# Uptake and release of preservatives from soft contact lens materials

by

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## Examining Committee Membership

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## 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
- Dr. Chau-Minh Phan
- Dr. David McCanna
- Dr. Karen Walsh
- Dr. Marc Schulze
- Miriam Heynen
- Vivian Chan

// Abstract

**Purpose:**

The purpose of this thesis was to examine the uptake and release of myristamidopropyl dimethylamine (MAPD) and polyhexamethylene biguanide (PHMB) from soft contact lens material using various *in vitro* models to determine the safety of the preservatives.

**Methods:**

- Chapter 3: The detection of radioactive and non-radioactive MAPD were determined with UV-Vis spectroscopy and a radioactive beta counter. MAPD was prepared in phosphate buffered saline (PBS) solutions and different vial materials (glass and polyethylene).
- Chapter 4: To determine the uptake and release kinetics of $^{14}C$ MAPD from reusable soft contact lens materials using radioactive labelling, five contemporary CLs were tested over a 1-day, and 7-day period. MAPD were extracted from contact lenses (CLs) with 2:1 chloroform:methanol at the end of each study. The radioactivity was measured using a beta scintillation counter.
- Chapter 5: To determine the uptake and release kinetics of $^{14}C$ PHMB from reusable soft contact lens materials using radioactive labelling, five contemporary CLs were tested over a 1-day, and 7-day period. CLs were soaked in PHMB for 8 hours, followed by a release in PBS for 16 hours. PHMB were extracted from CLs with methanol at the end of each study. The experimental design was similar to Chapter 4.

- Chapter 6: To evaluate the cytotoxicity of MAPD and PHMB (from Chapter 4 and 5) released from reusable soft CLs on immortalized corneal epithelial cells (ICEC) and human corneal epithelial cells (HCEC). CLs were soaked in PBS containing either PHMB or MAPD for 8 hours. After incubation period, the lenses were placed in fresh PBS for 16 hours. The release media was exposed to ICEC and HCEC for 16 hours. Afterwards, two multipurpose solutions, MAPD (2.5 µg/mL, 5 µg/mL, 10 µg/mL) and PHMB (1 µg/mL, 5 µg/mL, 10 µg/mL) concentrations were tested against ICEC and HCEC. Cell viability was then measured using the alamarBlue™ assay.

**Results:**

- Chapter 3: A mixture of radioactive and non-radioactive MAPD was able to be detected, resulting in a more cost-effective study. There were no differences in absorbance between PBS solutions. No differences in radioactivity were found between glass and polyethylene vials. However, polyethylene vials showed a more equal distribution of MAPD. The results suggest polyethylene vials are better suited for future radioactive kinetic studies (Chapter 4, Chapter 5).
- Chapter 4: Silicone hydrogel (SH) materials sorbed significantly more MAPD than the conventional hydrogel (CH) materials. However, the CH materials released a greater amount of MAPD than the SH materials. Over a 7-day period, similar results were found between SH and CH materials.
- Chapter 5: The CH material (etafilcon A) sorbed significantly more MAPD than the SH material (senofilcon A). Etafilcon A released more PHMB compared to all other

lens types within a 24-hr period. Over a 7-day period, all CLs continued to sorb more PHMB, with no signs of saturation. The CH materials released more PHMB than the SH materials.

- Chapter 6: The amount of PHMB or MAPD released from CLs did not have an impact on corneal epithelial cell viability. PHMB and MAPD at concentrations of 5 µg/mL and higher showed significantly reduced cell viability. Direct exposure to multipurpose solutions also significantly reduced cell viability.

**Conclusion:**

This thesis provided a chemical and biological assay for assessing the impact of MAPD and PHMB from CLs. Radioactive labelling provided a sensitive method for assessing the uptake and release of MAPD (Chapter 4) and PHMB (Chapter 5) from reusable soft CLs. The uptake and release of MAPD and PHMB were different based on their chemical structure and properties. In chapter 6, MAPD and PHMB released from CLs were not cytotoxic to corneal epithelial cells. Direct exposure of multipurpose solutions and increasing concentrations of MAPD and PHMB significantly reduced cell viability. These models provide a valuable tool to predict future adverse events for new multipurpose solutions.

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# Dedication

To my wife Taylor Brin, who supported me throughout this journey. I am so grateful to have you in my life. Very excited for our next journey in life.

To my parents and grandparents, for their sacrifice and hard work to provide me an education.

To future students, take a chance on yourself. Try something new, join a club, meet other leaders, and have fun learning from different people.

You never know when the next door opens.

“Use it or lose it”

“You gotta risk it to get the biscuit”

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## List of Abbreviations

| | |
|---|---|
| % | percent |
| $^{14}C$ | carbon-14 |
| $^{3}H$ | tritium |
| °C | degrees centigrade |
| g | gram |
| hrs | hours |
| L | liter |
| μg | microgram |
| mg | milligram |
| μL | microliter |
| BAK | benzalkonium chloride |
| CH | conventional hydrogel |
| CIEs | corneal infiltrative events |
| CL | contact lens |
| CLs | contact lenses |
| CPM | counts per minute |
| DMA | N,N-dimethylacrylamide |
| ECP | eye care practitioners |
| EDTA | ethylenediaminetetraacetic acid |
| EGDMA | ethylenegylcol dimethacrylate |
| FDA | Food and Drug Administration |

| | |
|---|---|
| HA | hyaluronic acid |
| HEMA | poly-2-hydroxyethyl methacrylate |
| HCEC | human corneal epithelial cells |
| HPLC | high-performance liquid chromatography |
| ICEC | immortalized corneal epithelial cells |
| ISO-PBS | phosphate buffered saline (International Organization for Standardization) |
| MAPD | myristamidopropyl dimethylamine |
| MPS | multipurpose solutions |
| NVA | N-vinyl aminobutyric acid |
| NVP | N-vinyl pyrrolidone |
| PAPB | polyaminopropyl biguanide |
| PBS | phosphate buffered saline |
| PBVC | poly[dimethysiloxy] di [silylbutanol] bis[vinyl carbamate] |
| PC | phosphorylcholine |
| PEG | polyethylene glycol |
| PG | propylene glycol |
| PI | povidone-iodine |
| pHEMA | poly(2-hydroxyethyl methacrylate) |
| PHMB | polyhexamethylene biguanide |
| PMMA | polymethylmethacrylate |
| PVP | poly(vinylpyrrolidone) |

| | |
|---|---|
| PQ-1 | polyquaternium-1 |
| SH | silicone hydrogel |
| SICS | solution induced corneal staining |
| TJ | tight junctions |
| TPVC | tris-[trimethylsiloxysilyl] propylvinyl carbamate |
| TRIS | trimethylsiloxy silane |
| WC | water content |

# Chapter 1. Literature review

## 1.1 Corneal epithelium

Located at the most anterior section of the eye is the cornea. The cornea is a transparent avascular connective tissue that acts as a physical barrier between the tear film and the intraocular environment.[1,2] The cornea is comprised of five layers, from anterior to posterior: the epithelium, Bowman's layer, stroma, Descemet's membrane, and endothelium (Figure 1.1).[3] The corneal epithelium is composed of stratified non-keratinized squamous cells that help the eye maintain a homeostatic environment under intraocular stress, atmospheric stress, tear film composition, and reduce the risk of infections.[4-6]

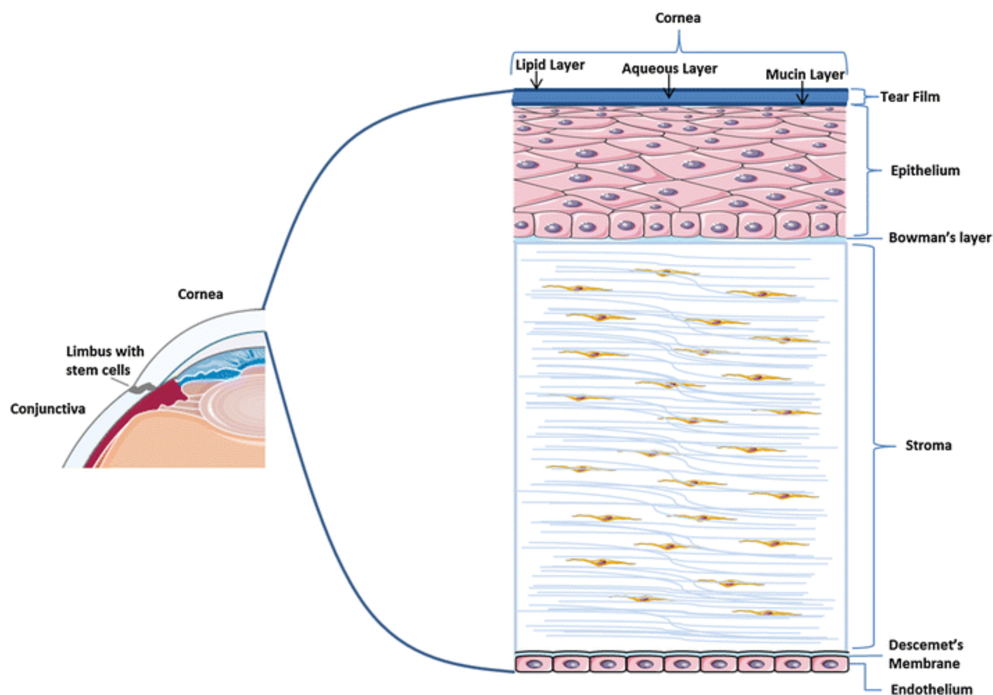


**Figure 1.1 The anatomy of the cornea and structures in the epithelium.** From "The role of lipids in corneal diseases and dystrophies: a systematic review." Rowsey TG & Karamichos D. *Clin Transl Med*. 2017;6(1):30. doi:10.1186/s40169-017-0158-1. This is an open access article distributed under the terms of the Creative Commons CC BY license, which permits unrestricted use, distribution, and reproduction in any medium. No changes were made. https://creativecommons.org/licenses/by/4.0/

The corneal epithelium is maintained and held together by a junctional complex.[1] The junctional complex is located at the most apical part of the epithelial layer and consist of three components: tight junctions (TJ), adheren junctions, and desmosomes (Figure 1.2).[7,8] The TJ consist of the proteins occludin, claudin, and the junctional adhesion molecule which play a role in supporting the structure and permeability of the cell.[9,10] The permeability of the corneal epithelial barrier is more susceptible to the hydrophilic, water soluble compounds that are transported across the tight junctions and intercellular spaces.[11,12]

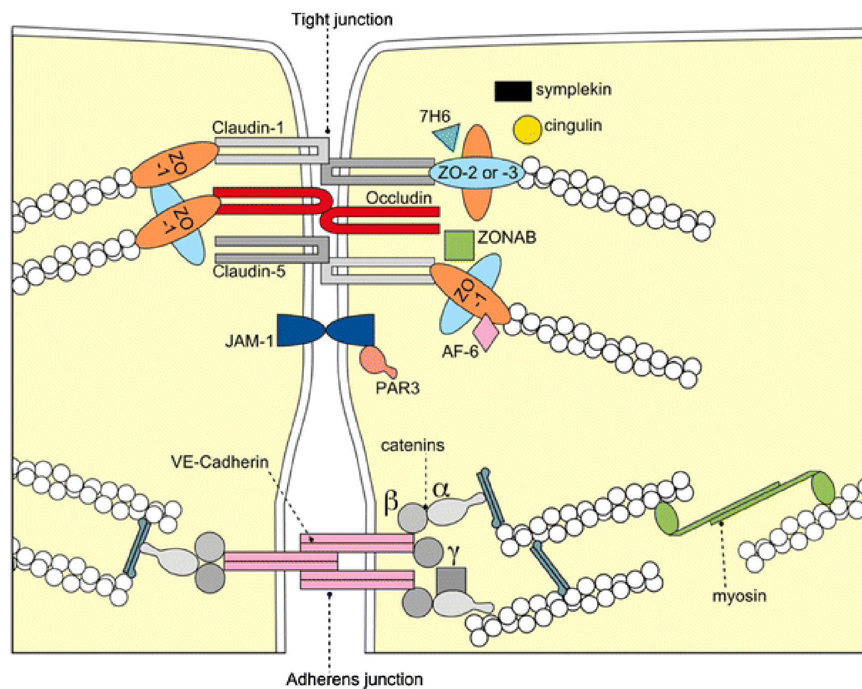


**Figure 1.2 The corneal epithelial tight junctions and various proteins influencing cell permeability.** From “Tight junctions and the modulation of barrier function in disease.” Förster, C. *Histochem Cell Biol.* 2018;130, 55–70. doi.org/10.1007/s00418-008-0424-9. This is an open access article distributed under the terms of the Creative Commons CC BY license, which permits unrestricted use, distribution, and reproduction in any medium. No changes were made. https://creativecommons.org/licenses/by/4.0/

The conjunctiva is an extension of the corneal epithelium. It is a thin transparent mucous layer that covers approximately 80% of the ocular surface including the upper and lower eyelid.[13] Within the conjunctival epithelium are goblet cells that produce mucins for the tear film.[14] The tear film is important in maintaining a healthy environment for the eye through the exchange of oxygen and nutrients, provide surface lubrication, and protection from dry eyes.[4,15,16] When irritants or drugs are applied to the surface of the cornea, it may be absorbed by the conjunctiva or drained with the tear fluid.[17-19] Thus, the impact of drugs on the cornea are greatly affected by the chemical properties of the drug and integrity of the epithelium.[13,17,18,20]

## 1.2 Contact Lens Materials

### 1.2.1 The History of Contact Lenses

Contact lenses (CLs) were first conceived by Leonardo da Vinci in 1508, in his manuscript "Codex of the Eye," in which he illustrated the possibility of correcting vision by submerging a person's head in a bowl of water.[21,22] In 1637, René Descartes "La Diotropique" described a lens with a fluid-filled tube that could be used to enhance the retinal image when placed on the cornea.[23] These early theorists were instrumental to the development of CLs.

More than 200 years later, Sir John F.W. Herschel suggested correcting irregular corneas with by placing a layer of transparent animal jelly onto the cornea as a refractive medium. [24] In 1846, Germany began to make contact lenses a reality. Carl Zeiss, the founder of the Zeiss

lens company, opened a workshop designed to make scientific tools and instruments such as telescopes and microscopes.[21] In 1887, the first scleral lenses were invented by Friedrich Müller and Albert Müller, who created an artificial glass shell to protect the patient's cornea and sclera.[21]

In the 19th century, ophthalmologists Fick and Kalt fitted glass scleral lenses on keratoconus eyes of patients. [21] However, the glass material severely impacted the permeability of oxygen and resulted in both substantial hypoxia and poor comfort during wear.[21] In 1934, a rigid, non-gas permeable polymethylmethacrylate (PMMA) material was suggested for CL wear.[21,25] The acrylate polymer material had the desired optical properties and biocompatibility with the cornea, but comfort and dryness remained a problem.[26] Today, the vast majority of CLs prescribed (>90%) are soft, flexible materials, either fitted as conventional hydrogel (CH) or silicone hydrogel (SH) materials.[27,28]

### 1.2.2 Conventional Hydrogel Material

Between 1952 and 1961, the first soft contact lens material was developed by Czechoslovak chemist Otto Wichterle and his assistant Drahoslav Lim.[21,24,25,29] The CL material they used was made from cross-linked poly(2-hydroxyethyl methacrylate) (pHEMA). [21,30] The main advantages of pHEMA were its biocompatibility with the eye and ability to absorb water. [31] Wichterle and Lim’s discovery of pHEMA led to the first mass production of CLs (SofLens) by Bausch and Lomb, with the first commercial offering being in 1971, exactly 50 years ago this year.[21,30]

The contact lenses made from pHEMA were popular but still presented challenges because of the insufficient exchange of oxygen to the eye.[25] The main source of oxygen to the eye comes from the atmosphere, with the oxygen then dissolving into the tear film layer and subsequently into the corneal tissues. The wearing of pHEMA lenses interferes with this passage of oxygen to the cornea, particularly when the lens is worn overnight, which increases the risk of hypoxia, inflammatory responses, and neovascularization.[25,32,33] Future versions of pHEMA-based materials added monomers such as methyl methacrylate (MMA) to increase material strength.[34] Other monomers include methacrylic acid (MA) and *N*-vinyl pyrrolidone (NVP) to increase the equilibrium water content (WC) and hence oxygen transport through the lens material.[34] An increased WC was thought that it may lead to improved wettability and comfort, but this also increased the dehydration of high water content hydrogel lenses and resulted in some clinical problems such as increased corneal staining and in some cases reduced comfort.[35-37] The inability to manage hypoxic complications with pHEMA-based materials, particularly during overnight wear or daily wear of thick lenses, resulted in the development of a new generation of contact lenses to address the issue of oxygen permeability.

### 1.2.3 Silicone Hydrogel Material

The next generation of materials was developed to increase the oxygen permeability and reduce the hypoxic events seen with previous extended-wear lenses. The first generation of SH lens materials was PureVision (balafilcon A, Bausch & Lomb) and Focus Night and Day (lotrafilcon A, Alcon).[25,38] The materials contain oxygen-permeable silicone monomers in combination with the hydrophilic benefits of hydrogel monomers that allow a significant amount of oxygen permeability and increased material softness.[39,40] Silicone elastomer lenses

exhibited extremely high oxygen permeability, but these materials had no water content and adhered to the ocular surface because of insufficient fluid and ion transport and thus were required to be merged with hydrophilic components to make commercially available siloxane-containing lens materials.[41] However, even when incorporated with hydrophilic components found in traditional hydrogel lenses, the hydrophobic nature of silicone demonstrated poor surface wettability,[41] increased lipid deposition,[42] and reduced comfort in the early SH materials.[43] To increase the hydrophilicity of these materials, surface modifications to balafilcon A and lotrafilcon A materials were required. The balafilcon A material were plasma oxidized through a process that created a wettable silicate "island" coating on the lens surface.[44] In comparison, lotrafilcon A was modified by applying a continuous non-siloxy plasma thin, high refractive index surface coating.[38,41]

The so-termed "second generation" of SH materials was Acuvue Oasys (senofilcon A, Johnson & Johnson) and Acuvue Advance (galyfilcon A, Johnson & Johnson). These materials incorporated the internal wetting agent polyvinylpyrrolidone (PVP) to improve the hydrophilicity of the CL. [41] The third generation of SH materials was Biofinity (comfilcon A, CooperVision). Instead of surface treatments or internal wetting agents, they incorporated siloxy macromers and hydrophilic monomers to produce inherently wettable moieties.[41,45] A new generation of SH materials include Dailies Total 1 (delefilcon A, Alcon), which consists of a central core SH material (33% WC) with a high water content hydrogel surface (80% WC) that improves wettability and overall lens comfort.[46]

### 1.2.4 Contact Lens Classification

The US Food and Drug Administration (FDA) has classified contact lens hydrogel materials into five groups (Table 1.1). Groups 1 – 4 are conventional hydrogel materials that are separated by their ionicity and water content. SH materials were originally grouped into Groups 1 – 4 based on water content and ionicity.[47] However, it quickly became apparent that a new group 5 category was necessary because the “behaviour” of SH materials could not be predicted solely on water content and ionicity.[28,47] The fifth group had to consider the various interactions between the SH materials (surface treatments, internal wetting agents, pore size), tear-film components (lipids, proteins), and CL solutions (multipurpose solutions, hydrogen peroxide).[28,47,48]

**Table 1.1 The FDA grouping system of CL materials is based on ionicity and water content.[28]**

| | Ionicity | Water Content | Lens material examples |
|---|---|---|---|
| Group 1 | Non-ionic | Low (<50%) | Polymacon |
| | | | Tetrafilcon A |
| Group 2 | Non-ionic | High (>50%) | Omafilcon A |
| | | | Nelfilcon A |
| | | | Alphafilcon A |
| Group 3 | Ionic | Low (<50%) | Phemfilcon A |
| Group 4 | Ionic | High (>50%) | Etafilcon A |
| | | | Ocufilcon A |
| | | | Methafilcon A |
| Group 5A | Ionic | No water specification | Delefilcon A |
| Group 5B | Non-ionic | High | Somofilcon A |
| Group 5Cm | Non-ionic | Low/surface treatment | Balafilcon A |
| | | | Lotrafilcon B |
| | | | Lotrafilcon A |
| Group 5C | Non-ionic | Low/no surface treatment | Samfilcon A |
| | | | Comfilcon A |
| | | | Enfilcon A |
| Group 5Cr | Non-ionic | Low/no surface treatment | Senofilcon A |
| | | | Narafilcon A |

### 1.2.5 Contact Lens Wear Modality (Contact Lens Distribution)

Contact lenses are used by approximately 175 million people around the world for vision correction purposes.[49] There has been a significant increase in the usage of SH around the world in the past 20 years. The increased use of CLs has been largely driven by significant

developments in soft contact lens materials, which now account for 90% of all lens fits globally.[50] As of 2021, the majority of CL wearers use SH lenses (72%), while the remaining wear CH lenses (28%).[50] Interestingly, soft reusable CLs were prescribed more than daily disposable CLs (54% vs 46% respectively) but this varies significantly between countries.[50] The trend towards SH lens wear continues to grow as technology further improves SH material design.

### 1.3 Contact Lens Solutions

The role of a CL solution is to clean, rinse, and disinfect soft reusable CLs. There are two main types of CL solutions currently available: hydrogen peroxide, and multipurpose solutions (MPS). In a recent survey, 35% of respondents used hydrogen peroxide, and 64% of users preferred MPS.[27]

#### 1.3.1 Hydrogen Peroxide

The broad-spectrum capabilities of hydrogen peroxide and relative lack of toxicity makes these systems the "gold-standard" for contact lens care.[51] Hydrogen peroxide is considered a relatively harmless chemical for CL disinfection because of its oxidative decomposition into non-toxic by-products of water and oxygen. The mode of action consists of hydrogen peroxide producing free hydroxyl radicals that act as oxidizing agents which react with lipids, proteins and nucleic acids.[52] Hydrogen peroxide systems must be neutralized prior to reinsertion of the lens and the neutralization process has, historically, been available in two different formats. The first is a two-step process whereby neutralization is achieved after

soaking the lens overnight in 3% peroxide by copiously rinsing the lens with saline in the morning or soaking it in a solution containing sodium pyruvate for 10-15 mins.[53] Patient compliance was challenging with a two-step process and thus one-step hydrogen peroxide systems became available, and this concept now accounts for virtually all peroxide disinfection systems globally. One-step hydrogen peroxide systems do not require a separate neutralization step. The neutralization is achieved in the lens case using a platinum-coated disc or soluble catalase tablet to render the hydrogen peroxide into water and oxygen.[54] A concentration of 3% (30 000 ppm) peroxide is the concentration used in all licensed disinfection products (Table 1.2). Hydrogen peroxide is considered highly effective against a wide range of microbial organisms, demonstrating a 4.0-6.0 log kill of bacteria within 1 hour and 3.6 log kill of Acanthamoeba cysts in 1 hour.[54] Another benefit of hydrogen peroxide is that it is preservative-free, which means they are suitable for people with a sensitivity to the preservatives found in MPS.

**Table 1.2 Commercially available one-step hydrogen peroxide systems.** Adopted from Yee et al, 2021 with permission.[55]

| | Alcon | Bausch + Lomb | CooperVision | Johnson & Johnson Vision |
|---|---|---|---|---|
| Product name | AOSEPT PLUS with HydraGlyde® | EasySept® | Refine One Step™ | Oxysept® Disinfecting Solution/Neutralizer Ultracare® Formula |
| Biocide (concentration) | Hydrogen peroxide (3%) | Hydrogen peroxide (3%) | Hydrogen peroxide (3%) | Hydrogen peroxide (3%) |
| Neutralization process | Platinum disc | Catalytic disc | Platinum disc | Catalase tablet |

### 1.3.2 Multipurpose Solutions (MPS)

MPS consist of a single solution designed to clean, disinfect and wet the CLs. To deliver this range of functions, MPS contain a wide variety of components, including preservatives, buffers, chelating agents, and surfactants/wetting agents (Table 1.3). It is important to ensure that MPS components in formulations are biocompatible with both the CL and the eye, along with achieving their primary objective of being efficacious against a wide range of microorganisms. In order for the solution to be effective and maintain the homeostasis

environment of the eye, it is important to understand the various components of a contact lens MPS.

**Table 1.3 Commonly available soft contact lens MPS products.** Adopted from Yee et al, 2021 with permission.[55]

| | Alcon | | |
|---|---|---|---|
| Product name | OPTI-FREE® Puremoist® MPS | OPTI-FREE® Replenish® MPS | OPTI-FREE® Express® MPDS |
| Biocide (concentration) | POLYQUAD® (PQ-1) (0.001%) ALDOX® (MAPD) (0.0006%) | POLYQUAD® (PQ-1) (0.001%) ALDOX® (MAPD) (0.0005%) | POLYQUAD® (PQ-1) (0.001%) ALDOX® (MAPD) (0.0005%) |
| Buffer | Borate buffer, sodium chloride, sorbitol | Boric acid, borate buffer, sorbitol | Boric acid, aminomethyl-propanol, sorbitol |
| Chelating agents | EDTA, citrate (citric acid) | Citrate (citric acid) | EDTA, citrate (citric acid) |
| Surfactant/wetting agents | Tetronic 1304 and HydraGlyde® Moisture Matrix | TearGlyde® (Tetronic 1304 and nonanoyl-EDTA) | Tetronic 1304 |

EDTA (ethylenediaminetetraacetic acid); MAPD (myristamidopropyl dimethylamine); PHMB (polyhexamethylene biguanide); PQ-1 (polyquaternium-1)

| | Bausch + Lomb | | CooperVision | | Johnson & Johnson Vision | |
|---|---|---|---|---|---|---|
| Product name | Biotrue® MPS | renu® Advanced Formula MPS | All In One Light™ MPDS | Hy-Care™ MPDS | ACUVUE™ RevitaLens MPDS | COMPLETE® MPS Easy Rub formula |
| Biocide (concentration) | Polyaminopropyl biguanide (0.00013%) polyquaternium (0.0001%) | Polyaminopropyl biguanide (0.00005%) polyquaternium (0.00015%) Alexidine (0.0002%) | Polyhexanide (PHMB) (0.0001%) | Polyhexanide (PHMB) (0.00013%) | PQ-1 (0.0003%) Alexidine (0.00016%) | PHMB (0.0001%) |
| Buffer | Sodium Borate, Sodium Chloride, Hyaluronic acid, Boric acid | Sodium borate, Sodium chloride, Boric acid, citrate, diglycine | Sodium chloride, sodium phosphate | Sodium chloride, sodium phosphate | Sodium borate, Sodium chloride, citrate | Sodium chloride, sodium phosphate |
| Chelating agents | EDTA | EDTA | EDTA | EDTA | EDTA | EDTA |
| Surfactant/ wetting agents | Poloxamine | Poloxamine, Poloxamer 181 | Poloxamer 188 | Tetronic 1304 and HydraGlyde® | Tetronic 904 | Poloxamer 237 |

#### 1.3.2.1 Biocides

##### 1.3.2.1.1 Early MPS preservatives (Chlorhexidine and Thimerosal)

Previous generation of biocides for hydrogel materials included chlorhexidine and thimerosal. One of the major concerns with older generation preservatives was the smaller molecular weight of these molecules. The smaller molecular weight of the molecules allowed them to be easily sorbed into the CL matrix, which upon putting the lens on the eye, would cause allergic and toxic effects to the eye.[56] Following chlorhexidine and thimerosal, a newer generation of preservatives are used to this day. This includes polyhexamethylene biguanide (PHMB), polyquaternium-1 (PQ-1), myristamidopropyl dimethylamine (MAPD), and povidone-iodine (PI) systems. All these systems have 5-10 times greater molecular weight compared to chlorhexidine and thimerosal.[56]

##### 1.3.2.1.2 Polyhexamethylene biguanide (PHMB)

PHMB was the first high molecular weight (1000-8000 g/mol) CL disinfectant and remains commonly used in many MPS products.[56] Other names include polyhexanide, polyaminopropyl biguanide (PAPB), Dymed® (Bausch+Lomb) and TrisChem® (AMO/J&J). PHMB is also used as a swimming-pool disinfectant, preservative in wet wipes, and to disinfect medical utensils.[56-58] PHMB is a relatively large molecule, cationic charge and has an amphipathic structure.[59,60] It is thought to work by selectively binding to phospholipids found in microbial plasma membranes, disrupting the cell membrane, leading to cellular lysis.[56,61,62]

##### 1.3.2.1.3 Polyquaternium (PQ-1)

PQ-1 is a quaternary ammonium molecule with a relatively high molecular weight (4000 – 11000 g/mol) biocide in comparison to other biocides.[56,62-64] PQ-1 is known to damage the potassium ion (K+) channel of the cell membrane of bacterial and fungal organisms.[63,65] Due to its high molecular weight, Polquaternium-1 has a lower uptake into hydrogel lenses compared to PHMB and thus a lower chance of exhibiting corneal toxicity.[56,62,63,66] Polyquad®, Alcon's tradename for polyquaternium-1, is included in all Alcon MPS products. PQ-1 is also used widely in ophthalmic preparations for dry eye disease and the treatment of glaucoma.[64]

##### 1.3.2.1.4 Amidoamine

Myristamidopropyl dimethylamine (MAPD) is a relatively low molecular weight biocide (312.54 g/mol) in comparison to PHMB and polyquaternium-1. The molecule contains both a lipophilic tail and hydrophilic head with a terminal amine group.[67] MAPD is found in the OPTI-FREE® brand products by Alcon under the tradename Aldox®.[56] MAPD is used in conjunction with polyquaternium-1 and is incorporated for its antifungal and antiprotozoal capabilities.[63,68-70] The mode of action is thought to disrupt the integrity of the cytoplasmic membrane, leading to leakage of the bacterial intracellular components.[68,70]

##### 1.3.2.1.5 Alexidine

Alexidine is a low molecular weight molecule, similar to chlorhexidine. The difference between alexidine and chlorhexidine are the additional two hydrophobic ethylhexyl terminal groups on chlorhexidine.[58,68] Alexidine is a bisbiguanide with antimicrobial properties effective against a wide range of microorganisms.[68,69] However, in 2004, widespread global cases of *Fusarium* keratitis that was directly linked to a MPS based on alexidine (renu®

MoistureLoc®; Bausch & Lomb) led to it being withdrawn from the market. It appeared that this failure of the biocide was due to poor stability of alexidine in the MPS bottles.[71,72]

1.3.2.1.6 Povidone-Iodine

Povidone-iodine (PI) is a relatively new type of contact lens disinfection system. Historically, PI has been used as an antiseptic to prevent skin infections or reduce the build-up of mucous membranes during surgery.[73-75] PI has been shown to be effective against a wide range of microbial organisms, has the ability to penetrate biofilms, and demonstrates low cytotoxicity.[74,76] The mode of action involves the PI complex delivering iodine into the solution which then targets bacterial cell membranes.[77] Iodine inhibits cellular mechanisms in bacteria by oxidizing and denaturing amino acids.[76,77] A limited number of studies have tested the efficacy of PI as a disinfectant in contact lens solutions.[54,73,74,78]

### 1.3.2.2 Surfactants

Surfactants are surface active agents that reduce the surface tension of water.[56] Surfactants serve two purposes. Firstly, surfactants are used to remove debris and deposits from lenses. Secondly, they aid lens wettability. Surfactants contain both a hydrophilic and hydrophobic end group. The hydrophobic portion binds to the contact lens surface while the hydrophilic portion binds to water, thus lowering the surface tension of the interface to allow the tear film to spread more easily.

Benzalkonium chloride (BAK) is a preservative and cationic surfactant which reduces surface tension.[79] The cationic surfactant can interrupt the metabolic processes of cells, cause cell lysis and kill microorganisms.[79] PHMB and MAPD are also cationic surface-active agents

with a lipophilic tail and hydrophilic head used to remove microorganisms.[67] The most common surfactants are Tetronic and Pluronic variations. Tetronics are octablock star copolymers and contain hydrophilic terminal blocks of polyethylene oxide/polypropylene oxide arms attached to an ethylenediamine core.[80] The properties of Tetronic have been clinically shown to aid in wetting, preservative uptake, and overall comfort for patients.[81]

#### 1.3.2.3 Chelating agents

Chelating agents are used to improve the disinfection efficacy of MPS and aid in the removal of proteins.[62] They help prevent calcium-bound proteins from depositing on the contact lens surface by attracting the proteins and allowing more active sites to be available for biocides.[82] There are various chelating agents such as ethylenediaminetetraacetic acid (EDTA), citrate, and hydroxyalkylphosphonate.

#### 1.3.2.4 Demulcents

Demulcents are agents used to manage inflamed or irritated areas of the epithelium by lubricating the mucous membrane.[56,83] They are most commonly used in artificial tears to manage dry eye patients. Demulcents are also used in various lens care disinfecting solutions to provide further lubrication. Common forms of demulcents in MPS include propylene glycol (PG) and polyethylene glycol (PEG) which are a subgroup of the liquid polyols.[83] Both of these demulcents are considered liquid polyols which are a sugar-like hydrogenated carbohydrate.[83] The FDA recognizes six categories of ophthalmic demulcents: cellulose derivatives, dextran 70, gelatin, liquid polyols, polyvinyl alcohol, and povidone.[83]

#### 1.3.2.5 Wetting agents

Hyaluronic acid (HA) is a wetting agent used in new hydrogel designs and MPS.[84-86] HA is a natural, anionic glycosaminoglycan polysaccharide with unique viscoelastic properties.[87] The properties of HA help in dry eye treatment by providing better water retention, lubrication, and improve tear stability thus providing better comfort. The agent helps the CL surface become more hydrophilic and to carry water to the hydrophobic sites.[87]

#### 1.3.2.6 Buffers

Buffering agents are necessary to help balance pH, tonicity, and osmolality of the MPS.[56] The tonicity of a solution depends on the salt. Common salts used in MPS are sodium chloride, sodium borate, boric acid, and various citrate buffers (Table 1.3).

### 1.3.3 Summary

In summary, the most commonly used cleaning solutions for soft contact lenses are hydrogen peroxide or MPS (Table 1.2, Table 1.3). In the past, first generation disinfectants such as chlorhexidine and thimerosal were used but many patients exhibited allergic and cytotoxic responses that led to corneal staining, hyperemia, corneal infiltrates, and palpebral conjunctivitis.[56] This has led to newer disinfectants with potentially less aversive and harmful consequences to the eye. These MPS contain an active disinfectant ingredient which are mainly PHMB, PQ-1, MAPD or alexidine as a solo or combinational disinfectant.[73,88,89] Moving forward, future products may include more disinfectants that cover a broader spectrum of microorganisms or additional components to improve comfort and wettability. Future studies

are needed to determine the safety of new disinfectant systems and its biocompatibility with lenses and lens cases.

## 1.4 Uptake and Release of MPS Components from Reusable Soft CLs

The uptake and release of MPS components from CLs are important in determining the safety and efficacy of the solution. When reusable soft CLs are soaked overnight in an MPS, a proportion of the biocides from the solution can be sorbed onto CLs and subsequent placement onto the eye may release the biocides.[60,67,90] The biocides in the solution need to be efficacious during the cleaning and disinfecting phase, but due to their potential toxicity to the eye, should ideally not be sorbed onto the lens material in extensive quantities and then released onto the ocular surface.[60,67,90] Thus, it is critical to determine the uptake and release profiles of biocides to demonstrate their ocular safety and biocompatibility.[60,67,90-93]

### 1.4.1 Material factors that drive uptake and release

The surface and the bulk matrix of soft reusable CLs are important to consider when evaluating uptake and release of MPS components to and from lenses. The adsorption of components onto the surface of the lens is primarily driven by the charge and hydrophilicity of both the solution and the lens material.[60,94,95] Modifications to the surface of the lens can improve lens wettability and comfort.[94] Absorption occurs when components enter the bulk of the material and may partition due to the hydrophobic phase of the lens material.[60] The pore size, charge, water content, and hydrophilic-lipophilic interaction are important material factors to consider.[41,60,67]

### 1.4.2 Biocide factors that drive uptake and release

The CL is capable of absorbing various components found in MPS and biocides such as PHMB, MAPD and PQ-1 are especially important since they disinfect the CL. As mentioned previously, the biocides have varying molecular size, hydrophobicity, and ionicity, all of which influence their uptake and release kinetics.[60,67] Biocides with higher molecular size and weights are less likely to penetrate and bind to the bulk matrix of the CL (Figure 1.3).[67,90,96] Both PHMB and MAPD are cationic molecules with lipophilic characteristics.[67] PHMB has a high positive charge-to-mass ratio which makes it prone to ionic interactions with any charged anionic surface.[67] PHMB oligomers vary in subunits with higher molecular weight oligomers binding more strongly to anionic surfaces.[67] PHMB sorption to anionic surfaces may be in common with sorption by highly cationic proteins, such as lysozyme which readily absorb to etafilcon A lenses.[67]Additionally, the interactions between biocides and other components of the MPS have to provide the ideal conditions of pH, ionic strength, and osmolarity to maintain lens parameters during lens wear.[67] These traits can be altered through interaction with other MPS components, which ultimately leads to different uptake and release kinetics compared to a stand-alone biocide.[60,97]

### 1.4.3 Methods and techniques that assess the uptake and release of MPS

Very few studies have assessed the uptake and release kinetics of MPS components from CL.[48,67,90,98] These studies have examined the kinetics using a solution containing only the biocide, commercially available MPS, or have compared both.

Different methods exist for assessing the rate of preservative uptake and release. The ISO 11986 guidelines for the rate of preservative uptake $K_n$, is expressed by the following equation for each time point ($T$).[99]

$$K_n = \frac{(U_n - U_{n-1})}{(T_n - T_{n-1})}$$

$U_n$ is the quantity of preservative taken up at a time point;

$U_{n-1}$ is the quantity of preservative taken up at the previous time point;

$T_n$ is the time the lens has been immersed in the solution for measurement, n;

$T_{n-1}$ is the time the lens has been immersed in the solution for measurement, n-1;

Similarly, the rate of preservative release $K'_n$, is expressed by the following equation for each time point ($T$):[99]

$$K_n = \frac{(R_n - R_{n-1})}{(T_n - T_{n-1})}$$

$R_n$ is the quantity of preservative released at a time point;

$R_{n-1}$ is the quantity of preservative taken up at a time point;

$T_n$ is the quantity of preservative taken up at a time point;

$T_{n-1}$ is the quantity of preservative taken up at a time point;

The Higuchi model is an equation that addresses the solutes (drug) rate of release from a matrix (polymer).[100,101]

$$M_t = \sqrt{DC_s(2A - C_s)t}$$

$M_t$ is the accumulative amount of solute release up to time ($t$)

$D$ is the diffusion coefficient in the matrix

$A$ is the loading of the solute

$C_s$ is the solubility from the matrix to surrounding fluid

The model divides the matrix into an inner region where undissolved particles exists, and an outer region where all the drug is dissolved.[102] Fick's second law considers the rate of release of the solute to the surrounding fluid.[103,104] Other important factors when determining the release of solutes include the shape of the material and the diffusion rates into the matrix compared to diffusion to the surrounding fluid.[104] Mathematical modelling[102-104] can provide a useful method to predict the uptake and release kinetics of drugs but is an expansive topic outside the scope of this thesis, and interested readers are referred to manuscripts that discuss this concept.[105]

#### 1.4.3.1 High-performance liquid chromatography (HPLC)

The most common analytical technique is high-performance liquid chromatography (HPLC), which separates the sample into its molecular components.[48,57,67,90] A high-pressure pump is used to push the solvent through the liquid mobile phase, which enhances the sample

separation.[106] The components of the sample will interact differently with the adsorbent material in the elution column, leading to various flow rates.[106] The technique can be modified to use different columns, detectors and solvents, depending on the type of sensitivity required.[48,57,67,90]

#### 1.4.3.2 UV-Vis Spectroscopy

UV-Vis spectroscopy is typically used with HPLC to detect and quantify molecules within a sample.[48,57,67,90] The technique excites a sample at a range of wavelengths, measures the absorbance, and compares the absorbance to a known standard molecule.[48,57,67,90]

Most studies used HPLC with UV detection to determine the uptake and release of MPS from CLs, and one study assessed a biocide-only solution.[48,67,90] Powell soaked CLs in MPS (3 – 100 mL) and subsequently released them in artificial tear fluid (2 mL).[67] The results showed that SH lenses sorbed the most MAPD, while CH lenses sorbed the most PHMB. Green and colleagues only assessed the uptake and release of MPS containing PHMB from CLs.[48] They tested the uptake of PHMB concentrations ranging from 1 – 10 ppm (2.5 mL) to assess a CL saturated with PHMB. The results by Green were in agreement with Powell, which showed hydrogel lenses (high ionicity and water content) sorbed more PHMB.[48,67] There were no data on the release kinetics. Morris assessed the uptake and release of a biocide-only solution or MPS from CLs.[90] The CLs were soaked in a biocide-only (PHMB, PQ-1) solution with significantly greater concentrations (500 µg/mL, 1.2 mL) than what is typically found in MPS.

#### 1.4.3.3 Radioactive labelling

Radioactive labelling is a technique that tracks an isotope ($^{3}H$, $^{14}C$, $^{125}I$) tagged on a molecule as it goes through a sequence of events.[107] Radioactive labelling has been frequently used to assess *in vitro* lipid and protein deposition from CLs.[108-111] However, this technique has not been widely used to assess the uptake and release of biocides. Chapman et al., was the first group to assess the uptake and release of the preservative benzalkonium chloride (BAK) using radioactive labelling.[112] They soaked soft lenses (etafilcon A; deltafilcon A) and hard lenses (PMMA, Boston II, Fluoroperm 90) in BAK (0.005%, 0.009%) for 7 days at room temperature and then placed the CLs in saline solution followed by an extraction of the remaining solution from the lenses. The results of the study showed that uptake of BAK was significantly greater for soft lenses (etafilcon A) compared to hard lenses. This was the first study using radioactive ($^{14}C$) to measure the uptake and release of biocides from CLs.[112] A recent study demonstrated that the uptake and release of MAPD can be measured using radioactive labelling with a high degree of sensitivity and accuracy.[98]

### 1.4.4 Limitations to the methods and techniques

The underlying limitations of UV-Vis and light scattering techniques are that they are both general purpose detection methods, which rely heavily on HPLC to be accurate. HPLC is a time-consuming and error-prone process, which could potentially result in low sensitivity.[111,113] Modifications to HPLC techniques and equipment were required to measure low biocide concentration levels.[57] A major barrier for radioactive studies is the cost of creating the isotope molecules and the required permits for radioactive use.

### 1.5 Cytotoxicity of MPS

MPS system consist of a single bottle of solution designed to clean, disinfect, and wet the CL. The lenses are removed from the eye, then rubbed and rinsed (or at the very least rinsed) with the MPS, soaked overnight in the MPS, and then inserted onto the ocular surface the next morning. Following insertion, any solution that may have been taken up by the lens material can be released over a period of time onto the ocular surface, which may cause adverse effects.

Early biocides such as chlorhexidine and thimerosal, although highly effective, are no longer used in CL care systems due to their potential to cause allergic and/or toxic reactions.[114-119] Their low molecular weight enable them to be easily absorbed into soft CL materials.[112,116,119,120] The subsequent release of these biocides onto the ocular surface following lens insertion led to a variety of allergic or toxic reactions, including red eyes, varying degrees of corneal staining, papillary conjunctivitis, reduced comfort, and corneal infiltrates.[114,115,118,121,122]

Contemporary MPS biocides have different chemical compositions and most commonly consist of PHMB, PQ-1, and MAPD. These biocides have significantly higher molecular weights than earlier biocides (Figure 1.3), which prevents the molecule from being absorbed into the CL in high quantities.[67,90,96] However, contemporary MPS still demonstrate cytotoxicity on corneal epithelial cells.[5,91-93] Cytotoxicity studies have used immortalized corneal epithelial cells (ICEC) and human corneal epithelial cells (HCEC).[93,123,124] ICEC are continuous cell lines that acquired the ability to proliferate through artificial modification.[125] The benefits of using ICEC is that it is more cost-effective and allows for genetic alterations

which provides a novel system for testing various pathogens.[126] HCEC are primary cells with a finite replicative lifespan but are more representative of human corneal cells.[125] *In vitro* studies examined the impact of MPS on human corneal epithelial cells (HCEC), and found that different concentrations of the MPS decreased the viability of HCEC.[93,127,128] The results conclude that at the lowest concentration, OPTI-FREE® Puremoist® exhibited greater cytotoxicity compared to other MPS systems. A number of studies have shown that certain combinations of MPS and lenses may cause various cytotoxic effects to HCEC, due to the release of preservatives from the CL.[91-93,129,130] It is important to consider the interactions between CL materials and solutions since it can lead to cytotoxic responses.

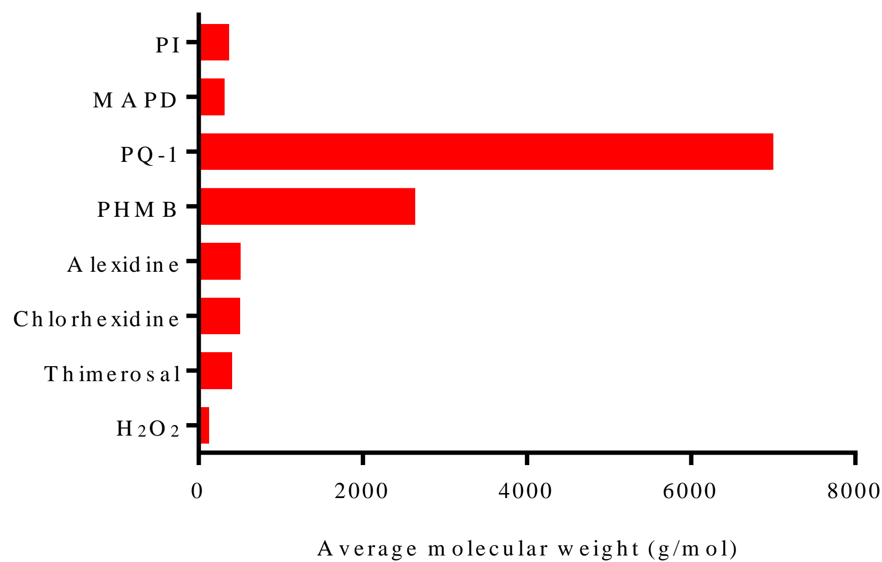


**Figure 1.3 Average moleculuar weight of biocides (g/mol).[28,131,132]**

## 1.6 Solution induced corneal staining (SICS)

Corneal staining is an important clinical observation that helps determine the integrity of the cornea, which acts as a barrier against infection.[5] Following lens removal, the observation of solution induced corneal staining (SICS) has been associated with certain combinations of MPS and CL materials and is thought to be an indicator of a compromised cornea.[133-138] The degree of observed SICS varies depending on the combination of lenses and solutions used.[133,135,139-141] SICS has been shown to be the greatest with MPS that contain PHMB during short term wear.[133,135,139-144] This was followed by MPS that contain PQ-1/MAPD, while hydrogen peroxide exhibited the least amount of corneal staining.[142] Long-term corneal staining investigations demonstrate increased staining with PQ-1/MAPD disinfectants.[144] A recent study suggest that fluorescein staining was attributed to the uptake of surfactants (Tetronic 1107) rather than the biocides.[143,145] Determining what combination of MPS work best with specific lenses is clinically valuable in minimizing cytotoxic effects and reducing corneal staining.

## 1.7 Patient compliance

A part of the following section has been published in:

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### 1.7.1 Hydrogen peroxide neutralization

Any $H_2O_2$ system must be neutralized before it is applied to the eye. In currently available peroxide systems this is achieved by one of two methods, either via the addition of a soluble catalase tablet, or with the inclusion of a platinum-coated disc in the lens case to neutralize the solution.[28,146] However, non-compliance from accidental misuse or incomplete neutralization exists.[147] The reports show insufficient neutralization time, which can cause acute discomfort through burning and stinging sensations.[148-150]

### 1.7.2 Topping up solution

Incorrect use of MPS has previously been implicated as one of the major factors contributing to a major global outbreak of *Fusarium* keratitis in 2005-6 and *Acanthamoeba* keratitis in 2007-8.[151-154] Wearers exhibited non-compliant behaviours that included topping off solutions, incomplete lens case closure and poor lens case cleaning. Topping off the solution prior to overnight soaking was found to lower disinfection ability of the MPS involved in the outbreak, as the preservative was shown to be absorbed into lenses.[96,155] Incomplete case closure can lead to solution evaporation, potentially altering its disinfection ability.[156-158] In fact, the efficacy of the MPS involved in the *Fusarium* keratitis outbreaks, renu® MoistureLoc® (Bausch + Lomb, US) was shown to be reduced under conditions simulating poor compliance.[156] This important work illustrates how use of the CL care system differs in the 'real world' compared to standard efficacy testing, and how non-compliant actions can

have a detrimental effect on the properties of the solution as seen with the *Acanthamoeba* keratitis outbreak.[152,159]

Topping up solution rather than replacing the solution fully each day is associated with a 2.5x increased risk of CL-related complications,[160] and it has been estimated that up to 30% of wearers perform this non-compliant behaviour.[161-163] A US-based survey of more than 6000 CL wearers, including 1618 adolescents, reported that regardless of age, 10-19% topped off their solution on a "regular basis".[163] Non-compliance may be associated with the time and routine required for managing lenses as patients have self-reported 'laziness' as a factor that led to topping up their solution.[164] The behaviour of topping off solutions by CL wearers continues even though they are aware of the risks. In a 2010 survey, 11% of patients reported topping up their solution despite being aware of the risk for CL-related complications.[165]

### 1.7.3 Generic brand solutions

A major consideration for reusable CL wearers is the cost of the CLs and cleaning solutions. Typically, eye care practitioners (ECP) will recommend a CL cleaning solution to complement the CLs. However, CL wearers can stray from the recommended CL cleaning solution, perhaps due to the price or cheaper alternative to the recommended product. The MPS composition from a generic brand is different than major company brands. CL wearers who use generic or store brand solutions were found to have the highest rate of complications including solution toxicity, infection, and discomfort.[166]

# Chapter 2. Objectives and Rationale

The advancement of soft CL materials, specifically SH materials, has greatly improved in the last two decades and has specifically reduced the risk of hypoxia and led to some improvements in comfort among soft CL wearers.[142,167] While SH lenses have some benefits over CH, situations have arisen where the combination of certain soft CL materials when used with some MPS has led to adverse events.[90,143,168] The concern is possibly even greater with certain SH lens materials and their interaction with MPS.[41,48,169] Thus, it is important to better understand the specific interactions that occur between various components of MPS, in particular the biocide, and soft CL materials.

The uptake of preservatives by soft CL materials has been a topic of great interest. The microbial efficacy of biocides was questioned when outbreaks of *Acanthamoeba* and *Fusarium* Keratitis were associated with the potential uptake of preservatives during overnight incubation of lenses.[96,154,170,171] When soft CL materials absorb biocides, a reduced amount of available biocide remains in the cleaning solution, potentially reducing their efficacy. Thus, it is important to assess the interaction between the biocides and the CL materials.

Previous studies demonstrated that HPLC and UV absorption were sensitive and specific enough to detect the biocides.[48,67] However, measuring biocides at lower levels was not suitable and modifications to the instrument were necessary.[57] Chapters 4 and 5 describes

*in vitro* studies using radioactive labelling, a highly sensitive and accurate technique,[110,112,172] to assess the interaction between biocides and CL materials.

After the lenses are removed from overnight soaking in MPS, they are directly placed onto the eye. During this time, the subsequent release of the sorbed solution may be cytotoxic to the ocular surface. Previous *in vitro* studies have found that the cytotoxicity to HCEC were affected by the combination of the type of lenses and the MPS used to place on the HCEC.[91-93,129,173] To our knowledge, no previous studies have assessed the cytotoxicity of only the biocides, a major component of the MPS. Chapter 6 aims to assess the impact of the biocides on primary HCEC *in vitro*. The importance of this work will help provide insight on the potential cytotoxicity of biocides alone, and whether this component in MPS may be responsible for corneal staining and CIEs. In this thesis, it is hypothesized that the uptake and release of biocides from reusable soft CLs will be different based on the chemical properties of the biocides and lens materials. Specifically, we predict that SH materials will uptake more MAPD but release less MAPD compared to CH materials. Furthermore, SH materials will uptake and release less PHMB compared to CH materials.

The overall aim of these chapters were to answer two main research questions:

1. Using an *in vitro* radioactive labelling technique, what are the factors that influence the uptake and release kinetics of biocides from contact lenses?
2. Does the release of the biocides (cytotoxicity) pose a threat for soft CL wearers?

The purpose of this thesis was to evaluate the uptake and release kinetics of MAPD and PHMB from commercially available soft CL materials using various *in vitro* models to determine the effect of the biocides.

# Chapter 3.Method optimization to quantify radioactive and non-radioactive myristamidopropyl (MAPD)

## 3.1 Objective

The aim of this chapter was to optimize the radioactive/non-radioactive proportions for future uptake and release studies.

## 3.2 Introduction

Radiolabeling is a technique used to track an isotope tagged on a molecule as it goes through a sequence of events triggered by light and ionizing radiation.[107] Common types of radioisotopes used are $^{3}H$ or $^{14}C$. Chapman et al., (1990) was the first group to determine the uptake and release of benzalkonium chloride (BAK) using radioactive labeling ($^{14}C$).[112] The study found that there were significant differences in BAK adsorption between soft and hard lenses, with rigid CLs taking up a smaller quantity of BAK than soft CLs. High water content CLs were shown to adsorb greater amounts of BAK (56 µg/mg of lens weight) compared to low water content CLs (30 µg/mg).[112] They showed that both high and low water content CLs had a very low percentage of BAK released within 24 hours (0.2-1.5%).[112] No studies have since used radioactive labelling to quantify the uptake and release of biocides from soft CLs.

Studies have assessed the uptake and release of biocides as either a biocide-only solution [90,98] or as multipurpose solutions (MPS).[48,67,90] Techniques used to quantify uptake and release of biocides include HPLC, UV-Vis spectroscopy, and radioactive labelling techniques.[48,57,67,98,112] Lucas et al., (2009) developed a new variation of HPLC to measure PHMB in MPS.[57] They developed a solid phase extraction method followed by HPLC analysis

using an evaporative light scattering detector.[57] The objective of their study was to extract PHMB from MPS using this technique and to determine the efficiency in recovering all PHMB. Though the study did not directly test uptake and release of PHMB into a CL, the potential method of measuring PHMB was promising. However, the authors noted that the repeatability and reproducibility of the light scattering detector was worse than UV-Vis due its low sensitivity.[57]

The underlying limitations of UV-Vis and light scattering techniques are that they are both general purpose detection methods, which rely heavily on the HPLC extraction to be accurate. HPLC is a time-consuming and error-prone step, which could potentially result in low sensitivity and selectivity.[111,113] Literature on the uptake and release of biocides and other tear components suggests high variability using these methods for quantification.[48,67,96,111] In the past, several studies have used radioisotopes to label cholesterol and phospholipids to determine their deposition on SH contact lenses.[111,113,172] Radiolabeling allowed for a direct quantification of sorbed lipids on the CLs and yielded a higher accuracy and precision than UV-Vis or light scattering techniques.[111] Numerous CL studies have suggested radiolabeling to be a highly sensitive and accurate method for quantifying binding of components to the lens material.[113,172,174]

The stability of MPS is important when storing a product, especially when the shelf-life of products are involved. MPS are typically contained in polyethylene bottles. Studies support polyethylene and polypropylene bottles as the most favorable material to contain MAPD and PHMB.[57,67] Polyethylene bottles were shown to minimize the loss of MAPD during release

measurements,[67] and polypropylene, a similar material, was superior to glass for maintaining the concentration of PHMB in MPS.[57]

Since differences in material properties were found, the chemical formulation of phosphate buffered saline (PBS) may also have different outcomes. The international organization for standardization (ISO) provides a general method for measuring the uptake and release of preservatives by CLs.[99] The standard is linked to ISO 18369-3, which outlines the formulation of the saline solution for testing.[175] For the remainder of this chapter, ISO-PBS refers to the PBS formulated following the ISO recommendations, while PBS refers to a company-formulated saline solution (Table 3.1).

Despite the apparent advantages of radioactive labelling, thus far only one study used radioactive labeling to evaluate the uptake and release of a biocide (BAK) from CLs.[112] To our knowledge, no study has examined the uptake and release of other radiolabeled biocides from CLs. Given the known sensitivity and specificity of radioactive labeling, this technique could be a valuable detection method to determine the uptake and release of biocides from soft CL materials.

The aim of this chapter was to optimize the radioactive/non-radioactive proportions for future uptake and release studies.

### 3.3 Method

The initial experiments tested various concentrations of MAPD (Toronto Research Chemicals, Toronto, ON, CA) with PBS (Lonza Biosciences, Walkersville, MD, USA) and ISO-PBS.[175] Both the PBS and ISO-PBS had pH of $7.4 \pm 0.1$ (Table 3.1).

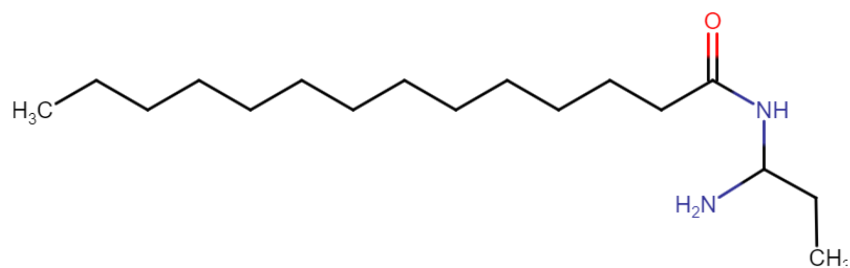


**Figure 3.1** Chemical structure of MAPD (312.53 g/mol). Appearance (white to off-white solid). Melting point (49-51°C). Solubility (Choloroform, methanol). https://www.trc-canada.com/prod-img/MSDS/A521000MSDS.pdf

**Table 3.1 Chemical components used for PBS and ISO-PBS**

| PBS (Lonza) | | ISO-PBS | |
|---|---|---|---|
| Chemical | Quantity | Chemical | Quantity |
| Sodium Chloride (NaCl) (Sigma Aldrich, St. Louis, USA) | 9.000 g/L | Sodium Chloride (NaCl) (Sigma Aldrich, St. Louis, USA) | 8.300 g/L |
| Sodium Phosphate Dibasic Anhydrous ($NaH_2PO_4$) (Sigma Aldrich, St. Louis, USA) | 0.795 g/L | Sodium Phosphate Dibasic Anhydrous ($NaH_2PO_4$) (Sigma Aldrich, St. Louis, USA) | 0.406 g/L |
| Potassium Phosphate Monobasic Anhydrous ($KH_2PO_4$) (Sigma Aldrich, St. Louis, USA) | 0.144 g/L | Disodium Phosphate Monobasic Anhydrous ($Na_2HPO_4$) (Sigma Aldrich, St. Louis, USA) | 2.376 g/L |
| Milli-Q water (grade 3) | 1 L | Milli-Q water (grade 3) | 1 L |

Serial dilutions were done at various concentrations ranging from 1000 µg/mL to 5 µg/mL in PBS and ISO-PBS. MAPD was dissolved in the saline solutions at a concentration of 500 µg/mL. The MAPD solution were prepared by solubilizing MAPD with the saline

solutions in a scintillation vial and vortexed for 60 seconds. A stir bar was added in the vial and placed on a stir plate in an incubator with temperatures between 28°C - 30°C overnight.

*Detection of non-radioactive MAPD*

Absorbance was measured using the SpectraMax M5 Spectrophotometer (Molecular Devices, Sunnyvale, CA) at 200 nm wavelength, as a previous study was able to detect MAPD in MPS.[67]

*Preparation and detection of radioactive MAPD*

The radiochemical purity for $^{14}C$ MAPD was 99.4% (Moravek Inc., Brea, USA). Radioactive MAPD was dissolved in PBS at a concentration of 5 µg/mL. Aliquots of the stock solution were obtained such that most of the product could be kept frozen when needed, instead of thawing and re-freezing the original stock solution, which could compromise the structural integrity of MAPD. Radioactive counts per minute (cpm) measures the detection rate of ionizing events per minute.

Preparations of radioactive MAPD and a solution combining radioactive & non-radioactive MAPD was undertaken in polyethylene vials. Radioactive MAPD was formulated by taking an aliquot of 2µL from one of the 1mL (500 µg/mL) stock solution. An additional 1.998 mL of ISO-PBS was added. The radioactive & non-radioactive MAPD was prepared by adding 2 µL of (500 µg/mL) radioactive MAPD to a 1.998 mL solution containing ISO-PBS and 5 µg/mL MAPD. Once the radioactive MAPD and the radioactive & non-radioactive MAPD were prepared, the next step was to undertake serial dilutions in scintillation vials. The

following thirteen serial dilutions had 1 mL of ISO-PBS for each vial. From the stock mixture, 1 mL was placed into the second vial and sonicated for 30 seconds. Afterwards, it was transferred to the next vial with the process continuing until the last vial. Once the serial dilution was completed, the samples were transferred to glass scintillation vials and 10 mL of Ultima Gold F scintillation fluor (PerkinElmer, Waltham, MA) was added. The samples were counted for their radioactive signal using the LS6500 Beckman Coulter liquid scintillation beta counter (Beckman Coulter, Mississauga, ON). Each sample was analyzed for 10 minutes.

*Statistical methods*

Statistical analysis and graphs were plotted using GraphPad Prism 6 software (GraphPad, La Jolla, CA). All data is reported as mean ± SD. A T-test was used to determine the differences between the treated conditions. In all cases, statistical significance was considered significant for a value of $p<0.05$.

### 3.4 Results

The UV absorbance of ISO-PBS was not significantly different compared to PBS ($p=0.73$, Figure 3.2). The UV absorbance between zero and 15 µg/mL were not significantly different for PBS ($p=0.76$) or ISO-PBS ($p=0.60$). This suggests that UV absorbance may not be sensitive enough to detect lower concentrations of MAPD.

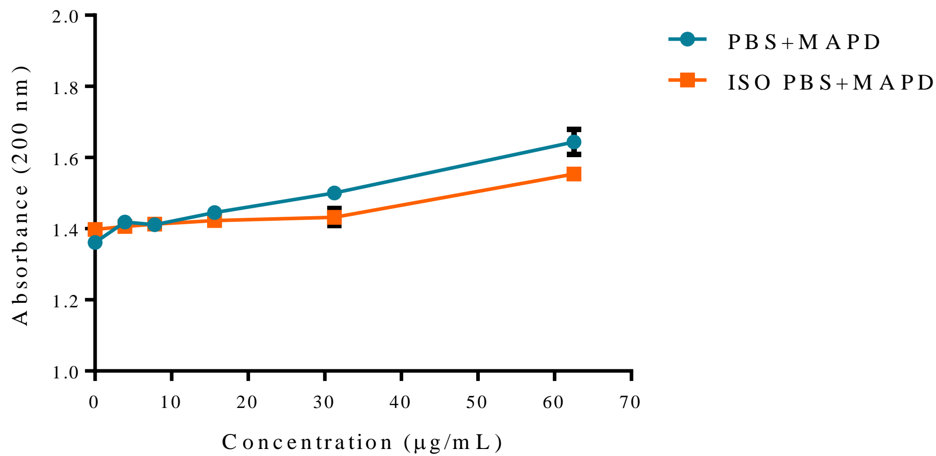


**Figure 3.2 Absorbance spectrum of MAPD.** A comparison between PBS and ISO-PBS with equal concentrations of MAPD.

Preliminary tests of pure radioactive MAPD were compared to a mixture of radioactive & non-radioactive MAPD (Figure 3.3). A higher cpm indicates a greater level of radioactivity was present. Starting at 1 µg/mL, every subsequent dilution was half the concentration. The radioactive counts per minute (cpm) were not significantly different between the purely radioactive MAPD and mixed solution ($p$=0.38, Figure 3.3).

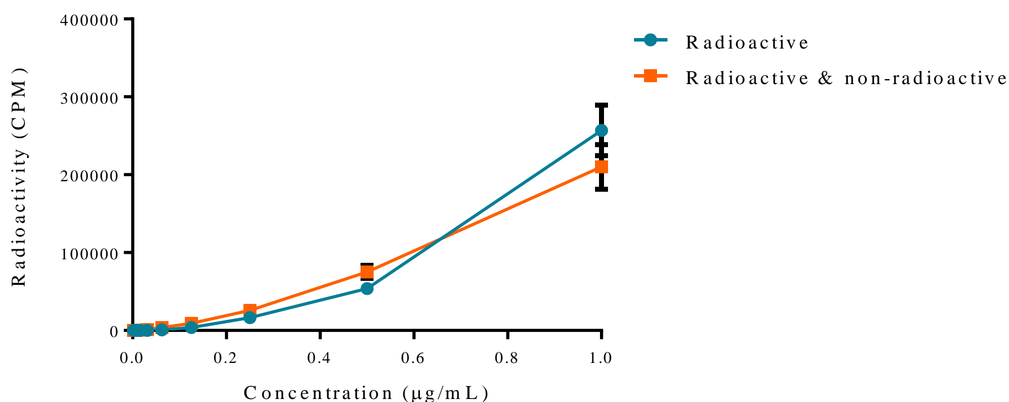


**Figure 3.3 Serial dilutions were compared between radioactive MAPD and a soution of radioactive & non-radioactive MAPD.** There were no significant diferences between radioactive and radioactive & non-radioactive MAPD ($p$=0.38).

The radioactive & non-radioactive MAPD mixtures were used with glass and polyethylene vials. Glass and polyethylene vials showed similar radioactive cpm ($p$=0.38, Figure 3.4). However, serial dilutions with polyethylene vials showed a more equal distribution of MAPD. The results suggest polyethylene vials are better suited for future radioactive kinetic studies.

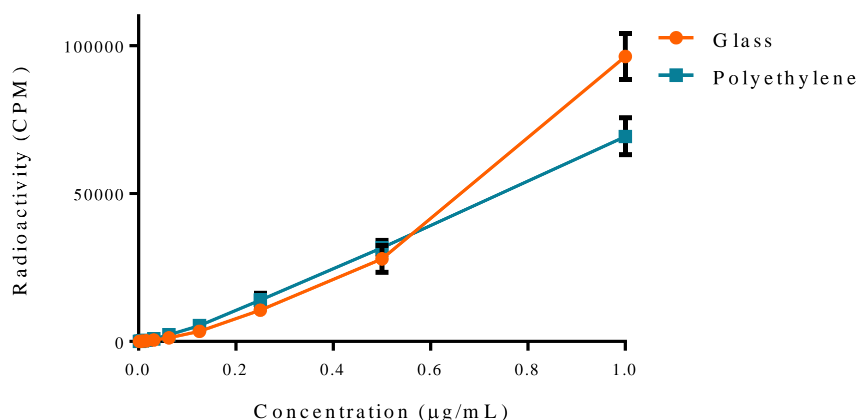


**Figure 3.4 Serial dilutions of radioactive & non-radioactive MAPD were compared between glass and polyethylene vials.** There were no significant differences between glass and polyethylene vials ($p$=0.38). Polyethylene vials showed more consistent results after subsequent dilutions.

## 3.5 Discussion

There were limitations to using UV-Vis spectroscopy in detecting lower concentrations of MAPD (Figure 3.2). Radioactive labeling of MAPD offers a highly sensitive method of assessing the kinetic profiles. MAPD was able to be detected at low concentrations using radioactive labelling as shown in Figure 3.3. The results suggest that there was no significant advantage to using only radioactive MAPD, and a mixture was more cost-effective because of less radioactivity needed. Requiring less radioactive material also reduces the amount of radioactive waste needed to be disposed. Future experiments will use a mixture of radioactive & non-radioactive MAPD.

The results from the BAK study supports the use of glass scintillation vials containing radioactive solution.[112] Glass vial studies were undertaken to ensure that BAK adsorption to glass was minimal, since this has been shown to occur with chlorhexidine.[176] Chlorhexidine showed a cyclical sorption phenomenon where preservative was taken up by the lens followed by minimal release, with a continued buildup of chlorhexidine.[176] These patterns were shown to be similar to BAK.[112] Thus, the studies support the use of glass scintillation vials for the uptake and release studies described in this thesis.

A comparison of glass and polyethylene vials were tested to determine whether the signal (cpm) was lost due to the material properties (Figure 3.4).[57,67] The study showed that there were no differences in radioactive CPM using glass or polyethylene vials at lower concentrations. Moving forward, polyethylene vials are a suitable choice for uptake and release studies with radioactive labelled MAPD. Even though some MAPD may be absorbed, ISO 11986 acknowledges that as long as the absorbed preservative does not reduce the concentration of the preservative in the test solution to less than 25% of the initial test concentration, the method of testing is considered acceptable.[99] In the following chapters, ISO-PBS, a mixture of radioactive & non-radioactive MAPD, and polyethylene vials were chosen for testing the uptake and release kinetics from CLs.

# Chapter 4. Uptake and release of a multipurpose solution biocide (MAPD) from hydrogel and silicone hydrogel contact lenses using a radiolabel methodology

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https://journals.lww.com/claojournal/Abstract/2021/05000/Uptake_and_Release_of_a_Multipurpose_Solution.4.aspx

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| Author | Concept/Design | Data Acquisition | Analysis | Writing/Publication |
|---|---|---|---|---|
| Yee | Y | Y | Y | Y |
| Phan | Y | | | Y |
| Chan | Y | | | |
| Heynen | Y | | | |
| Jones | Y | | | Y |

## 4.1 Outline

### 4.1.1 Objective

To evaluate the uptake and release of radiolabelled myristamidopropyl dimethylamine (MAPD) on reusable daily wear contact lenses (CLs) over 7 days.

### 4.1.2 Methods

Three silicone hydrogel (SH) CL materials (lotrafilcon B, balafilcon A, senofilcon A) and two conventional hydrogel (CH) materials (etafilcon A, omafilcon A) were tested. A short-term (experiment 1, N=4) and a longer-term (experiment 2, N=3) study was conducted. In experiment 1, the CLs were incubated in 2 mL phosphate buffered solution (PBS) containing $^{14}C$ MAPD (5µg/mL) for 8 hours. The release of $^{14}C$ MAPD was measured at t = 0.25, 0.5, 1, 2, 4, 8, and 24 hours in PBS. In experiment 2, the CLs were incubated in the $^{14}C$ MAPD solution for 8 hours followed by a 16-hour release in PBS. This cycle was repeated daily for 7 days. At the end of both experiments, lenses were extracted to determine the total uptake of MAPD. The radioactivity was measured using a beta scintillation counter.

### 4.1.3 Results

In experiment 1, all three SH lenses sorbed similar amounts of MAPD (p=0.99), all of which were higher than the two CH materials ($p<0.01$). However, the CH materials released a greater amount of MAPD than the SH materials ($p<0.01$). In experiment 2, the uptake of MAPD in SH materials increased over 7 days, while the amount of MAPD remained constant

in the CH materials ($p$=0.99). Similar to experiment 1, the CH lenses released more MAPD than SH lenses after 7 days ($p$<0.01).

#### 4.1.4 Conclusion

The SH materials absorbed greater amounts of MAPD compared to CH materials. However, the CH materials released the greatest amount of MAPD. Radioactive labelling of MAPD offers a highly sensitive method of assessing the uptake and release profiles of biocides to CL materials.

## 4.2 Introduction

Contact lenses (CLs) are used by approximately 140 million wearers worldwide for vision correction.[177] Because these biomedical devices are in close contact with bacteria-prone surfaces, they are a potential route for ocular infections and such contact lens related infections occur due to a wide variety of organisms, including bacteria, amoeba and fungal species.[178-181] These infections, when left untreated, can lead to substantial and irreversible vision loss.

When worn as reusable lenses, biocides within disinfection solutions provide CLs with appropriate disinfection during overnight soaking before the lenses are re-worn. A biocide commonly used in commercial multipurpose solutions is myristamidopropyl dimethylamine (MAPD).[28,56] MAPD is used as an antifungal and antiprotozoal agent in a range of commercial multipurpose solutions (MPS) from Alcon under the registered trade name of Aldox®.[63,66,182] The antimicrobial mode of action is damage to the cell plasma membrane, causing leakage of the cell.[63,66]

One important factor to consider in the design of contact lens (CL) disinfecting solutions relates to the uptake and release of these biocides into the bulk and onto the surface of the CL material under disinfection.[60,67,90,183] The key characteristics that differentiate SH materials from CH materials include: a non-linear relationship between oxygen permeability and water content, an ionic charge, surface modifications, and whether there is an internal wetting agent.[184] Within SH materials, sub-categories were included to the classification because the structure of the material, added monomers or surface treatments may differ between SH materials and ultimately impacts how it interacts with disinfecting solutions.[28] Previous studies have found that MAPD in OPTI-FREE® Express® (Alcon) exhibited

increased cytotoxicity and therefore an increased risk in ocular discomfort.[185,186] In addition to the potential toxicity and reduced efficacy, the sorption of biocides, including MAPD have been associated with undesirable clinical outcomes such as fluorescein staining.[135,187,188] The biocides need to be efficacious during the cleaning and disinfecting phase, but because of their potential toxicity to the ocular surface, should not be absorbed onto the lens material and then subsequently released in large quantities onto the ocular surface when placed on the eye after overnight soaking. Thus, the uptake and release profiles of CL solution biocides are critical in demonstrating their ocular safety and biocompatibility.[91,93,183]

The uptake and release of MAPD and other biocides present in MPS from CLs has been previously examined using HPLC (high-performance liquid chromatography), in combination with either UV detection at 200 nm[67] or evaporative light scattering.[57] One advantage of UV-Vis and light scattering techniques is that they are able to identify and separate tear components such as lipids and cholesterol.[189] Powell et al.[67] demonstrated that HPLC and UV absorption were sensitive and specific enough to detect MAPD and polyhexamethylene biguanide (PHMB), two common biocides used in MPS. However, Lucas et al.[57] used a modified HPLC technique to measure PHMB since HPLC using UV absorption was not sensitive enough to measure low levels of PHMB. The literature on the uptake and release of biocides and other tear components suggests that light scattering techniques are suitable methods of quantification but a degree of variability still remains.[111,190,191]

Another method to quantify the deposition of components on CLs uses radiolabeling. A study using radioisotopes to label cholesterol and phospholipids demonstrated that radiolabelling allowed for a direct quantitation of sorbed lipids on CLs, and also yielded a high

degree of accuracy and precision.[111] Numerous other CL studies using radiolabeled lipids and proteins have suggested this method is highly sensitive and accurate.[110,113,172,174,192,193] Thus far, only one study has used radioactive labelling to evaluate the uptake and release of a biocide from CLs[112] and no study has examined the uptake and release of radiolabelled MAPD from contemporary lens materials. The purpose of this study was to evaluate the release kinetics of radiolabelled MAPD from contemporary conventional hydrogel (CH) and silicone hydrogel (SH) contact lenses over 7 days of use.

## 4.3 Materials and Methods

### 4.3.1 Reagents

Radioactive ($^{14}C$) MAPD was purchased from Moravek Inc. (California, USA). International Organization for Standardization (ISO) guidelines were followed for saline solution formulation for contact lens testing. The guideline was followed to ensure the compliance with standardized testing methods of either a contact lens or a contact lens material (ISO 18369-3). The phosphate buffered solution (PBS) mentioned in this experiment was formulated to comply with the ISO guideline.[194]

### 4.3.2 Uptake experimental methods

In experiment 1, three SH CL materials (lotrafilcon B, balafilcon A, senofilcon A) and two CH materials (etafilcon A, omafilcon A) of -3.00 diopters were tested (N=4 per material) (Table 4.1). In experiment 2, the same lenses were used (N=3 per material). Prior to each experiment, CLs were removed from their blister packs and placed in a 24 well plate containing

2 mL of PBS. The plates were sealed with parafilm and placed on a shaker overnight at room temperature (23±2°C) to remove any blister pack components. The lenses were removed from the plates and dabbed on lens paper to remove excess PBS prior to further testing.

**Table 4.1 Contact lenses and manufacturing details used in this study.**

| | Air Optix Aqua | PureVision | Acuvue Oasys | Acuvue 2 | Proclear |
|---|---|---|---|---|---|
| **Material type** | Silicone hydrogel | Silicone hydrogel | Silicone hydrogel | Conventional hydrogel | Conventional hydrogel |
| **United States Adopted Name (USAN)** | lotrafilcon B | balafilcon A | senofilcon A | etafilcon A | omafilcon A |
| **Water content** | 33% | 36% | 38% | 58% | 62% |
| **Oxygen transmissibility ($10^{-9}$, -3.00D)** | 138 | 101 | 147 | 20 | 25 |
| **Surface treatment** | Plasma coating | Plasma oxidation | PVP as an internal wetting agent | None | None |
| **Principal monomers** | DMA, TRIS | NVP, TPVC, NVA, PBVC | HEMA, EGDMA, PVP | HEMA, MA | HEMA, PC |

DMA (N,N-dimethylacrylamide); EGDMA (ethylenegylcol dimethacrylate); HEMA (poly-2-hydroxyethyl methacrylate); MA (methacrylic acid); NVA (N-vinyl aminobutyric acid); NVP (N-vinyl pyrrolidone); PBVC (poly[dimethysiloxy] di [silylbutanol] bis[vinyl carbamate]); PC (phosphorylcholine); PVP (poly(vinylpyrrolidone)); TPVC (tris-[trimethylsiloxysilyl] propylvinyl carbamate); TRIS (trimethylsiloxy silane).

### 4.3.3 Uptake and release experiment 1

The uptake and release of MAPD from contact lenses within a 24-hr period was measured. The lenses were incubated in 2 mL of PBS containing radioactive MAPD (5μg/mL) for 8 hrs (N=4). The concentration of 5μg/mL was chosen to match the amount of MAPD in commercially available products of 5 ppm. The lenses were then placed into 2 mL of PBS and the release was measured at t = 0.25, 0.5, 1, 2, 4, 8, and 24-hr time points. At the end of the 24-hr period, the remaining MAPD was extracted from the lenses. The first three extractions used 4:1 hexane:isopropanol and the fourth extraction using 2:1 chloroform:methanol. Four extractions were undertaken to ensure that all MAPD was removed from the lens and that subsequent extractions reduced the CPM to be as low as possible. The extraction mixture was shaken at 1600 rpm for 30 minutes at room temperature.

The total amount of preservative uptake was calculated as follows:

$$A: Total\ uptake = Total\ amount\ released + Total\ amount\ extracted$$

The percent released was calculated as follows:

$$Percent\ Released = \frac{Total\ amount\ released}{A} \times 100$$

### 4.3.4 Uptake and release experiment 2

In the second study, the uptake and release of MAPD from contact lenses was measured over 7 days. The lenses were incubated in a fresh 2 mL of $^{14}C$ MAPD (5μg/mL) for 8 hours, followed by a release period in PBS for 16 hours. This cycle was repeated each day to mimic the daily wearing and overnight soaking cycle that a wearer would expose their lenses to. At

the end of the study period, the remaining amount of MAPD was extracted from the lenses using the same method as described in the first experiment.

The total amount of preservative uptake was calculated as follows:

$$B: Total\ uptake = Total\ amount\ released + Total\ amount\ extracted$$

The percent released was calculated as follows:

$$Percent\ released = \frac{Total\ amount\ released}{B} \times 100$$

### 4.3.5 Radioactivity detection

The uptake, release, and extraction samples were measured independently. First, the uptake and release samples from each experiment were added separately to 10 mL of Scintillation fluor (PerkinElmer, MA, USA) and placed in a 20 mL scintillation vial. Secondly, the extraction samples with the respective solvents were added to 10 mL of Scintillation fluor (PerkinElmer, MA, USA) and placed in a 20 mL scintillation vial. The samples were loaded into the LS6500 Beckman Coulter liquid scintillation beta counter (Beckman Coulter, ON, CA) and counted for their radioactive signal (counts per minute, CPM). Each sample was analyzed for 10 minutes. A standard curve was used to convert CPM to µg of MAPD. The standard curve concentration ranged from 0.01 – 10 µg/mL.

### 4.3.6 Statistics

Statistical analysis and graphs were plotted using GraphPad Prism 6 software (GraphPad, La Jolla, CA). All data is reported as mean ± SD for experiment 1 (N=4) and experiment 2 (N=3). A two-way analysis of variance was used to determine the differences between contact lens material and time. Post-hoc Tukey multiple comparison tests were used when necessary. In all cases, statistical significance was considered significant for a value of $p<0.05$.

## 4.4 Results

### 4.4.1 Experiment 1: Short-term (1-day) uptake & release

The total uptake of MAPD for the SH materials (lotrafilcon B (6.32 ± 0.14µg); balafilcon A (6.13 ± 0.38 µg); senofilcon A (6.45 ± 0.21 µg)) was significantly greater than for the two CH materials (etafilcon A (4.00 ± 0.35 µg); omafilcon A (2.45 ± 0.04µg)) after the 8 hour incubation period ($p<0.01$; Figure 4.1, Table 4.2). There were no significant differences in the amount of MAPD sorbed between the SH materials ($p=0.99$). However, etafilcon A sorbed significantly more MAPD than omafilcon A ($p<0.01$).

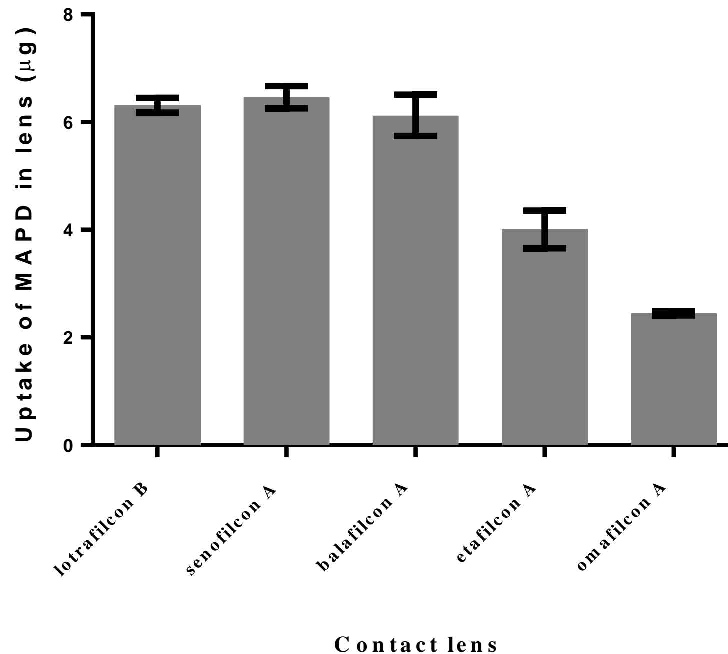


**Figure 4.1 Total uptake of MAPD in lenses (mean ± SD) after 8 hour incubation (5ug/mL).** A total of 10 µg was available for uptake. The total uptake was significantly greater for the SH materials than the CH materials ($p<0.01$). There were no significant differences between the SH materials ($p=0.99$). Etafilcon A sorbed significantly more MAPD than omafilcon A ($p<0.01$).

**Table 4.2 Summary of results for experiment 1.**

| | | Experiment 1 (1 day) | | |
|---|---|---|---|---|
| **Lens type** | **Total uptake (mean±SD)** | **Total release (mean±SD)** | **Extraction amount** | **Percent release,%** |
| **Lotrafilcon B** | 6.32±0.14 µg | 0.67±0.04 µg | 5.80 µg | 11% |
| **Balafilcon A** | 6.13±0.38 µg | 0.55±0.03 µg | 5.73 µg | 9% |
| **Senofilcon A** | 6.45±0.21 µg | 0.57±0.02 µg | 6.05 µg | 9% |
| **Etafilcon A** | 4.00±0.35 µg | 1.66±0.11 µg | 2.49 µg | 42% |
| **Omafilcon A** | 2.45±0.04 µg | 1.78±0.03 µg | 0.82 µg | 73% |

Figure 4.2 and table 4.2 show the average amount of MAPD released at each time point across the 24-hour period. As shown in Figure 2, the release kinetics of MAPD were

significantly different between the materials ($p<0.01$). The CH materials released significantly more MAPD than the SH materials at all time points ($p<0.01$).

There were no significant differences between the three SH materials at all time points ($p=0.99$). There was an initial burst release of approximately 0.23 μg for all SH materials, followed by a slower rate of release. The results suggest that the release of MAPD from SH materials plateaus after 2 hours.

Between 0.25 to 2-hour (inclusive), omafilcon A released significantly more MAPD than etafilcon A ($p<0.01$; Figure 4.2). A burst release was also observed for CH materials, followed by a slower rate of release. The results suggest the release kinetics of MAPD from SH and CH materials are different ($p<0.01$).

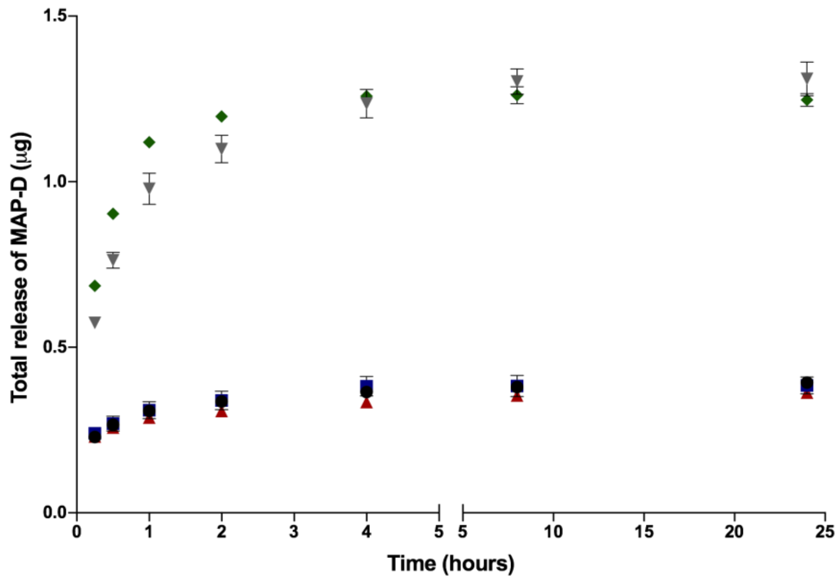


**Figure 4.2 Release kinetics of MAPD over a 24 hour period (mean±S.D).** ●=lotrafilcon B, ■=senofilcon A, ▲= balafilcon A, ▼=etafilcon A, ◆ = omafilcon A. The release kinetics were significantly different between the materials ($p<0.01$). There was a significant difference between the SH materials and the CH materials at all time points ($p<0.01$). There were no significant differences between SH materials ($p=0.99$). Omafilcon A released significantly more MAPD than etafilcon A up until 4 hours ($p<0.01$).

Figure 3 shows the percent release of MAPD from the CLs after 24 hours in PBS. The CH materials (etafilcon A and omafilcon A) had a significantly higher total percentage release of MAPD than the three SH materials ($p<0.01$). There were no significant differences in the percentage of MAPD released between SH materials ($p=0.99$). For the CH materials, omafilcon A released a significantly higher percentage of MAPD than etafilcon A ($p<0.01$).

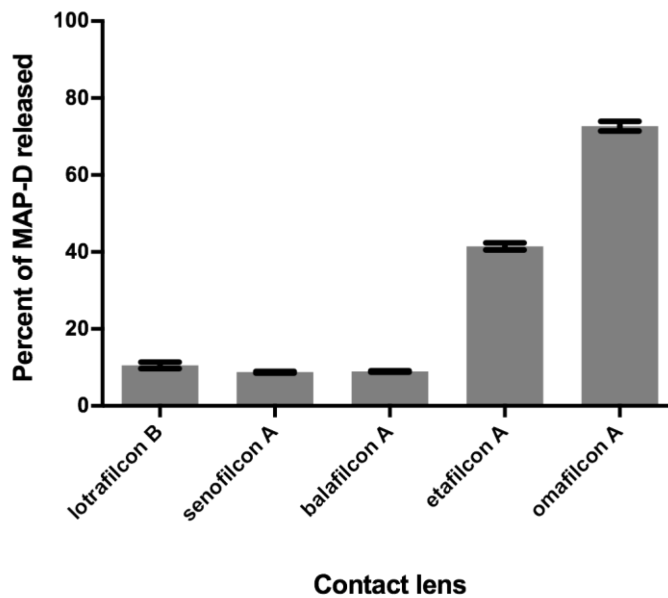


**Figure 4.3 Percent of MAPD released after 24 hours in PBS (mean±S.D).** The two CH materials (etafilcon A and omafilcon A) had a significantly higher percentage overall release of MAPD than the three SH materials ($p<0.01$). Omafilcon A had a significantly higher release of MAPD than etafilcon A ($p<0.01$). There were no significant differences between SH materials ($p=0.99$).

### 4.4.2 Experiment 2: Long-term (7-days) uptake & release

In an attempt to mimic the process that occurs over a week of typical wear by a patient who is using their lenses on a reusable basis, the CLs were cycled in an alternating uptake (soak) and release environment.

Figure 4.4 shows the uptake and release of MAPD over a 7 day period. The SH lenses continued to sorb more MAPD over 7 days whereas the sorption on CH lenses remained the same ($p<0.01$). The SH lenses sorbed significantly more MAPD than the CH lenses at all time points ($p<0.01$). There were no differences in the amount of MAPD sorbed between the SH lenses at all time points ($p=0.99$). Between CH materials, etafilcon A sorbed significantly more MAPD than omafilcon A ($p<0.01$).

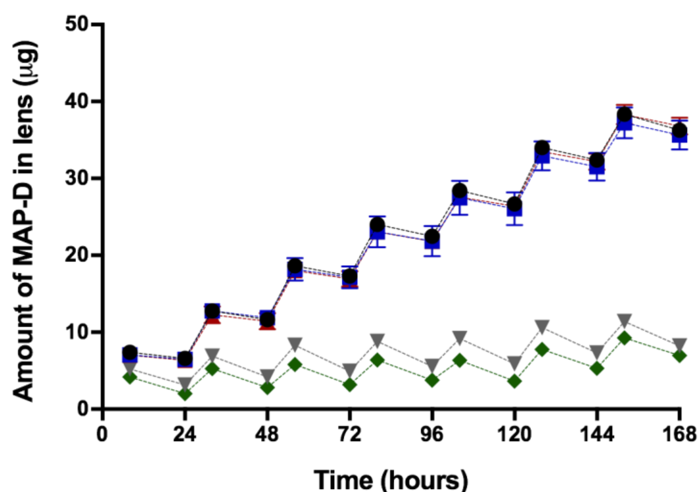


**Figure 4.4 Uptake and release profile of MAPD in a 7 day period. Initial uptake at 8 hours followed by a 16 hour release period for each daily cycle.** ●=lotrafilcon B, ■=senofilcon A, ▲=balafilcon A, ▼=etafilcon A, ◆=omafilcon A. The SH lenses sorbed more MAPD than the CH lenses at all time points and these differences were significant ($p<0.01$). A post-hoc analysis showed that etafilcon A sorbed significantly more MAPD than omafilcon A at all time points ($p<0.01$). There were no differences in the amount of MAPD sorbed between the SH lenses ($p=0.99$). After 8 hours, the initial uptake was significantly greater between the SH lenses compared to the CH materials ($p<0.05$).

Figure 4.5 shows that the amount of MAPD released after each day over the 7 day period ($p<0.05$). The CH materials had a higher total release of MAPD than SH materials ($p<0.01$). From day 4 to day 7, lotrafilcon B released significantly more MAPD than senofilcon A ($p<0.05$) and balafilcon A ($p<0.05$). There were no differences between senofilcon A and balafilcon A at all time points. On day 3, etafilcon A released significantly more MAPD than omafilcon A and this continues to day 7 ($p<0.01$). On day 1, the CH materials released approximately more than twice the amount of MAPD than SH materials and this trend continued to day 4.

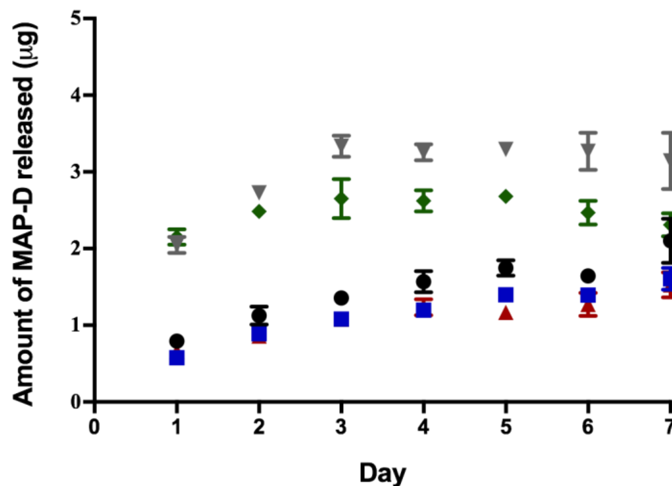


**Figure 4.5 Amount of MAP-D released each day in a 7 day period.** ●=lotrafilcon B, ■=senofilcon A, ▲= balafilcon A, ▼=etafilcon A, ◆ = omafilcon A. The amount of MAPD released after each day was significantly greater for CH lenses compared to SH lenses ($p<0.05$). On day 1, the CH materials released approximately twice the amount of MAPD and this trend continued to day 4. By day 7, the lenses continued to release more MAPD, with omafilcon A exhibiting a slower rate of release than etafilcon A ($p<0.01$). A post-hoc analysis showed CH lenses released more MAPD than SH lenses up to day 7 ($p<0.05$). On day 7, there was no significant difference between omafilcon A and lotrafilcon B ($p=0.34$).

There was a significant difference in the percent of MAPD released between the SH and CH groups ($p<0.01$; Table 4.3). There were no significant differences between the percentage of MAPD released from the SH materials. However, omafilcon A released a significantly greater percentage of MAPD than etafilcon A ($p<0.01$). The percent of MAPD released in the 7 day study was very similar to the 1 day study.

**Table 4.3 Summary of results for experiment 2.**

| | **Experiment 2 (7 days)** | | | |
|---|---|---|---|---|
| **Lens type** | **Total uptake (mean±SD)** | **Total release (mean±SD)** | **Extraction amount** | **Percent release, %** |
| **Lotrafilcon B** | 44.05±10.63 µg | 10.15±0.49 µg | 33.90 µg | 25% |
| **Balafilcon A** | 43.55±1.08 µg | 8.11±0.53 µg | 35.44 µg | 19% |
| **Senofilcon A** | 42.57±1.20 µg | 8.00±0.43 µg | 34.57 µg | 19% |
| **Etafilcon A** | 26.88±0.57 µg | 21.44±0.71 µg | 5.44 µg | 80% |
| **Omafilcon A** | 18.85±0.42 µg | 17.60±0.36 µg | 1.25 µg | 93% |

After one day, the SH materials had approximately 5 µg of MAPD remaining in the lens, while the CH materials had less than half that amount remaining in the lens (Table 4.2). After 7 days, approximately 33-35 µg of MAPD remained in the SH materials (Table 4.3), an almost 7-fold increase in comparison to the remaining amount from the 1 day experiment. In the CH materials, 1-5 µg of MAPD remained after the 7 day experiment (Table 4.3), which was approximately 1.5-2 times more than the amount of MAPD remaining in the CH materials after the 1 day experiment. These results clearly show that the accumulation of MAPD over time is greater for the SH materials, and that the amount of MAPD taken up over time for the CH materials is much lower.

**Table 4.4 The percent of MAPD extracted from each extraction phase.**

| | Extraction efficiency | | | |
|---|---|---|---|---|
| **Lens type** | **Extraction 1, %** | **Extraction 2, %** | **Extraction 3, %** | **Extraction 4, %** |
| **Lotrafilcon B** | 63.2 | 12.0 | 1.3 | 0.4 |
| **Balafilcon A** | 69.1 | 10.5 | 1.3 | 1.2 |
| **Senofilcon A** | 67.7 | 11.9 | 1.2 | 0.4 |
| **Etafilcon A** | 15.7 | 2.8 | 0.6 | 1.2 |
| **Omafilcon A** | 4.4 | 0.9 | 0.6 | 0.8 |

## 4.5 Discussion

The two studies undertaken showed that the uptake and release of MAPD can be measured using a radiolabeling method. The experiments showed several trends that are worthy of discussion.

The SH lenses sorbed significantly greater amounts of MAPD in comparison to CH lenses over both short and longer periods of time, as shown in Figures 4.1 and 4.4. These results are similar to a previous study, where SH lenses were shown to sorb greater amounts of MAPD compared to CH lenses.[67] Interestingly, the CH lenses released more MAPD than the SH lenses, as shown in Figures 4.2 and 4.5. As a consequence, the percentage of MAPD released from CH lenses was the highest (Figure 4.3 and Tables 4.2 and 4.3). The amount of MAPD released from SH lenses plateaued after the initial release at 15 minutes, whereas the CH lenses demonstrated a burst release for the first 15 minutes but continued to release MAPD over the 24 hour testing period. The SH lenses released approximately 10% of the MAPD that was taken up, which indicates that the majority of MAPD remained within the lens. In comparison, CH released 40-75% of MAPD that was sorbed (Figure 4.3).

Throughout the 7 day study, the SH materials were capable of taking up more MAPD, with no evidence of an equilibrium being achieved, compared to the CH lenses (Figure 4.4). At the end of each day, CH lenses released more MAPD than SH lenses (Figure 4.5). However, by day 7, omafilcon A exhibited less release of MAPD, to the point that it released similar amounts of MAPD as lotrafilcon B ($p$=0.34). Based on this trend, SH lenses may release more MAPD as each day progresses, while the release from CH lenses plateaus, with a consistent release profile.

The first extraction showed a significant amount of MAPD had remained in the SH materials compared to CH materials (Table 4.4). The extraction efficiency of MAPD demonstrate that by the fourth extraction, the amount of MAPD remaining in the lens accounted for <2% of the total amount sorbed. Each extraction was performed consecutively and subsequent extractions reduced the radioactivity CPM by a factor of 10. The extraction efficiency was similar to previous extraction studies of non-polar compounds from CLs.[110,174]

The overall trends of our results are similar to a previous study conducted by Powell et al, which measured the uptake and release of MAPD using HPLC and UV spectroscopy.[67] In their study, they found that etafilcon A sorbed the least MAPD but also released a higher amount of MAPD compared to SH.[67] The hypothesis was that the weak hydrogen bonds between the lens polymer and MAPD were highly reversible.[67] In contrast, SH sorbed the highest amount of MAPD, while releasing the least amount of MAPD. This is likely due to the hydrophobic and lipophilic domains of the SH material creating strong, irreversible interactions with MAPD.[67]

The amount of MAPD sorbed and released from the lenses in the current study was lower than that reported by Powell et al.[67] This variation is likely due to the differences in the concentration of the uptake solution. In the current study, 5 µg of MAPD was in the uptake solution, which was 3 times less than the 15 µg in the previous study.[67] The rationale for using 5 µg was to match the concentration found in commercially available MPS containing MAPD. The concentrations of MAPD in commercial products are similar between OPTI-FREE® Replenish® (5µg/mL), OPTI-FREE® Express® (5µg/mL) and OPTI-FREE® PureMoist® (6µg/mL).[195]

Several papers have described in detail the various bulk and surface properties that differ between the SH and CH lenses used in this study.[34,41,47,48,169,196,197] All three SH materials had high uptake levels of MAPD and there were no significant differences between them, even though these lens types have very different surface properties. For instance, lotrafilcon B has a plasma coating, balafilcon A is plasma oxidized, whereas senofilcon A is not surface treated.[41,169] Based on these results, the surface chemistry of the SH lenses does not seem to have a major impact on the absorption of MAPD and it appears that absorption is primarily driven by the bulk monomer composition of the lenses.

Previous studies have shown that an increased uptake of MAPD into a lens may decrease the biocidal efficacy of contact lens solutions.[65,96] During the disinfection step, a reduction in biocides, due to the uptake of biocides into the lens material, may lower its ability to kill bacteria, fungi and acanthamoeba.[65,96,198] In 2006, an outbreak of fungal keratitis was in part due to the uptake of the biocide into the lens matrix, thus reducing its efficacy in solution.[96,198,199] In 2007, an outbreak of acanthamoeba keratitis was related to the use of

Advanced Medical Optics' Complete Moisture Plus solution.[152,200] In both cases, there was a failure of biocidal efficacy of the solution to specific organisms during cleaning and storage of the CL.[69,201-204]

Studies have found that SH lenses have been associated with approximately two times greater risk for corneal infiltrative events (CIEs) when using MPS compared with CH lenses.[202-204] CIEs are described as risk factors associated with soft CL wear that include MPS, bacterial bioburden, and reusable lenses.[205] Clinically, cytotoxicity levels and CIEs are greater with SH materials compared to CH materials when they are exposed to MPS, with varying degrees of risk from different material and solution combinations.[136,206] Several animal studies have demonstrated that corneal exposure to MPS during lens wear can adversely impact the corneal epithelium.[173,207,208] Solution induced corneal staining (SICS) has been associated with soft contact lens wear and certain MPS products, with varying degrees of staining based on the CL and MPS combinations used.[135,144] Solutions which contain polyquaternium-1 and MAPD show significantly higher levels of SICS at 30 minutes compared to two hours after lens insertion.[188] Several studies have suggested that the release of MPS components onto the ocular surface could lead to cytotoxicity and corneal staining issues.[145,209,210] Other theories suggest that non-pathological staining can occur as a result of the secondary binding of fluorescein to the biocide, resulting in a transient phenomenon that may not be toxic.[9,187]

Based on clinical findings, *in vitro* experiments and the results from the current study, it appears that clinical findings of increased CIEs and SICS found with SH materials cannot be directly linked to the release of MAPD from these materials, which release lower levels than CH materials. While it is plausible that such events could be related to their higher uptake, on

the basis that SH lenses containing higher amounts of MAPD are held in close proximity to the ocular surface, this would seem unlikely given that the degree of 'tear flushing' that occurs behind the lens would be similar between SH and CH materials and some degree of contact between the preservative and ocular surface would be required to initiate such events.

In conclusion, the uptake and release kinetics of MAPD appear to be driven primarily by the siloxane content within the CL materials. The results of the experiments showed that the uptake of MAPD was highest for all SH materials. However, CH materials released the greatest amount of biocide despite absorbing significantly less MAPD than SH materials. As a result, the percentage release of MAPD from CH materials was significantly higher than SH materials. The clinical implications of these findings remain unclear given the *in vitro* nature of this experiment, and further investigation is necessary to determine whether the results from this experiment may help explain the association between CIEs and SICS found with certain combinations of lens materials and care systems.

# Chapter 5. Uptake and release of polyhexamethylene biguanide from hydrogel and silicone hydrogel contact lenses using a radiolabel methodology

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| **Author** | **Concept/Design** | **Data Acquisition** | **Analysis** | **Writing/Publication** |
|---|---|---|---|---|
| Yee | Y | Y | Y | Y |
| Phan | Y | | | Y |
| Jones | Y | | | Y |

## 5.1 Outline

### 5.1.1 Objective

To evaluate the uptake and release of radiolabelled polyhexamethylene biguanide (PHMB) on reusable daily wear contact lenses (CLs) over 7 days.

### 5.1.2 Methods

Three silicone hydrogel (SH) contact lens materials (lotrafilcon B, balafilcon A, senofilcon A) and two conventional hydrogel (CH) materials (etafilcon A, omafilcon A) were examined. Two experiments were conducted. In experiment 1 (1-day study), CLs were soaked in 2 mL of phosphate buffered solution (PBS) containing radiolabelled $^{14}C$ PHMB (1µg/mL) for 8 hours. The release kinetics of $^{14}C$ PHMB from the CLs was measured at t = 0.25, 0.5, 1, 2, 4, 8, and 24 hours in fresh 2 mL PBS. In experiment 2 (7-day study), the CLs were soaked in the $^{14}C$ PHMB (1µg/mL) solution for 8 hours followed by a 16-hour release in 2 mL PBS. The lens cycle was repeated daily for 7 days. After both experiments, the residual amount of PHMB remaining within the lenses was extracted to determine the total uptake of PHMB. The radioactivity was measured using a beta scintillation counter.

### 5.1.3 Results

In experiment 1, the total uptake of PHMB for etafilcon A was significantly greater than senofilcon A ($p$=0.01). There were no significant differences in total uptake of PHMB between other lens materials ($p$>0.05). Etafilcon A released more PHMB compared to all other lens types over a 24-hr period ($p$<0.001). In experiment 2, all CL materials continued to sorb

more PHMB over time ($p<0.001$). By day 7, the amount of PHMB sorbed by etafilcon A was significantly greater than senofilcon A ($p=0.02$). After day 2, the CH materials released significantly more PHMB than the SH materials ($p<0.01$).

### 5.1.4 Conclusion

The CL materials continued to sorb PHMB with no signs of saturation after 7 days. There was a higher uptake and release of PHMB from the etafilcon A material compared to senofilcon A. All lens materials released a consistent amount of PHMB each day. Radioactive labelling provides a highly sensitive method of assessing the uptake and release of PHMB from CL materials.

### 5.2 Introduction

Contact lenses (CLs) are used by approximately 175 million wearers worldwide for vision correction.[49] Currently, reusable soft CLs account for 44% of annual contact lens fits worldwide.[50] When wearing reusable CLs, it is essential that they are disinfected overnight with an appropriate disinfecting solution, with almost 90% of wearers using a one-bottle multipurpose solution (MPS).[50] The main purpose of an MPS is to clean and remove deposited tear film components and various microorganisms (bacteria, amoeba, and fungal species) from the lenses to ensure their comfortable and safe use by the wearer.[178-180,211]

Biocides are essential constituents of all MPS products and are the components directly responsible for killing any adherent microorganisms and ensuring that lenses are appropriately disinfected prior to re-application to the eye.[56] A commonly used biocide in commercial MPS is polyhexamethylene biguanide (PHMB),[28,56] which has been available within contact lens MPS since the late 1980's. The biocide selectively binds to phospholipids found in microbial plasma membranes, which disrupts the cell membrane and consequently causes cell lysis.[61,212,213]

Soft CLs can be broadly classified as either conventional hydrogel (CH) or silicone hydrogel (SH) lenses.[47] The United States Food and Drug Administration (FDA) categorizes CH lenses into one of four groups (I-IV), based on the water content and ionicity of the lens material.[214] In comparison, SH lenses, which contain a siloxane component to increase their oxygen permeability, are classified as group V materials.[47] The CH and SH lenses that are approved for reusable daily wear all require disinfection overnight in an approved CL care solution. Understanding the uptake and release profile of the biocide in the MPS in which the

lens material is placed is critical in ensuring safe CL wear, as significant uptake of biocides (including PHMB) into CL materials has been shown to reduce the microbial efficacy of the MPS.[90,96,195,215] The reduction of PHMB in the solution has been linked to a loss of efficacy against both *Staphylococcus aureus*[65] and *Fusarium solani*.[216] Once placed onto the eye, the subsequent release of PHMB from the CL material has been linked to increased corneal staining and CL discomfort,[81,133-135,217-222] although more recent studies would suggest that the corneal staining may not be directly linked to the biocide.[145]

Several methods have been used to analyze the uptake and release kinetics of PHMB from CL materials, including high-performance liquid chromatography (HPLC) and light scattering techniques.[48,57,67,90] These techniques are able to separate and quantify various components of MPS or the tear film.[57,67,189] However, previous studies report differences in sensitivity and specificity for the uptake and release of PHMB from CLs.[48,57,67,90] Recently, a radioactive labelling technique has been used to assess the uptake and release of the biocide myristamidopropyl dimethylamine (MAPD) from reusable CL materials with a high degree of sensitivity.[98] Radioactive labelling offers a highly sensitive and accurate method for measuring the interaction of both biocides and tear film components with CL materials.[98,111-113,174,193]

The purpose of this study was to assess the uptake and release of PHMB from contemporary soft reusable CL materials over a 7-day period using a novel radiolabelling method recently reported.[98]

## 5.3 Materials and Methods

### 5.3.1 Reagents

Radioactive ($^{14}C$) PHMB was purchased from Moravek Inc. (California, USA). International Organization for Standardization (ISO) guidelines were followed for saline solution formulation for contact lens testing (ISO 18369-3).[223] Three SH materials (lotrafilcon B, balafilcon A, senofilcon A) and two CH materials (etafilcon A, omafilcon A), all of -3.00 diopters back vertex power, were tested (n = 4 per material). The solvents used were 2 mL of 100% methanol for each lens.

### 5.3.2 Experimental Procedures

The experiment followed identical procedures to that previously described.[98] ISO guidelines were followed to ensure compliance with standardized testing of CL materials and care products (ISO 11986).[99] Prior to each experiment, CL were removed from their blister packs and placed in a 24 well plate containing 2 mL of PBS. The plates were sealed with parafilm and placed on a shaker overnight at room temperature (23±2°C) to remove any blister pack components. The lenses were removed from the plates and dabbed on lens paper to remove excess PBS prior to further testing.

### 5.3.3 Experiment 1 (1-day study)

The uptake and release of PHMB from contact lenses was measured within a 24-hour period. The lenses were incubated in 2 mL of PBS containing radioactive PHMB (1 µg/mL) for 8 hours (n = 4). The concentration of 1 µg/mL (1 ppm) was chosen to simulate the amount

of PHMB typically found in commercially available care products.[55] The lenses were then placed into 2 mL of PBS and the release measured at t = 0.25, 0.5, 1, 2, 4, 8, and 24-hour time points. At the end of the 24-hour period, the remaining PHMB was extracted from the lenses, denoted in this study as the residual amount remaining within the lens. The three extractions, each using 2mL of 100% methanol with shaking at 1600 rpm for 30 minutes at room temperature, ensured that all of the PHMB was extracted from the lenses. The percent released was determined by the following calculation below. A greater amount of PHMB extracted from the lenses (more PHMB remaining within the lenses) would reduce the percent released. Similarly, a decrease in total amount released would reduce the overall percent released.

The total amount of preservative uptake was calculated as follows:

$$Total\ uptake = Total\ amount\ released + Total\ residual\ amount\ remaining\ on\ lens$$

The percent released was calculated as follows:

$$Percent\ Released = \frac{Total\ amount\ released}{Total\ uptake} \times 100$$

### 5.3.4 Experiment 2 (7-day study)

The uptake and release of PHMB from contact lenses was measured over 7 days. Each day, the lenses were incubated in a fresh 2 mL of 14C PHMB (1 µg/mL) for 8 hours, followed by a release period in PBS for 16 hours. This uptake and release cycle was repeated every day to mimic the daily wearing and overnight soaking of reusable contact lenses. At the end of the

study period, the remaining residual amount of PHMB on the lenses was extracted using the same method as described in Experiment 1.

### 5.3.5 Radioactivity detection

The radioactive uptake, release, and residual amount of PHMB remaining within the lenses were measured independently using a LS6500 Beckman Coulter liquid scintillation beta counter (Beckman Coulter, ON, CA). In each experiment, the uptake and release samples were added separately to 10 mL of Scintillation fluor (PerkinElmer, MA, USA) and placed in a 20 mL scintillation vial. The PHMB remaining within the lenses were extracted with methanol. The extract was then added to 10 mL of Scintillation fluor (PerkinElmer, MA, USA) and placed in a 20 mL scintillation vial. The samples were loaded into the LS6500 Beckman Coulter liquid scintillation beta counter (Beckman Coulter, ON, CA) and counted for their radioactive signal (CPM). Each sample was analyzed for 10 minutes. A standard curve was used to convert CPM to µg of PHMB. The standard curve concentration ranged from 0.01 – 1 µg/mL.

### 5.3.6 Statistics

Statistical analysis and graphs were plotted using GraphPad Prism 6 software (GraphPad, La Jolla, CA). The data is reported as mean ± SD for experiment 1 (n = 4) and experiment 2 (n = 4). A one-way analysis of variance (ANOVA) was used to determine the differences in PHMB uptake. A repeated measures two-way ANOVA was used to determine the differences in PHMB release between contact lens material and time. Post-hoc Tukey multiple comparison tests were used when necessary. A value of $p<0.05$ was considered statistically significant.

## 5.4 Results

### 5.4.1 Experiment 1: Uptake & release (1-day study)

Table 5.1 summarizes the results of the total uptake and release of PHMB from lenses. The total uptake of PHMB for etafilcon A (0.22±0.01 μg) was significantly greater than senofilcon A (0.14±0.03 μg) after the 8-hour incubation period ($p$=0.01). There were no significant differences in the total uptake of PHMB between the other lens materials ($p$>0.05)

**Table 5.1 Summary of results for experiment 1 (1-day study).**

| Lens type | Total uptake (mean±SD) | Total release (mean±SD) | Residual amount remaining within the lens (mean±SD) | Percent release |
|---|---|---|---|---|
| **Lotrafilcon B** | 0.17±0.05 μg | 0.14±0.04 μg | 0.03±0.01 μg | 82% |
| **Senofilcon A** | 0.14±0.03 μg | 0.11±0.02 μg | 0.03±0.01 μg | 79% |
| **Balafilcon A** | 0.18±0.01 μg | 0.16±0.01 μg | 0.03±0.02 μg | 89% |
| **Omafilcon A** | 0.17±0.01 μg | 0.15±0.01 μg | 0.02±0.01 μg | 88% |
| **Etafilcon A** | 0.22±0.02 μg | 0.20±0.01 μg | 0.02±0.01 μg | 91% |

The release kinetics of PHMB over the 24-hr period is shown in Figure 5.1. As time increased, the total release of PHMB was greater for all lens materials ($p$<0.001). Post-hoc analysis show that etafilcon A released more PHMB compared to all other lens types ($p$<0.001). CH lens materials, as a group, also released significantly more PHMB than SH lens materials ($p$=0.03). Within SH lens materials, balafilcon A released more PHMB than senofilcon A ($p$=0.03). There were no significant differences between the CH materials ($p$=0.19). The release of PHMB, as shown in Figure 5.1, followed a burst-plateau profile.

Approximately 0.14-0.18 μg of PHMB was released within the first 2 hours, followed by a slower rate of release rate thereafter.

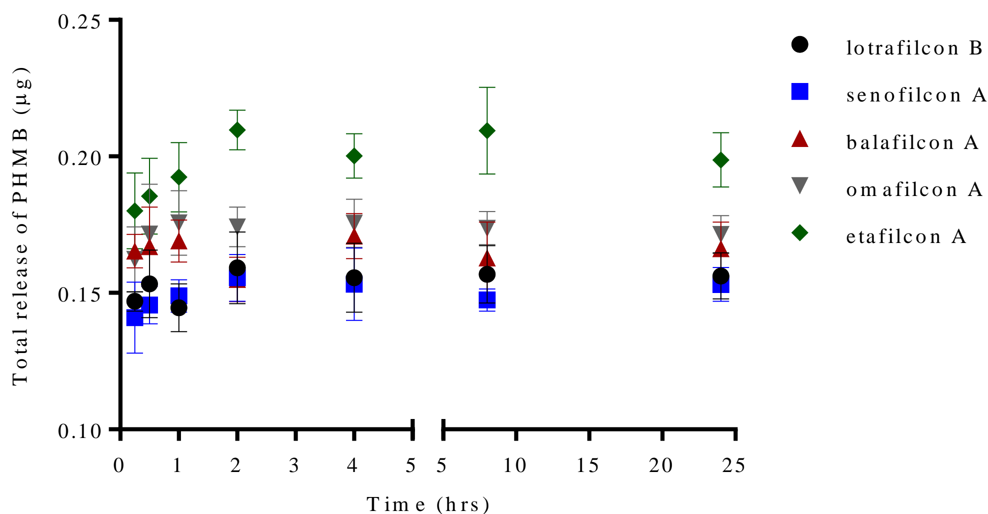


**Figure 5.1 Release kinetics of PHMB over 24 hours (mean±S.D).** The release kinetics of PHMB were significantly different between etafilcon A and all other lens types ($p$<0.001). Within SH lens materials, balafilcon A released more PHMB than senofilcon A ($p$=0.03). There was no significant difference between the CH materials ($p$=0.19).

The percent release of PHMB from the CLs after 24 hrs in PBS, shown in Table 5.1, suggests that the CH lens materials release a higher percentage of PHMB than SH materials ($p$<0.01). In particular, etafilcon A (91%, $p$=0.01) and omafilcon A (88%, $p$=0.02), demonstrated a significantly higher percent release of PHMB compared to senofilcon A (79%). The percent release was not significantly different between the two CH materials ($p$=0.99). Within SH materials, balafilcon A (89%) had a higher percent release of PHMB compared to lotrafilcon B (82%, $p$=0.02) and senofilcon A (79%, $p$=0.02).

### 5.4.2 Experiment 2: Uptake & release (7-day cycling study)

The contact lenses were cycled over a 7-day period in an alternating uptake and release cycle to mimic contact lens wear on a reusable basis. Figure 5.2 illustrates the uptake and release of PHMB over a 7-day period. All lens materials continued to sorb more PHMB over time ($p$<0.001). After the 7-day period, the amount of PHMB remaining in the etafilcon A material was significantly greater than senofilcon A ($p$=0.02). There were no significant differences between the other lens materials ($p$>0.05).

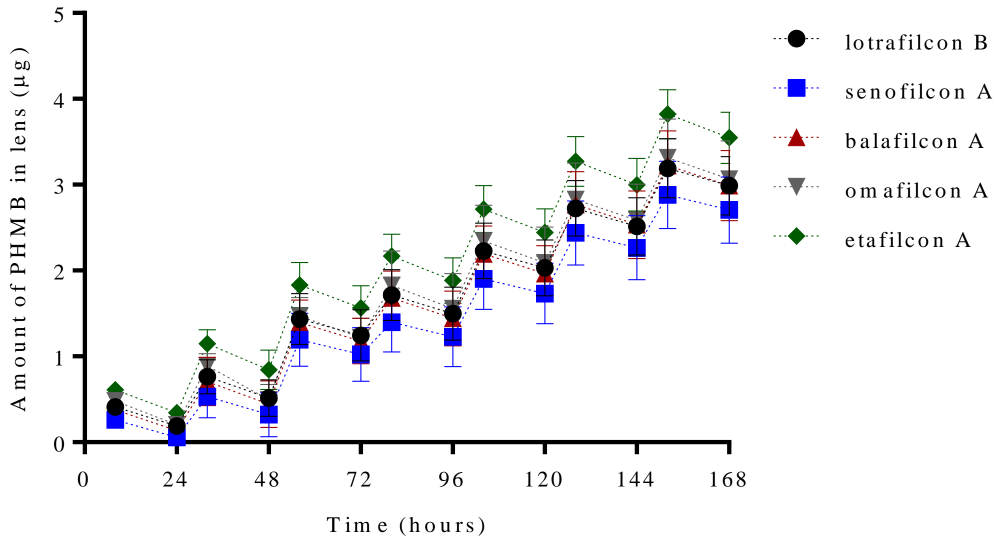


**Figure 5.2 The uptake and release profile of PHMB in a 7-day period.** An initial uptake at 8 hrs followed by a 16-hr release period for each daily cycle. There was a significant increase in PHMB uptake over time ($p$<0.001). The etafilcon A material sorbed significantly more PHMB compared to senofilcon A ($p$=0.02). There were no significant differences between the other lens materials ($p$>0.05).

Figure 5.3 shows the amount of PHMB released after each day over the 7-day period ($p$=0.02). After day 2, the CH materials (etafilcon A and omafilcon A) released significantly

more PHMB than the SH materials (lotrafilcon A, senofilcon A, and balafilcon A) ($p<0.01$). Within the CH materials, there were no significant differences in the amount of PHMB released ($p=0.55$). Within the SH materials, balafilcon A released more PHMB each day compared to senofilcon A ($p<0.01$). On days 2, 4, and 6, senofilcon A released more PHMB compared to lotrafilcon A ($p<0.01$). There were no differences between balafilcon A and lotrafilcon A ($p=0.28$). Visual inspection of Figure 5.3 shows that each lens material released a relatively consistent amount of PHMB each day, despite the fact that Figure 5.2 shows that over time the lens materials accumulate more PHMB, implying that irreversible binding of PHMB occurs over time

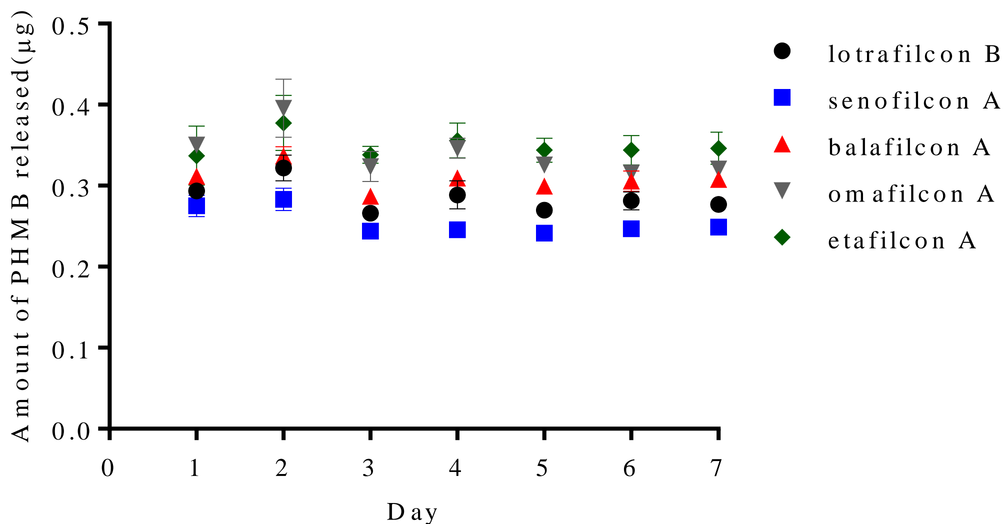


**Figure 5.3 Amount of PHMB released each day in a 7-day period.** The CH materials released significantly more PHMB compared to SH materials ($p<0.001$). There were no significant differences between CH materials ($p=0.55$). Within the SH materials, only balafilcon A released significantly more PHMB each day compared to senofilcon A ($p<0.01$).

By the end of the 7-day cycle, the total uptake of PHMB from etafilcon A was significantly greater than the SH lens materials (Table 2, $p<0.05$). Additionally, omafilcon A (2.87±0.04 µg, $p=0.01$) and balafilcon A (2.67±0.01 µg, $p=0.03$) sorbed significantly more PHMB than senofilcon A (2.26 ±0.02 µg). There were no differences between the other lens types ($p>0.10$). The total amount of PHMB released was significantly different between all lens materials ($p<0.01$) with both etafilcon A and omafilcon A releasing the most PHMB (Table 5.2). There was no significant difference between the CH materials ($p=0.56$).

In the 1-day study, the remaining PHMB in all lenses was approximately 0.03 µg (Table 5.1), whereas in the 7-day study the remaining PHMB was approximately 0.50 µg for all lenses (Table 5.2). There was more than a 15-fold increase in the amount of PHMB remaining in the lenses after a 7-day period in comparison to a 1-day wear period. There were no significant differences in the percent of PHMB released between all lens types after 7 days ($p=0.99$). The amount of PHMB remaining within the lenses from each extraction phase is shown in Table 5.3 and Table 5.4.

**Table 5.2 Summary of results for experiment 2 (7-day study).**

| Lens type | Total uptake (mean±SD) | Total release (mean±SD) | Residual amount remaining within the lens (mean±SD) | Percent release |
|---|---|---|---|---|
| **Lotrafilcon B** | 2.50±0.03 µg | 2.00±0.04 µg | 0.50±0.01 µg | 80% |
| **Senofilcon A** | 2.26±0.02 µg | 1.79±0.01 µg | 0.47±0.02 µg | 79% |
| **Balafilcon A** | 2.67±0.03 µg | 2.16±0.01 µg | 0.51±0.02 µg | 80% |
| **Omafilcon A** | 2.87±0.04 µg | 2.38±0.03 µg | 0.48±0.02 µg | 83% |
| **Etafilcon A** | 2.98±0.03 µg | 2.45±0.08 µg | 0.48±0.05 µg | 82% |

**Table 5.3 Experiment 1.** The amount of PHMB remaining within the lenses from each extraction phase. The extraction was carried out after the 1-day release period.

| Lens Type | Extraction 1, µg | Extraction 2, µg | Extraction 3, µg | Total extraction, µg |
|---|---|---|---|---|
| **Lotrafilcon B** | 0.03 | BDL | BDL | 0.03 |
| **Senofilcon A** | 0.03 | BDL | BDL | 0.03 |
| **Balafilcon A** | 0.02 | BDL | BDL | 0.02 |
| **Omafilcon A** | 0.02 | BDL | BDL | 0.02 |
| **Etafilcon A** | 0.02 | BDL | BDL | 0.02 |

BDL: Below detection limit. The limits of detection for extracted values were below 0.01 µg.

**Table 5.4 Experiment 2.** The amount of PHMB remaining within the lenses from each extraction phase. Lenses were exposed to fresh PHMB after each day. The extraction was carried out at the end of the 7-day release period.

| Lens Type | Extraction 1, µg | Extraction 2, µg | Extraction 3, µg | Total extraction, µg |
|---|---|---|---|---|
| **Lotrafilcon B** | 0.38 | 0.12 | BDL | 0.50 |
| **Senofilcon A** | 0.41 | 0.06 | BDL | 0.47 |
| **Balafilcon A** | 0.42 | 0.09 | BDL | 0.51 |
| **Omafilcon A** | 0.38 | 0.10 | BDL | 0.48 |
| **Etafilcon A** | 0.39 | 0.09 | BDL | 0.48 |

BDL: Below detection limit. The limits of detection for extracted values were below 0.01 µg.

### 5.5 Discussion

This study demonstrated that the uptake and release of PHMB could be determined reliably using a radioactive labelling method. In the 1-day study, etafilcon A sorbed significantly greater amounts of PHMB compared to senofilcon A ($p$=0.01), as shown in Table 1. The release of PHMB were significantly different between the CL materials. Etafilcon A released more PHMB than all other lens types ($p$<0.001). The results suggest that the majority of PHMB absorbed into the CLs were released within a 24-hr period for all materials.

In the 7-day study, both the CH materials (etafilcon A, omafilcon A) sorbed significantly more PHMB than SH materials (senofilcon A, balafilcon A, lotrafilcon A) with no evidence of saturation occurring for any of the lens materials, as shown in Figure 2. At the end of each day, all lenses released a relatively similar amount of PHMB, that remained fairly consistent for each lens material (Figure 5.3). The CH lenses appeared to release approximately 0.1 µg more PHMB per day compared to the SH lenses. After day 2, the CH materials demonstrated a significantly higher total release of PHMB compared to SH materials ($p$<0.01). Given the consistent release of PHMB after 7-days, further cycling of the lenses may demonstrate similar release patterns.

The greater uptake of PHMB by the etafilcon A material may be due to the ionicity and material properties of the lens.[48,67,90] PHMB is a positively charged, highly cationic molecule that is soluble in water and alcohol.[224] The negative charge of methacrylic acid (MA) found in etafilcon A promotes higher uptake of the positively charged PHMB.[90] Non-MA containing lens materials (omafilcon A and balafilcon A) show the next highest uptake of PHMB.[90] The surface treatment of certain SH lenses may also influence the uptake and release kinetics of

PHMB. Balafilcon A and lotrafilcon A lenses are treated to increase surface wettability and hydrophilicity, but the weakly charged surfaces resulted in low uptake of PHMB yet rapid release of PHMB.[48,90] This may suggest that the bulk matrix of the CL is more important for the uptake of PHMB.

Table 5.1 and Table 5.2 show that more than 80% of PHMB absorbed into the lens materials were released from the lenses. These results suggest that although PHMB does bind to CL materials, it is readily released from CLs when incubated in a PBS solution. Interestingly, in the 7-day study, there was a significant decline in the percent released of PHMB for balafilcon A, omafilcon A, and etafilcon A to similar levels as senofilcon A, and lotrafilcon A. This would suggest that over time, the uptake and release of PHMB behaved similarly for all the CL materials tested. Overall, these results indicate that the ionicity, water content, and surface treatment, may have an affect on the initial uptake and release of PHMB, but at longer time intervals, these factors have relatively minimal impact.

The current study shows that PHMB exhibits a very different uptake and release profile compared to another common biocide, MAPD.[98] In a previous study with radioactive MAPD, it was observed that SH materials sorbed greater amounts of MAPD compared to CH materials.[98] Interestingly, the percent release of MAPD from SH materials after 7 days (< 25%) was significantly less than CH materials (> 80%).[98] The results with MAPD contrasts those with PHMB in the current study, in which the overall uptake and release pattern of PHMB were very similar for both CH and SH materials.

The differences in the results between PHMB and MAPD uptake and release can be attributed to their chemical structure and properties. MAPD is a small molecular weight (312 g/mol) compound, with a surfactant-like structure, containing a lipophilic tail and a hydrophilic positively charged group.[67] As a result, the biocide was able to easily penetrate the lens materials and irreversibly bind with the silicone domains of SH materials, resulting in a higher absorption.[67,98] It was shown that SH materials absorbed as much as 40 μg of MAPD over 7 days,[98] which was significantly greater than the amount of PHMB absorbed in the current study (2.5 - 4 μg) over the same period of time.

In comparison, PHMB has a higher molecular weight (8000 g/mol) and is a highly cationic molecule with multiple branched polymeric chains.[56] Based on its structure, we hypothesize that it would have a more difficult time penetrating the bulk of the lens material. The adsorption may occur on the surface or sub-surface of the lens. Furthermore, due to its hydrophilic structure, PHMB likely does interacts minimally with the lipophilic domains of SH materials but has a higher degree of interaction with more hydrophilic HEMA (poly-2-hydroxyethyl methacrylate) material.[67] For this reason, there was a higher uptake and release of PHMB from etafilcon A as compared to senofilcon A.

In the 7-day study, the CL materials were cycled to resemble lens storage (uptake) and lens wear (release) throughout the day. The results show that lenses are not saturated by day 7 and that more PHMB could potentially be sorbed onto the lens materials with further wear cycles, increasing the degree of potential exposure of the cornea to PHMB over increasing wear cycles.

Previous studies have demonstrated that an increased uptake of PHMB into a CL material may reduce the biocidal efficacy of the CL solutions.[96,215,225,226] The total uptake of PHMB by etafilcon A in the 1-day study was 11%, while fresh cycling of PHMB in the 7-day study had 22% sorbed. The increased uptake of PHMB during lens storage may lower the MPS ability to kill bacteria, fungi, and acanthamoeba.[96,225,226] Prior outbreaks of fungal keratitis and acanthamoeba were linked to the uptake of biocides into the lens material, leading to a reduced efficacy of the solution.[96,152,155,200]

Studies have shown that MPS with various lens materials have varying degrees of cytotoxicity, oxidative stress, and "damage" to epithelial cells.[91,93,186,227,228] Various MPS have been linked to the development of corneal infiltrative events (CIEs).[204-206,228] Clinically, various combinations of MPS and CL materials have also demonstrated solution-induced corneal staining (SICS).[133,135] However, it remains unclear whether CIEs related to wearing reusable soft lenses are associated with corneal staining or indeed could be influenced by this cyclical uptake and release of MPS components.[136,179,205,229]

Daily wear of CL exposes the lens to various tear components, which could impact the uptake and release kinetics of PHMB due to these components blocking potential adsorption sites, resulting in competition for binding.[174,230-233] Fluorescein staining has been directly linked to the type of surfactant (tetronic 1107) used in an MPS.[145] Other studies suggest that non-pathological staining may occur from the secondary binding of fluorescein to the biocide bound to the corneal epithelium, indicating a transient binding.[9,187]

The clinical findings, *in vitro* studies, and findings from the current study, suggest that the clinical findings of CIEs and SICS may not be directly linked to the release of PHMB from the CL materials. SICS has been suggested as a benign phenomenon not indicative of compromised cells[138,145,234] and perhaps occurs due to the other components (surfactants, borate buffer) of the solution.[93,235] PHMB-based MPS are commonly associated with SICS but no histological changes to the cornea were observed.[128] There has been no direct link that microbial keratitis is associated with SICS, yet one study found patients with SICS were more likely to have an inflammatory CIEs.[136] The uptake and release issues are believed to be a solution and materials interaction, while SICS is considered to be a transient solution induced phenomenon that may not imply corneal toxicity.[28]

In conclusion, radioactive labelling provides an alternative and sensitive method to assess PHMB uptake and release at concentrations found in commercial MPS. The uptake of PHMB is likely driven primarily by the ionic charge and water content of the lens material. The results support this concept, since the uptake and release of PHMB was highest for group 4 hydrogel materials. However, the interaction between PHMB and the tested CL materials were fairly weak, as all lenses exhibited similar release profiles of PHMB. Over a 7-day period, the amount of PHMB within all lens types increased, with no evidence of a plateau. The clinical implications of these findings remain unclear given the *in vitro* nature of the study does not entirely mimic the conditions of the eye. Further investigation is required to understand the clinical significance of biocides, CL materials, and their interaction with the ocular surface.

# Chapter 6. Evaluating the cytotoxicity of polyhexamethylene biguanide (PHMB) and myristamidopropyl dimethylamine (MAPD) released from contact lenses on cell viability

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| Author | Concept/Design | Data Acquisition | Analysis | Writing/Publication |
|---|---|---|---|---|
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| McCanna | Y | | | Y |
| Phan | | | | Y |
| Jones | Y | | | Y |

### 6.1 Outline

#### 6.1.1 Objective

To evaluate the cytotoxicity of polyhexamethylene biguanide (PHMB) and myristamidopropyl dimethylamine (MAPD) released from reusable soft contact lenses (CLs) on human corneal epithelial cells (HCEC).

#### 6.1.2 Methods

Three silicone hydrogel CLs (lotrafilcon B, balafilcon A, senofilcon A) and two conventional hydrogel CLs (etafilcon A, omafilcon A) were soaked in 2 mL of phosphate buffered solution (PBS) containing either PHMB (1 µg/ml) or MAPD (5 µg/ml) for 8 hours (n=4). After the incubation period, the lenses were placed in 2 mL of PBS for 16 hours. 0.5 mL of this release media was exposed to immortalized corneal epithelial cells (ICEC) and HCEC for 16 hours. Afterwards, 0.5 mL of two multipurpose solutions (renu® fresh™ and OPTI-FREE® Replenish®), PHMB at 1 µg/mL, 5 µg/mL, 10 µg/mL and MAPD at 2.5 µg/mL, 5 µg/mL, 10 µg/mL were tested against ICEC and HCEC. PBS was used as the control condition. Cell viability was then measured using the alamarBlue™ assay.

#### 6.1.3 Results

The amount of PHMB or MAPD released from CLs did not have an impact on cell viability for either ICEC or HCEC cells ($p>0.05$). PHMB and MAPD at concentrations of 5 µg/mL and higher showed significantly reduced cell viability ($p<0.05$). OPTI-FREE® Replenish®, which contains 5 µg/mL of MAPD reduced cell viability of ICEC to 30%

($p<0.001$) and HCEC to 1% ($p<0.001$) as compared to the control. At similar concentrations, MAPD appears to be more cytotoxic than PHMB ($p<0.05$).

### 6.1.4 Conclusion

Cell metabolic activity provides a useful endpoint to measure the cytotoxicity for chemicals commonly used in ophthalmic products. The study showed that the uptake and release of PHMB and MAPD from CLs did not significantly reduce cell viability after a 1-day incubation-wear cycle. However, it should be noted that a high concentration of biocides can cause cytotoxic effects.

## 6.2 Introduction

The majority of reusable soft contact lens (CL) wearers replace their lenses after 2-4 weeks of wear.[160,162] Prior to reinsertion, lenses need to be cleaned and disinfected, with most wearers using multipurpose solutions (MPS).[28,55] MPS consist of a single bottle of solution designed to clean, disinfect, and adequately wet reusable CLs. MPS are comprised of a complex combination of biocides, surfactants, buffering agents, wetting agents, and chelating agents.[28,55,132] Two commonly encountered biocides are polyhexamethylene biguanide (PHMB) and myristamidopropyl dimethylamine (MAPD).[65,90,96,236]

PHMB is a cationic molecule with broad spectrum activity against both gram-positive and gram-negative bacteria.[59] The mode of action disrupts the plasma membrane of bacterial cell walls leading to cell lysis.[59] MAPD is also a cationic molecule, which is effective against bacteria, fungal and amoebic organisms.[63,66,69] The antimicrobial mode of action is via damage to the cell plasma membranes, causing leakage of the cell.[63,66] The same mechanisms for PHMB and MAPD to kill microbes may also be toxic to human corneal epithelial cells (HCEC) when used at concentrations greater than the specified concentration in MPS.[66,69,91,93]

Previous studies have shown that MAPD and PHMB can accumulate on CLs over time.[48,60,67,90,98] Silicone hydrogel (SH) lens materials were able to sorb more MAPD compared to conventional hydrogel (CH) lens materials,[67,98] yet, CH materials released more MAPD than SH lenses.[67,98] CH materials (etafilcon A) sorbed more PHMB than a variety of SH materials,[48,67,90,98] but SH materials released more PHMB compared to CH materials.[67] Reusable soft CL materials can uptake various components of MPS during the cleaning and disinfecting phase and subsequently release them onto the corneal surface during lens

wear,[48,60,67,90,98] and their release may result in cytotoxic effects.[91,93,124,129,227,237,238] Clinically, certain combinations of lenses and solutions demonstrate increased amounts of corneal staining[132-134,137] and reports of patient discomfort, papillary conjunctivitis, and an increased risk of corneal infiltrative events have been linked with these findings.[28,134,204,237]

Most studies have assessed MPS as a whole rather than the individual components (biocides, surfactants, buffering agents).[5,91,93,124,129] A number of studies have shown that surfactants[129,145] and buffering agents[5,93,124,129,239] can also reduce cell viability. To our knowledge, no studies have assessed the impact of a biocide-only solution against HCEC. The purpose of this study was to evaluate the cytotoxicity of PHMB and MAPD released from soft reusable CLs on HCEC.

## 6.3 Material and Methods

### 6.3.1 Soft contact lens materials

Three SH materials (lotrafilcon B, senofilcon A, balafilcon A) and two CH materials (omafilcon A, etafilcon A), all -3.00 diopters, were investigated (n=4 per material). The CLs were taken out of the blister packs and then rinsed and soaked in 2 mL PBS (ISO 11986) for 8 hours to remove any excess blister pack solution.[99]

### 6.3.2 Multipurpose and biocide solutions

Two commercially available MPS, renu® fresh™ (Bausch & Lomb) and OPTI-FREE® Replenish® (Alcon) MPS were evaluated (Table 1). The concentration of PHMB and MAPD

in a commercially available MPS is 1 ppm (1 µg/mL) and 5 ppm (5 µg/mL) respectively, as shown in Table 6.1. In this study, three concentrations of PHMB (1 µg/mL, 5 µg/mL, 10 µg/mL) and MAPD (2.5 µg/mL, 5 µg/mL, 10 µg/mL) in phosphate buffered saline (PBS) were also evaluated. The PBS was formulated based on ISO testing guidelines.[175]

**Table 6.1 Components of the multipurpose solutions (MPS) evaluated in this study.**

| MPS | Manufacturer | Biocide | Other components |
|---|---|---|---|
| renu® fresh™ | Bausch + Lomb | PHMB at 1 µg/mL (1 ppm) | Borate, boric acid, poloxamine 1107, hyaluronan, sodium chloride, EDTA |
| OPTI-FREE® Replenish® | Alcon | Polyquad® (PQ-1) at 10 µg/mL (10 ppm)<br>Aldox® (MAPD) at 5 µg/mL (5 ppm) | Sodium borate, sodium citrate, sorbitol, sodium chloride, propylene glycol, TearGlyde® (Tetronic® 1304 and nonanoyl-EDTA) |

EDTA (ethylenediaminetetraacetic acid); MAPD (myristamidopropyl dimethylamine); PHMB (polyhexamethylene biguanide); PQ-1 (polyquaternium-1)

### 6.3.3 *In vitro* cell culture

Immortalized corneal epithelial cells (ICEC) (University of Ottawa, Ottawa, ON, Canada) and primary human corneal epithelial cells (HCEC) (Millipore, CA, USA) were cultured in serum-free EpiGRO™ Human Ocular Epithelia Media (Millipore, CA, USA), with supplemental kit components: L-Glutamine (15 mL, 6 mM), EpiFactor O (1 mL), Epinephrine (0.5 mL, 1.0 µM), EpiFactor P (2 mL, 0.4%), rh Insulin (0.5 mL, 5 µg/mL), Apo-Transferrin

(0.5 mL, 5 µg/mL), and Hydrocortisone hemisuccinate (0.5 mL, 100 ng/mL). The cells were propagated in 75 $cm^2$ Collagen 1-coated BioCoat™ culture flasks (Corning, NY, USA) and grown to 80% confluency in a 37°C incubator with 5% $CO_2$. EpiGRO™ media was replaced every 2-3 days.

The adherent cells were dissociated from the culture flask using 10 mL TrypLE™ Express (Invitrogen, CA, USA) without phenol red. Cells were centrifuged (5 mins, 500 rpm) in 50 mL Falcon tubes (BD Falcon, Mississauga, ON) and the supernatant discarded. Cells were resuspended with EpiGRO media and a hemacytometer was used to count the cells.

The cells were then seeded onto a 48-well Collagen 1 BioCoat™ coated culture plate (Corning, NY, USA) at $10^5$ cells per well with 0.5 mL of EpiGRO™ media. Cells were incubated at 37°C with 5% $CO_2$ for 48 hrs to allow for adherence and formation of a cell monolayer. After the formation of a monolayer, the old media was removed from the wells and replaced with 0.5 mL of fresh media. These cell culture plates were then ready to be used for testing the cytotoxicity of the biocide solutions.

### 6.3.4 Cytotoxicity of biocide uptake and release from contact lenses

The commercial CLs were soaked in a 2 mL PBS solution containing either PHMB (1 µg/ml) or MAPD (5 µg/ml) for 8 hours (n=4), which simulates a standard overnight CL soaking procedure. After the incubation period, the lenses were placed in 2 mL of PBS for 16 hours to simulate the release of the biocides from a CL over a typical wear period. 0.5 mL of this release media was exposed to the ICEC and HCEC cell culture plates for 16 hours at 37°C with 5% $CO_2$ (n=4). Following the exposure period, cell viability was determined using the

alamarBlue™ assay. 0.5 mL of MPS solutions (renu® fresh™ and OPTI-FREE® Replenish®), PHMB at 1 µg/mL, 5 µg/mL, 10 µg/mL and MAPD at 2.5 µg/mL, 5 µg/mL, 10 µg/mL in PBS were also exposed to the cell cultures. 0.5 mL of PBS was used as the control solution.

### 6.3.5 AlamarBlue™ cell viability assay

The non-toxic alamarBlue™ cell viability reagent (Molecular Probes, Life Technologies, ON) was used to measure the cytotoxic effects of the solutions. The alamarBlue™ solution (10% w/v) was prepared in DMEM/F12 (Invitrogen, ON, CA) without phenol red or serum. The alamarBlue™ solution (0.5 mL) was placed into each well (48-well plate). The 48-well plates were incubated for 4 hrs at 37°C and 5% $CO_2$. A change in fluorescence indicated the metabolic activity of the cells.[93] The fluorescence was measured with a Cytation 5 Cell Imaging Multi-Mode Reader (BioTek Instruments Inc., USA) with excitation/emission wavelengths set at 540±20 nm/590±20 nm. The results were shown as a percentage relative to the control.

### 6.3.6 Statistics

Statistical analysis and graphs were plotted using GraphPad Prism 6 software (GraphPad, CA, USA). A one-way analysis of variance (ANOVA) was used to determine the differences in cell viability. Post-hoc Tukey multiple comparison tests were used to compare treatments. All data were expressed as mean ± SD and $p<0.05$ was considered statistically significant.

## 6.4 Results

### 6.4.1 Effect of PHMB solutions on cell viability

The effects of various biocide solutions containing PHMB on cell viability are shown in Figure 6.1. The results indicate that the release of PHMB from CLs did not have an impact on cell viability for either ICEC ($p>0.85$) or HCEC ($p>0.27$). ICEC exposed to PHMB released from CLs retained 92-96% cell viability (Figure 6.1A), whereas HCEC retained 86-93% of cell viability (Figure 6.1B). The release of PHMB from CH materials omafilcon A (87%) and etafilcon A (90%) appear to impact HCEC slightly more than the SH materials, but this difference was not statistically significant ($p>0.94$, Figure 6.1B). ICEC and HCEC had reduced cell viability for concentrations of PHMB at 5 µg/mL and higher ($p<0.05$). The renu® fresh™ MPS showed reduced ICEC (72%, $p<0.001$) and HCEC viability (44%, $p<0.001$) as compared to the control.

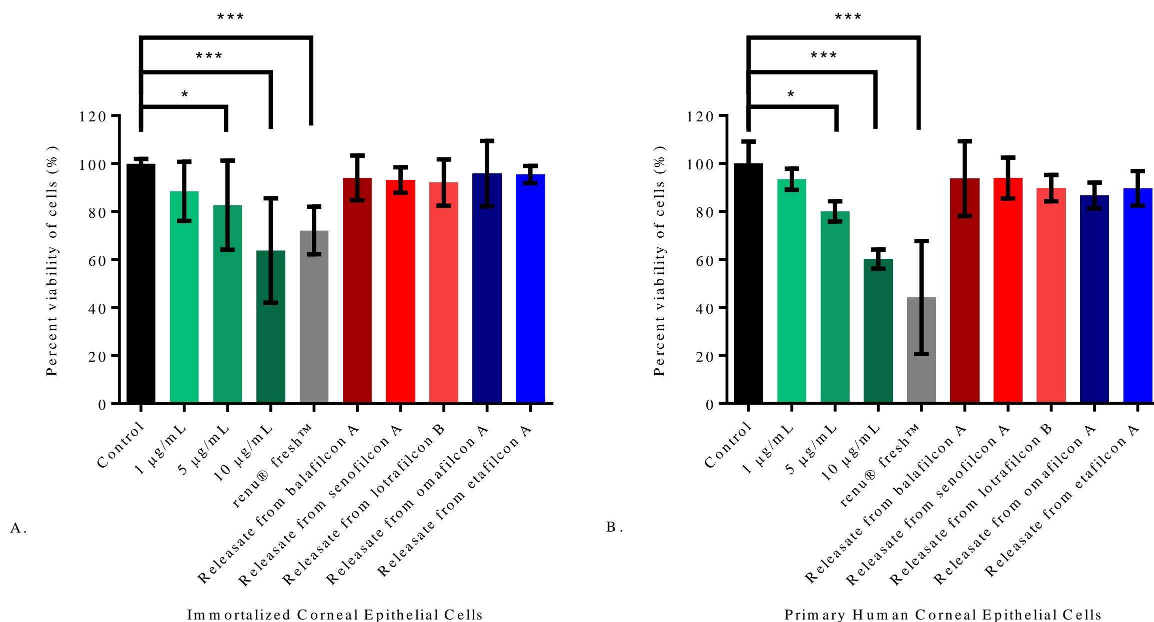


**Figure 6.1 Effect of various solutions on corneal epithelial cell viability.** Viability was measured by alamarBlue™ assay and is expressed as a percentage relative to the control. **A** ICEC and **B** HCEC were exposed to 0.5 mL of various solutions containing PHMB (n=4). The PHMB eluted from CLs did not significantly impact ICEC ($p$>0.85) and HCEC ($p$>0.27). PHMB concentrations at 5 µg/mL ($p$<0.05),10 µg/mL ($p$<0.001) and renu® fresh™ MPS solution ($p$<0.001) had significantly reduced cell viability.

### 6.4.2 Effects of MAPD solutions on cell viability

The effects of various biocide solutions containing MAPD on cell viability are shown in Figure 6.2. The results show that the release of PHMB from CLs did not have an impact on cell viability for either ICEC ($p$>0.98) or HCEC ($p$>0.68). ICEC exposed to MAPD released from CLs retained 92-96% cell viability (Figure 6.2A) whereas HCEC retained 90-95% of cell viability (Figure 6.2B). Increasing the concentration of MAPD showed a dose-dependent effect on cell metabolic activity for ICEC and HCEC, with significantly lower cell viability at concentrations 5 µg/mL and higher ($p$<0.05). At 5 µg/mL, ICEC viability was 80% ($p$=0.03)

and HCEC viability was 55% ($p<0.001$). At 10 µg/mL, ICEC viability was 30% ($p<0.001$, Figure 6.2A) and HCEC viability was 1% ($p<0.001$, Figure 6.2B). The OPTI-FREE® Replenish® MPS containing MAPD at 5 µg/mL showed reduced cell viability of ICEC to 30% ($p<0.001$) and HCEC to 1% ($p<0.001$) as compared to the control.

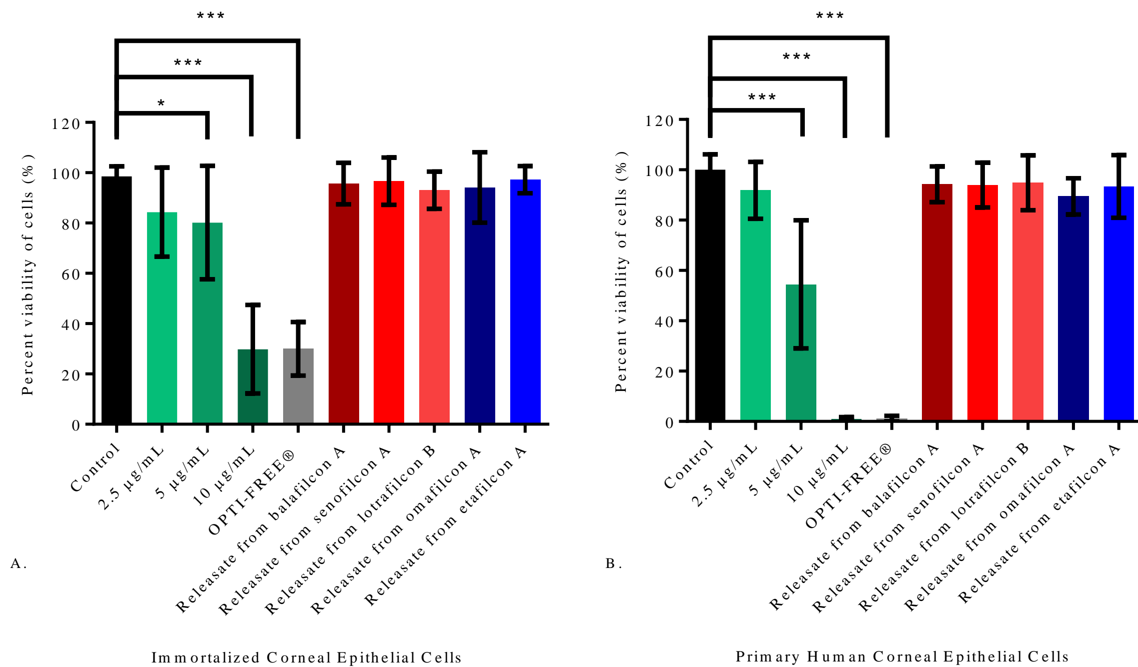


**Figure 6.2 Effect of various solutions on corneal epithelial cell viability.** Viability was measured by alamarBlue™ assay and is expressed as a percentage relative to the control. **A** ICEC and **B** HCEC were exposed to 0.5 mL of various solutions containing MAPD (n=4). The MAPD eluted from CLs did not significantly impact ICEC ($p>0.98$) and HCEC ($p>0.68$). MAPD concentrations at 5 µg/mL ($p<0.05$),10 µg/mL ($p<0.001$) and OPTI-FREE® Replenish® ($p<0.001$) had significantly reduced cell viability.

### 6.5 Discussion

The results showed that PHMB and MAPD at concentrations below 1 µg/mL and 2.5 µg/mL respectively were not cytotoxic to the cells. However, higher concentrations of biocides resulted in significant cytotoxicity. Biocides released from CLs after a typical 1-day daily wear cycle did not have any significant impact on cell viability ($p>0.05$). These results suggest that the biocides released from CLs were well below the cytotoxic thresholds of PHMB (1 µg/mL) and MAPD (2.5 µg/mL).

PHMB at 1µg/mL did not reduce cell viability compared to renu® fresh™ (PHMB 1µg/mL), which suggests that the reduction in cell viability may be related to the other components contained in renu® fresh™.[92,145,240] In this study, PHMB at concentrations of 5 µg/mL and higher resulted in reduced cell viability. PHMB has been shown to disrupt microbial cell membranes leading to cell lysis.[59] Additionally, it can reduce epithelial cell proliferation and therefore reduce the number of viable cells.[241]

Several studies have shown the cytotoxicity of MAPD in MPS against microbial organisms,[65,66,69,70,242] but to our knowledge, none have shown the impact of a biocide-only solution against corneal epithelial cells. The results show that MAPD at concentrations of 5 µg/mL and higher resulted in reduced cell viability ($p<0.05$). This same concentration of MAPD is also present in OPTI-FREE® Replenish® MPS, which was as expected to be also cytotoxic to the cells. Interestingly, the MPS had a higher reduction in cell viability than the same concentration of MAPD in PBS, which suggests that other components of the MPS solution may also be contributing to this cytotoxic effect, reducing cell viability.[91,93,145,183,240]

OPTI-FREE® Replenish® (containing MAPD at 5 µg/mL) and direct application of the MAPD solution at 5µg/mL significantly reduced cell viability ($p<0.05$). While both were shown to reduce cell viability, the MPS had a significantly higher reduction. MPS contain a variety of components aside from biocides (such as buffers, surfactants, etc) that also may be cytotoxic to epithelial cells. Previous studies have shown that borate buffer used in some MPS can cause cytotoxic damage.[93,124,235] Surfactants, such as tetronics and poloxamers, were also shown to exhibit cytotoxic effects on the ocular surface.[129,239,243-245] Biocides and other components of a MPS (such as buffers) can impact corneal epithelial cells, including its microbial efficacy,[246] and this fine balance is important to minimizing cytotoxicity on the ocular surface.

The direct application of OPTI-FREE® Replenish® and renu® fresh™ significantly reduced ICEC and HCEC viability. One study found that OPTI-FREE® Express®, which contains the same biocides found in OPTI-FREE® Replenish®, significantly decreased the number of mitochondria in the superficial and intermediate epithelium compared to renu® fresh™.[247] The authors suggested that the ingredients in OPTI-FREE® Express® can penetrate the epithelium more deeply than those found in renu® fresh™. OPTI-FREE® Replenish® contains a second biocide, PQ-1 (10 µg/mL), which is effective against gram-negative bacteria.[65,91,124,130,248] In cell survival studies, MPS containing PQ-1 significantly reduced cell proliferation compared to other solutions.[91,238]

A recent study from our group (Chapter 4) assessed the uptake and release of MAPD from CL following the chemical assay recommendations.[98,99] Within the first 24-hrs, CH lenses (etafilcon A, omafilcon A) released between 0.8-0.9 µg/mL of MAPD while SH lenses

(lotrafilcon B, balafilcon A, senofilcon A) released between 0.25-0.35 µg/mL of MAPD.[98] Within the current experimental conditions, the results suggest that the amount of MAPD released from any CL within 24 hours is well below the cytotoxic limit at 2.5 µg/mL. In Chapter 5, all CLs released 0.05-0.10 µg/mL PHMB within 24 hours, which supports the fact that PHMB released from CLs in this study should not reduce cell viability.

In this model, biocides were released in 2 mL of PBS solution, which dilutes the biocides prior to exposure on the cells. A 2 mL release solution was used because that is the average volume of tears released during an average day (1-3 µL/min).[249-251] However, if the release solution were reduced to levels typically found in the tear film (3-5 µL),[4,251,252] then the concentration of the biocide would significantly increase, thereby potentially increasing cytotoxicity. In contrast, tear replenishment and tear flow would help remove the biocides from the cornea and reduce toxicity.[250,252,253] Future studies will attempt to validate the release of biocides in a more complex model that better mimics the ocular surface

*In vitro* cell culture studies offer a rapid, safe, and affordable model to determine whether the solution or material is potentially harmful to a biological system. The current study used similar protocols as the chemical assay (Chapter 4 & 5) while following ISO recommendations and determined that for short-term use, the biocides released from CLs are not cytotoxic.[98,99,175] The chemical and toxicity assay provides an important foundation for a better comparison of different assays which may include future microbiological testing. Further research should consider testing a lens-solution combination with additional complexities to the system, which will better mirror clinical outcomes.[183,254,255]

# Chapter 7. Discussion and Conclusion

Soft CL materials (specifically SH lens materials) have continued to grow in popularity over the last two decades, as they have greatly improved oxygen transmissibility and demonstrate improved comfort in some subjects.[142,167] The introduction of SH lens materials has provided significant benefits over CH lens materials, however, the combination of certain soft CL materials with MPS can lead to discomfort and certain complications. [90,143,168] Therefore, it is important to understand the interaction between reusable soft CL materials and MPS, and in particular the biocides (MAPD and PHMB), which act as the main disinfecting agent in a wide range of commercially available MPS.

In this thesis, radioactive labelling provided a sensitive method for assessing the uptake and release of MAPD (Chapter 4) and PHMB (Chapter 5) from soft CLs. SH lens materials sorbed significantly greater amounts of MAPD in comparison to CH lenses. Interestingly, CH lenses released more MAPD compared to SH lenses. Previous uptake and release studies on MPS containing PQ-1 and MAPD showed that MAPD was also readily taken up by all lens types, with SH lenses sorbing the most. However, a very minimal release of MAPD was shown from any of the CLs. In comparison, CH lens materials sorbed more PHMB than SH materials. All lenses released a relatively similar amount of PHMB, which remained consistent for each lens material.

The uptake and release of MAPD and PHMB were different based on their chemical structure and properties.[48,67,90] MAPD is a smaller molecule (312 g/mol), with a surfactant-like structure, and a lipophilic tail and hydrophilic charged group.[67] These properties allow MAPD

to penetrate the lens materials and irreversibly bind to the silicone domains of SH materials, which led to higher absorption and reduced desorption.[67] PHMB is a significantly larger molecule (8000> g/mol) and is highly cationic with multiple polymeric chains.[56] Thus, it would be more difficult for PHMB to penetrate the bulk of the lens material. The CL materials ionicity, water content, and surface treatment of certain lenses (lotrafilcon A and balafilcon A) can also influence the uptake and release of biocides.

One major drawback with using radioactive labelling are the high costs associated with the synthesis process.[256] Typically, $^{14}C$ or $^{3}H$ are the radioisotopes of choice. The advantages of $^{14}C$ compared to $^{3}H$ is that $^{14}C$ is integrated within the molecular framework, thus reducing the chance of free-floating radioisotope.[256] Alternatives to the high cost associated with $^{14}C$ may be to optimize and minimize the use of it to quantities that are detectable. Other radioactive studies with CLs have been successful using $^{125}I$ tagged to proteins or lipids.[111,113,172,257] Radiolabeling using $^{125}I$ is sensitive enough to measure low levels of protein, however, a major drawback is $^{125}I$ can dissociate from the protein and bind to other test materials reducing its accuracy.[113]

Pharmacokinetic modelling of the uptake and release kinetics would provide supportive data to Chapters 4 and 5. Depending on the type of model used, a Higuchi model or a first-order kinetic rate model could be used to determine the rate of drug release from the CL matrix and compare it to the experimental data shown. A kinetic model could account for the different partitioning behavior between the SH and CH materials. Additional complexities would be the biocides chemical properties (molecular weight and charge). However, given the current experimental design, only the release data was measured at various time points, and a further

set of experiments would be required to measure uptake at the respective time points to provide accurate modelling. Due to the high costs associated with radioactive labelling, further experiments may not be feasible.

Biocides and MPS have previously demonstrated a significant amount of cytotoxicity to corneal epithelial cells. In chapter 6, the study showed that biocides released from CLs were not cytotoxic to corneal epithelial cells. When direct exposure of biocide concentrations 2-10X normally found in MPS were applied to the cells, a significant reduction in cell viability was observed. Cell viability is a common indicator in many *in vitro* MPS studies correlating to cell metabolism or membrane integrity.[91-93,123,130] However, direct comparisons between studies are difficult since exposure times, concentrations, and cell confluency vary significantly.[91-93,123,130] The results may indicate that some of these events may not be due to cytotoxicity (i.e. cell death) but rather due to cell damage. Additionally, mechanisms of cell death, either apoptosis (programmed cell death) or necrosis (pathological process), are important pathways to differentiate as MPS (PHMB and polyquaternium-1) has been shown to induce necrosis.[227] Studies using corneal epithelial cells in a monolayer have been criticized to be overly sensitive to MPS because of the absence of underlying cells, which play an important role in wound-healing.[183] In certain cell survival studies, cells that were exposed to MPS (PHMB and polyquaternium-1) were shown to proliferate less compared to other solutions.[91,238]

Very few studies have assessed the effect of lens-solution combination on corneal epithelial cells,[92,183,239] yet it provides a good representation of the *in vivo* environment. A few studies have incorporated “onlay” models with direct CL exposure to the cells, which incorporates mechanical wear as another factor to consider.[124,183,254,255] In chapter 6, CLs were

not directly placed on cell cultures because testing the biocides released from CLs would provide a worst-case scenario where all biocides are released at once. The main purpose of this study was to determine the potential cytotoxicity of biocides (PHMB or MAPD) against corneal epithelial cells. Future studies should consider *in vitro* models using lens-solution combinations and additional complexities to the system such as tear components and tear flushing to better approximate to clinical situations.

While *in vitro* models, either a solutions-only or solution-lens combination, can provide valuable information, there are limitations based on the type of cell culture used (monolayer vs stratified).[183] The solutions are typically released onto the cells and remain in the incubation medium with corneal epithelial cells for an extended period of time. Thus, current *in vitro* models may actually overestimate the impact of solutions on corneal epithelial cells. Many *in vitro* models do not account for tears which may dilute and wash away the solution released from the lens. Tear secretion and blinking are physiological and mechanical properties that help maintain tear film stability and homeostasis of the ocular surface.

The biocompatibility between the solution, lens, and ocular surface are important for improving the comfort of wearing CLs. However, the biocides must be efficacious enough to remove microbial organisms while maintaining the homeostasis of the ocular surface. Though this thesis did not assess the biocidal efficacy of MAPD and PHMB against microbial organisms, it is an important endpoint related to the uptake and release of biocides and a required step in microbiological testing. Uptake of PHMB by different materials significantly reduced the fungicidal activity of MPS, with authors concluding that "the ISO committee should consider adding 'soaking experiments' to quantify the effect that CL materials have on

the performance of MPS."[216] The addition of organic matter also impacts the uptake and release of biocides, and the cleaning efficacy. Solutions tested using the addition of an organic load designed to simulate tears, showed reduced efficacy compared to standard testing without the presence of tear-like fluid.[259] The addition of a model tear organic soil significantly reduced the disinfecting activity of certain solutions containing various combinations of PHMB, MAPD, PQ-1, and alexidine. Future studies should include CLs in ISO testing requirements to advance current *in vitro* models. Doing so will allow us to better connect the uptake and release kinetics to the ocular toxicity studies.

The clinical findings, *in vitro* studies, and findings from the current study suggest that the clinical findings of CIEs and SICS may not be directly linked to the release of PHMB from the CL materials. SICS has been suggested as a benign phenomenon not indicative of compromised cells[138,145,234] and perhaps occurs due to the other components (surfactants, borate buffer) of the solution.[93,235] PHMB-based MPS are commonly associated with SICS but no histological changes to the cornea are observed.[128] There has been no direct link that microbial keratitis is associated with SICS, yet one study found patients with SICS were more likely to have an inflammatory CIEs.[136] The uptake and release issues are believed to be caused by an interaction between the solution and materials, while SICS is considered to be a transient solution-induced phenomenon that may not imply corneal toxicity.[28]

Though the introduction of SH lenses has helped reduce corneal hypoxic complications such as epithelial microcysts, corneal oedema, neovascularization, and hyperaemia, the overall incidence of CIEs has not reduced.[260-265] Studies have shown that reusable SH materials have double the risk of CIEs compared to pHEMA hydrogel materials.[202,204,265-267] The increased

risk (1.85-2.18 fold) has been consistent with SH lens materials[265] but one study reported that the risk can vary depending on the combination of lens material and solutions.[143] Increased risk of CIEs associated with SH lenses may be related to bacterial adhesion properties towards the low water and hydrophobic properties of SH materials.[205] The rate of bacterial adhesion increases inversely to the water content of lenses, and hydrophobic surfaces attach more bacteria than hydrophilic surfaces.[268] Although the risk of developing a CIE using SH materials is higher, the CIEs that occur have a shorter duration of complication and are less severe compared to hydrogel materials.[269]

CL wearers are unlikely to be fully compliant with all steps relating to safe wear and care of their lenses. Some non-compliant practices, for example topping up solutions, using tap water, or not closing the lens case fully can directly affect the efficacy of the MPS.[55] Other factors such as irregular lens case replacement or poor case cleaning can expose the solution to increased bacterial bioburden. The cleaning, disinfection, and storage methods may be indirectly associated with increased risk of CIEs.[205] Many reports show an increased risk of CIEs with the use of MPS compared to peroxide-based solutions.[143,202,203,270] MPS has been associated with increased corneal staining and eyes that demonstrated solution toxicity were three times more likely to develop a CIE.[270] The association between solutions and CIE development may be a secondary problem because of residual case contamination.[271] Case contamination has been reported in 23-81% of CL cases[165,272,273] and lens cases six months or older resulted in an eight-fold increased risk of CIEs.[203] It is plausible that residual bacteria found in a lens storage case may be the trigger for a lens-solution combination driven CIE.[205] However, if that were the case (given that no papers have shown there to be an increased degree

of case-contamination for SH lenses compared with hydrogels) then CIE rates would be the same between materials.

The safety and testing of care solutions is addressed in many ISO standards, ranging from guidelines on fundamental requirements, to the guidance on microbiological requirements and biocompatibility of the care solution in toxicity (*in vitro* and animal) models.[274] In terms of solution efficacy, ISO 14729:2017 relates to the microbiological requirements and test methods for products and regimens for disinfection of CLs and is the most relevant standard.[275] The first part of ISO 14729 requires the care solution to pass a 'stand-alone' test.[275] The solution is challenged against five standard test organisms and must demonstrate a minimum of 3-log reduction of bacteria and a 1-log reduction of fungi within the manufacturer's recommended soaking time. No CL or case is used in this protocol. If the minimum requirements of log reduction of microorganisms are met, the solution has passed the stand-alone test and can be labelled as a multipurpose disinfecting solution. A regimen test is required should the solution not pass the stand-alone test. In the regimen test, a full cleaning routine based on manufacturers recommended guidelines are incorporated. The physical mechanisms of rub and rinse has been shown to reduce a significant amount of bacterial and fungal organisms bound to a CL surface.[276]

The standards outlined above only include a CL for the regimen part of the test, and do not include a CL case or the presence of 'organic soil' (which is intended to mimic the impact of the tear film) as part of the testing protocol. These omissions do not reflect the real-world situation and recommendations have been made to either make changes to the standard, or to add further tests in addition to basic efficacy testing.[275,277,278] The *Acanthamoeba* keratitis

outbreaks highlighted the need for additional test methods and testing efficacy in the presence of 'real world' factors that includes using lens cases and storage times.[278]

Uptake of PHMB by different materials significantly reduce the fungicidal activity of MPS, with the authors concluding that "the ISO committee should consider adding 'soaking experiments' to quantify the effect that CL materials have on the performance of MPS".[216] Differences in kill rates of standard challenge organisms have also been reported for the chlorhexidine-based solution depending on the type of lens materials present.[237]

The addition of organic matter also impacts efficacy. Solutions tested using the addition of an organic load designed to simulate tears, showed reduced efficacy compared to standard testing without the presence of the tear-like fluid.[259] The authors recommended "that the test conditions for ISO 14729 should be revised in order to create more realistic conditions".[259] A recent paper demonstrated that the disinfecting activity of povidone-iodine or hydrogen peroxide solutions was not affected by the presence of various organic soils.[236] However, the addition of a model tear organic soil significantly reduced the disinfecting activity of certain solutions containing various combinations of PHMB, PQ-1, alexidine, and MAPD. The reduced disinfecting capabilities of biocides against various organic soils and clinical isolates was an indication that more rigorous testing may be beneficial and could be incorporated into new regulatory standards.[279-281]

CL materials and lens cases are an integral part of the system. A number of additions to CL materials and lens cases have been explored to help confer antibacterial properties, thereby reducing risk of infection and in part, mitigating for some of the non-compliant behaviors that wearers perform.[282-286] Xiao and colleagues produced a recent comprehensive

review of some of the strategies under investigation.[287] These include using silver, free-radical producing agents, antimicrobial peptides (melamine) or by employing passive surface modification approaches (PEG coatings). The antimicrobial properties of silver are the most widely researched in relation to CLs and silver-impregnated CL cases are already commercially available.[287] Selenium coated lenses or cases also show promise, along with coated CLs and cases which hinder microbial adhesion to surfaces.[287] Xiao concludes with a reminder that CLs and cases are exposed to a wide variety of organisms, which may lead to a combination of antimicrobial technologies ultimately being the most effective approach.

Solution efficacy testing continues to evolve to better meet the needs of the CL care system and material technology, along with improving representation of the real-world use, where care systems may be challenged beyond the limits of currant standard protocols. It is important that those changes in standards are timely, keeping up with the pace of change in available technology, and keeping in line with the needs of the end user to best promote their safe use.[274] Standard testing could be extended to mandate inclusion of additional variables such as lens cases, CL materials, organic soil, and efficacy against *Acanthamoeba*. Some recommendations are in place, but opportunity exists to reevaluate global standards to ensure consistency in all markets. Future innovation may further support increased safety in reusable lens wear through novel antimicrobial additions to both CL materials and cases.

Overall, CL solutions, and specifically biocides, continue to be safe and effective when used correctly following the packaged instruction. CL solutions testing continues to evolve and developing new methods for assessing the safety and efficacy of contact lens solutions is a difficult process that requires researchers, clinicians, and manufacturers to work together. The

current thesis developed a chemical and biological assay to determine the impact of biocides on CLs. The chemical assay provided a sensitive method for measuring the uptake and release of biocides from CLs. The biological assay provided a model to predict ocular toxicity while comparing the results to the chemical assay. These models may help to better predict any future adverse events for new MPS.

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**Author:** Alan Yee,Karen Walsh,Marc Schulze,Lyndon Jones

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