eye drop instillation. Thus, for the control lens to be effective in eradicating bacterial growth, the application of the contact lens would need to follow the schedule seen with eye drops. Repeated removal of worn lenses and replacement with loaded lenses would have been necessary to provide the proper dose, negating any practical advantages of the drug delivery system.

Recently, there has been a report by a research group demonstrating the feasibility of an extended antibiotic releasing contact lens for the prevention of ocular infections. [50] In their model of bacterial endopthalmitis, infection was achieved through anterior chamber injection of a methicillin resistant strain of *S. aureus*. [50] Untreated, after 24 hours approximately $10^5$ CFU/mL of bacteria were recovered from the experimental eyes, while treatment with topical fluoroquinolone eye drops only reduced the number of recovered bacteria to approximately $10^4$ CFU/mL. In contrast, immediate treatment after bacterial injection with their experimental

gatifloxacin releasing contact lenses completely prevented growth of microorganisms, proving the utility of their lenses in potentially preventing postoperative infections of the globe. The results of the current study extend the application of antibiotic releasing contact lenses even further, with the aim of treatment of infection rather than mere prevention. Delay in treatment of the exposed animals with contact lenses or eye drops allow for the clinical signs and symptoms of an infection to occur, framing the results from these trials in the context of treatment of ocular treatments rather than prevention.

In conclusion, in this study, the development of a slow release ciprofloxacin contact lens system was achieved using a molecular imprinting strategy. Evaluations *in vitro* show the potential of these materials to release clinically relevant amounts of the antibiotic while retaining critically important contact lens material properties, and evidence from *in vivo* testing show that they can perform similarly to antibiotic drop therapy in models of MK. Application of these materials may be useful for future treatment paradigms of MK.

## 5.6 ACKNOWLEDGEMENTS

The authors would like to thank Ajay Vijay and Denise Lawler at the University of New South Wales for their aid in performing the animal studies. This study was supported by the Natural Science and Engineering Research Council of Canada (NSERC) 20/20 Network for the Development of Advanced Ophthalmic Materials. AH is supported by an NSERC Alexander Graham Bell Doctoral Scholarship, the Ezell Fellowship from the American Optometric Foundation and the Endeavour Research Fellowship from the Australian Government.

# CHAPTER 6 - GENERAL DISCUSSION AND CONCLUSIONS

The aim of developing contact lens materials for drug delivery is an ambitious and difficult task. Within the course of this thesis, several conclusions on the potential for contact lenses as pharmaceutical delivery devices were made, and suggest that CLs as a drug delivery platform for antibiotics may be a viable strategy to treat ocular infections.

Experiments with commercially available contact lenses (Chapter 3) demonstrated a few key findings for lenses which are already on the market. Even though all of the lenses being used had the same dioptic power, and relatively the same size and shape, there were significant differences which arose between the amount of drug taken up and released, and the time needed for drug plateaus to be reached *in vitro*. This serves to highlight the fact that the material itself has some influence on drug release properties. There was some evidence from this study of the types of parameters that appeared to be contributory to the differences in properties. The water content of the lenses appeared to have an effect, with higher water content lenses releasing more of the antibiotic. This would make sense, as it is likely that some of the drug was dissolved within the aqueous component of the lenses themselves, and thus the lenses with greater water content would be expected to release more than those with lower concentrations. The surface charge of the lenses also had an effect. It was no coincidence that the lens which took up the most ciprofloxacin in this study (etafilcon A) was also negatively charged. Ciprofloxacin, by nature of having two ionizable groups (a carboxylic acid group and an amino group), exists as a positively charged molecule in the uptake solution at pH of 4.0. This would naturally lead to higher affinity and higher loading with the negatively charged materials. For the silicone hydrogels, it is interesting to examine the amount of drug taken up versus the eventual amount

that was released. On the whole, the silicone hydrogel materials did not appear to have any difficulty in loading ciprofloxacin, as there were several examples of silicone hydrogel materials (such as senofilcon A and comfilcon A) taking up a comparable amount of ciprofloxacin compared to the hydrogels, but the amount that was eventually released into solution was significantly lower. It is illustrative to examine Table 3-5 for the percentage of drug released relative to the amount loaded. For the silicone hydrogels, there was a significant amount of the loaded drug which never left the lens. Whether this is due to binding of the drug to the material polymer is unknown. The final major conclusion from this study was that a limitation to how much ciprofloxacin can realistically be loaded into a contact lens exists if it is eventually to be worn comfortably on the ocular surface. The change in pH as lenses went from the loading solution to the release solution caused complete precipitation of the ciprofloxacin found within the highly loaded etafilcon A material, and was seen to a lesser degree in some other lenses. While the lens may have had an advantageous drug release profile, as evidenced by Figure 3-3 the inability to see through the lens when worn would render it unusable. There have been reports within the literature of extended ciprofloxacin drop topical treatment leading to ciprofloxacin concretions developing within the cornea. These typically disappear after a few days, as the ciprofloxacin is eventually dissolved and removed. [1]

Ultimately, the main limitation with commercial lenses was their inability to release ciprofloxacin for significant periods of time. In the treatment of ocular infections, it would be required that a drug delivery device be able to release clinically relevant amount of antibiotic for extended periods of time. Ideally, continued release over the course of 24 hours, or even several days would be beneficial to continually combat against the growth of microorganisms. None of the commercially available lenses tested in this study were able to sustain release for more than a

few hours, suggesting that while antibiotic concentrations were reached *in vitro*, they would be unable to be sustained over the long period when worn in situ. The usefulness of commercial contact lenses as drug delivery devices in simple soak and release techniques may be for pharmaceuticals whose dosing frequency is not high. It is easy to envision use of these lenses for such treatments such as seasonal allergy or early glaucoma, where once a day dosing is sufficient. We have already performed some studies on the potential of commercial lenses for the delivery of an anti-allergy agent ketotifen fumarate, envisaged to be used in a daily disposable context. [2]

The main focus of Chapter 4 was the creation of contact lens model materials which would have more favorable drug release kinetics. To do this, a molecular imprinting strategy was employed. The results from this study demonstrated several characteristics of imprinted materials, and the challenges in forming materials which would have slow releasing drug release characteristics. During the molecular imprinting process, the proper choice of the functional monomer is crucial to effectively create the "memory" within the cavities. In this study, two functional monomers were tested, acetic and acrylic acid. While the two molecules are similar, acrylic acid is ultimately a more useful functional monomer because of the presence of a double bond within the molecule. This double bond allows for the molecule to be incorporated within the growing polymerization chains, making the imprinted cavities to be a permanent feature of the material. Acetic acid lacks this double bond, and thus would be expected to eventually be removed from the material over time. The ratio of the functional monomer to the template molecule in the polymerization mixture is also critical. Too much or too little of the functional monomer affects the efficiency of creating the imprinted areas within the material, and thus a decrease in the ability of the material to slow down the drug release. One of the studies also

varied the overall concentration of the functional monomer within the polymerization mixture as a whole. While this did have an effect, the effect was not particularly pronounced in this current study.

The greatest contribution of the molecular imprinted materials created in Chapter 4 was demonstration that there can be a significant difference between the amount and time that ciprofloxacin could be released from the molecular imprinted materials versus non imprinted control materials. Monitoring for up to two weeks of release time showed a continued increase in concentration for solutions with imprinted materials within them, and a relatively flat plateau reached extremely early on for the control materials. It was also demonstrated that the greatest effect of the molecular imprinting process versus an unmodified material can be seen when the materials are loaded with relatively low concentration solutions. The reason for this is that when there is a low amount of ciprofloxacin within the solution, materials which are imprinted have a higher affinity for the low amounts of drug within the solution, and will thus load more and release more when it is eventually placed within the release media. This has been demonstrated in other imprinted materials. [3] The non imprinted materials have only a concentration gradient pushing towards loading the ciprofloxacin, and this gradient is not very high due to the low loading concentration.

The limitations to the studies performed in Chapter 4 relate to the form of the materials created and the release medium. All of the materials created in Chapter 4 were made from components critical for silicone hydrogel contact lenses - pHEMA, EGDMA, TRIS, but they were not formed into contact lenses. Instead, the materials created were small, flat discs which were punched out of larger pieces of polymerized material. The thickness, shape and size of the materials thus did not completely match that of contact lenses. The medium into which the lenses

were releasing the drug was also static. It was sampled and returned during each of the time points monitored, but there was no attempt to replace the media at different times to stimulate tear flow and drainage, which would presumably favor a greater amount of release by inducing a continuous concentration gradient from the lens to the release medium.

The goals of Chapter 5 were to establish the feasibility of molecular imprinted contact lenses for the treatment of infection *in vivo*. The methods and successes which were developed within Chapters 3 and 4 were needed to pave the way for manufacturing of molded contact lenses, and these materials needed to be evaluated not only for their favorable drug release characteristics, but also their relevant contact lens properties. Some modification of the protocol was needed to achieve desirable contact lens properties. The ciprofloxacin needed to be pre dissolved with acrylic acid in chloroform to allow for acceptable light transmission properties. The addition of PVP was needed to improve the wettability of the lens surface. Lens molds were needed to achieve the shape, size and thickness of commercially available contact lenses.

These lenses were tested for their ability to eradicate replicating bacteria within the corneas of rabbits infected with a particularly virulent strain of *P. aeruginosa*. Here, the imprinting process demonstrated their worth, as they were successful in significantly reducing the number of viable bacteria recovered from excised corneas after only a short eight hour treatment period. This was in contrast to the non imprinted control lenses, whose bacterial counts were similar to non-treated infected corneas.

There are several follow up experiments which could be done to further flesh out the effectiveness of this drug delivery system *in vivo*. Multiple strains of bacteria could be used to generate keratitis responses, and the results of treatments with these lenses could be demonstrated with multiple MK causing organisms. This would be more of a function of the

antimicrobial properties of ciprofloxacin itself, but would be necessary before these lenses could possibly be used for human cases of MK. The endpoint of the *in vivo* experiments was limited ethically to only 24 hours post scratch and bacteria introduction. The outcome measure in this study was the number of bacteria recovered per cornea, but this does not mimic what is seen clinically to define whether any treatment is successful or not. Future studies using a less virulent strain of bacteria which would still allow for a MK response to be seen, but the possibility of a milder clinical course, would open up the possibility of using a more relevant clinical endpoint such as a comparison of the time needed to resolve the infiltrate and re-reepithelialise the corneal surface.

There results of this thesis are encouraging for the development of contact lenses as drug delivery devices. The knowledge gained is suggestive of other potential future projects and engineering challenges. For example, do the results for imprinting with ciprofloxacin translate into imprinting for other fluoroquinolone antibiotics such as gatifloxacin, moxifloxacin and besifloxacin, the newest generation and more commonly used fluoroquinolone antibiotics? Can the sustained release of ciprofloxacin from these materials overcome fluoroquinolone-resistant bacterial strains? There is also a very interesting question regarding regulatory and prescribing authorities. While the majority of eye care professionals are able to prescribe contact lenses and antibiotics, there could a case by regulatory authorities where further certification or training would be required before one can prescribe these sustained release devices.

In this thesis, a sustained ciprofloxacin releasing contact lens system was developed and evaluated. *In vitro* and *in vivo* data generated is very compelling regarding the potential ability of these lenses to be valuable in the control of ocular infections caused by *P. aeruginosa*. The

challenge, if these lenses are ever to be used in clinical practice, may lie in convincing the eye care practitioner of the utility of these devices in the management of ocular infections.

# REFERENCES

## CHAPTER 1 REFERENCES


1. Huebsch N, Mooney DJ. Inspiration and application in the evolution of biomaterials. *Nature* 2009;462:426-432.
2. Anderson DG, Burdick JA, Langer R. Smart biomaterials. *Science* 2004;305:1923-1924.
3. Ratner BD, Bryant SJ. Biomaterials: Where we have been and where we are going. 2004:41-75.
4. Williams D. The golden anniversary of titanium biomaterials. *Medical Device Technology* 2001;12:8-11.
5. Yamanaka A, Yokoyama Y, Iwamoto H. Biomaterials in ophthalmology, 2005. *Journal of Artificial Organs* 2006;9:67-70.
6. Tathe A, Ghodke M, Nikalje AP. A brief review: Biomaterials and their apllication. *International Journal of Pharmacy and Pharmaceutical Sciences* 2010;2:19-23.
7. Swanson MW. A cross-sectional analysis of U.S. contact lens user demographics. *Optometry and Vision Science* 2012;89:839-848.
8. Pearson RM. A review of the limitations of the first hydrogel contact lenses. *Clinical and Experimental Optometry* 2010;93:15-25.
9. Bowden T. *Contact lenses : the story : a history of the development of contact lenses*. Gravesend, Kent: Bower House; 2009:xli, 743 p., [712] p. of plates.
10. McMahon TT, Zadnik K. Twenty-five years of contact lenses: The impact on the cornea and ophthalmic practice. *Cornea* 2000;19:730-740.
11. Nixon G. Contact Lens Materials Update 2008. *Contact Lens Spectrum* 2008;23:33-40.
12. Stapleton F, Stretton S, Papas E, Skotnitsky C, Sweeney DF. Silicone hydrogel contact lenses and the ocular surface. *Ocul Surf* 2006;4:24-43.
13. Dart J, Stapleton F, Minassian D. Contact Lenses and keratitis. *Lancet* 1991;338:1146-1147.
14. Carlson KH, Ilstrup DM, Bourne WM, Dyer JA. Effect of silicone elastomer contact lens wear on endothelial cell morphology in aphakic eyes. *Cornea* 1990;9:45-47.
15. Cutler SI, Nelson LB, Calhoun JH. Extended wear contact lenses in pediatric aphakia. *J Pediatr Ophthalmol Strabismus* 1985;22:86-91.
16. Dahl AA, Brocks ER. The use of continuous-wear silicone contact lenses in the optical correction of aphakia. *Am J Ophthalmol* 1978;85:454-461.
17. Gurland JE. Use of silicone lenses in infants and children. *Ophthalmology* 1979;86:1599-1604.
18. Sweeney DF, Holden BA. Silicone elastomer lens wear induces less overnight corneal edema than sleep without lens wear. *Curr Eye Res* 1987;6:1391-1394.
19. Tighe B. Silicone hydrogels: Structure, properties and behaviour. In: Sweeney D (ed), *Silicone Hydrogels: Continuous Wear Contact Lenses.* Oxford: Butterworth-Heinemann; 2004:1 - 27.
20. Tighe B. Contact Lens Materials. In: Phillips A, Speedwell L (eds), *Contact Lenses.* Edinburgh: Butterworth-Heinemann; 2006:59 - 78.
21. Guillon M. Are silicone hydrogel contact lenses more comfortable than hydrogel contact lenses? *Eye and Contact Lens* 2013;39:86-92.

22. Efron N, Morgan PB, Woods CA. International survey of rigid contact lens fitting. *Optometry and Vision Science* 2013;90:113-118.
23. Morgan PB, Efron N, Woods CA. An international survey of contact lens prescribing for presbyopia. *Clin Exp Optom* 2011;94:87-92.
24. Morgan PB, Efron N, Woods CA. An international survey of toric contact lens prescribing. *Eye and Contact Lens* 2013;39:132-137.
25. Efron N, Morgan PB, Woods CA. International survey of contact lens prescribing for extended wear. *Optometry and Vision Science* 2012;89:122-129.
26. Efron N, Morgan PB, Woods CA, et al. An international survey of daily disposable contact lens prescribing. *Clinical and Experimental Optometry* 2013;96:58-64.
27. Morgan PB, Efron N, Helland M, et al. Twenty first century trends in silicone hydrogel contact lens fitting: An international perspective. *Contact Lens And Anterior Eye* 2010;33:196-198.
28. Chryssolor A. Guest editorial. Contact lens manufacturing - past and present. *Journal of the British Contact Lens Association* 1988;11:7.
29. Gupta H, Aqil M. Contact lenses in ocular therapeutics. *Drug Discov Today* 2012;17:522-527.
30. Wichterle O, Lim D. Method for producing shaped articles from three-dimensional hydrophilic polymers. US patent # 2 976 576; 1961.
31. Maldonado-Codina C, Efron N. Impact of manufacturing technology and material composition on the surface characteristics of hydrogel contact lenses. *Clinical and Experimental Optometry* 2005;88:396-404.
32. McDermott S, Walsh JE, Howard RG. A novel application of UV-LEDs in the contact lens manufacturing process. In: Byrne HJ, Lewis E, MacCraith BD, McGlynn E (eds). Dublin; 2005:119-130.
33. Guy JS. *Pharmacology for the prehospital professional*. St. Louis, Mo.: Mosby/Elsevier; 2010:xvi, 456 p.
34. Brenner GM, Stevens CW. *Pharmacology*. 3rd ed. Philadelphia, PA: Saunders/Elsevier; 2010:vii, 540 p.
35. Waldman SA, Terzic A. *Pharmacology and therapeutics : principles to practice*. Philadelphia, PA: Saunders/Elsevier; 2009:xix, 1378 p.
36. Wecker L, Crespo LM, Brody TM. *Brody's human pharmacology : molecular to clinical*. 5th ed. Philadelphia, PA: Mosby/Elsevier; 2010:xviii, 688 p.
37. McGavock H. *How drugs work : basic pharmacology for healthcare professionals*. 3rd ed. Oxford ; New York: Radcliffe Pub.; 2011:ix, 193 p.
38. Choonara YE, Pillay V, Danckwerts MP, Carmichael TR, Du Toit LC. A review of implantable intravitreal drug delivery technologies for the treatment of posterior segment eye diseases. *JPharmSci* 2010;99:2219-2239.
39. Gaudana R, Ananthula HK, Parenky A, Mitra AK. Ocular drug delivery. *The AAPS journal* 2010;12:348-360.
40. Bourlais CL, Acar L, Zia H, Sado PA, Needham T, Leverge R. Ophthalmic drug delivery systems--recent advances. *Progress in Retinal Eye Research* 1998;17:33-58.
41. Lee SS, Hughes P, Ross AD, Robinson MR. Biodegradable implants for sustained drug release in the eye. *PharmRes* 2010;27:2043-2053.
42. Rawas-Qalaji M, Williams CA. Advances in ocular drug delivery. *Current Eye Research* 2012;37:345-356.

43. Yasukawa T, Ogura Y, Kimura H, Sakurai E, Tabata Y. Drug delivery from ocular implants. *Expert Opinion on Drug Delivery* 2006;3:261-273.
44. Mitra AK. *Ophthalmic drug delivery systems*. 2nd ed. New York, NY: Marcel Dekker; 2003:xiv, 727 p.
45. Ghate D, Edelhauser HF. Ocular drug delivery. *Expert Opinion on Drug Delivery* 2006;3:275-287.
46. Kuno N, Fujii S. Recent advances in ocular drug delivery systems. *Polymers* 2011;3:193-221.
47. Garg A. *Clinical applications of antibiotics and anti-inflammatory drugs in ophthalmology*. New Delhi, India

Philadelphia: Jaypee Brothers Medical Publishers ;

Lippincott Williams & Wilkins; 2007:xviii, 598 p.
48. Pearson RM, Hopkins GA. *Ophthalmic drugs : diagnostic and therapeutic uses*. 5th ed. Edinburgh ; London ; New York: Butterworth Heinemann : Elsevier; 2007:vii, 331 p.
49. Borase Manoj B, Kale Sachin S, Baviskar Dheeraj T, Jain Dinesh K. Comparative review on conventional advanced occular drug delivery system. *International Journal of Research in Pharmaceutical Sciences* 2012;3:215-223.
50. Doughty MJ. *Ocular pharmacology and therapeutics : a primary care guide*. Oxford ; Boston: Butterworth-Heinemann/Optician; 2001:132 p.
51. Duvall B, Kershner RM. *Ophthalmic medications and pharmacology*. 2nd ed. Thorofare, NJ: SLACK Inc.; 2006:130 p.
52. Clark AF, Yorio T. Ophthalmic drug discovery. *Nature Reviews Drug Discovery* 2003;2:448-459.
53. Bourges JL, Bloquel C, Thomas A, et al. Intraocular implants for extended drug delivery: Therapeutic applications. *AdvDrug DelivRev* 2006;58:1182-1202.
54. Novack GD. Ophthalmic drug delivery: development and regulatory considerations. *Clin Pharmacol Ther* 2009;85:539-543.
55. Short BG. Safety evaluation of ocular drug delivery formulations: Techniques and practical considerations. *Toxicologic Pathology* 2008;36:49-62.
56. Schwartz GF, Plake KS, Mychaskiw MA. An assessment of readiness for behaviour change in patients prescribed ocular hypotensive therapy. *Eye (Lond)* 2009;23:1668-1674.
57. Nordstrom BL, Friedman DS, Mozaffari E, Quigley HA, Walker AM. Persistence and adherence with topical glaucoma therapy. *Am J Ophthalmol* 2005;140:598-606.
58. Behar-Cohen F. Drug delivery to the eye: Current trends and future perspectives. *Therapeutic Delivery* 2012;3:1135-1137.
59. White CJ, Byrne ME. Molecularly imprinted therapeutic contact lenses. *Expert Opinion on Drug Delivery* 2010;7:765.
60. Ciolino JB, Dohlman CH, Kohane DS. Contact lenses for drug delivery. *SeminOphthalmol* 2009;24:156.
61. Saettone MF, Salminen L. Ocular inserts for topical delivery. *AdvDrug DelivRev* 1995;16:95-106.
62. Sihvola P, Puustjarvi T. Practical problems in the use of Ocusert®-pilocarpine delivery system. *Acta Ophthalmologica* 1980;58:933-937.
63. Greenwald Y, Kleinmann G. Use of collagen shields for ocular-surface drug delivery. *Expert Review of Ophthalmology* 2008;3:627-633.

64. Vasantha V, Sehgal PK, Rao KP. Collagen ophthalmic inserts for pilocarpine drug delivery system. *IntJPharm* 1988;47:95-102.
65. Unterman SR, Rootman DS, Hill JM, Parelman JJ, Thompson HW, Kaufman HE. Collagen shield drug delivery: Therapeutic concentrations of tobramycin in the rabbit cornea and aqueous humor. *Journal of Cataract and Refractive Surgery* 1988;14:500-504.
66. Sawusch MR, O'Brien TP, Dick JD, Gottsch JD. Use of collagen corneal shields in the treatment of bacterial keratitis. *American Journal of Ophthalmology* 1988;106:279-281.
67. O'Brien TP, Sawusch MR, Dick JD, Hamburg TR, Gottsch JD. Use of collagen corneal shields versus soft contact lenses to enhance penetration of topical tobramycin. *Journal of Cataract and Refractive Surgery* 1988;14:505-507.
68. Sawusch MR, O'Brien TP, Updegraff SA. Collagen corneal shields enhance penetration of topical prednisolone acetate. *Journal of Cataract and Refractive Surgery* 1989;15:625-628.
69. Schwartz SD, Harrison SA, Engstrom Jr RE, Bawdon RE, Lee DA, Mondino BJ. Collagen shield delivery of amphotericin B. *American Journal of Ophthalmology* 1990;109:701-704.
70. PRODUCT MONOGRAPH: AzaSite® (azithromycin ophthalmic solution) 1%. Inspire Pharmaceuticals.
71. Ariturk N, Oge I, Erkan D, Sullu Y, Sahin M. The effects of nasolacrimal canal blockage on topical medications for glaucoma. *Acta Ophthalmol Scand* 1996;74:411-413.
72. Bartlett JD, Boan K, Corliss D, Gaddie IB. Efficacy of silicone punctal plugs as adjuncts to topical pharmacotherapy of glaucoma--a pilot study. Punctal Plugs in Glaucoma Study Group. *J Am Optom Assoc* 1996;67:664-668.
73. Chee SP. Moxifloxacin punctum plug for sustained drug delivery. *Journal of Ocular Pharmacology and Therapeutics* 2012;28:340-349.
74. Mohammad Zaki AJ, Patil SK, Baviskar DT, Jain DK. Implantable drug delivery system: A review. *International Journal of PharmTech Research* 2012;4:280-292.
75. Sanborn GE, Anand R, Torti RE, et al. Sustained-release ganciclovir therapy for treatment of cytomegalovirus retinitis: Use of an intravitreal device. *Arch Ophthalmol* 1992;110:188-195.
76. del Amo EM, Urtti A. Current and future ophthalmic drug delivery systems. A shift to the posterior segment. *Drug Discovery Today* 2008;13:135-143.
77. Martin DF, Parks DJ, Mellow SD, et al. Treatment of cytomegalovirus retinitis with an intraocular sustained- release ganciclovir implant: A randomized controlled clinical trial. *Arch Ophthalmol* 1994;112:1531-1539.
78. Jaffe GJ, Martin D, Callanan D, Pearson PA, Levy B, Comstock T. Fluocinolone Acetonide Implant (Retisert) for Noninfectious Posterior Uveitis. Thirty-Four-Week Results of a Multicenter Randomized Clinical Study. *Ophthalmology* 2006;113:1020-1027.
79. Driot JY, Novack GD, Rittenhouse KD, Milazzo C, Pearson PA. Ocular pharmacokinetics of fluocinolone acetonide after Retisert™ intravitreal implantation in rabbits over a 1-year period. *Journal of Ocular Pharmacology and Therapeutics* 2004;20:269-275.
80. Mohammad DA, Sweet BV, Elner SG. Retisert: Is the new advance in treatment of uveitis a good one? *Annals of Pharmacotherapy* 2007;41:449-455.
81. Carle MV, Chu TG, Boyer DS. Fluocinolone acetonide (Iluvien) extended-release intravitreal implant for diabetic macular edema. *Expert Review of Ophthalmology* 2014;9:81-88.
82. Sarao V, Veritti D, Boscia F, Lanzetta P. Intravitreal steroids for the treatment of retinal diseases. *The Scientific World Journal* 2014;2014.

83. Sejpal P, Scott-Weideman J. Surgical intervention of steroid-induced ocular hypertension from ozurdex. *Optometry and Vision Science* 2013;90:e24-e30.
84. Kuppermann BD, Blumenkranz MS, Haller JA, et al. Randomized controlled study of an intravitreous dexamethasone drug delivery system in patients with persistent macular edema. *Arch Ophthalmol* 2007;125:309-317.
85. Sedlacek J. Possibility of the application of ophthalmic drugs with the use of gel contact lenses. *Ceska a Slovenska Oftalmologie* 1965;21:509-512.
86. Gasset AR, Kaufman HE. Therapeutic uses of hydrophilic contact lenses. *Am J Ophthalmol* 1970;71:1185 - 1189.
87. Kaufman HE, Gasset AR. Therapeutic soft bandage lenses. *Int Ophthalmol Clin* 1970;10:379-385.
88. Waltman S, Kaufman H. Use of hydrophilic contact lenses to increase ocular penetration of drugs. *Invest Ophthalmol Vis Sci* 1970;9:250 - 255.
89. Hoare TR, Kohane DS. Hydrogels in drug delivery: Progress and challenges. *Polymer* 2008;49:1993-2007.
90. Alvarez-Lorenzo C, Hiratani H, Concheiro A. Contact lenses for drug delivery: Achieving sustained release with novel systems. *American Journal of Drug Delivery* 2006;4:131-151.
91. Bengani LC, Hsu KH, Gause S, Chauhan A. Contact lenses as a platform for ocular drug delivery. *Expert Opinion on Drug Delivery* 2013;10:1483-1496.
92. Jung Jung H, Chauhan A. Ophthalmic drug delivery by contact lenses. *Expert Review of Ophthalmology* 2012;7:199-201.
93. Momose T, Ito N, Kanai A, Watanabe Y, Shibata M. Adsorption of levocabastine eye drops by soft contact lenses and its effects in rabbit eyes. *CLAO Journal* 1997;23:96-99.
94. Hehl EM, Beck R, Luthard K, Guthoff R, Drewelow B. Improved penetration of aminoglycosides and fluoroquinolones into the aqueous humour of patients by means of Acuvue contact lenses. *European Journal of Clinical Pharmacology* 1999;55:317-323.
95. Tian X, Iwatsu M, Kanai A. Disposable 1-Day Acuvue® contact lenses for the delivery of lomefloxacin to rabbits' eyes. *CLAO Journal* 2001;27:212-215.
96. Salz JJ, Reader Iii AL, Schwartz LJ, Van Le K. Treatment of corneal abrasions with soft contact lenses and topical diclofenac. *Journal of Refractive and Corneal Surgery* 1994;10:640-646.
97. Karlgard CCS, Jones LW, Moresoli C. Survey of bandage lens use in North America, October-December 2002. *Eye and Contact Lens* 2004;30:25.
98. Guzman-Aranguez A, Colligris B, Pintor J. Contact lenses: Promising devices for ocular drug delivery. *Journal of Ocular Pharmacology and Therapeutics* 2013;29:189-199.
99. Bengani L, Chauhan A. Are contact lenses the solution for effective ophthalmic drug delivery? *Future Medicinal Chemistry* 2012;4:2141-2143.
100. Hillman JS. Management of acute glaucoma with pilocarpine-soaked hydrophilic lens. *Br J Ophthalmol* 1974;58:674-679.
101. Hillman JS, Marsters JB, Broad A. Pilocarpine delivery by hydrophilic lens in the management of acute glaucoma. *Trans Ophthalmol Soc U K* 1975;95:79-84.
102. Busin M, Spitznas M. Sustained gentamicin release by presoaked medical bandage contact lenses. *Ophthalmol* 1988;95:796 - 798.

103. Cerulli L, Cimmino PT, Ravagnan GP. The pharmacokinetics of antibiotics vehiculed by soft contact lenses: An experimental approach. *Contact and Intraocular Lens Medical Journal* 1981;7:39-49.
104. Tanaka I. An experimental study on intracorneal transfer of an antibiotic in the application of soft contact lens. *Yokohama Medical Journal* 1988;39:35-42.
105. Zatloukal Z, Dolezal P, Sklubalova Z. Release of pilocarpin from therapeutic lenses. *Cesko-Slovenska Farmacie* 1986;35:318-321.
106. Karlgard CC, Jones LW, Moresoli C. Ciprofloxacin interaction with silicon-based and conventional hydrogel contact lenses. *Eye Contact Lens* 2003;29:83-89.
107. Karlgard CC, Wong NS, Jones LW, Moresoli C. In vitro uptake and release studies of ocular pharmaceutical agents by silicon-containing and p-HEMA hydrogel contact lens materials. *Int J Pharm* 2003;257:141-151.
108. Boone A, Hui A, Jones L. Uptake and release of dexamethasone phosphate from silicone hydrogel and group I, II, and IV hydrogel contact lenses. *Eye & Contact Lens* 2009;35:260.
109. Soluri A, Hui A, Jones L. Delivery of ketotifen fumarate by commercial contact lens materials. *Optom Vis Sci* 2012;89:1140-1149.
110. Winterton LC, Lally JM, Sentell KB, Chapoy LL. The elution of poly (vinyl alcohol) from a contact lens: the realization of a time release moisturizing agent/artificial tear. *J Biomed Mater Res B Appl Biomater* 2007;80:424-432.
111. Peng CC, Kim J, Chauhan A. Extended delivery of hydrophilic drugs from silicone-hydrogel contact lenses containing vitamin E diffusion barriers. *Biomaterials* 2010;31:4032-4047.
112. Peng CC, Burke MT, Chauhan A. Transport of topical anesthetics in vitamin e loaded silicone hydrogel contact lenses. *Langmuir* 2012;28:1478-1487.
113. Peng CC, Chauhan A. Extended cyclosporine delivery by silicone-hydrogel contact lenses. *JControlled Release* 2011;154:267-274.
114. Kim J, Conway A, Chauhan A. Extended delivery of ophthalmic drugs by silicone hydrogel contact lenses. *Biomaterials* 2008;29:2259.
115. Kim J, Peng CC, Chauhan A. Extended release of dexamethasone from silicone-hydrogel contact lenses containing vitamin E. *JControlled Release* 2010;148:110-116.
116. Peng CC, Ben-Shlomo A, MacKay EO, Plummer CE, Chauhan A. Drug delivery by contact lens in spontaneously glaucomatous dogs. *Current Eye Research* 2012;37:204-211.
117. Ciolino JB, Hoare TR, Iwata NG, et al. A drug-eluting contact lens. *Invest Ophthalmol Vis Sci* 2009;50:3346-3352.
118. Ciolino JB, Hudson SP, Mobbs AN, et al. A prototype antifungal contact lens. *Invest Ophthalmol Vis Sci* 2011;52:6286-6291.
119. Alexander C, Andersson HS, Andersson LI, et al. Molecular imprinting science and technology: A survey of the literature for the years up to and including 2003. *Journal of Molecular Recognition* 2006;19:106-180.
120. Byrne ME, Park K, Peppas NA. Molecular imprinting within hydrogels. *AdvDrug DelivRev* 2002;54:149.
121. Cunliffe D, Kirby A, Alexander C. Molecularly imprinted drug delivery systems. *AdvDrug DelivRev* 2005;57:1836-1853.
122. Alvarez-Lorenzo C, Hiratani H, Gómez-Amoza JL, Martínez-Pacheco R, Souto C, Concheiro A. Soft contact lenses capable of sustained delivery of timolol. *JPharmSci* 2002;91:2182.

123. Hiratani H, Alvarez-Lorenzo C. Timolol uptake and release by imprinted soft contact lenses made of N,N-diethylacrylamide and methacrylic acid. *JControlled Release* 2002;83:223.
124. Alvarez-Lorenzo C, Yañez F, Barreiro-Iglesias R, Concheiro A. Imprinted soft contact lenses as norfloxacin delivery systems. *JControlled Release* 2006;113:236.
125. Andrade-Vivero P, Fernandez-Gabriel E, Alvarez-Lorenzo C, Concheiro A. Improving the loading and release of NSAIDs from pHEMA hydrogels by copolymerization with functionalized monomers. *JPharmSci* 2007;96:802.
126. Ali M, Horikawa S, Venkatesh S, Saha J, Hong JW, Byrne ME. Zero-order therapeutic release from imprinted hydrogel contact lenses within in vitro physiological ocular tear flow. *JControlled Release* 2007;124:154.
127. White CJ, McBride MK, Pate KM, Tieppo A, Byrne ME. Extended release of high molecular weight hydroxypropyl methylcellulose from molecularly imprinted, extended wear silicone hydrogel contact lenses. *Biomaterials* 2011;32:5698-5705.
128. Venkatesh S, Saha J, Pass S, Byrne ME. Transport and structural analysis of molecular imprinted hydrogels for controlled drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics* 2008;69:852.
129. Venkatesh S, Sizemore SP, Byrne ME. Biomimetic hydrogels for enhanced loading and extended release of ocular therapeutics. *Biomaterials* 2007;28:717.
130. Malaekeh-Nikouei B, Ghaeni FA, Motamedshariaty VS, Mohajeri SA. Controlled release of prednisolone acetate from molecularly imprinted hydrogel contact lenses. *Journal of Applied Polymer Science* 2012;126:387-394.
131. Malaekeh-Nikouei B, Vahabzadeh SA, Mohajeri SA. Preparation of a molecularly imprinted soft contact lens as a new ocular drug delivery system for dorzolamide. *Curr Drug Deliv* 2013.
132. Hiratani H, Alvarez-Lorenzo C. The nature of backbone monomers determines the performance of imprinted soft contact lenses as timolol drug delivery systems. *Biomaterials* 2004;25:1105-1113.
133. Tieppo A, White CJ, Paine AC, Voyles ML, McBride MK, Byrne ME. Sustained in vivo release from imprinted therapeutic contact lenses. *JControlled Release* 2012;157:391-397.
134. Hiratani H, Fujiwara A, Tamiya Y, Mizutani Y, Alvarez-Lorenzo C. Ocular release of timolol from molecularly imprinted soft contact lenses. *Biomaterials* 2005;26:1293-1298.
135. Alvarez-Lorenzo C, Yañez F, Concheiro A. Ocular drug delivery from molecularly-imprinted contact lenses. *Journal of Drug Delivery Science and Technology* 2010;20:237-248.
136. Lorenzo RA, Carro AM, Alvarez-Lorenzo C, Concheiro A. To remove or not to remove? The challenge of extracting the template to make the cavities available in molecularly imprinted polymers (MIPs). *International Journal of Molecular Sciences* 2011;12:4327-4347.
137. Madigan MT, Martinko JM, Brock TD. *Brock biology of microorganisms*. 11th ed. Upper Saddle River, N.J.: Pearson Prentice Hall; 2006:1 v. (various pagings).
138. Brooks GF, Jawetz E, Melnick JL, et al. Jawetz, Melnick & Adelberg's medical microbiology. [New York]: McGraw-Hill; 2013.
139. Harvey RA, Champe PC, Fisher BD, Strohl WA. *Microbiology*. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 2007:ix, 438 p.
140. Prescott LM, Harley JP, Klein DA. *Microbiology*. 6th ed. Boston: McGraw-Hill Higher Education; 2005:1 v. (various pagings).
141. Willey JM, Sherwood L, Woolverton CJ, Prescott LM. *Prescott, Harley, and Klein's microbiology*. 7th ed. New York: McGraw-Hill Higher Education; 2008:xx, 1088, [1107] p.

142. Jones DS, Podolsky SH, Greene JA. The burden of disease and the changing task of medicine. *N Engl J Med* 2012;366:2333-2338.
143. Behlau I, Gilmore MS. Microbial biofilms in ophthalmology and infectious disease. *Arch Ophthalmol* 2008;126:1572-1581.
144. Mah FS. New antibiotics for bacterial infections. *Ophthalmology Clinics of North America* 2003;16:11-27.
145. Rodríguez-Rojas A, Rodríguez-Beltrán J, Couce A, Blázquez J. Antibiotics and antibiotic resistance: A bitter fight against evolution. *International Journal of Medical Microbiology* 2013;303:293-297.
146. Laxminarayan R, Duse A, Wattal C, et al. Antibiotic resistance—the need for global solutions. *The Lancet Infectious Diseases* 2013;13:1057-1098.
147. MacGowan A, Macnaughton E. Antimicrobial therapy: principles of use. *Medicine* 2013;41:635-641.
148. Drusano GL. Antimicrobial pharmacodynamics: Critical interactions of 'bug and drug'. *Nature Reviews Microbiology* 2004;2:289-300.
149. López-Cabezas C, Muner DS, Massa MR, Pueyo JMM. Antibiotics in endophthalmitis: Microbiological and pharmacokinetic considerations. *Current Clinical Pharmacology* 2010;5:47-54.
150. Kowalski RP, Karenchak LM, Romanowski EG. Infectious disease: Changing antibiotic susceptibility. *Ophthalmology Clinics of North America* 2003;16:1-9.
151. Baum J, Barza M. The evolution of antibiotic therapy for bacterial conjunctivitis and keratitis: 1970-2000. *Cornea* 2000;19:659-672.
152. Tananuvat N, Suwanniponth M. Microbial keratitis in Thailand: A survey of common practice patterns. *Journal of the Medical Association of Thailand* 2008;91:316-322.
153. Daniell M. Overview: Initial antimicrobial therapy for microbial keratitis. *Br J Ophthalmol* 2003;87:1172-1174.
154. Utine CA. Update and critical appraisal of the use of topical azithromycin ophthalmic 1% (AzaSite®) solution in the treatment of ocular infections. *Clinical Ophthalmology* 2011;5:801-809.
155. Federici TJ. The non-antibiotic properties of tetracyclines: Clinical potential in ophthalmic disease. *Pharmacological Research* 2011;64:614-623.
156. Salamon SM. Tetracyclines in ophthalmology. *Survey of Ophthalmology* 1985;29:265-275.
157. Zhanel GG, Fontaine S, Adam H, et al. A review of new fluoroquinolones: Focus on their use in respiratory tract infections. *Treatments in Respiratory Medicine* 2006;5:437-465.
158. Olson R. Zymar as an ocular therapeutic agent. *International Ophthalmology Clinics* 2006;46:73-84.
159. Scoper SV. Review of third-and fourth-generation fluoroquinolones in ophthalmology: in-vitro and in-vivo efficacy. *Adv Ther* 2008;25:979-994.
160. Schlech BA, Alfonso E. Overview of the potency of moxifloxacin ophthalmic solution 0.5% (VIGAMOX®). *Survey of Ophthalmology* 2005;50:S7-S15.
161. Schlech BA, Blondeau J. Future of ophthalmic anti-infective therapy and the role of moxifloxacin ophthalmic solution 0.5% (VIGAMOX®). *Survey of Ophthalmology* 2005;50:S64-S67.
162. Bertino Jr JS. Impact of antibiotic resistance in the management of ocular infections: The role of current and future antibiotics. *Clinical Ophthalmology* 2009;3:507-521.

163. Azari AA, Barney NP. Conjunctivitis: a systematic review of diagnosis and treatment. *Jama* 2013;310:1721-1729.
164. Dalhoff A, Ambrose PG, Mouton JW. A long journey from minimum inhibitory concentration testing to clinically predictive breakpoints: Deterministic and probabilistic approaches in deriving breakpoints. *Infection* 2009;37:296-305.
165. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. *Clinical Infectious Diseases* 2009;49:1749-1755.
166. Kronvall G, Giske CG, Kahlmeter G. Setting interpretive breakpoints for antimicrobial susceptibility testing using disk diffusion. *International Journal of Antimicrobial Agents* 2011;38:281-290.
167. Tenover FC. Antibiotic Susceptibility Testing. In: Editor-in-Chief: Moselio S (ed), *Encyclopedia of Microbiology (Third Edition)*. Oxford: Academic Press; 2009:67-77.
168. Kiska DL. In vitro testing of antimicrobial agents. *Seminars in Pediatric Infectious Diseases* 1998;9:281-291.
169. Turnidge J, Paterson DL. Setting and revising antibacterial susceptibility breakpoints. *Clinical Microbiology Reviews* 2007;20:391-408.
170. Keay L, Edwards K, Dart J, Stapleton F. Grading contact lens-related microbial keratitis: relevance to disease burden. *Optom Vis Sci* 2008;85:531-537.
171. Edwards K, Keay L, Naduvilath T, Snibson G, Taylor H, Stapleton F. Characteristics of and risk factors for contact lens-related microbial keratitis in a tertiary referral hospital. *Eye (Lond)* 2009;23:153-160.
172. Panda A, Vanathi M. Management approach for bacterial and fungal corneal ulcers. *Expert Review of Ophthalmology* 2008;3:471-479.
173. Bourcier T, Thomas F, Borderie V, Chaumeil C, Laroche L. Bacterial keratitis: predisposing factors, clinical and microbiological review of 300 cases. *Br J Ophthalmol* 2003;87:834-838.
174. Dahlgren MA, Lingappan A, Wilhelmus KR. The clinical diagnosis of microbial keratitis. *Am J Ophthalmol* 2007;143:940-944.
175. Bennett H, Hay J, Kirkness C, Seal D, Devonshire P. Antimicrobial management of presumed microbial keratitis: guidelines for treatment of central and peripheral ulcers. *Brit J Ophthalmol* 1998;82:137-145.
176. Schein OD, Glynn RJ, Poggio EC, Seddon JM, Kenyon KR. The relative risk of ulcerative keratitis among users of daily-wear and extended-wear soft contact lenses. A case-control study. Microbial Keratitis Study Group. *N Engl J Med* 1989;321:773-778.
177. Lam DS, Houang E, Fan DS, Lyon D, Seal D, Wong E. Incidence and risk factors for microbial keratitis in Hong Kong: comparison with Europe and North America. *Eye* 2002;16:608-618.
178. Szczotka-Flynn L, Ahmadian R, Diaz M. A re-evaluation of the risk of microbial keratitis from overnight contact lens wear compared with other life risks. *Eye Contact Lens* 2009;35:69-75.
179. Stapleton F, Keay L, Edwards K, et al. The incidence of contact lens-related microbial keratitis in Australia. *Ophthalmology* 2008;115:1655-1662.
180. Dart JK, Radford CF, Minassian D, Verma S, Stapleton F. Risk factors for microbial keratitis with contemporary contact lenses: a case-control study. *Ophthalmology* 2008;115:1647-1654, 1654 e1641-1643.

181. Dart JK, Stapleton F, Minassian D. Contact lenses and other risk factors in microbial keratitis. *Lancet* 1991;338:650-653.
182. Wong T, Ng T, Fong K, Tan D. Risk factors and clinical outcomes between fungal and bacterial keratitis: a comparative study. *Clao J* 1997;23:275-.
183. Miedziak AI, Miller MR, Rapuano CJ, Laibson PR, Cohen EJ. Risk factors in microbial keratitis leading to penetrating keratoplasty. *Ophthalmology* 1999;106:1166-1170; discussion 1171.
184. Bharathi MJ, Ramakrishnan R, Meenakshi R, Padmavathy S, Shivakumar C, Srinivasan M. Microbial keratitis in South India: influence of risk factors, climate, and geographical variation. *Ophthalmic Epidemiol* 2007;14:61-69.
185. Green M, Apel A, Stapleton F. Risk factors and causative organisms in microbial keratitis. *Cornea* 2008;27:22-27.
186. Saeed A, D'Arcy F, Stack J, Collum LM, Power W, Beatty S. Risk factors, microbiological findings, and clinical outcomes in cases of microbial keratitis admitted to a tertiary referral center in ireland. *Cornea* 2009;28:285-292.
187. Young AL, Leung KS, Tsim N, Hui M, Jhanji V. Risk factors, microbiological profile, and treatment outcomes of pediatric microbial keratitis in a tertiary care hospital in Hong Kong. *Am J Ophthalmol* 2013;156:1040-1044 e1042.
188. Keay L, Stapleton F. Development and evaluation of evidence-based guidelines on contact lens-related microbial keratitis. *Cont Lens Anterior Eye* 2008;31:3-12.
189. PRODUCT MONOGRAPH: CILOXAN® 0.3% Ciprofloxacin Hydrochloride Ophthalmic Solution and Ointment (0.3% as ciprofloxacin) Antibacterial Agent. ALCON CANADA INC.
190. Hanet MS, Jamart J, Chaves AP. Fluoroquinolones or fortified antibiotics for treating bacterial keratitis: systematic review and meta-analysis of comparative studies. *Can J Ophthalmol* 2012;47:493-499.
191. Shah VM, Tandon R, Satpathy G, et al. Randomized clinical study for comparative evaluation of fourth-generation fluoroquinolones with the combination of fortified antibiotics in the treatment of bacterial corneal ulcers. *Cornea* 2010;29:751-757.
192. Constantinou M, Daniell M, Snibson GR, Vu HT, Taylor HR. Clinical efficacy of moxifloxacin in the treatment of bacterial keratitis: a randomized clinical trial. *Ophthalmology* 2007;114:1622-1629.
193. O'Brien TP. Management of bacterial keratitis: beyond exorcism towards consideration of organism and host factors. *Eye (Lond)* 2003;17:957-974.
194. Fleiszig SMJ, Evans DJ. Pathogenesis of Contact Lens-associated microbial keratitis. *Optometry and Vision Science* 2010;87:225-232.
195. Fleiszig SMJ, Evans DJ. The pathogenesis of bacterial keratitis: Studies with Pseudomonas aeruginosa. *Clinical and Experimental Optometry* 2002;85:271-278.
196. Morgan PB, Efron N, Hill EA, Raynor MK, Whiting MA, Tullo AB. Incidence of keratitis of varying severity among contact lens wearers. *Br J Ophthalmol* 2005;89:430-436.
197. Evans DJ, Fleiszig SMJ. Why does the healthy cornea resist pseudomonas aeruginosa infection? *American Journal of Ophthalmology* 2013;155:961-970.e962.
198. Carnt N, Willcox MD, Keay L, Flanagan J, Stapleton F. Pathogenesis of contact lens-associated microbial keratitis. *Optom Vis Sci* 2010;87:612-613; author reply 613-614.
199. Marquart ME. Animal models of bacterial keratitis. *J Biomed Biotechnol* 2011;2011:680642.

200. Cowell BA, Wu C, Fleiszig SMJ. Use of an animal model in studies of bacterial corneal infection. *ILAR Journal* 1999;40:43-50.
201. Dajcs JJ, Thibodeaux BA, Marquart ME, Girgis DO, Traidej M, O'Callaghan RJ. Effectiveness of ciprofloxacin, levofloxacin, or moxifloxacin for treatment of experimental Staphylococcus aureus keratitis. *Antimicrobial Agents and Chemotherapy* 2004;48:1948-1952.
202. Wu X, Jiang H, Xu Y, et al. Efficacy of gemifloxacin for the treatment of experimental staphylococcus aureus keratitis. *Journal of Ocular Pharmacology and Therapeutics* 2012;28:420-427.
203. Aswad MI, Baum J, Barza M. The effect of cleaning and disinfection of soft contact lenses on corneal infectivity in an animal model. *American Journal of Ophthalmology* 1995;119:738-743.
204. Blaylock WK, Yue BYJT, Robin JB. The use of concanavalin A to competitively inhibit Pseudomonas aeruginosa adherence to rabbit corneal epithelium. *CLAO Journal* 1990;16:223-227.
205. Frucht-Pery J, Golan G, Hemo I, Zauberman H, Shapiro M. Efficacy of topical gentamicin treatment after 193-nm photorefractive keratectomy in an experimental Pseudomonas keratitis model. *Graefe's Archive for Clinical and Experimental Ophthalmology* 1995;233:532-534.
206. Koch JM, Refojo MF, Hanninen LA, Leong FL, Kenyon KR. Experimental Pseudomonas aeruginosa keratitis from extended wear of soft contact lenses. *Arch Ophthalmol* 1990;108:1453-1459.
207. Mannis MJ. The use of antimicrobial peptides in ophthalmology: An experimental study in corneal preservation and the management of bacterial keratitis. *Transactions of the American Ophthalmological Society* 2002;100:243-271.
208. Michalová K, Moyes AL, Cameron S, et al. Povidone-iodine (betadine) in the treatment of experimental Pseudomonas aeruginosa keratitis. *Cornea* 1996;15:533-536.
209. Davis SD, Sarff LD, Hyndiuk RA. Staphylococcal keratitis. Experimental model in guinea pigs. *Arch Ophthalmol* 1978;96:2114-2116.
210. Cole N, Hume EBH, Vijay AK, Sankaridurg P, Kumar N, Willcox MDP. In vivo performance of melimine as an antimicrobial coating for contact lenses in models of CLARE and CLPU. *InvestOphthalmolVisual Sci* 2010;51:390-395.
211. Schechter JE, Warren DW, Mircheff AK. A lacrimal gland is a lacrimal gland, but rodent's and rabbit's are not human. *Ocular Surface* 2010;8:111-134.

# CHAPTER 2 REFERENCES


1. Klein R, Klein BEK. The prevalence of age-related eye diseases and visual impairment in aging: Current estimates. *InvestOphthalmolVisual Sci* 2013;54:ORSF5-ORSF13.
2. White CJ, Byrne ME. Molecularly imprinted therapeutic contact lenses. *Expert Opinion on Drug Delivery* 2010;7:765.
3. Ciolino JB, Dohlman CH, Kohane DS. Contact lenses for drug delivery. *SeminOphthalmol* 2009;24:156.
4. Borase Manoj B, Kale Sachin S, Baviskar Dheeraj T, Jain Dinesh K. Comparative review on conventional advanced occular drug delivery system. *International Journal of Research in Pharmaceutical Sciences* 2012;3:215-223.
5. Ghate D, Edelhauser HF. Ocular drug delivery. *Expert Opinion on Drug Delivery* 2006;3:275-287.
6. Kuno N, Fujii S. Recent advances in ocular drug delivery systems. *Polymers* 2011;3:193-221.
7. Hillman JS. Management of acute glaucoma with pilocarpine-soaked hydrophilic lens. *Br J Ophthalmol* 1974;58:674-679.
8. Busin M, Spitznas M. Sustained gentamicin release by presoaked medical bandage contact lenses. *Ophthalmol* 1988;95:796 - 798.
9. Cerulli L, Cimmino PT, Ravagnan GP. The pharmacokinetics of antibiotics vehiculed by soft contact lenses: An experimental approach. *Contact and Intraocular Lens Medical Journal* 1981;7:39-49.
10. Tanaka I. An experimental study on intracorneal transfer of an antibiotic in the application of soft contact lens. *Yokohama Medical Journal* 1988;39:35-42.
11. Edwards K, Keay L, Naduvilath T, Snibson G, Taylor H, Stapleton F. Characteristics of and risk factors for contact lens-related microbial keratitis in a tertiary referral hospital. *Eye (Lond)* 2009;23:153-160.
12. Panda A, Vanathi M. Management approach for bacterial and fungal corneal ulcers. *Expert Review of Ophthalmology* 2008;3:471-479.
13. Bourcier T, Thomas F, Borderie V, Chaumeil C, Laroche L. Bacterial keratitis: predisposing factors, clinical and microbiological review of 300 cases. *Br J Ophthalmol* 2003;87:834-838.

# CHAPTER *3* REFERENCES


1. Lesher GA, Gunderson GG. Continuous drug delivery through the use of disposable contact lenses. *Optom Vis Sci.* Dec 1993;70(12):1012-1018.
2. Sedlacek J. Possibilities of application of eye drugs with the aid of gel-contact lenses. *Cesk Slov Oftalmol.* 1965;21:509 - 512.
3. Fonn D, Bruce AS. A review of the Holden-Mertz criteria for critical oxygen transmission. *Eye Contact Lens.* Nov 2005;31(6):247-251.
4. Holden BA, Sankaridurg PR, Sweeney DF, et al. Microbial keratitis in prospective studies of extended wear with disposable hydrogel contact lenses. *Cornea.* Mar 2005;24(2):156-161.
5. Stapleton F, Stretton S, Papas E, et al. Silicone hydrogel contact lenses and the ocular surface. *Ocul Surf.* Jan 2006;4(1):24-43.
6. Sweeney D, du Toit R, Keay L, et al. Clinical performance of silicone hydrogel lenses. In: Sweeney D, ed. *Silicone hydrogels: Continuous wear contact lenses*. 2nd ed. Oxford: Butterworth-Heinemann; 2004:164 - 216.
7. Lim L, Tan DT, Chan WK. Therapeutic use of Bausch & Lomb PureVision contact lenses. *CLAO J.* Oct 2001;27(4):179-185.
8. Ambroziak AM, Szaflik JP, Szaflik J. Therapeutic use of a silicone hydrogel contact lens in selected clinical cases. *Eye Contact Lens.* Jan 2004;30(1):63-67.
9. Bendoriene J, Vogt U. Therapeutic use of silicone hydrogel contact lenses in children. *Eye Contact Lens.* Mar 2006;32(2):104-108.
10. Karlgard CC, Jones LW, Moresoli C. Ciprofloxacin interaction with silicon-based and conventional hydrogel contact lenses. *Eye Contact Lens.* Apr 2003;29(2):83-89.
11. Karlgard CC, Wong NS, Jones LW, et al. In vitro uptake and release studies of ocular pharmaceutical agents by silicon-containing and p-HEMA hydrogel contact lens materials. *Int J Pharm.* May 12 2003;257(1-2):141-151.
12. Hehl EM, Beck R, Luthard K, et al. Improved penetration of aminoglycosides and fluoroquinolones into the aqueous humour of patients by means of Acuvue contact lenses. *Eur J Clin Pharmacol.* 1999;55(4):317-323.
13. Marchese AL, Slana VS, Holmes EW, et al. Toxicity and pharmacokinetics of ciprofloxacin. *J Ocul Pharmacol.* Spring 1993;9(1):69-76.
14. Smith A, Pennefather PM, Kaye SB, et al. Fluoroquinolones: place in ocular therapy. *Drugs.* 2001;61(6):747-761.
15. Bower KS, Kowalski RP, Gordon YJ. Fluoroquinolones in the treatment of bacterial keratitis. *Am J Ophthalmol.* Jun 1996;121(6):712-715.
16. Stevens SX, Fouraker BD, Jensen HG. Intraocular safety of ciprofloxacin. *Arch Ophthalmol.* Dec 1991;109(12):1737-1743.
17. Bedard J, Bryan LE. Interaction of the fluoroquinolone antimicrobial agents ciprofloxacin and enoxacin with liposomes. *Antimicrob Agents Chemother.* Aug 1989;33(8):1379-1382.
18. Ross D, Riley C. Aqueous solubilities of some variously substituted quinolone antimicrobials. *Int J Pharm.* 1990;63:237 - 250.
19. Wilhelmus KR, Abshire RL. Corneal ciprofloxacin precipitation during bacterial keratitis. *Am J Ophthalmol.* Dec 2003;136(6):1032-1037.
20. Efron N, Young G, Brennan NA. Ocular surface temperature. *Curr Eye Res.* Sep 1989;8(9):901-906.

21. Oliveira AD, D'Azevedo PA, Francisco W. In vitro activity of fluoroquinolones against ocular bacterial isolates in Sao Paulo, Brazil. *Cornea.* Feb 2007;26(2):194-198.
22. Ruben M, Watkins R. Pilocarpine dispensation for the soft hydrophilic contact lens. *Br J Ophthalmol.* Aug 1975;59(8):455-458.
23. Matoba AY, McCulley JP. The effect of therapeutic soft contact lenses on antibiotic delivery to the cornea. *Ophthalmology.* Jan 1985;92(1):97-99.
24. Momose T, Ito N, Kanai A, et al. Adsorption of levocabastine eye drops by soft contact lenses and its effects in rabbit eyes. *Clao J.* Apr 1997;23(2):96-99.
25. Baker D, Tighe B. Polymers in contact lens applications (VIII) the problem of biocompatibility. *Contact Lens J.* 1981;10(3):3-14.
26. Lopez-Alemany A, Compan V, Refojo MF. Porous structure of Purevision versus Focus Night&Day and conventional hydrogel contact lenses. *J Biomed Mater Res.* 2002;63(3):319-325.
27. Foulks GN, Harvey T, Raj CV. Therapeutic contact lenses: the role of high-Dk lenses. *Ophthalmol Clin North Am.* Sep 2003;16(3):455-461.
28. Arora R, Jain S, Monga S, et al. Efficacy of continuous wear PureVision contact lenses for therapeutic use. *Cont Lens Anterior Eye.* Mar 2004;27(1):39-43.
29. Montero J, Sparholt J, Mely R, et al. Retrospective case series of therapeutic applications of lotrafilcon a silicone hydrogel soft contact lenses. *Eye Contact Lens.* Apr 2003;29(2):72-75.
30. Montero J, Sparholt J, Mely R. Retrospective case series of therapeutic applications of a lotrafilcon A silicone hydrogel soft contact lens. *Eye Contact Lens.* Jan 2003;29(1 Suppl):S54-56; discussion S57-59, S192-194.
31. Szaflik JP, Ambroziak AM, Szaflik J. Therapeutic use of a lotrafilcon A silicone hydrogel soft contact lens as a bandage after LASEK surgery. *Eye Contact Lens.* Jan 2004;30(1):59-62.
32. Mely R. Therapeutic and cosmetic indications of lotrafilcon a silicone hydrogel extended-wear lenses. *Ophthalmologica.* 2004;218 Suppl 1:29-38; discussion 45-26.
33. Ozkurt Y, Rodop O, Oral Y, et al. Therapeutic applications of lotrafilcon a silicone hydrogel soft contact lenses. *Eye Contact Lens.* Nov 2005;31(6):268-269.
34. Subbaraman LN, Glasier MA, Senchyna M, et al. Kinetics of in vitro lysozyme deposition on silicone hydrogel, PMMA, and FDA groups I, II, and IV contact lens materials. *Curr Eye Res.* Oct 2006;31(10):787-796.
35. Jones L, Subbaraman LN, Rogers R, et al. Surface treatment, wetting and modulus of silicone hydrogels. *Optician.* 2006;232(6067):28 - 34.

# CHAPTER 4 REFERENCES


1. Riley, C.; Young, G.; Chalmers, R., Prevalence of ocular surface symptoms, signs, and uncomfortable hours of wear in contact lens wearers: The effect of refitting with daily-wear silicone hydrogel lenses (senofilcon A). *Eye and Contact Lens* 2006, *32*, 281-286.
2. White, C. J.; Byrne, M. E., Molecularly imprinted therapeutic contact lenses. *Expert Opinion on Drug Delivery* 2010, *7*, 765.
3. Villani, E.; Ceresara, G.; Beretta, S.; Magnani, F.; Viola, F.; Ratiglia, R., In vivo confocal microscopy of meibomian glands in contact lens wearers. *Investigative ophthalmology & visual science* 2011, *52*, 5215-5219.
4. Hoare, T. R.; Kohane, D. S., Hydrogels in drug delivery: Progress and challenges. *Polymer* 2008, *49*, 1993-2007.
5. Lesher, G. A.; Gunderson, G. G., Continuous drug delivery through the use of disposable contact lenses. *Optom Vis Sci* 1993, *70*, 1012-1018.
6. Reynolds, A.; Moore, J. E.; Naroo, S. A.; Moore, C. T.; Shah, S., Excimer laser surface ablation - a review. *Clinical and Experimental Ophthalmology* 2010, *38*, 168-182.
7. Boone, A.; Hui, A.; Jones, L., Uptake and release of dexamethasone phosphate from silicone hydrogel and group I, II, and IV hydrogel contact lenses. *Eye and Contact Lens* 2009, *35*, 260.
8. Hui, A.; Boone, A.; Jones, L., Uptake and release of ciprofloxacin-HCl from conventional and silicone hydrogel contact lens materials. *Eye and Contact Lens* 2008, *34*, 266.
9. Karlgard, C. C. S.; Jones, L. W.; Moresoli, C., Ciprofloxacin interaction with silicon-based and conventional hydrogel contact lenses. *Eye and Contact Lens* 2003, *29*, 83.
10. Karlgard, C. C. S.; Wong, N. S.; Jones, L. W.; Moresoli, C., In vitro uptake and release studies of ocular pharmaceutical agents by silicon-containing and p-HEMA hydrogel contact lens materials. *International journal of pharmaceutics* 2003, *257*, 141.
11. Kim, J.; Peng, C. C.; Chauhan, A., Extended release of dexamethasone from silicone-hydrogel contact lenses containing vitamin E. *Journal of Controlled Release* 2010, *148*, 110-116.
12. Peng, C. C.; Kim, J.; Chauhan, A., Extended delivery of hydrophilic drugs from silicone-hydrogel contact lenses containing vitamin E diffusion barriers. *Biomaterials* 2010, *31*, 4032-4047.
13. Weiner, A. L.; Gilger, B. C., Advancements in ocular drug delivery. *Veterinary Ophthalmology* 2010, *13*, 395-406.
14. Xu, J.; Li, X.; Sun, F., Cyclodextrin-containing hydrogels for contact lenses as a platform for drug incorporation and release. *Acta Biomaterialia* 2010, *6*, 486.
15. Danion, A.; Arsenault, I.; Vermette, P., Antibacterial activity of contact lenses bearing surface-immobilized layers of intact liposomes loaded with levofloxacin. *Journal of pharmaceutical sciences* 2007, *96*, 2350-2363.
16. Alvarez-Lorenzo, C.; Concheiro, A., Molecularly imprinted polymers for drug delivery. *J Chromatogr B Analyt Technol Biomed Life Sci* 2004, *804*, 231-245.
17. Hiratani, H.; Alvarez-Lorenzo, C., Timolol uptake and release by imprinted soft contact lenses made of N,N-diethylacrylamide and methacrylic acid. *Journal of Controlled Release* 2002, *83*, 223.
18. Alvarez-Lorenzo, C.; Concheiro, A., Molecularly imprinted polymers for drug delivery. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* 2004, *804*, 231.

19. Ali, M.; Byrne, M. E., Controlled release of high molecular weight hyaluronic acid from molecularly imprinted hydrogel contact lenses. *Pharmaceutical research* 2009, *26*, 714.
20. Alvarez-Lorenzo, C.; Yañez, F.; Barreiro-Iglesias, R.; Concheiro, A., Imprinted soft contact lenses as norfloxacin delivery systems. *Journal of Controlled Release* 2006, *113*, 236.
21. Andrade-Vivero, P.; Fernandez-Gabriel, E.; Alvarez-Lorenzo, C.; Concheiro, A., Improving the loading and release of NSAIDs from pHEMA hydrogels by copolymerization with functionalized monomers. *Journal of pharmaceutical sciences* 2007, *96*, 802.
22. Venkatesh, S.; Sizemore, S. P.; Byrne, M. E., Biomimetic hydrogels for enhanced loading and extended release of ocular therapeutics. *Biomaterials* 2007, *28*, 717.
23. Ali, M.; Horikawa, S.; Venkatesh, S.; Saha, J.; Hong, J. W.; Byrne, M. E., Zero-order therapeutic release from imprinted hydrogel contact lenses within in vitro physiological ocular tear flow. *Journal of Controlled Release* 2007, *124*, 154.
24. White, C. J.; McBride, M. K.; Pate, K. M.; Tieppo, A.; Byrne, M. E., Extended release of high molecular weight hydroxypropyl methylcellulose from molecularly imprinted, extended wear silicone hydrogel contact lenses. *Biomaterials* 2011, *32*, 5698-5705.
25. Bedard, J.; Bryan, L. E., Interaction of the fluoroquinolone antimicrobial agents ciprofloxacin and enoxacin with liposomes. *Antimicrob Agents Chemother* 1989, *33*, 1379-1382.
26. Marchese, A. L.; Slana, V. S.; Holmes, E. W.; Jay, W. M., Toxicity and pharmacokinetics of ciprofloxacin. *J Ocul Pharmacol* 1993, *9*, 69-76.
27. Smith, A.; Pennefather, P. M.; Kaye, S. B.; Hart, C. A., Fluoroquinolones: place in ocular therapy. *Drugs* 2001, *61*, 747-761.
28. Bower, K. S.; Kowalski, R. P.; Gordon, Y. J., Fluoroquinolones in the treatment of bacterial keratitis. *Am J Ophthalmol* 1996, *121*, 712-715.
29. PRODUCT MONOGRAPH: CILOXAN® 0.3% Ciprofloxacin Hydrochloride Ophthalmic Solution and Ointment (0.3% as ciprofloxacin) Antibacterial Agent. http://www.alcon.ca/pdf/Product_pharma/Product_pharma_ciloxan_eng.pdf (December 22, 2011),
30. Ross, D. L.; Riley, C. M., Aqueous solubilities of some variously substituted quinolone antimicrobials. *International journal of pharmaceutics* 1990, *63*, 237-250.
31. Wilhelmus, K. R.; Abshire, R. L., Corneal ciprofloxacin precipitation during bacterial keratitis. *Am J Ophthalmol* 2003, *136*, 1032-1037.
32. Oliveira, A. D. D.; D'Azevedo, P. A.; Francisco, W.; Höfling-Lima, A. L., In vitro activity of fluoroquinolones against ocular bacterial isolates in São Paulo, Brazil. *Cornea* 2007, *26*, 194-198.
33. Hiratani, H.; Mizutani, Y.; Alvarez-Lorenzo, C., Controlling drug release from imprinted hydrogels by modifying the characteristics of the imprinted cavities. *Macromolecular Bioscience* 2005, *5*, 728.
34. Kowalski, R. P.; Dhaliwal, D. K.; Karenchak, L. M.; Romanowski, E. G.; Mah, F. S.; Ritterband, D. C.; Gordon, Y. J., Gatifloxacin and moxifloxacin: An in vitro susceptibility comparison to levofloxacin, ciprofloxacin, and ofloxacin using bacterial keratitis isolates. *American Journal of Ophthalmology* 2003, *136*, 500-505.
35. Schultz, C.; Breaux, J.; Schentag, J.; Morck, D., Drug delivery to the posterior segment of the eye through hydrogel contact lenses. *Clin Exp Optom* 2011, *94*, 212-218.
36. Van Beek, M.; Weeks, A.; Jones, L.; Sheardown, H., Immobilized hyaluronic acid containing model silicone hydrogels reduce protein adsorption. *Journal of Biomaterials Science. Polymer edition* 2008, *19*, 1425-1436.

37. Lorentz, H.; Heynen, M.; Kay, L.; Dominici, C.; Khan, W.; Ng, W.; Jones, L., Contact lens physical properties and lipid deposition in a characterized artificial tear solution. *Molecular Vision* 2011, *Accepted December 8, 2011*.

# CHAPTER 5 REFERENCES


1. Keay L, Stapleton F. Development and evaluation of evidence-based guidelines on contact lens-related microbial keratitis. *Cont Lens Anterior Eye* 2008;31:3-12.
2. Keay L, Stapleton F, Schein O. Epidemiology of contact lens-related inflammation and microbial keratitis: a 20-year perspective. *Eye Contact Lens* 2007;33:346-353, discussion 362-343.
3. Stapleton F, Carnt N. Contact lens-related microbial keratitis: how have epidemiology and genetics helped us with pathogenesis and prophylaxis. *Eye (Lond)* 2012;26:185-193.
4. Edwards K, Keay L, Naduvilath T, Snibson G, Taylor H, Stapleton F. Characteristics of and risk factors for contact lens-related microbial keratitis in a tertiary referral hospital. *Eye (Lond)* 2009;23:153-160.
5. Stapleton F, Keay L, Edwards K, et al. The Incidence of Contact Lens-Related Microbial Keratitis in Australia. *Ophthalmology* 2008;115:1655-1662.
6. Young AL, Leung KS, Tsim N, Hui M, Jhanji V. Risk factors, microbiological profile, and treatment outcomes of pediatric microbial keratitis in a tertiary care hospital in Hong Kong. *Am J Ophthalmol* 2013;156:1040-1044 e1042.
7. White CJ, Byrne ME. Molecularly imprinted therapeutic contact lenses. *Expert Opinion on Drug Delivery* 2010;7:765.
8. Ghate D, Edelhauser HF. Barriers to glaucoma drug delivery. *J Glaucoma* 2008;17:147-156.
9. Saeed A, D'Arcy F, Stack J, Collum LM, Power W, Beatty S. Risk factors, microbiological findings, and clinical outcomes in cases of microbial keratitis admitted to a tertiary referral center in ireland. *Cornea* 2009;28:285-292.
10. Keay L, Edwards K, Dart J, Stapleton F. Grading contact lens-related microbial keratitis: relevance to disease burden. *Optom Vis Sci* 2008;85:531-537.
11. Sedlácek J. Possibility of the application of ophthalmic drugs with the use of gel contact lenses. *Ceskoslovenska oftalmologie* 1965;21:509-512.
12. Sellergren B, Allender CJ. Molecularly imprinted polymers: A bridge to advanced drug delivery. *AdvDrug DelivRev* 2005;57:1733-1741.
13. Sulley A. Practitioner and patient acceptance of a new silicone hydrogel contact lens. *Optician* 2005;230:15 - 17.
14. Covey M, Sweeney DF, Terry R, Sankaridurg PR, Holden BA. Hypoxic effects on the anterior eye of high-Dk soft contact lens wearers are negligible. *Optom Vis Sci* 2001;78:95-99.
15. Karlgard CC, Jones LW, Moresoli C. Survey of bandage lens use in North America, October-December 2002. *Eye Contact Lens* 2004;30:25-30.
16. Hui A, Boone A, Jones L. Uptake and release of ciprofloxacin-HCl from conventional and silicone hydrogel contact lens materials. *Eye & Contact Lens* 2008;34:266.
17. Boone A, Hui A, Jones L. Uptake and release of dexamethasone phosphate from silicone hydrogel and group I, II, and IV hydrogel contact lenses. *Eye & Contact Lens* 2009;35:260.
18. Karlgard CCS, Wong NS, Jones LW, Moresoli C. In vitro uptake and release studies of ocular pharmaceutical agents by silicon-containing and p-HEMA hydrogel contact lens materials. *IntJPharm* 2003;257:141.
19. Soluri A, Hui A, Jones L. Delivery of ketotifen fumarate by commercial contact lens materials. *Optom Vis Sci* 2012;89:1140-1149.

20. Peng CC, Kim J, Chauhan A. Extended delivery of hydrophilic drugs from silicone-hydrogel contact lenses containing vitamin E diffusion barriers. *Biomaterials* 2010;31:4032-4047.
21. Peng CC, Ben-Shlomo A, MacKay EO, Plummer CE, Chauhan A. Drug delivery by contact lens in spontaneously glaucomatous dogs. *Current Eye Research* 2012;37:204-211.
22. Ciolino JB, Hoare TR, Iwata NG, et al. A drug-eluting contact lens. *InvestOphthalmolVisSci* 2009;50:3346.
23. Ciolino JB, Hudson SP, Mobbs AN, et al. A prototype antifungal contact lens. *Invest Ophthalmol Vis Sci* 2011;52:6286-6291.
24. Alvarez-Lorenzo C, Concheiro A. Molecularly imprinted polymers for drug delivery. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* 2004;804:231.
25. Byrne ME, Park K, Peppas NA. Molecular imprinting within hydrogels. *AdvDrug DelivRev* 2002;54:149.
26. White CJ, Tieppo A, Byrne ME. Controlled drug release from contact lenses: A comprehensive review from 1965-present. *Journal of Drug Delivery Science and Technology* 2011;21:369-384.
27. Hiratani H, Alvarez-Lorenzo C. The nature of backbone monomers determines the performance of imprinted soft contact lenses as timolol drug delivery systems. *Biomaterials* 2004;25:1105.
28. Alvarez-Lorenzo C, Yañez F, Barreiro-Iglesias R, Concheiro A. Imprinted soft contact lenses as norfloxacin delivery systems. *JControlled Release* 2006;113:236.
29. White CJ, McBride MK, Pate KM, Tieppo A, Byrne ME. Extended release of high molecular weight hydroxypropyl methylcellulose from molecularly imprinted, extended wear silicone hydrogel contact lenses. *Biomaterials* 2011;32:5698-5705.
30. Malaekeh-Nikouei B, Ghaeni FA, Motamedshariaty VS, Mohajeri SA. Controlled release of prednisolone acetate from molecularly imprinted hydrogel contact lenses. *Journal of Applied Polymer Science* 2012;126:387-394.
31. Hiratani H, Alvarez-Lorenzo C. Timolol uptake and release by imprinted soft contact lenses made of N,N-diethylacrylamide and methacrylic acid. *JControlled Release* 2002;83:223.
32. Ali M, Byrne ME. Controlled release of high molecular weight hyaluronic acid from molecularly imprinted hydrogel contact lenses. *PharmRes* 2009;26:714.
33. Ali M, Horikawa S, Venkatesh S, Saha J, Hong JW, Byrne ME. Zero-order therapeutic release from imprinted hydrogel contact lenses within in vitro physiological ocular tear flow. *JControlled Release* 2007;124:154.
34. Hui A, Sheardown H, Jones L. Acetic and Acrylic Acid Molecular Imprinted Model Silicone Hydrogel Materials for Ciprofloxacin-HCl Delivery. *Materials* 2012;5:85-107.
35. Malaekeh-Nikouei B, Vahabzadeh SA, Mohajeri SA. Preparation of a molecularly imprinted soft contact lens as a new ocular drug delivery system for dorzolamide. *Curr Drug Deliv* 2013.
36. Menzies KL, Jones L. The impact of contact angle on the biocompatibility of biomaterials. *Optometry and Vision Science* 2010;87:387-399.
37. Menzies KL, Rogers R, Jones L. In vitro contact angle analysis and physical properties of blister pack solutions of daily disposable contact lenses. *Eye and Contact Lens* 2010;36:10-18.

38. Cole N, Willcox MD, Fleiszig SM, et al. Different strains of Pseudomonas aeruginosa isolated from ocular infections or inflammation display distinct corneal pathologies in an animal model. *Curr Eye Res* 1998;17:730-735.
39. Tenover FC. Antibiotic Susceptibility Testing. In: Editor-in-Chief: Moselio S (ed), *Encyclopedia of Microbiology (Third Edition)*. Oxford: Academic Press; 2009:67-77.
40. Karlgard C, Jones L, Moresoli C. Uptake and release of Acular from silicone-hydrogel and conventional hydrogel contact lens materials. *Optom Vis Sci* 2000;77:179.
41. Karlgard C, Jones L, Moresoli C. Uptake and release of ciloxan from silicone-hydrogel and conventional hydrogel contact lens materials. *Invest Ophthalmol Vis Sci* 2001;42:s592.
42. Hiratani H, Mizutani Y, Alvarez-Lorenzo C. Controlling drug release from imprinted hydrogels by modifying the characteristics of the imprinted cavities. *Macromolecular Bioscience* 2005;5:728.
43. Drusano GL. Antimicrobial pharmacodynamics: Critical interactions of 'bug and drug'. *Nature Reviews Microbiology* 2004;2:289-300.
44. Nightingale J. Clinical limitations of in vitro testing of microorganism susceptibility. *American Journal of Hospital Pharmacy* 1987;44:131-137.
45. Parry MF, Neu HC. Effect of N acetylcysteine on antibiotic activity and bacterial growth in vitro. *Journal of Clinical Microbiology* 1977;5:58-61.
46. Menzies KL, Rogers R, Jones L. In vitro contact angle analysis and physical properties of blister pack solutions of daily disposable contact lenses. *Eye Contact Lens* 2010;36:10-18.
47. Marquart ME. Animal models of bacterial Keratitis. *Journal of Biomedicine and Biotechnology* 2011;2011.
48. Hume EB, Dajcs JJ, Moreau JM, Sloop GD, Willcox MD, O'Callaghan RJ. Staphylococcus corneal virulence in a new topical model of infection. *Invest Ophthalmol Vis Sci* 2001;42:2904-2908.
49. Wu X, Jiang H, Xu Y, et al. Efficacy of gemifloxacin for the treatment of experimental staphylococcus aureus keratitis. *Journal of Ocular Pharmacology and Therapeutics* 2012;28:420-427.
50. Kakisu K, Matsunaga T, Kobayakawa S, Sato T, Tochikubo T. Development and efficacy of a drug-releasing soft contact lens. *Invest Ophthalmol Vis Sci* 2013;54:2551-2561.

# CHAPTER 6 REFERENCES

1. Wilhelmus KR, Abshire RL. Corneal ciprofloxacin precipitation during bacterial keratitis. *Am J Ophthalmol* 2003;136:1032-1037.
2. Soluri A, Hui A, Jones L. Delivery of ketotifen fumarate by commercial contact lens materials. *Optom Vis Sci* 2012;89:1140-1149.
3. Alvarez-Lorenzo C, Yañez F, Barreiro-Iglesias R, Concheiro A. Imprinted soft contact lenses as norfloxacin delivery systems. *JControlled Release* 2006;113:236.

# APPENDIX 1 REFERENCES


1. Yang R, Fu Y, Li LD, Liu JM. Medium effects on fluorescence of ciprofloxacin hydrochloride. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy* 2003;59:2723-2732.
2. Ulu ST. Spectrofluorimetric determination of fluoroquinolones in pharmaceutical preparations. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy* 2009;72:138-143.
3. Kaur K, Kumar A, Malik AK, Singh B, Rao ALJ. Spectrophotometric methods for the determination of fluoroquinolones: A review. *Critical Reviews in Analytical Chemistry* 2008;38:2-18.
4. Kaur K, Singh B, Malik AK. Chemiluminescence and spectrofluorimetric methods for determination of fluoroquinolones: A review. *Analytical Letters* 2011;44:1602-1639.
5. Belal F, Al-Majed AA, Al-Obaid AM. Methods of analysis of 4-quinolone antibacterials. *Talanta* 1999;50:765-786.
6. Jin W, Jin J. Quantitative determination of ciprofloxacin hydrochloride tablets by fluorescence spectrophotometry. *Chinese Journal of Antibiotics* 1996;21:195-197.
7. Ross DL, Riley CM. Aqueous solubilities of some variously substituted quinolone antimicrobials. *IntJPharm* 1990;63:237-250.
8. PRODUCT MONOGRAPH: CILOXAN® 0.3% Ciprofloxacin Hydrochloride Ophthalmic Solution and Ointment (0.3% as ciprofloxacin) Antibacterial Agent. ALCON CANADA INC.
9. Marquart ME. Animal models of bacterial keratitis. *J Biomed Biotechnol* 2011;2011:680642.
10. Oo TZ, Cole N, Garthwaite L, Willcox MDP, Zhu H. Evaluation of synergistic activity of bovine lactoferricin with antibiotics in corneal infection. *Journal of Antimicrobial Chemotherapy* 2010;65:1243-1251.

# APPENDIX 1 - METHOD DEVELOPMENT AND TROUBLESHOOTING

## CIPROFLOXACIN SOLUTIONS

Ciprofloxacin, the antibiotic used in this thesis, is a second generation fluoroquinolone antibiotic. While it is useful clinically, its preparation within the laboratory requires some knowledge of the chemical properties of the drug. The family of fluoroquinolones all contain similar structures of a fluorinated quinolone ring, which is shown in Figure A-1a. The structure of ciprofloxacin is shown in Figure A-1b. There have been numerous publications describing laboratory techniques to detect and quantify fluoroquinolones.[1-5]

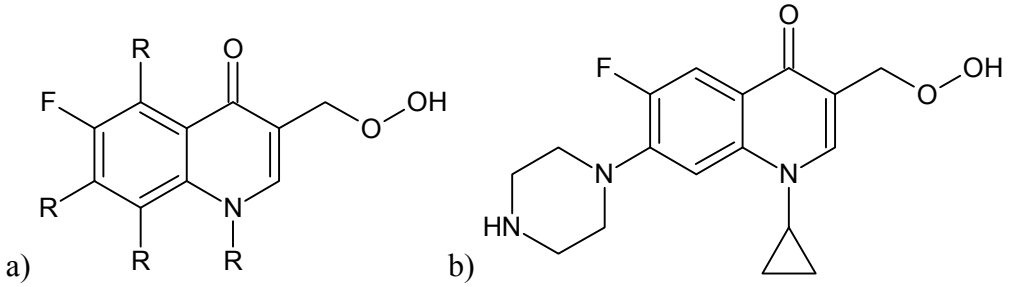



**Figure A-1 Chemical Structure of Fluoroquinolone Rings and Ciprofloxacin**
a) Generalized structure of Fluoroquinolone rings and b) ciprofloxacin. The -R subgroups of the general fluoroquinolone can be substituted with various different chemical entities which will have effects on the chemical properties and antibacterial spectrum of the resultant molecule.

Spectrophotometry based analysis for the detection of ciprofloxacin has been found to be a sensitive and inexpensive technique to determine the concentration of ciprofloxacin within a solution. [6] Spectrophotometric analysis relies on the natural fluorescence of a molecule. For ciprofloxacin, when a liquid sample is exposed to light of wavelength of 270 to 280 nm, it will fluoresce and emit light with a wavelength peak of 420 nm. [6] The amount of light emitted at 420 nm can be correlated to the concentration of ciprofloxacin within the solution. Use of this

technique requires a sensitive fluorescence spectrophotometer, such as the Hitachi F-4500 (Hitachi Ltd, Tokyo, Japan) or the Spectramax M5 Microplate reader (Molecular Devices, Sunnyvale, California). There are two ionizable groups on ciprofloxacin, a carboxylic acid group and an amino group, which can be alternatively protonated/deprotonated at different pH levels. The listed pKa values for ciprofloxacin are 6.00 and 8.80. [7] The molecule exists as a zwitterion (a molecule with a positive charge on one portion of the molecule and a negative charge on the other portion, leading to an overall neutral charge) at physiological pH, leading to very poor aqueous solubility at that pH. In acidic or basic pH, there is a significant increase in ciprofloxacin solubility. Thus, the majority of ophthalmic preparations of ciprofloxacin are adjusted for pH to be slightly acidic. Ciprofloxacin ophthalmic drops are listed on their product monograph of having a pH of approximately 4.0. [8] Preparation within the laboratory must take pH into account if a stable solution is to be created. A stable solution is necessary if spectrophotometry is used to determine the concentration of the ciprofloxacin within the solution, as unreliable readings would result if a precipitated solution was examined. Heating of ciprofloxacin within a neutral buffer such as phosphate buffered saline (PBS) will increase solubility of the molecule, but after the solution cools the ciprofloxacin with simply precipitate out of solution. As detailed in the experimental chapters, to combat this ciprofloxacin solutions were created in acidic media. The most useful was a pH 4.0 acetic acid-sodium acetate buffer, as the pKa of acetic acid is 4.76.

## CONTACT LENS MANUFACTURING

Several difficulties needed to be overcome to manufacture the molecular-imprinted discs into “true” contact lenses. The majority of these challenges did not become apparent until the

experiments described in Chapter 5, when actual contact lenses were being formed, versus the thicker discs which were formed during Chapter 4. First, as model silicone hydrogel lenses were being formed, some means were necessary to increase the wettability of the ocular surface. As the equipment and technology needed for plasma oxidation or plasma coating employed by Bausch and Lomb and CIBA Vision for their lenses were unavailable, it was decided to utilize the internal wetting agent strategy employed by VISTAKON by incorporating polyvinyl pyrrolidone (PVP) into the reaction mixture. Secondly, the light transmission of lenses which had ciprofloxacin dissolved within them was very poor. The reaction under the UV light when ciprofloxacin was directly loaded into the polymerization mixture led to an opaque yellow coloration to the thin contact lenses. To combat this, the ciprofloxacin was initially dissolved in chloroform with the acrylic acid functional monomer. Ciprofloxacin has significantly better solubility within chloroform compared to water, and the acidic pH imparted by the acrylic acid enhances the solubility even further. This mixture could then be added to the polymerization mixture before being placed within the UV oven. The third challenge to contact lens manufacturing was the curing time. A delicate balance is needed for the amount of time that the lenses are cured. If left curing for too long, the lenses became incredibly dried out, and developed holes where the material had pulled away from the mold. Too short of a curing time and the lenses produced were extremely fragile and floppy, which were signs that the polymerization reaction was incomplete, and there still existed liquid monomers which had not fully polymerized. A time of five minutes was found to be optimal for the creation of the molecular imprinted materials. The exact time of curing is dependent on the UV light emission power from the curing system. As the running age of the UV bulb increases, as well as the number of times the bulb has been switched on and off, the power output of the bulb decreased,

and the curing times had to be increased accordingly. Finally, removal of the cured contact lenses from the contact lens molds was occasionally problematic. The lens sometimes stuck to the polypropylene contact lens mold and because of their brittleness, was liable to crack or break if forcibly removed with tweezers or other means. This was alleviated partially by two modifications to the protocol. The cure time was decreased accordingly to five minutes to prevent overexposure and brittleness development, and lenses which were cured that still were difficult to remove from the molds were soaked in saline buffer. The buffer hydrated the lens and the lens eventually released from the mould into the solution.

## RABBIT MODELS OF MICROBIAL KERATITIS

There were several considerations when we considered the design of our model of Microbial Keratitis (MK). Methods of injection into the corneal stroma with a bacterial solution have the advantage of a more consistent MK response, as the bacteria have bypassed many of the ocular barriers and defence mechanisms. [9] This was not preferable for our experiments due to the artificial nature of the infection process, leading us to choose using a corneal epithelial scratch model of infection, as this was deemed to be more representative of the clinical reality of patients who develop MK.

The choice of experimental organism to test in our model of MK was also critical. There were many strains available which had been isolated from human cases of microbial keratitis. Unfortunately, for the majority of them tested, we were unable to generate a reliable MK response within the rabbit model using a epithelial scratch. Only isolate *P. aeruginosa* strain 6294 allowed for consistent MK responses to be generated with this model. *P. aeruginosa* strain 6294 was a particularly virulent and aggressive isolate from a human case of MK in the USA. The downside to using 6294 for our experiments was that the strain is ciprofloxacin sensitive; a

very low concentration of ciprofloxacin (0.4 μg/mL) is able to completely limit the growth of the bacteria *in vitro*. It would have proved useful to test the performance of the ciprofloxacin releasing contact lenses in the rabbit MK model caused by a ciprofloxacin resistant strain of bacteria, to see if the amount and duration of ciprofloxacin release could have overcome acquired bacterial resistance. Unfortunately, none of the ciprofloxacin resistant (MIC > 16 μg/mL ) [10] isolates available were able to generate a consistent MK response with the rabbit scratch MK model.

The incubation time after bacterial introduction within the rabbit corneas was also elucidated with some trial and error. Initial protocols submitted for animal ethical approval had interventions with the contact lenses or drops occurring 8 hours post scratch/bacterial introduction. Unfortunately, after 8 hours there were little or no signs of active infection as the bacterium did not have enough time to establish and replicate on the rabbit corneas. The protocol was modified to allow the incubation time to be extended to 16 hours before intervention, allowing a repeatable MK response to be seen in the trials.

# APPENDIX 2 - COPYRIGHT PERMISSIONS

## COPYRIGHT: NATIONAL EYE INSTITUTE (FOR FIGURE 1-2)

NIH National Eye Institute
*Research Today...Vision Tomorrow*

Search

S M L Text size

About NEI | Health Information | News & Events | Grants & Funding | Research at NEI | Education Programs | Training & Jobs

Home » NEI Photos and Images

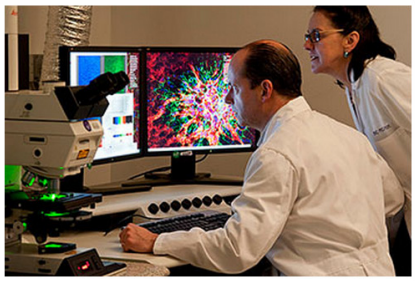
### NEI Photos and Images

#### Use of Images

The NEI Photos and Images Catalog is provided as a source of free audiovisuals. Permission is granted to use these items for educational, news media or research purposes, provided the source for each image is credited. The NEI Photos and Images catalog may not be used to promote or endorse commercial products or services. Use by non-profit organizations in connection with fundraising or product sales is considered commercial use.

Permission to use NEI website graphics found any place other than the NEI Photos and Images catalog is granted on a case-by-case basis. Some are public domain, some are created by NEI contractors, some are copyrighted and some are used by NEI with specific permission granted by the owner. Therefore, the logos, photos and illustrations found on the NEI website should not be reused without permission.

For information about the copyright holders of a given photo or illustration on the NEI website; how the owners can be contacted; and what, if any, use those owners allow of their material; please contact the NEI Website Manager and provide the URL, file name, and intended use.

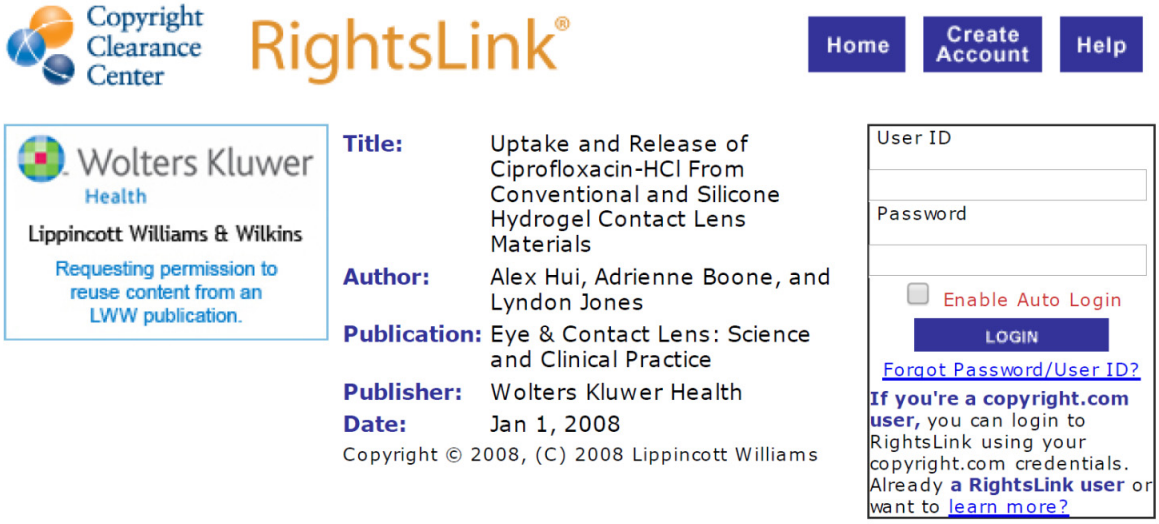


Two scientists observe ocular tissue samples with microscopic detail. (Laboratory Research Ref#:LAB30)

Granting the right to use a graphic from the website does not explicitly or implicitly convey NEI's endorsement of the site where it is used.

Search NEI Photos and Images

## COPYRIGHT: EYE AND CONTACT LENS




This reuse is free of charge. No permission letter is needed from Wolters Kluwer Health, Lippincott Williams & Wilkins. We require that all authors always include a full acknowledgement. Example: AIDS: 13 November 2013 - Volume 27 - Issue 17 - p 2679-2689. Wolters Kluwer Health Lippincott Williams & Wilkins© No modifications will be permitted.

BACK CLOSE WINDOW



## COPYRIGHT: MATERIALS

**Re: Reproduction of Manuscript for Thesis**

Materials Editorial Office [materials@mdpi.com]

**Sent:** February 8, 2014 1:57 AM
**To:** Alex Hui

Dear Hui,

Sorry for the late reply because I was out of office.

It is fine. You can reproduce the published papers in your doctoral thesis with citing it as a reference.

Best regards,
Xiaohua Zhu
Managing Editor
Materials (http://www.mdpi.com/journal/materials)

On 2014/2/5 0:29, Alex Hui wrote:
> Dear /Materials /Editorial Office,
>
> I hereby request permission to reproduce the manuscript that has
> previously been published in /Materials/ for inclusion in my Doctoral
> Thesis at the University of Waterloo.
>
> Hui A, Sheardown H, Jones L. Acetic and Acrylic Acid Molecular Imprinted
> Model Silicone Hydrogel Materials for Ciprofloxacin-HCl Delivery.
> Materials 2012;5:85-107.
>
> The original article will be cited within the thesis chapter that this
> manuscript is being reproduced. I should also note that at the
> University of Waterloo, all completed theses are publicly available on
> the internet in electronic form.
>
> I thank you for your consideration, and look forward to hearing from you.
>
> - Alex
>
> _____
>
> Alex Hui, OD
>
> Ph.D Candidate, Biological Sciences Laboratory
> Centre for Contact Lens Research
> School of Optometry and Vision Science, Rm 228
> University of Waterloo
> 200 University Avenue West
> Waterloo, ON, N2L 3G1
> Canada
>
> Phone: +1-519-888-4567 ext. 37009 <tel:%2B1-519-888-4567%20ext.%2037009>
> (lab)
>