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Comfort and Compatibility of Silicone Hydrogel Contact Lenses

Ngai Keung Tam

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

Master of Science

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ABSTRACT

Comfort and Compatibility of Silicone Hydrogel Contact Lenses

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Master of Science

Silicone Hydrogel (SiHy) contact lenses are highly successful compared to previous soft lenses; they were developed to provide superior oxygen permeability. However, the hydrophobic natures of the silicone segments enhance lipid sorption which may diminish the lens surface wettability, clarity and comfort. While lens and lens care product are designed to remove lipid deposition, there is lack of experimental evidence to evaluate the actual performances with respect to lipid removal. An *in vitro* model using an artificial tear fluid containing radiolabeled lipids was employed in this thesis research to evaluate the efficacy of different multi-purpose lens care solutions in removing lipids from SiHy contact lenses. Additional rubbing with the lens care solution is often encouraged by professionals. Part of this research evaluated the effect of additional rubbing process on lipid removal.

Overall, a multi-purpose solution (MPS) for lens care, Opti-Free PureMoist®, removed the most lipid deposition from lenses (senofilcon A, comfilcon A, and balafilcon A and one conventional hydrogel lens polymacon). The overall removal percentages were approximately 55% of DPPC and 28% of cholesterol from a conventional hydrogel. However, the MPSs did not remove lipids effectively from SiHy lenses. The highest percentages of removal were 3.08% of DPPC and 0.76% of cholesterol from SiHy lotrafilcon B lenses with Opti-Free PureMoist. The rubbing process increased the amount of removal in some MPSs, but the effects were small. The lack of removal of lipid suggests that the surfactants in the MPSs are not hydrophobic enough to remove lipids from SiHy lenses. Apparently a majority of deposited lipids absorbed into the lens matrix as rubbing did not enhance removal significantly. Future study on determining the concentration profile of lipid sorption throughout the lens thickness is encouraged.

Another topic in this research thesis is the use of hydrogel lenses to deliver comfort agents or lubricating molecules from lenses. A screening study was performed in this research to select possible agents to be loaded into several SiHy macromer formulations. Experiments showed that comfort agents PNVP and Kollidon were the best candidates for such a procedure.

Keywords: silicone hydrogel, lipid deposition, multi-purpose lens care solutions, comfort agents

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CHAPTER 1 INTRODUCTION

The wearing of contact lenses is possibly the most ubiquitous application of a medical hydrogel in our society. A hydrogel is a cross-linked polymer containing hydrophilic units which normally dissolve in water. However, crosslinking prevents the polymer from dissolving. In recent years, silicone hydrogel (SiHy) contact lenses have become the mainstream product in the contact lens market because of their superior oxygen permeability, which is 3 to 6 times greater than conventional poly-HEMA based hydrogel lenses [1]. Since oxygen is able to transfer easily through the silicone units in SiHy lenses, the oxygen permeability is enhanced significantly and is not limited by the water content as conventional hydrogel lenses are. The improvement allows a high level of comfort and improved corneal health during extended wear (even overnight wear) by maintaining a sufficient level of oxygen concentration at the ocular surface. One study estimated that the U.S. soft contact lens market in 2010 was $2.1 billion, the worldwide market was about $6.1 billion, and it will reach $11.7 billion by 2015 [2].

While SiHy lens are highly successful, the adsorption of tear components to contact lenses remains a major challenge of contact lens wear that can lead to user discomfort and decreased visual acuity [3-6], and ultimately leads to discontinuation of lens wear [6-9]. Consequently, contact lenses and lens-care products are often engineered to reduce deposition and improve wear comfort. Of special note in this thesis, a multi-purpose solution (MPS) for lens care is often used to store, to disinfect and to clean lenses by removing deposited proteins, lipids and other debris. While many MPSs claim to be able to reduce or remove sorbed lipid, there is a lack of studies to

quantify their efficacy, particular with SiHy lenses. One of the objectives in this research is to evaluate the efficiency of different MPSs in reducing lipid sorption and to compare their performances with a simple buffered solution.

Unfortunately, the beneficial hydrophobic segments in SiHy lenses also reduce surface wettability, which is highly correlated with wear comfort. Irritation and dry eye syndrome are induced when tear films are unable to spread across the hydrophobic lens surfaces. Thus, the lens material itself is a predominate factor to the level of comfort and the tendency to sorb tear components. For example the surfaces of lotrafilcon B lenses are plasma coated a continuous ultra-thin hydrophilic layer which improves the wettability [10], and which also lowers the lipid deposition significantly.

While using hydrogel lenses to deliver therapeutic drugs to eyes is not a new idea, little research has been done in controlled release of comfort agents or lubricants molecules from lenses. A screening study was performed in this thesis research to select possible agents to be loaded into several SiHy macromer formulations, which opens the door to produce lenses that can deliver an additional level of comfort over many hours.

CHAPTER 2 LITERATURE REVIEW

This chapter reviews contact lenses, the sorption (adsorption and/or absorption) of lipids and proteins, and the role of a multipurpose solution (MPS) for lens care. Contact lens materials are named by a generic name of the material, such as lotrafilcon, senofilcon etc. Each material has a unique chemistry. It is sometimes subcategorized as "A" or "B" representing small chemical or processing differences. A manufacturer usually has a patent on the material (such as lotrafilcon A) and produces a brand name contact lens from the material (such as CIBAVISION Focus Night & Day). This thesis will usually use the generic chemical name.

2.1 Lipid deposition in silicone hydrogel contact lenses

The hydrophobic silicone components in silicone hydrogel (SiHy) lenses enhance oxygen transfer but also enhance lipid deposition. In general, SiHy lenses are more apt to sorb lipids, while traditional hydrogels are more likely to adsorb proteins [11,12]. One study suggested that initial deposition of phospholipid on SiHy lenses may stabilize the tear film and promote the overall wettability; but eventually it may become deleterious as more lipid accumulates on the lens surfaces, resulting in reductions in visual clarity, surface wettability, and comfort [13].

2.1a Reduction of lipid deposition in MPS

A general concern is the uncertainty in the effectiveness of lipid removal by MPSs. Several studies have been performed to evaluate the efficiency of lens care solutions in reducing protein

sorption [14-19], but only a few evaluated lipid removal [20,21]. For example, Lorentz et al. recently reported that a hydrogen peroxide lens care solution containing Pluronic 17R4 (a di-functional block copolymer surfactant with terminal secondary hydroxyl groups) removed more lipid from lenses when compared to a non-surfactant hydrogen peroxide solution [20]. One study analyzed lipid sorption on several SiHy lenses and reported that lotrafilcon B lenses accumulated the least amount of cholesterol (CH) regardless of the lens care solution used [21]. This study also showed that the lipid deposition process is dependent on lens materials and contact lens care solutions [21].

2.1b Removal of deposition in MPS with a rubbing process

MPSs are sterile, buffered solutions containing various surfactants and preservatives to clean, store and disinfect contact lenses. While some MPSs emphasize that no rubbing is needed, two MPSs used in this study recommend the consumer to rub both sides of the lens with the solution for 20 seconds [22,23]. Regarding the needs of rubbing for effective cleaning, the FDA stated, "Several professional groups that represent optometrists and ophthalmologists recommend rubbing each lens in the palm of the hand with a few drops of solution, even if using a "no rub" product [24]. Dr. Townsend mentioned in an article [25], "We're inclined to tell patients to rub their lenses after removal for two reasons. First, some studies have demonstrated that even minimal rubbing reduces the bacterial population by approximately 3 log units. Second, with the increasing popularity of silicone hydrogel lenses, we're seeing more problems with lipid deposits." These statements suggested that a significant amount of tear deposit and other debris could possibly be removed from the lens surface by rubbing with the MPSs. There are several studies that investigated the effects of rubbing. Nichols reported that rubbing and rinsing lenses decreased the amount of deposition in general [26]. Previous studies have shown that rubbing in the presence of MPS removes some proteins, and that protein removal by rubbing is greater on

conventional hydrogels than on SiHy lenses [14]. Another study indicated that the use of a manual rubbing step is more effective than rinsing or soaking alone in removing pathogenic microbes from SiHy lenses [27]. Many professionals suggested that rubbing will help remove lipid deposits from SiHy lenses, but there are no specific evaluations reported in the literature. Thus, the effect on lipid deposition by the addition of rubbing was quantified and is discussed in this thesis.

2.2 Methods of quantifying lipid deposition

Lipid sorption is commonly quantified by chromatographic methods, as this allows for simultaneous measurement of multiple classes of lipids from worn lenses or from complicated lipid/protein solutions. Several studies of lipid sorption on contact lenses using chromatography techniques have been reported. In 2003, Jones et al.[11] confirmed that lipid deposition on SiHy lenses (lotrafilcon A and balafilcon A) are significantly greater than on conventional hydrogel (etafilcon) contact lenses. The composition of these lipid depositions was determined by a high-performance liquid chromatography (HPLC) technique. In 2006, Maziarz et al.[28] quantified the sorption of oleic acid, oleic acid methyl ester, and cholesterol (CH) on commercial SiHy contact lenses; they also compared two HPLC methods. In 2008, Iwata et al.[29] claimed that chromatography/mass spectrometry analytical methods are more accurate and sensitive than standard HPLC techniques. In 2009, Zhao et al.[21] used thin layer chromatography to measure CH sorption on lotrafilcon B, balafilcon A, senofilcon A, and galyfilcon A lenses after 30 days of wear and reported that both the lens type and the MPS had an effect upon sorption, which averaged from 0.1 to 8.2 µg per lens. In 2011, Heynen et al.[3] used HPLC to measure lipid sorption on senofilcon A lenses and reported that less total lipid sorbed when a MPS was used compared with another no-rub hydrogen peroxide system.

When comparing sorption data from various labs on similar lenses, often one sees similar general trends but variations in the details of the amount sorbed. Lab-to-lab reproducibility is not always obtained. For example, Jones et al.[11] reported high levels of lipid adsorption to balafilcon lenses. However, Maziarz et al.[28] reported much lower levels and also demonstrated that small differences in sample extraction and HPLC methods can yield substantially different results. Lorentz et al.[29,30] showed that when measuring sorption from laboratory solutions that simulate tear, small variations in solution composition can produce statistical differences in amounts sorbed.

In addition to chromatographic methods, radiolabeling techniques are sometimes used to quantitate protein and lipid sorption from artificial tears. While radiolabels have excellent accuracy and precision, they are less broadly applicable for studying simultaneous sorption of multiple lipids and proteins because each species of lipid and/or protein requires a different radioisotope (or a different experiment for each different species). Quantitation is independent of complexation with proteins or other lipids that sometimes makes chromatography challenging due to differences in retention between complexed and single lipids. Furthermore, radioisotope methods can be used to validate and support chromatographic methods. There are several radioisotopes such as ^{125}I, ^{3}H and ^{14}C that were used in previous studies to investigate lipid/protein deposition on contact lenses. With ^{125}I-labeled chemicals, no extractions are required as ^{125}I emits highly penetrating gamma radiation, so the quantifying of such a labeled substance can be accomplished with direct measurement methods, such as putting the lens directly into scintillation fluid (SF). However, ^{125}I labels are limited to proteins, and cannot be easily applied to lipids. Other common radioactive elements, ^{3}H and ^{14}C, have a half-life of 12 years and 5730 years respectively, and their low penetrating beta radiation allows relatively

simpler experiments and reduces the risk of radiation hazards. However, the extraction process becomes necessary as the lens itself will absorb some of beta particles so they cannot be quantified by direct measurement techniques. In 1997 Prager and Quintana [30] reported uptake on traditional hydrogels of ^{14}C-dioleoyl phosphatidylcholine and ^{3}H-cholesteryl oleate from a multi-component artificial tear fluid (ATF). Since then, little has been published on the use of radiolabels to study protein and lipid sorption to contact lenses. Only recently, Lorentz et al.[31,32] quantified the deposition of lipids on SiHy contact lenses using ^{14}C-labeled cholesterol (CH) and phosphatidylcholine (PC). Their data showed that the quantities of CH and PC deposited on balafilcon A and omafilcon A lenses are much less than reported in previous studies by Iwata et al.[29], Carney et al.[12], and Pucker et al.[33]. Another key finding from Lorentz's studies is that variation in compositions of laboratory tear fluid had a large influence upon the amount of lipid deposition [31,32]; they reported that both CH and PC deposition significantly decreased when lactoferrin and immunoglobulin G were not included in the ATF [31].

While chromatographic methods have become the norm, few such studies have also utilized independent techniques such as radiochemistry to validate extraction procedures or chromatographic results. The majority of chromatographic studies of deposition on contact lenses required extraction steps prior to the quantification process, and chloroform:methanol (Chlf:MeOH) solutions are often used as the extraction solvents. Zhao et al.[21] reported that the recovery percentage of their extraction technique with a 50% Chlf/50% MeOH solution was 72.7% to 95.5%, and that the efficiencies were dependent on the lens type and the representative tear components, which implied that the actual amount of lipid deposition may not have been accurately quantified unless calibrations were done on each combination of lens and lipid type. Lorentz et al. [31] extracted the lipid deposition 2 times with 2 mL of 66% Chlf/33% MeOH

solution for three hours at 37 °C while shaking on an orbital shaker. Jones et al.[11] employed a similar technique where lenses were placed in a 50% Chlf/50% MeOH extraction solvent, and the extracted solution was evaporated and re-suspended in a HPLC buffer solution before performing the quantitation procedure. Despite laboratory skills and careful extraction procedures, solvent selection is the predominant factor for extraction efficiency, and it will greatly alter the accuracy of an experimental result, with actual sorption higher than calculated due to extraction inefficiency. Pitt et al.[34] recently developed a 3 stage n-propanol extraction method that captures 99% of dipalmitoylphosphatidylcholine (DPPC) deposition. Although the extraction process is more laborious, the extraction efficiency is significantly higher than the common one-stage Chlf/MeOH method. While considering using radiolabeling techniques, n-propanol has an advantage over Chlf/MeOH extraction in that n-propanol does not interfere with scintillation counting and thus does not need to be evaporated prior to performing the counting procedure.

2.3 Controlled release from silicone hydrogel lenses

Besides providing vision correction, contact lenses are able to deliver drug molecules to the ocular surface. In 1965, hydrogel contact lenses were invented by Otto Wycherley, and he mentioned the potential for hydrogel contact lenses to act as a drug delivery platform [35]. There are various techniques that have been developed to control the release rate of a loaded drug from a hydrogel polymer matrix. Among those methods, the molecular imprinting technique is capable of increasing drug loading and extending the period of a relatively constant release rate [35,36]. In 2008, Kim et al. developed SiHy contact lenses that deliver ophthalmic drugs (timolol, dexamethasone, and dexamethasone 21-acetate) for an extended period of time from 6 days to 4 weeks; they proved that the variation of drug loading and elution kinetics greatly depend on the

compositions of hydrophobic and hydrophilic components of SiHy lenses [36]. In 2012, Tieppo et al. performed another successful in vivo study to extend the release of a therapeutic molecule (ketotifen fumarate) from molecularly imprinted contact lenses [37]. They stated that for hydrophilic substrates, the solubility limit of the drug in solution is the major factor determining the loading capacity.

Dry eye syndrome is a major contributor to discomfort during contact lens wear, and eye drops which contain comfort agents are commonly applied to relieve dryness. However, ocular tear flow reduces the residence time of comfort agents within the tear fluid, so repeated application of eye drops becomes necessary. Applying novel techniques to embed and elute comfort agents in SiHy lenses may make it possible to maintain consistent high levels of comfort during wear. Nevertheless, no comparative studies are reported or found in the literature except from Pitt et al. [38,39]. The general ocular tear film drug concentration based on delivery methods are shown in Figure 1, where A, B, and C represent the drug concentration profiles for applying eye drops, using drug soaked lenses, and employing molecularly imprinted lenses, respectively [37]. In 2011, Pitt et al. reported that it is possible to polymerize a SiHy lenses containing the comfort agent 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) [39]. They later discovered that the DMPC concentration reached an equilibrium of 6 μg/lens at 122 ^{o}C while they were investigating the effect of autoclaving and temperature on DMPC elution rate [38]. Their findings open future research opportunities in evaluating the possibility of loading DMPC during the autoclaving process, and also identifying comfort agents that can be present during lens polymerization. Ultimately, development of contact lenses that release comfort agents to the eye with profitable manufacturing cost was achieved based on the above investigations.

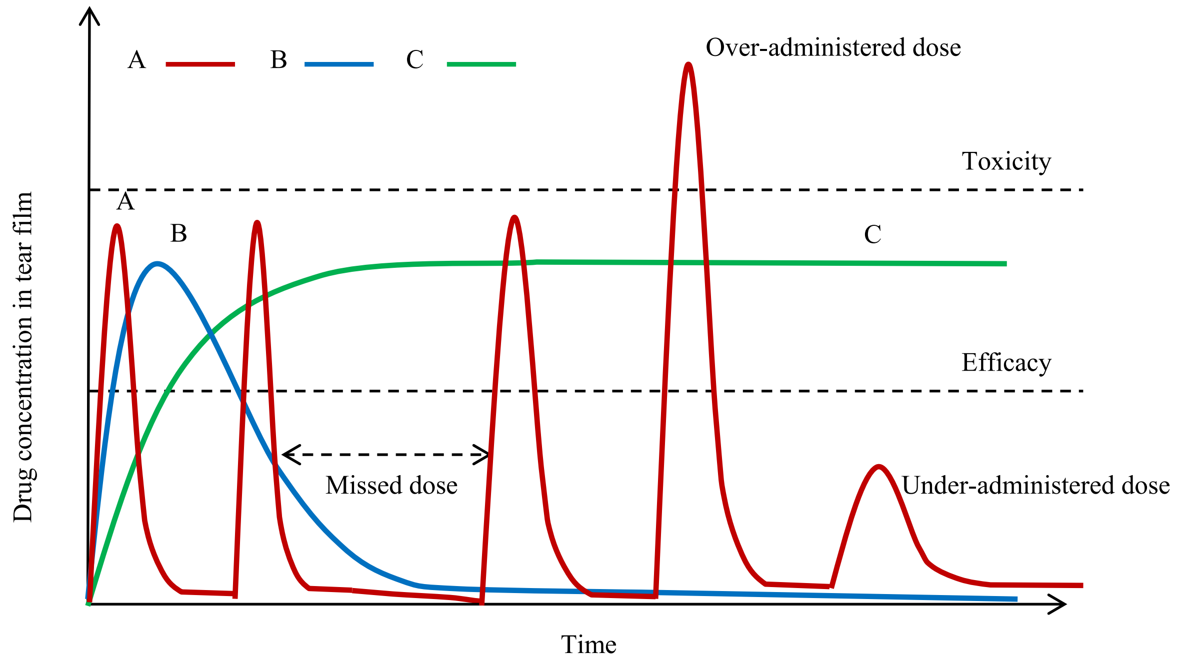

Figure 1: Ocular tear film drug concentration based on delivery method. A, B, and C represent the drug concentration profiles for applying eye drops, drug soaked lenses, and imprinted lenses respectively. This figure is adapted from Tieppo et al. [37].

CHAPTER 3 OBJECTIVES

The objective of this research is to improve comfort and compatibility of silicone hydrogel (SiHy) contact lenses. Specifically, the objectives are **1)** to evaluate the efficacy of lens care multi-purpose solutions (MPS) in preventing lipid sorption to SiHy lenses; **2)** to evaluate the performance of MPSs in removing sorbed lipids; **3)** to evaluate the performance of MPS solutions in removing sorbed lipids with additional rubbing; **4)** to determine the efficiency of an experimental peroxide-based solution in removing lipid deposits; **5)** to estimate the solubility limits of various comfort agents in SiHy marcromer formulations. This last study will determine the potential comfort agents that could be loaded into the macromer formulations without significant decrease of visual clarity.

For the first four objectives, an *in vitro* model was used to mimic the actual lipid sorption process in human tears. Lenses were incubated in an artificial tear fluid (ATF) containing radiolabeled lipids. The lipid sorptions to lenses were extracted by n-propanol and quantified by a liquid scintillation method. For objective 5, optical densities of several silicone macromer solutions were determined when loaded with various wt% of comfort agents. The solubility limits of the comfort agents were estimated by analyzing the optical density.

CHAPTER 4 PREVENTION OF LIPID DEPOSITION BY MPS

4.1 Experimental approach

In this study, we used a radiolabeling technique to evaluate the effectiveness of three multi-purpose solutions (MPSs) – OPTI-FREE® PureMoist® (PureMoist), Biotrue™ and an experimental MPS (BLS) developed by Bausch & Lomb – in preventing the depositions of cholesterol (CH) and dipalmitoylphosphatidylcholine (DPPC) on various commercial silicone hydrogel (SiHy) lenses and one conventional hydrogel lens. We also used borate buffered saline (BBS) as a control having no surfactants. CH and DPPC are model components that represent respectively non-polar and polar lipid components in human tear films (Figure 2).

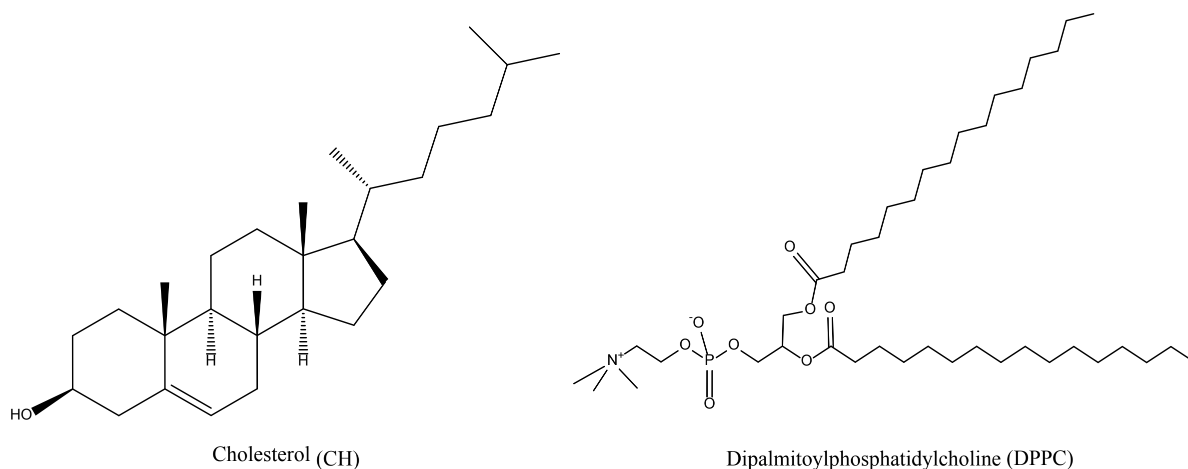

Figure 2: Chemical Structures of the lipids. Cholesterol (CH) and dipalmitoylphosphatidylcholine (DPPC).

4.1a Materials

The active components of the three MPSs are presented in Table 2. Four commercial SiHy lenses — senofilcon A, comfilcon A, lotrafilcon B ,and balafilcon A and one conventional hydrogel lens polymacon — were used in this study (information of the lenses were obtained from Jones et al. and Real et al.[10,11], see Table 1).

Table 1: Characteristics of the contact lens materials.

Lens material	Commercial name	Manufacturer	Principal components [10,11]	Surface treatment
Senofilcon A	Acuvue Oasys	Johnson & Johnson	mPDMS, DMA, HEMA, SiGMA, TEGDMA, PVP	No surface treatment. Internal wetting agent (PVP) throughout the matrix that also coats the surface
Comfilcon A	Biofinity	CooperVision	M3U, FMM, TAIC, IBM, NMNVA, NVP, HOB	None (inherently wettable)
Balafilcon A	PureVision	Bausch & Lomb	NVP, TPVC, NVA, PBVC	Plasma oxidation process
Lotrafilcon B	AIR OPTIX® AQUA	CIBA VISION®	DMA, TRIS, fluorine-containing siloxane macromer	25nm plasma coating with high refractive index
Polymacon	Soflens	Bausch & Lomb	HEMA	None (conventional hydrogel)

PVP: poly(vinyl pyrrolidone); **mPDMS**: monofunctional methacryloxypropyl terminated polydimethylsiloxane; **DMA**: N,Ndimethylacrylamide; **HEMA**: hydroxyethyl methacrylate; **TEGDMA**: tetraethyleneglycol dimethacrylate; **TRIS**: methacryloxypropyl tris(trimethyl siloxy)silyane; **NVP**: N-vinyl pyrrolidone **TPVC**: tris-(trimethyl siloxysilyl) propylvinyl carbamate; **NVA**: N-vinyl amino acid; **PBVC**: poly(dimethysiloxy) di(silylbutanol) bis(vinyl carbamate); **M3U**: αω-bis(methacryloyloxyethyl iminocarboxy ethyloxypropyl)-poly(dimethylsiloxane)-poly(trifluoropropylmethylsiloxane)-poly(methoxy-poly(ethyleneglycol)propylmethyl-siloxane; **FMM**: α-methacryloyloxyethyl iminocarboxyethyloxypropyl-poly(dimethylsiloxy)-butyldimethylsilane; **TAIC**: 1,3,5-triallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione; **IBM**: isobornyl methacrylate; **HOB**: 2-hydroxybutyl methacrylate; **NMNVA**: N-methyl-N-vinyl acetamide; **SiGMA**: 2- propenoic acid, 2-methyl, 2-hydroxy-3-(3-(1,3,3,3-tetramethyl-1-(trimethylsilyl)oxy)disiloxanyl)propoxypropyl ester.

Table 2: Components of the multi-purpose lens care solutions.

Solution	Manufacturer	Wetting agents/Surfactants	Preservatives
Opti-free® Pure Moist®	ALCON	TETRONIC® 1304 (poloxamine), HydraGlyde (EOBO)*	POLYQUAD® (polyquaternium-1) 0.001%, ALDOX® (myristamidopropyl dimethylamine) 0.0006%
Biotrue™	Bausch & Lomb	hyaluronan, sulfobetaine, poloxamine	polyaminopropyl biguanide 0.00013%, polyquaternium 0.0001%.
Experimental MPS**	Bausch & Lomb	poloxamine	polyaminopropyl biguanide

***EOBO**: poly (ethylene oxide)-poly (butylene oxide)
**This experimental MPS is not yet in commercial production.

4.1b Preparation of artificial tear fluid

Table 3 shows the composition of the artificial tear fluid (ATF) used in this study, which includes lipids, proteins and buffer. The composition used herein differs somewhat from other published compositions [32,40,41]. Compared to others, it contains methyl-β-cyclodextrin (to achieve higher lipid concentrations), DPPC as the phospholipid, reduced protein concentrations, and all lipid components increased (compared to other ATFs), with the goal of accelerating the lipid spoliation process. As there is currently no consensus composition presented in the literature or used uniformly in industry, the composition chosen for this work was selected because it deposits lipids and proteins on SiHy lenses overnight at levels similar to deposits found on worn lenses after several weeks of wear [34].

Borate buffered saline (BBS), the aqueous base for the ATF, was first prepared by dissolving boric acid, sodium borate and sodium chloride in distilled deionized water (DDH_2O); the pH of the solution was adjusted to 7.3 by adding NaOH or HCl.

The ATF contained radiolabeled CH and DPPC for quantitative analysis of their sorptions. It was prepared by adding appropriate amounts of ^{14}C-CH in ethanol, ^{3}H-DPPC in ethanol/toluene, as well as unlabeled CH and DPPC in Chlf, methyl-β-cyclodextrin in Chlf, cholesteryl linoleate

in Chlf, oleic acid in Chlf, methyl oleate in Chlf, and triolein in Chlf to a 100-mL volumetric flask. The solvent was evaporated under a nitrogen stream for 2 hours and followed by 2 hours of vacuum drying. Then 50 mL of BBS was added to the flask and magnetically stirred for 6 hours at room temperature (21°C) with a 1.5-cm Teflon stir bar at 650 rpm. This hydrated the dried lipids and created a suspension most probably of micelles and liposomes. Solid powdered protein components were added to the solution in the sequence of lysozyme, lactoferrin, albumin and mucin. Then the mixture was stirred for an additional 8 hours at room temperature. An additional 50 mL of BBS was added to the vial before final stirring for 20 minutes. The ATF was then used immediately for incubation of lenses in the sorption studies. ATF stored for more than a few hours tended to give less reproducible results.

Table 3: Composition of the artificial tear fluid.

Ingredients	Manufacturer	Product number	mg/mL
Borate buffered saline			
Boric Acid	Mallinckrodt	2549-04	10
Sodium Borate	Mallinckrodt	7457-06	1.2
Sodium Chloride	Mallinckrodt	7581-06	4
Proteins			
Chicken Egg White Lysozyme	USB	18645	0.02648
Bovine Lactoferrin	USB	18177	0.03584
Bovine Albumin	USB	9048-46-8	0.087
Porcine Mucin	Sigma	M1778	0.1
Lipids			
DPPC	Avanti	850355P	0.032
Methyl-β-cyclodextrin	Sigma	C45555	0.08
Cholesteryl Oleate Esters	Sigma	C0289	0.066
Oleic Acid	Sigma	1008	0.004
Methyl Oleate	Sigma	311111	0.088
Triolein	Sigma	T7146	0.01
^{3}H-DPPC	ARC	ART 0284A	0.000026
CH	Avanti	700000P	0.032
^{14}C-CH	PerkinElmer	NEC018250UC	0.001679

DPPC: dipalmitoylphosphatidylcholine, **CH**: cholesterol; **ARC**: American Radiolabelled Chemicals

4.1c Quantitation procedure

Following sorption, a three-stage n-propanol extraction was used to extract the lipid sorbed on the lenses, as a previous study confirmed the high extracting efficiency of this procedure [34]. Each lens was placed in a 20-mL glass scintillation vial containing 2 mL of n-propanol and placed on a rotary table shaker at 60 rpm for one hour at 37°C. Lenses were then held with soft tweezers above the extraction solution in the scintillation vial and rinsed with 1 mL of n-propanol into the same vial; then the lens was transferred to a second extraction vial containing another 2 mL of n-propanol. After one hour, it was rinsed again the same way and the lens was transferred to a third extraction vial. Following the third one-hour extraction and rinsing procedure, the lens was transferred to a final vial filled with 10 mL of scintillation fluid (SF, Ecoscint™ A, National Diagnostics, Atlanta, Georgia) to measure any residual radioactivity in the lens. All blanks, standards and samples were submitted in the same batch in identical scintillation vials to a LS 6500 scintillation counter (Beckman Coulter) and simultaneously counted twice using a program that counted both ^{14}C and ^{3}H.

4.1d Pre-conditioning of lenses with MPS and mimicking the lipid sorption process

Senofilcon A, comfilcon A, balafilcon A, lotrafilcon B, and polymacon lenses were removed from their blister packages and blotted dry on Kim Wipes®. The lenses were then placed into 5-mL glass vials containing 2 mL of BBS, PureMoist or Biotrue at room temperature without shaking or rubbing the lenses. After 16 hours, lenses were removed from vials, rinsed in BBS and lightly blotted dry with a Kimwipe to remove excess liquid before being transferred to another set of vials containing 2 mL of ATF containing radiolabeled lipids. A set of control lenses (without pre-treatment) were removed from their blister packages, blotted dry, and placed into ATF at the same time. To mimic the sorption process, lenses were placed in an incubator at

37°C on an orbital shaker at 60 rpm for 16 hours. Lenses were then removed from the vials containing ATF, rinsed with distilled deionized water (DDH_2O) and placed in a set of plastic wells filled with DDH_2O. The contact lenses were then taken out of the first set of wells, rinsed with DDH_2O again, and placed in a second set of wells. Lenses were removed from the second set of well, rinsed again, and blotted dried. Finally each lens was placed in a glass scintillation vial containing 2 mL of n-propanol to begin the quantitation procedure which is described in previous section. Two sets of calibration standard vials were prepared at the time of incubation; the first standard was 100 μL of ATF in 10 mL of SF, and the second standard was 100 μL of ATF, 3 mL of n-propanol and 10 mL of SF. This second standard was prepared because the presence of n-propanol slightly changes the efficiency of β-emission capture; it was used in quantifying the extracts containing 3 mL of n-propanol. See Figure 3 for the general experimental process.

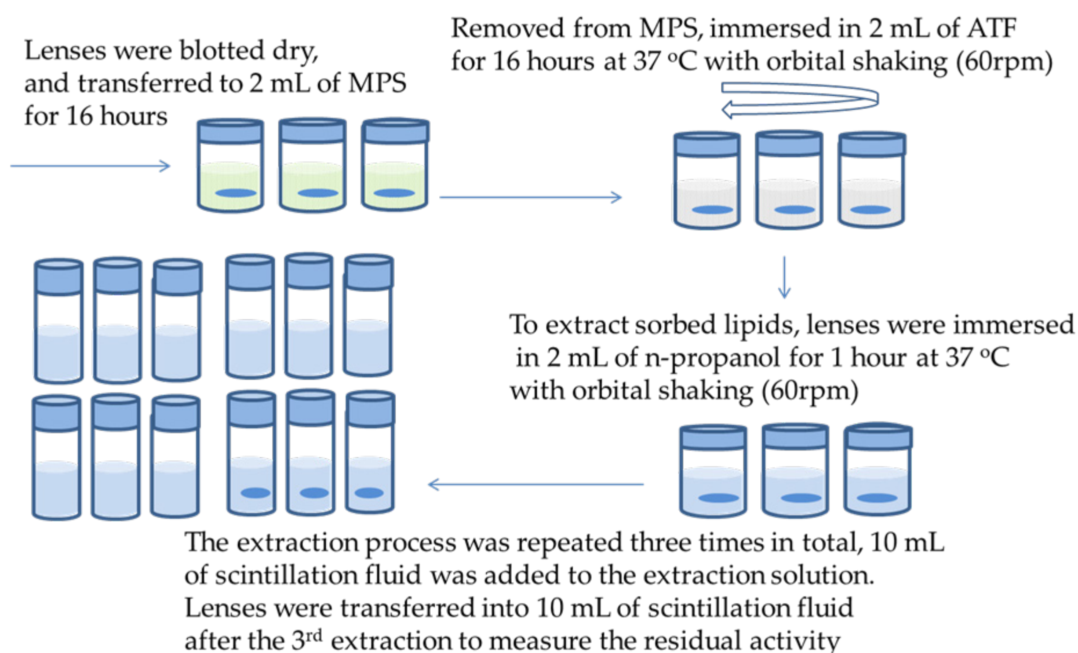

Figure 3: A general flow diagram of experiments in chapter 4.

4.2 Results

In this study, we used a radiolabeling technique to evaluate the effectiveness of three MPSs – PureMoist, Biotrue, and an experimental MPS made by Bausch and Lomb (BLS) – in reducing the depositions of CH and DPPC on various commercial SiHy lenses and one conventional hydrogel lens. CH and DPPC are model components that represent respectively non-polar and polar lipid components in human tears.

After the 16 hours of incubation in the ATF, with or without any pre-soaking in an MPS, 4 to 6 μg of DPPC and 6 to 8 μg of CH were sorbed to senofilcon A, comfilcon A, and balafilcon A. Approximately 1 μg of DPPC and 1 μg of CH were sorbed to lotrafilcon B, and about 0.2 μg of DPPC and 0.1 μg of CH were sorbed to polymacon.

Figure 4 presents the amounts of DPPC and CH depositions on each lens material when pre-conditioned with different solutions. In general, the DPPC sorption is consistently lower than the CH sorption for all lenses except for lotrafilcon B lenses and polymacon lenses. Both DPPC and CH depositions follow the same pattern for each combination of lens material and pre-soak solution.

4.2a Analysis by lens type

There are significant differences in the amount of lipid sorbed on SiHy lenses while pre-soaking with the same lens care solution. When lenses are preconditioned with PureMoist, DPPC sorption on senofilcon A lenses is 20.3% lower than on balafilcon A lenses ($p<0.001$) and 18.7% lower than on comfilcon A lenses ($p<0.01$). With the BBS pre-soak, the CH deposition on senofilcon A is 13.2% higher than on balafilcon A lenses ($p<0.001$) and 15.7% higher than comfilcon A lenses ($p<0.0005$). However, while statistically significant, these differences are not

large. The type of preconditioning solution made less difference in the amount of lipid sorbed to lotrafilcon A and polymacon lenses. Both the DPPC sorption and CH sorption to these lenses were significantly lower than the other lens types ($p<0.0005$), and polymacon lenses sorbed the least amount of DPPC and CH overall (see Figure 4).

4.2c Analysis by presoaking solution

Lipid sorption on the same lens material is also somewhat dependent on the lens care solution. For example DPPC sorption on senofilcon A lenses is 19.4% lower when the lenses are preconditioned with PureMoist ($p<0.001$), and 25.1% lower when pre-conditioned with Biotrue ($p<0.00005$) compared to those preconditioned with the BBS. CH sorption on senofilcon A is also 20.3% lower when the lenses are presoaked in PureMoist ($p<0.001$), and 26.0% lower when pre-conditioned in Biotrue ($p<0.0005$). Both DPPC and CH depositions on senofilcon A lenses without prior exposure to any lens care solutions are 25.4% and 23.7% lower respectively than lenses exposed to BBS ($p<0.00005$). However, there are no differences on DPPC and CH sorptions ($p>0.05$) when senofilcon A was preconditioned with BLS compared with those preconditioned in BBS.

There are no statistical differences ($p>0.05$) for both DPPC and CH sorptions when balafilcon A, comfilcon A, and polmacon lenses were pre-conditioned with PureMoist, Biotrue, BLS or BBS.

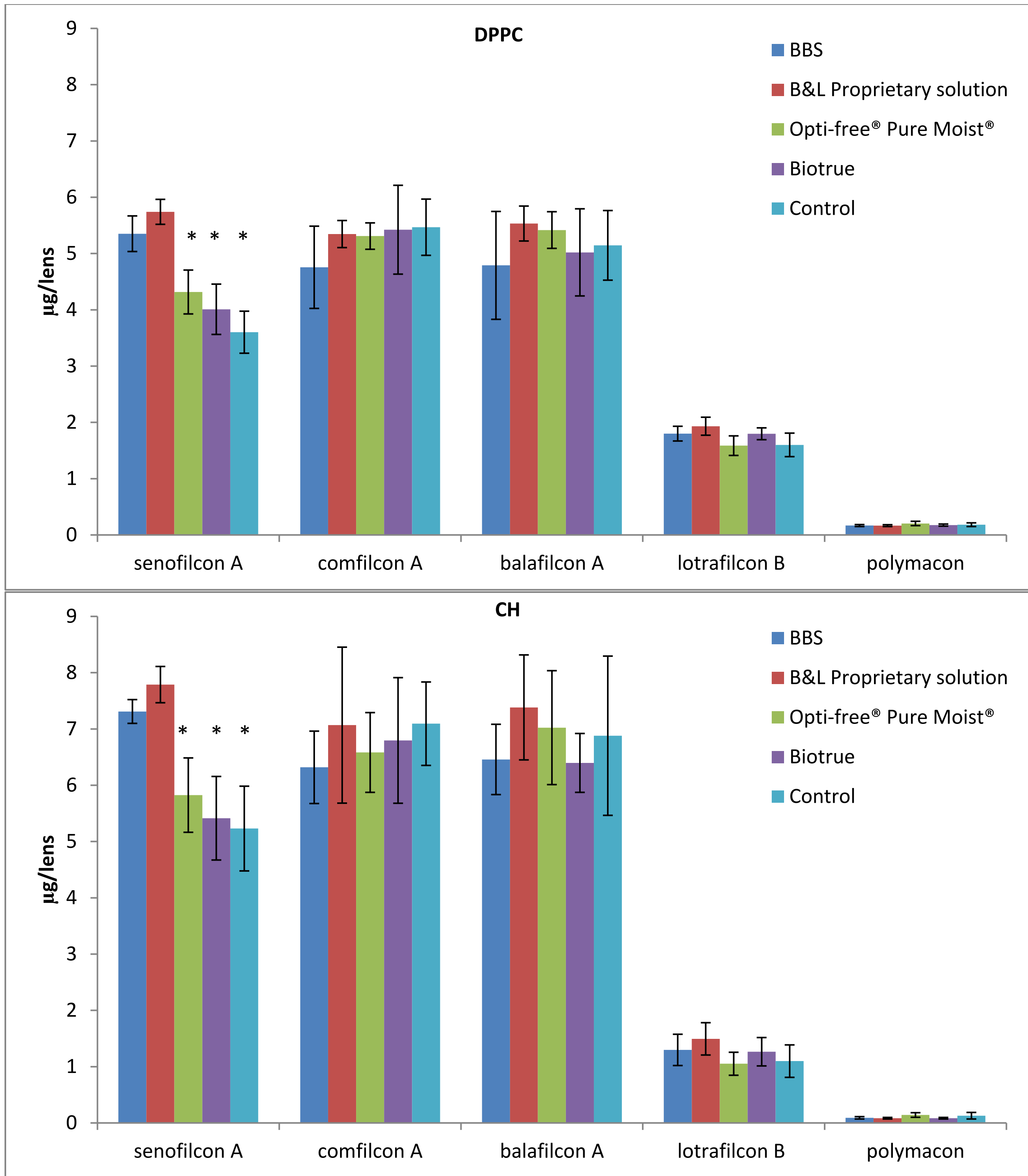

Figure 4: Lipids sorption with preconditioning in lens care solutions. Error bars represent the 95% confidence intervals (n>=6). * indicates statistical differences from BBS ($p<0.05$).

4.3 Discussion

The objective of this study presented in this chapter was to evaluate the efficacy of the MPSs in reducing lipid sorption on SiHy lenses. The data show that only PureMoist and Biotrue perform better than BBS in reducing lipid sorption to senofilcon A lenses, reducing the CH and DPPC sorption by approximately 20 to 25% compared with lenses preconditioned in BBS.

Lens and solution manufactures hoped that MPSs would reduce lipid sorption. Their proposed mechanism was that the polymeric surfactants in MPS would adsorb to the lens material and block the hydrophobic sorption sites, thus reducing the level of lipid deposition. However, sorption of DPPC and CH occurred generally to about the same amount to all lenses irrespective of preconditioning the lenses with MPS or buffer or not at all. There were a few statistically significant differences that may give hints as to mechanisms and directions for future improvements. For example, there was some dependency on lens chemistry. This study showed that both DPPC and CH sorption on senofilcon A are greater when lenses were preconditioned in BBS instead of in the PureMoist and Biotrue; but there are no significant effects of solutions on comfilcon A and balafilcon A lenses (Figure 4). A previous study also concluded that the efficiencies of MPS on reduction of lipid deposition are somewhat dependent on lens material [21].

The non-surfaced-treated senofilcon A lenses are the only lens materials in this study that used an internal wetting agent, polyvinyl pyrrolidone (PVP), to improve wettability and hopefully reduce surface deposition [42]. Our current speculation is that some of the PVP on the senofilcon A lenses was removed during the 16 hour of preconditioning in BBS and the MPSs; this may have reduced the hydrophilicity of the surface region and reduced the ability of the lens surface to block lipid deposition with the very hydrophilic PVP. PureMoist and BioTrue both contain various surfactants and wetting agents, and those substances may have replaced some of

the desorbed PVP in senofilcon A lenses, producing a reduction in sorption compared to sorption with BBS pretreatment, but about the same sorption as non-pretreated control senofilcon A lenses. Obviously this hypothesis needs to be substantiated in future studies. On the other hand, comfilcon A material is inherently wettable, having more hydrophilic units in its chemistry and thus requires no surface treatment [10]. Balafilcon A lenses are treated with a plasma oxidation that converts the TRIS structure on the surface into islands of hydrophilic silicate [42]. Lotrafilcon B lenses surface are coated with a uniform wettable layer which acts as a barrier to lipid sorption. Since these materials are engineered to permanently enhance hydrophilicity of the surface, the surfactants in the MPSs may have less additional influence on the surface chemistry with respect to reducing lipid deposition.

To summarize, none of the MPSs (which all contain polymers and surfactants) are much better than borate buffered saline in preventing lipid sorption. In fact in some cases preconditioning with an MPS may increase lipid sorption most probably by removing hydrophilic polymers from the contact lens.

CHAPTER 5 REMOVAL OF LIPID DEPOSITION BY MPS

5.1 Experimental approach

In this chapter, we report on using a radiolabeling technique to evaluate the effectiveness of three multi-purpose solutions (MPSs) – OPTI-FREE® PureMoist® (PureMoist), Biotrue™ and an experimental MPS (BLS) – in removing the depositions of cholesterol (CH) and dipalmitoylphosphatidylcholine (DPPC) on various commercial silicone hydrogel (SiHy) lenses and one conventional hydrogel lens. CH and DPPC are model components that represent respectively non-polar and polar lipid components in human tear films.

5.1a Materials

The three MPSs are described in section 4.1a. Table 2 shows the active components of the solutions. Four commercial SiHy lenses — senofilcon A, comfilcon A, lotrafilcon B, and balafilcon A and one conventional hydrogel lens polymacon — were used in this study (see Table 1).

5.1b Preparation of artificial tear solution

Table 3 of section 4.1a shows the composition of the artificial tear fluid (ATF) used in the study of this chapter, which includes lipids, proteins and buffer. As mentioned previously, the composition used herein differs somewhat from other published compositions [32,40,41]. Compared to others, it contains methyl-β-cyclodextrin (to achieve higher lipid concentrations), DPPC as the

phospholipid, and lower protein concentrations, and higher lipid concentrations compared to natural tears, with the goal of accelerating the lipid spoliation. As there is currently no consensus composition for tear film presented in the literature or used uniformly in industry, the composition chosen for this work was selected because it deposits lipids and proteins on SiHy lenses overnight at levels similar to deposits found on worn lenses after several weeks wear [34].

The preparation method of the ATF is presented in section 4.1b. Briefly, the aqueous base of the ATF, borate buffered saline (BBS), was prepared by mixing boric acid, sodium borate and sodium chloride in distilled deionized water (DDH_2O), and the pH of the solution was adjusted to 7.3 by adding NaOH or HCl. The ATF was prepared by adding appropriate amounts of various lipids in organic solvent in a 100-mL volumetric flask. The solvent was evaporated under a nitrogen stream for 2 hours and followed by 2 hours of vacuum drying. Then 50 mL of BBS was added to the flask and magnetically stirred for 6 hours at room temperature (21°C) with a 1.5-cm Teflon stir bar at 650 rpm. Solid powdered protein components were added to the solution. The mixture was stirred for additional 8 hours at room temperature. An additional 50 mL of BBS was added to the vial before final stirring for 20 minutes. The ATF was then used immediately for incubation of lenses in the sorption studies. ATF stored for more than a few hours tended to give less reproducible results.

5.1c Quantitation procedure

Following sorption, the three-stage n-propanol extraction was used to extract the lipid sorbed on the lenses. The detailed extraction procedure is described in section 4.1c. In brief, each lens was placed in a 20-mL glass scintillation vial containing 2 mL of n-propanol and placed on a rotary table shaker at 60 rpm for one hour at 37°C. Lenses were then held above the extraction

solution and rinsed with 1 mL of n-propanol into the same vial. It was placed in another container of n-propanol for 1 hour. Then, it was rinsed again the same way and the lens was transferred to the third extraction vial. Following the third one-hour extraction and rinsing procedure, the lens was transferred to a final vial filled with 10 mL of scintillation fluid to measure any residual radioactivity in the lens. All blanks, standards and samples were submitted in the same batch in identical scintillation vials to a LS 6500 scintillation counter (Beckman Coulter) and simultaneously counted twice using a program that counted both ^{14}C and ^{3}H.

5.1d Soaking lenses with sorbed radiolabeled lipids in MPS

Lenses were removed from their blister packs, rinsed in BBS, lightly blotted dry with a Kimwipe and immersed in glass vials each containing 2 mL of ATF. The vials were placed in an incubator at 37°C on an orbital shaker at 60 rpm for 16 hours. Lenses were then removed from the vials containing ATF, rinsed with DDH_2O as described above. After rinsing, the lenses were placed into 5-mL glass vials containing 2 mL of PureMoist, 2 mL of Biotrue or 2 mL of BBS. To measure the kinetics of the elution of CH and DPPC, 200 µL samples were transferred from each glass vial into scintillation vials containing 10 mL of SF at 0, 1, 2, 4 and 8 hours. After the 8 hours of desorption, the lenses were transferred into scintillation vials with 2 mL of n-propanol to be extracted as described above.

Since the radioactivity of the aqueous samples was very low, accuracy was ensured by preparing blank solutions and calibration solutions that were adjusted to have the same amount of BBS as the samples. Thus the capture efficiency of β-particles was the same in samples, standards and blanks. See section 4.1d for the composition of each blank solution. See Figure 5 for the general experimental process which is described above.

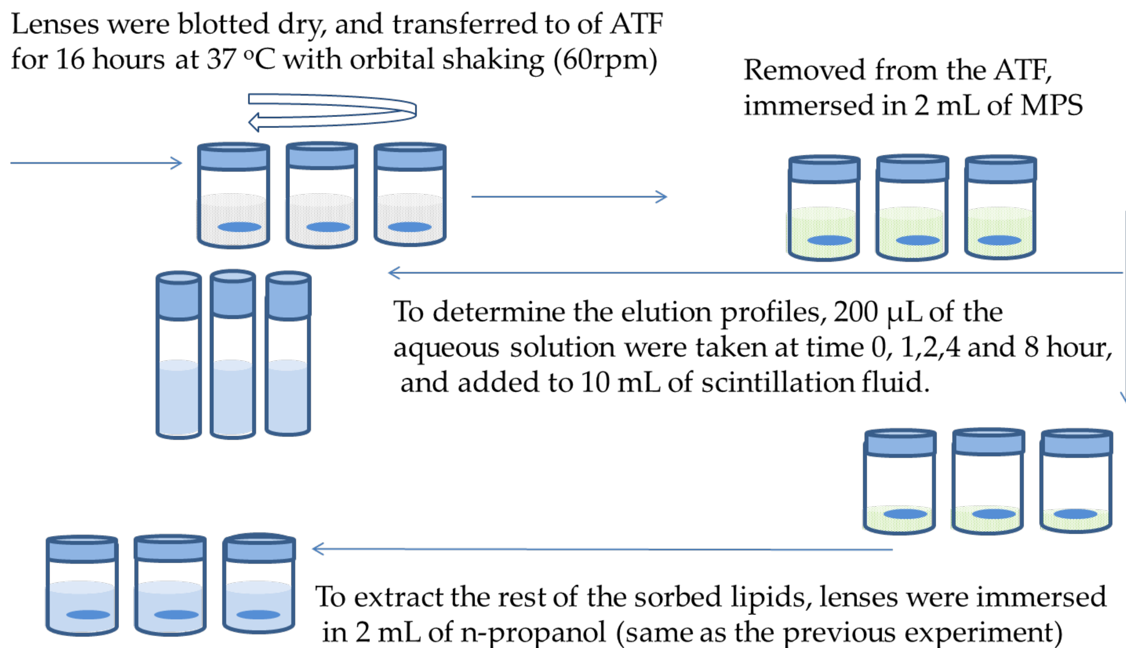

Figure 5: A general flow diagram of experiments in chapter 5.

5.2 Results

In this study, we used a radiolabeling technique to evaluate the effectiveness of three MPSs – PureMoist, Biotrue, and BLS – in removing the depositions of CH and DPPC on various commercial SiHy lenses and one conventional hydrogel lens. As mentioned, CH and DPPC are model components that represent respectively non-polar and polar lipid components in human tears.

Overall, DPPC was desorbed slowly in all the MPSs and also in BBS. However, little to no CH was eluted from lenses to the solutions and the BBS. The overall removal percentages that compare the amount of lipid deposition before and after soaking in MPSs or BBS for 8 hours were calculated (Table 4). PureMoist solution removed the highest percentage of both CH (0.1% to 28.2%) and DPPC (1.0% to 54.8%), from all lenses. The highest fractions of lipid were removed from polymacon lenses (28.2% of CH and 54.8% of DPPC).

Table 4: Percentages of removal of sorbed lipids by the multi-purpose lens care solutions. Values are the mean % ± 95% intervals which are calculated by propagation of errors.

Percentage of removal	DPPC				
	Senofilcon A	**Comfilcon A**	**Balafilcon A**	**Lotrafilcon B**	**Polymacon**
BBS	0.37(±0.16)	0.45(±0.32)	0.59(±0.17)	1.52(±0.71)	14.29(±4.63)
Experimental MPS (BLS)	0.47(±0.16)	0.63(±0.17)	0.82(±0.36)	1.85(±0.51)	24.07(±3.55)
Opti-free® Pure Moist®	1.03(±0.31)	1.3(±0.46)	1.61(±0.31)	3.08(±0.77)	54.81(±12.04)
Biotrue™	0.49(±0.13)	0.65(±0.10)	0.81(±0.21)	1.93(±0.32)	24.92(±4.71)
	CH				
	Senofilcon A	**Comfilcon A**	**Balafilcon A**	**Lotrafilcon B**	**Polymacon**
BBS	0.03(±0.11)	-0.05(±0.10)	0.00(±0.11)	-0.04(±0.27)	-0.6(±3.49)
B&L Experimental MPS	-0.02(±0.13)	-0.01(±0.06)	-0.06(±0.12)	-0.06(±0.32)	5.19(±1.93)
Opti-free® Pure Moist®	0.12(±0.11)	0.45(±0.26)	0.17(±0.12)	0.76(±0.76)	28.17(±10.77)
Biotrue™	-0.04(±0.03)	-0.04(±0.12)	0.03(±0.13)	0.04(±0.47)	8.05(±3.93)

5.2a Analysis by lens type

Data of DPPC and CH elution profiles from the various lenses to MPSs are presented in Figure 6. In general, the DPPC is slowly eluted from all lenses, but there are no statistically significant differences between balafilcon A, senofilcon A and comfilcon A lenses. Lotrafilcon B lenses eluted comparatively less DPPC (but it sorbed less to begin with), and polymacon lenses eluted the highest amounts of DPPC and CH to the solution compared with other lens types, and these lenses sorbed the least. This is noteworthy since lotrafilcon B and polymacon lenses sorbed less lipids than the other three lenses.

5.2b Analysis by MPS type

The elution data of sorbed DPPC and CH from lenses are solution dependent. For example PureMoist removed the greatest amount of DPPC and CH, Biotrue and BLS removed both lipids at similar levels, and BBS removed the least (Figure 6). The percentage of removal of DPPC and

CH deposition after 8 hours in various solutions are presented in Table 4. The percentages were calculated by comparing the amounts of lipids desorbed from the lenses at the end of the soaking procedure with the total amounts of sorbed lipid in control sets (control lenses were extracted directly after incubation in ATF without any presoaking in MPSs or BBS, n = 7). After 8 hours of soaking in MPSs, PureMoist removed greater amount of DPPC from senofilcon A, comfilcon, balafilcon A , lotrafilcon B and polymacon lenses than did BBS statistically ($p<0.05$). The differences were 0.03µg, 0.05µg, 0.04µg, 0.02µg and 0.7µg respectively. Biotrue and BLS both statistically removed greater amounts of DPPC from polymacon lenses compared with BBS, and the removals were 0.02µg more for both solutions.

Significant amount of CH deposits were removed from all lenses in PureMoist (confidence intervals are above zero) during the 8 hours soaking period. The average removals from senofilcon A, comfilcon A, balafilcon A, lotrafilcon B and polymacon were 0.0087 µg, 0.0286 µg, 0.0115µg, 0.0002 µg and 0.0324 µg respectively. For polymacon lenses, BLS and Biotrue were also able to remove some of the CH sorption (Figure 6), but the amounts were significantly less than removal by PureMoist ($p<0.05$).

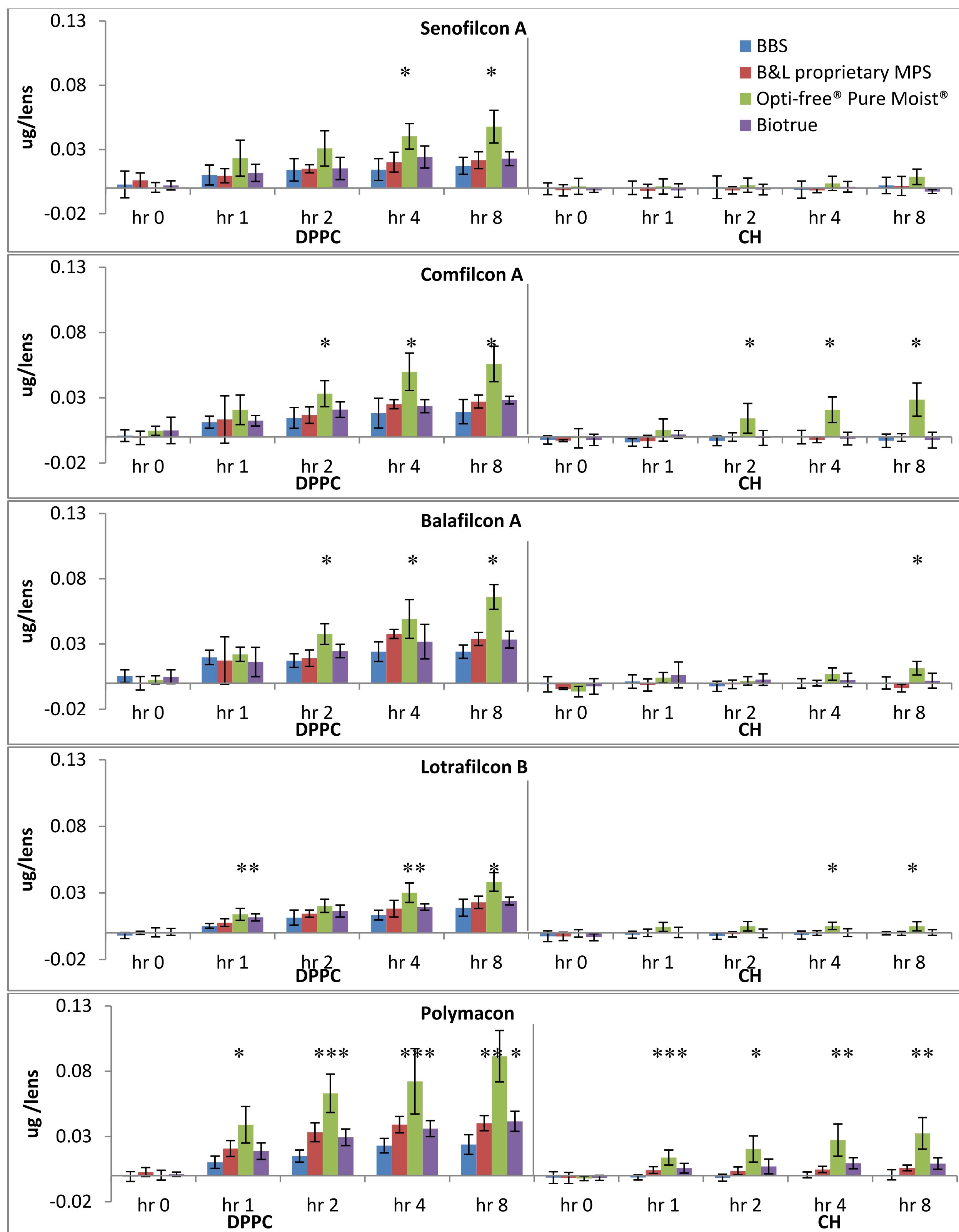

Figure 6: The elution profiles of sorbed lipids. DPPC (left) and CH (right) in solutions. Error bars represent the 95% confidence intervals (n>=6). *indicates statistical difference from BBS at the same the point (p<0.05).

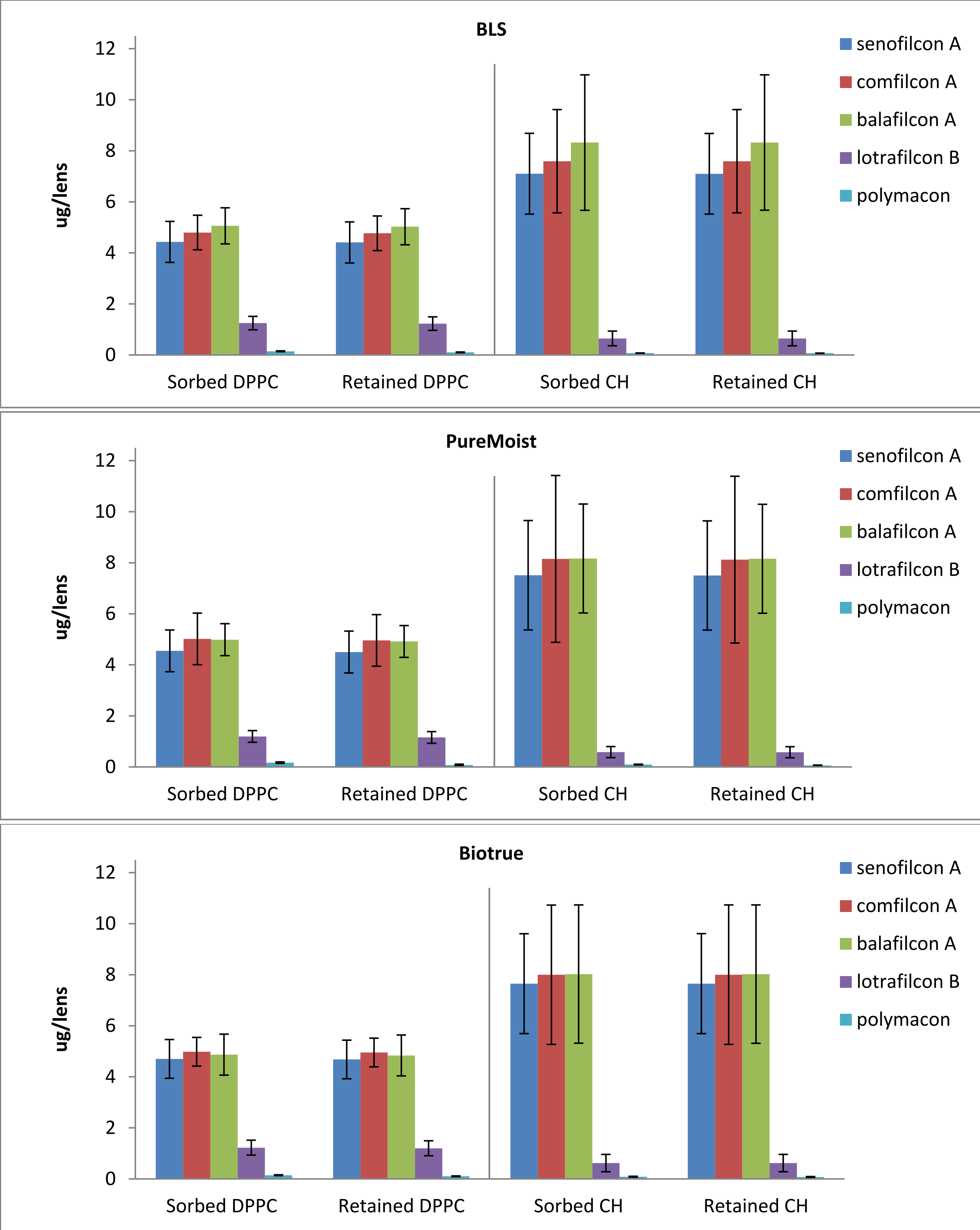

Figure 7: Initial sorption of DPPC and CH, and retained sorption. Error bars represent the 95% confidence intervals (n=6).

5.3 Discussion

The MPSs were generally able to remove more lipids than BBS. PureMoist removed the largest amount of DPPC and CH from all lenses. However, even with PureMoist, less than 4% of DPPC and less than 1% of CH were removed from SiHy lenses (senofilcon A, comfilcon A, balafilcon A, and lotrafilcon B). PureMoist removed approximately 55% of DPPC and 28% of CH from polymacon lenses, which are non-hydrophobic conventional hydrogel lenses.

SiHy materials have some very hydrophobic silicone segments so lipids are thermodynamically driven to partition from the aqueous solution to the hydrophobic polymer segments of the SiHy lenses. On the other hand, no such hydrophobic segments are present in poly-HEMA based hydrogels; lipid deposits are small and are effectively removed by the surfactants in the MPS. The chemicals and surfactants present in MPSs have to be mild enough to be compatible with and comfortable to the eyes, so it is a difficult task to develop a multi-purpose solution to remove lipid and protein deposits and yet remain biocompatible with human eyes at the same time. Apparently the surfactants in the MPSs are not hydrophobic enough to remove lipids, especially removal of non-polar lipids from the hydrophobic segments in SiHy lenses.

5.3a Removal of sorbed lipids by the MPS

If one only examines the amounts of lipids that remain on the lenses before and after the attempt to remove DPPC and CH by soaking in MPS, the scatter intrinsic to these experiments hides the fact that there is a small but detectable desorption of DPPC. Thus while it appears that there are no significant differences between the initial deposition and the retained sorption on each lens type for both of the MPSs, some desorption actually occurs. For example, see Figure 7

which shows the amount of sorbed lipid and the retained lipid for lenses with preconditioning in BLS, PureMoist and Biotrue. Thus it falsely appears that there are no significant differences between the initial deposition and the retained sorption on these lens type for the MPSs. Less sensitive techniques, such as chromatography might also lead to the incorrect assumption that these MPSs do not remove sorbed lipid. However, because of the sensitivity of the radiolabeling technique used, the DPPC and the CH elution into the solutions could be accurately measured, showing that DPPC and CH elute slightly from some of the lenses under the conditions of these experiments. However, the composition of the eluting solution, whether BBS or an MPS, appears to make little difference on removing lipids from SiHy lenses (See Figure 6 and Table 4). While some marketing schemes might tout the fact that PureMoist removes 3% of DPPC and 0.8% CH from lotrafilcon B lenses, while statistically significant, there is little to cheer about. The observation that the multi-purpose solutions clean only slightly better than BBS suggests that the surfactants employed are not particularly effective for SiHy lenses. The small fraction of sorbed DPPC was being extracted slowly with time in all solutions. In contrast, little to no significant amount of CH was eluted in both MPSs and BBS after 8 hours of soaking. The difference in removal might be attributed to the more polar nature of the DPPC compared to the non-polar CH. This lack of desorption of CH hints that other non-polar tear components, such as CH esters, fatty acid esters and triolein may also have little to no desorption from a lens by soaking in an MPS.

Despite the small removal of lipids, there are other essential benefits using MPSs to clean and store contact lenses. All soft contact lenses – both SiHy lenses and conventional hydrogel lenses – need to be stored in MPS lens care solutions while not in use for at least 2 reasons. First, storing in MPS rehydrates the lenses and replenished the lubricants and wetting agents to keep

the lenses moist and comfortable. Second, the preservatives eliminate harmful microorganisms to prevent infections [24,43,44]. As mentioned, rubbing has been shown to remove microorganisms and protein deposits [14,27]. Such benefits should always be considered when evaluating the overall performance of an MPS.

CHAPTER 6 EFFECTS OF RUBBING AND A PEROXIDE-BASED SOLUTION

While experiments in previous chapters were performed without rubbing, rubbing has been shown to remove proteins and bacterial from contact lenses [14,27]. Most commercial multi-purpose solutions (MPSs) for lens care and some professionals indicate that rubbing should be included during the process of cleaning silicone hydrogel (SiHy) lenses [24,25]. However, there is currently no experimental evidence in the literature to support the additional rubbing process removes significant amounts of lipid from SiHy lenses. Thus, the effect of rubbing was investigated and is discussed in this chapter.

Hydrogen peroxide-based solutions for lens care are sometimes used to disinfect lenses. The cleaning process of such solutions often involves a neutralization step to convert all the peroxide to water, so the lens is ready to be worn without causing irritation. The ·OH radicals in hydrogen peroxide solutions kill microorganisms, and it might also enhance lipid removal. A study reported that a hydrogen peroxide lens care solution containing a surfactant removed more lipid from lenses when compared to the non-surfactant hydrogen peroxide solution [20]. This chapter also reports data for a peroxide-based experimental solution that was investigated for its lipid removal efficacy and compared with a simple borate buffered saline (BBS).

6.1 Experimental approach

In this study, the experimental setup including the artificial tear fluid (ATF) preparation was identical with the pervious experiments of removal and reduction of lipids by the MPSs (chapter

4 and 5), except there is an additional rubbing step after the incubation in ATF and before soaking lenses in MPS. The rubbing procedure was designed to mimic the effect on lipid removal by rubbing the lens with the finger in the palm of the hand. The detail of the procedure will be discussed in the follow sections.

6.1a Materials

Senofilcon A, balafilcon A and a proprietary SiHy lens (Zeta) in development by Bausch and Lomb were used in this study. Zeta is an experimental lens material as a next-generation, non-plasma modified silicone hydrogel that has a chemistry that does not require a second step to apply a hydrophilic coatings such as is done with lotrafilcon B and balafilcon A lenses. Two commercial MPSs, SEEDO Softcare (SEEDO) and Opti-free® Pure Moist® (PureMoist), and one experimental peroxide-based solution (BLP) were evaluated and compared with the performance of the BBS.

6.1b Removal of sorbed lipids in MPSs and BLP

Six different removal methods were evaluated in this chapter. They are 1) rub and soak in SEEDO, 2) soak only in SEEDO, 3) rub and soak in PureMoist 4) soak only in BLP, 5) rub and soak in BBS and 6) soak only in BBS. With three lens types (senofilcon A, Zeta and balafilcon A), there were 18 combinations overall, and 6 replicates were performed in each combination.

The experimental procedure for combinations that only involve soaking in solutions is the same as the pervious experiment which is presented in section (5.1d), except that those lenses were soaked in BLP used the special vials containing platinum-plated neutralizers rather than using the 5-ml glass vials (Figure 9). Since BLP is a peroxide-based solution, neutralizers were

placed in the vials to convert H_2O_2 to H_2O during the cleaning process. Two lenses and 10 mL of BLP were placed into each vial. Similar to other lenses, 200 μL of solution were taken from the vials at 0, 1, 2, 4 and 8 hours to determine the desorption kinetics of CH and DPPC. After the 8 hours of desorption, the lenses were transferred into scintillation vials with 2 mL of n-propanol to be extracted by the standard three stages of n-propanol extraction as described in section 4.1c

With those lenses that were rubbed prior to soaking in MPSs, the process is described as follow. First, lenses were incubated in ATF like other lenses (section 5.1d). Following the 16-hour incubation process, lenses were transferred from the vials containing ATF, and rinsed with distilled deionized water (DDH_2O) twice. After rinsing, 400 μL of SEEDO, PureMoist or BBS were pipetted into 10-mL polyethylene zip lock bags. The bags were labeled correspondingly. Lenses with sorbed radioactive lipids were placed into the center of the bags, and the bags were closed completely with care to exclude nearly all air. The rubbing step began with placing the bag on the left palm, with the palm facing up. The lenses were rubbed through the bag using the index finger for 20 times in a circular motion, and the finger traced the peripheral region of the lenses. 200 μL of MPS sample were taken from the bags to scintillation vials filled with 10 mL of SF. The lenses were rinsed with DDH20 and blotted dry with a Kimwipe®, and placed in 5-mL glass vials containing 2 mL of the various "removal" solutions. 200 μL samples were taken from each glass vial into scintillation vials containing 10 mL of SF at 0, 1, 2, 4 and 8 hours to determine the desorption kinetics of CH and DPPC after the rubbing procedure. After the 8 hours of desorption, the lenses were transferred into scintillation vials with 2 mL of n-propanol to be extracted by the standard three stages of n-propanol extraction as described in section 4.1c.

After transferring the lenses to MPS, the bags were rinsed with DDH_2O two times to make sure no lipids were left in the aqueous phase. Then 2 mL of n-heptanol was transferred to the

bags, the air trapped in the bags was carefully removed by softly pushing. The bags were then closed and placed flat at room temperature on a clean paper towel. After an hour, the solvent in the bags was transferred to scintillation vials filled with 10 mL of SF. The bags were rinsed with additional 1mL of n-heptanol into the same scintillation vials. The second extraction was performed by transferring another 2 mL of n-heptanol to the bags and repeat the process described above. After the third extraction, the bags were filled with 10mL of SF and placed into a set of new scintillation vials for counting any residual radioactivity on the bags. This is called "direct counting" of the bags. N-heptanol was selected for the bag extraction after trying several solvents such as n-propanol, octane, hexane, n-hexanol, n-heptanol, and tetrahydrofuran. It was found that alkanes diffuse through the bags quickly, and thus are not ideal as extraction solvents, and n-heptnaol extracted most of the sorbed lipids from the bags (lowest direct counting values). The direct counting of the bags was still slightly above the background count but of the same order of magnitude. Thus the amount of detected lipid β emission was multiplied by two while quantifying the lipids from the direct counting stage. This adjustment was made by assuming half of the beta emissions from lipid depositions were registered by the SF, and half of the lipid depositions were sorbed into the bags polymer and were not registered by the SF.

Upon the completion of the lens extraction and the bag extraction, all blanks, standards and samples were submitted to a LS 6500 scintillation counter (Beckman Coulter) and counted using a program that counted both ^{14}C and ^{3}H simultaneously. This was a tremendously complex experiment that required careful timing, much extraction (bags and lenses) and the consumption of about 1500 scintillation vials that were each counted twice. See Figure 8 for the general flow diagram of the experiment.

Table 5: Characteristics of the contact lens materials.

Lens material	Commercial name	Manufacturer	Principal components	Surface treatment
Senofilcon A	Acuvue Oasys	Johnson & Johnson	mPDMS, DMA, HEMA, SiGMA, TEGDMA, PVP	No surface treatment. Internal wetting agent (PVP) throughout the matrix that also coats the surface
Balafilcon A	PureVision	Bausch & Lomb	NVP, TPVC, NVA, PBVC	Plasma oxidation process
N/A	Zeta*	Bausch & Lomb	Unpublished	None (inherently wettable)

PVP: poly(vinyl pyrrolidone); **mPDMS**: monofunctional methacryloxypropyl terminated polydimethylsiloxane; **DMA**: N,Ndimethylacrylamide; **HEMA**: hydroxyethyl methacrylate; **TEGDMA**: tetraethyleneglycol dimethacrylate; **TRIS**: methacryloxypropyl tris(trimethyl siloxy)silyane; **NVP**: N-vinyl pyrrolidone **TPVC**: tris-(trimethyl siloxysilyl) propylvinyl carbamate; **NVA**: N-vinyl amino acid; **PBVC**: poly(dimethysiloxy) di (silylbutanol) bis(vinyl carbamate); **SiGMA**: 2- propenoic acid, 2-methyl, 2-hydroxy-3-(3-(1,3,3,3-tetramethyl-1-(trimethylsilyl)oxy)disiloxanyl)propoxypropyl ester.
* This experimental lens is not yet in commercial production.

Table 6: Components of the multi-purpose lens care solutions.

Solution	Manufacturer	Wetting agents/surfactants	Preservatives
Opti-free® Pure Moist®	Alcon	TETRONIC® 1304 (poloxamine), HydraGlyde (EOBO)*	POLYQUAD® (polyquaternium-1) 0.001%, ALDOX® (myristamidopropyl dimethylamine) 0.0006%
SEEDO	SEED Co.,Ltd	2-methacryroyloxyethyl phosphorylcholine	Unpublished
Experimental peroxide-based solution	Bausch and Lomb**	Unpublished	Unpublished

***EOBO**: poly (oxyethylene)-poly (oxybutylene); BLP: Properiatory peroxide based solution
** This experimental solution is not yet in commercial production.

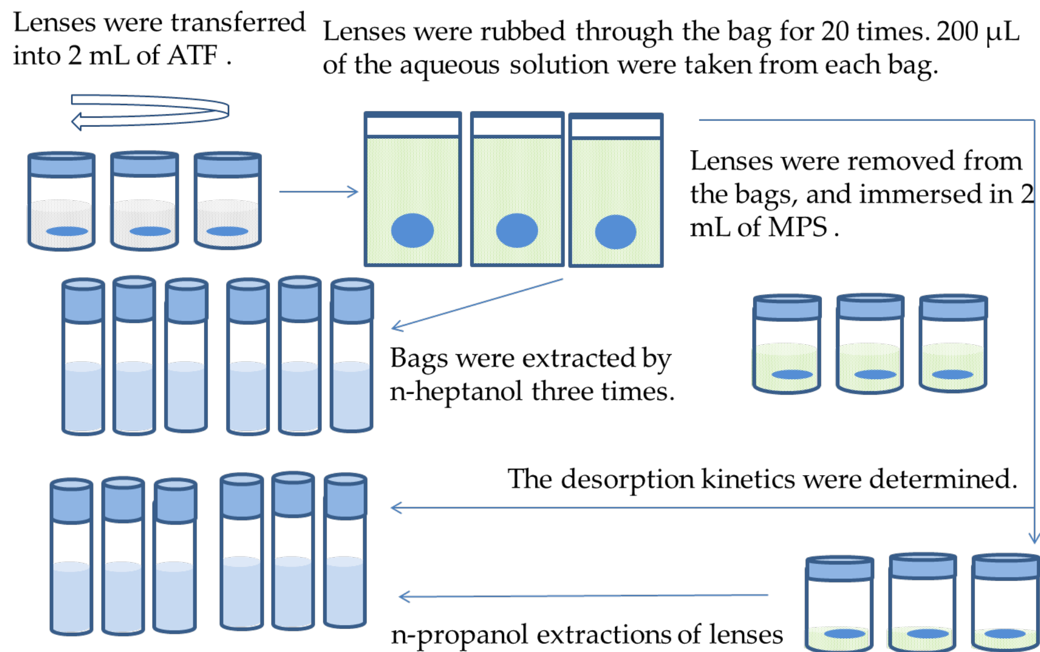

Figure 8: A general flow diagram of experiments in chapter 6.

Figure 9: The proprietary vial for using the peroxide-based solution.

6.2 Results

In this study, we used a radiolabeling technique to evaluate the effectiveness of three MPSs – PureMoist, SEEDO and BLP – in removing the depositions of CH and DPPC on various SiHy lenses. In additional to soaking in MPS, the effects of rubbing were determined with SEEDO, PureMoist and BBS.

6.2a Effects by additional rubbing and the peroxide-based solution

There were no significant differences in the amount of removal of lipids between lenses. Surprisingly the BLP did not remove any lipids from any lenses after 8 hours of soaking (Figure 10). Overall, more lipids were removed when lenses were treated with the additional rubbing procedure compared with "soak only" procedure, and the removal is statistically independent of solution types ($p>0.05$), as they all performed identically to BBS (Figure 10). The percentages of removal of both lipids by rub and soak procedure were between 0.5% and 2.0% (Table 7).

The effect of the additional rubbing step in SEEDO and BBS were evaluated. Both solutions statistically removed greater amounts of DPPC and CH from all lenses. While using SEEDO with rubbing, approximately 5.3, 9.0 and 2.7 times more ($p<0.05$) DPPC and 8.4, 6.6 and 6.6 times more CH were removed respectively from senofilcon A, Zeta, and balafilcon A lenses compared with lenses that were soaked only. While using BBS with rubbing, approximately 26.5, 9.9 and 7.2 fold more of DPPC sorption and 14.3, 14.1 and 34.5 fold more of CH was removed ($p<0.05$) from lenses compared to those were only soaked in BBS (Figure 10).

The rubbing effect on senofilcon A and balafilcon A lenses when using PureMoist are also evaluated and compared with other solutions (Figure 11) where the data of "PureMoist Soak only" were generated from experiments of chapter 5. There were no significant differences in DPPC

removal between "PureMoist Rub" and "PureMoist soak only" (P>0.05) for both lenses. Approximately 6.6 and 5.0 times more (p<0.05) CH was removed respectively form senofilcon A and balafilcon A lenses when these lenses were cleaned with additional rubbing step.

The complete elution profiles of lipids of lenses in different solutions are showed in Figure 12. Additional rubbing did not affect the elution kinetic of lipids as there were no significant differences (p>0.05) in removal along the 8 hours period between "SEEDO rub and soak" and "SEEDO soak only", and "BBS rub and soak" and "BBS soak only".

Table 7: Percentages of removal of the sorbed lipids by multi-purpose lens care solutions. Parentheses indicate 95% intervals which are calculated by propagation of errors, n=6.

Percentage of removal	DPPC			CH		
	Senofilcon A	Zeta	Balafilcon A	Senofilcon A	Zeta	Balafilcon
SEEDO rub and soak	0.96(±0.64)	1.2(±0.40)	0.84(±0.40)	0.74 (±0.57)	0.81(±0.43)	0.57(±0.39)
SEEDO soak only	0.18(±0.10)	0.13(±0.07)	0.30(±0.09)	0.09(±0.05)	0.12(±0.05)	0.09(±0.07)
PureMoist rub and soak	1.27(±0.68)	1.18(±0.75)	1.22(±0.59)	0.91(±0.72)	0.94(±0.76)	0.87(±0.48)
BLP soak only	0.04(±0.14)	0.03(±0.12)	0.02(±0.05)	-0.02(±0.09)	0.01(±0.06)	0.02(±0.04)
BBS rub and soak	1.94(±1.98)	1.26(±0.76)	0.83(±0.36)	1.24(±1.06)	1.13(±0.69)	0.64(±0.17)
BBS soak only	0.07(±0.07)	0.13(±0.19)	0.12(±0.05)	0.09(±0.03)	0.08(±0.06)	0.02(±0.05)

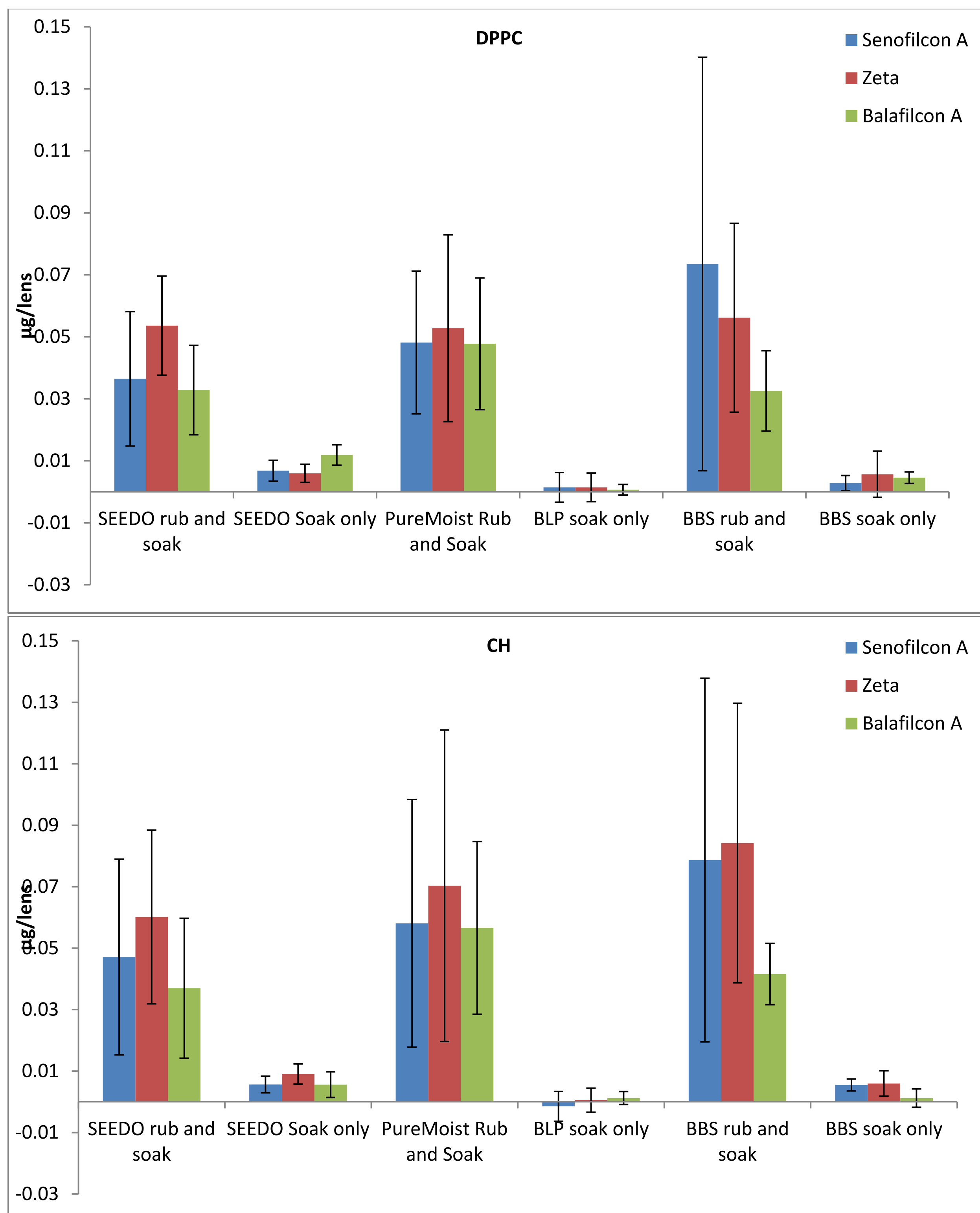

Figure 10: The amount of sorbed DPPC (top) and CH (bottom) removed in multi-purpose lens care solutions and boric buffered saline, with or without rubbing. Error bars represent the 95% confidence intervals (n=6). All rubbing removes DPPC and CH at a greater level ($p<0.05$) than without rubbing.

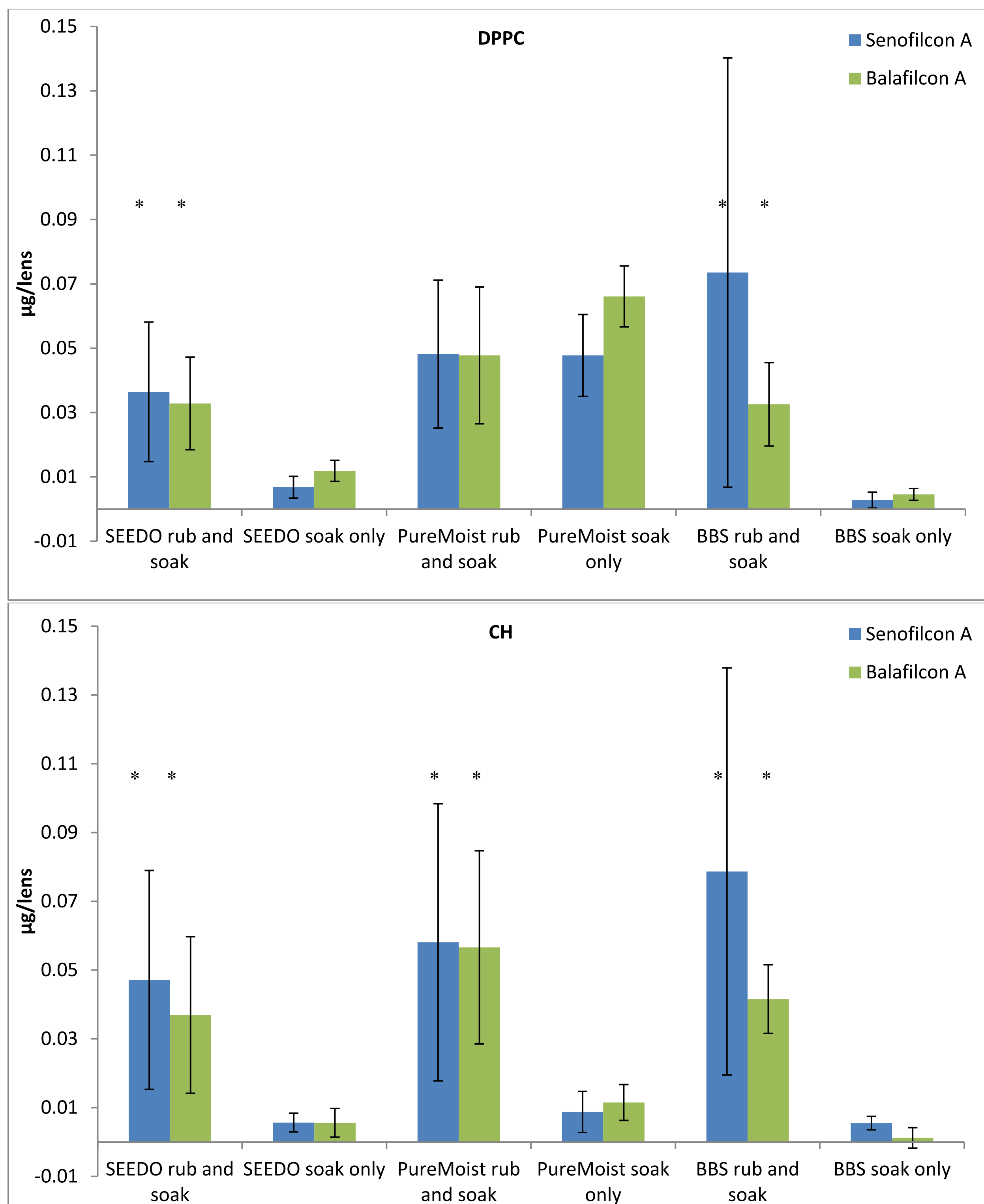

Figure 11: The amount of sorbed DPPC (top) and CH (bottom) removed from senofilcon A and balafilcon A lenses in various solutions, comparing between rub and no rub. Error bars represent the 95% confidence intervals ($n \geq 6$). Data of "PureMoist Soak only" were generated from experiments of chapter 5. * indicates a significant improvement in removal when rubbing compared to removal in the same MPS without rubbing ($p < 0.05$).

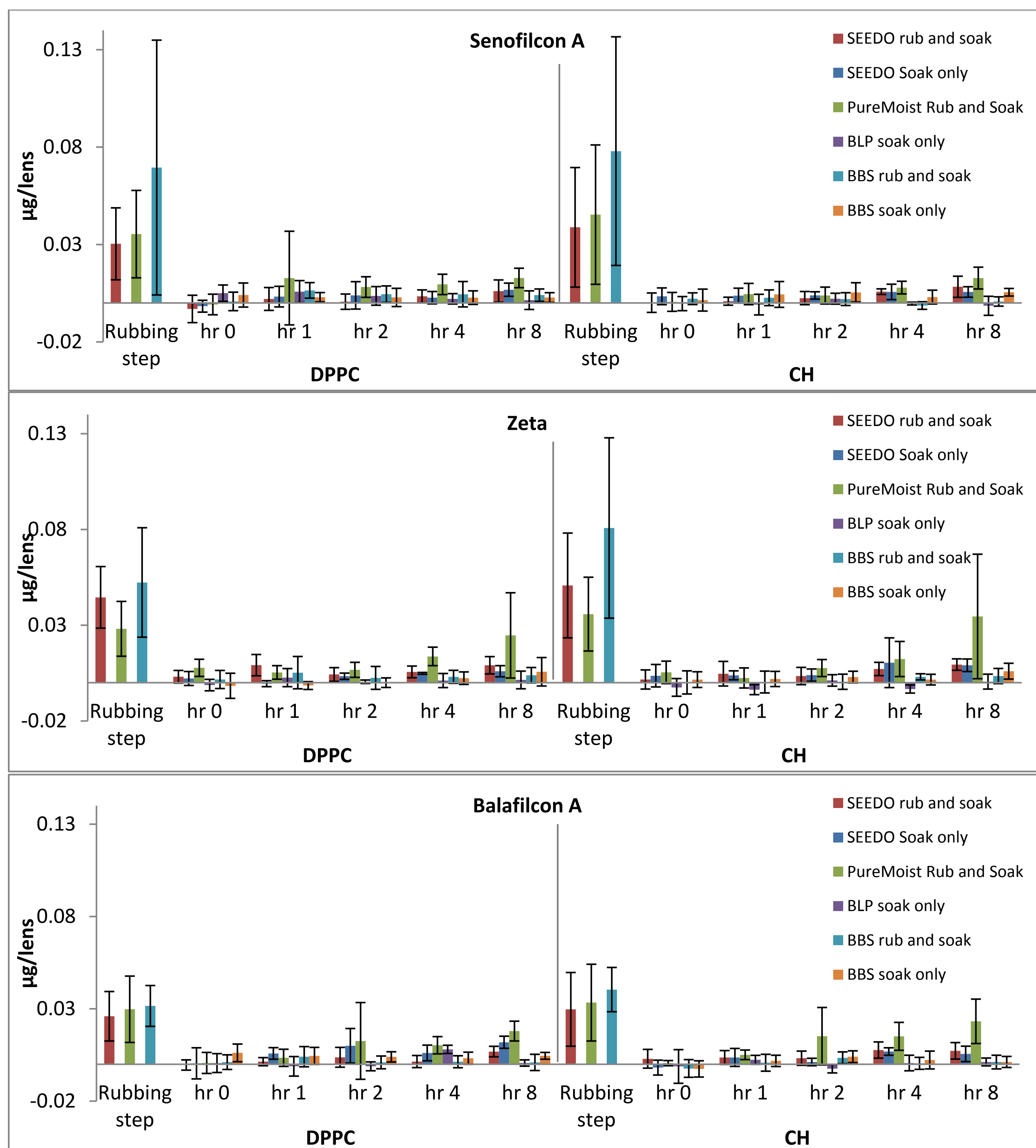

Figure 12: The elution profiles of sorbed lipids in solutions. DPPC (left) and CH (right). Error bars represent the 95% confidence intervals (n=6).

6.3 Discussion

This particular study examined the effect of rubbing on lipid removal. Based on literature reports of protein and bacterial removal by rubbing, we expected to see significant lipid removal by rubbing. However, this was not observed in all cases. My current hypothesis as to why lipids are not removed significantly by rubbing is that lipids are small compared to proteins. Thus the majority of lipids could be absorbed into the lens material deeper than just the surface adsorption. As rubbing should only affect the surface of the lens, less removal by rubbing was expected on lenses with hydrophilic surfaces since less lipid should adsorb. Lipids that sorbed to the more hydrophilic polymacon lenses could be easily be removed by just soaking in MPSs, as was reported in chapter 5.

While it is true that additional rubbing in most cases removes more lipid deposition from the SiHy lenses compared with the “soak only” treatment, the benefit is might not be significant on SiHy lenses as the overall removal percentages of both DPPC and CH are less than 2% (Table 7) . We should also consider that vigorous or repeated rubbing could possibly remove the thin hydrophilic treated surface on particular lenses such as lotrafilcon and balafilcon, and thereby expose the underlying hydrophobic polymer and accelerate the lipid deposition process. For example, the average thickness of the hydrophilic surface layer of a lotrafilcon B lens is only 25 nm. To investigate the possible damage to the hydrophilic layer by rubbing, one could rub half of a set of lenses before the incubation process in ATF and measure the total lipid sorption. If the hydrophilic layer of the lens is damaged, more lipid deposition would be observed in rubbed lenses compared to lenses that are not rubbed.

In conclusion, rubbing did improve the lipid removal efficacy of the MPSs. However, the overall removal percentages are still low (≤2%). Vigorous or repeat rubbing of lens might

destroy the hydrophilic layer in some surface-treated lenses such as lotrafilcon B lenses and balafilcon A lenses. Before suggesting whether consumers to employ rubbing in their lens care regimens, further studies are encouraged that investigate the long term effects on lipid sorption and wettability of rubbing various surface-treated SiHy lenses.

CHAPTER 7 CLINICAL APPLICABILITY OF THE IN VITRO MODEL AND THE SOURCES OF ERROR

The result at chapters 4, 5 and 6 may be disappointing to the industrial sponsors of this research who hoped to show that their lens care solutions could prevent or reverse lipid sorption.

To temper the bleak results of chapter 4, 5 and 6, we must acknowledge that these results may not directly represent the actual effectiveness of these MPSs in actual clinical wear. It is always a challenge to extrapolate the results of *in vitr*o experiments to *in vivo* performance. There are obvious differences between our experimental model and the conditions that exist on the eye. First, tear fluid in a human eye is constantly flowing, and throughout the day there are physical shear stresses on the tear film due to eye movement and blinking. Second, the composition of tears changes with external stimuli and often varies for each individual. Third, the actual tear film on an ocular surface is thought to be a multi-layer structure of a few microns in thickness, rather than a homogenous solution in a beaker. On the eye a complex lipid layer containing both polar and non-polar lipids forms the outer-most layer and serves to prevent excessive evaporation and to stabilize the tear film. An aqueous middle layer consist of proteins, salts, electrolytes, and a relatively thick mucin layer lies on the bottom adjacent to the corneal cells [45-48]. In addition, these tear film layers should not be segregated into various experiments examining only one layer at a time because components from all these layers, including proteins and lipids, were found in contact lens deposits [11,29,49]. The system becomes even more complex when including the interactions of different types of contact lenses; for example one study

confirms that wearing contact lenses leads to higher tear film evaporation rates for up to one day after removal of the lenses [50].

Although there are challenges in mimicking the actual ocular deposition process, the use of *in vitro* models allows researchers to control variables easily, and such studies require a less complex experimental design. Many *in vitro* studies involve incubation of lenses in artificial tear solutions at body temperature with gentle shaking to predict the amount of lipid deposition to contact lenses. Much of the published data from *in vitro* experiments are somewhat different than the clinical observations. However they do show consistency in the general trends, such as lotrafilcon materials sorb less lipid than other commercial SiHy lenses [12,33,51]. Other general trends are that DPPC sorption is less than CH sorption for SiHy lenses, and SiHy lenses sorb relatively more lipids and less proteins, while conventional poly-HEMA based hydrogels sorb relatively more proteins and less lipids [11,12,31]. These general trends, observed in clinically worn lenses, are also observed in these *in vitro* studies using artificial tear fluids and simulated soaking and care procedures. Thus we are confident that the main observations of these *in vitro* studies in this thesis research reflect the general behavior of worn lenses: in a no-rub situation, polar lipids may elute very slowly from SiHy lenses into MPSs, but non-polar lipids probably have little to no desorption. While rubbing may remove bacteria and perhaps some proteins, our studies show that very little lipid is removed by rubbing, particularly on lenses with hydrophobic character.

This study produced other valuable observations and data that were not previously published. For example, all SiHy lenses in this study swelled in n-propanol due to their intrinsic hydrophobic properties, reaching a maximum size (50% increases in diameter) in about 4 minutes. While all SiHy lenses swelled in n-propanol, the edges of lotrafilcon B lenses did not flare in a “scallop” border, as did balafilcon A and senofilcon A lenses. Additionally, the

lotrafilcon B lenses were more robust than the other SiHy lenses, as they did not easily break during handling while swollen in n-propanol. As lenses become fragile with swelling, they could easily be broken into small pieces between extraction steps. The extraction efficiency is reduced when some of the pieces of the lens do not get transferred to the next extraction vial, leading to challenges in quantitation. Thus lotrafilcon B lenses were easiest to use in this study. Although the conventional hydrogel polymacon lenses do not swell in the n-propanol solvent as do the SiHy lenses, they have a tendency to stick on the inner surface of glass vials. As a result, those lenses could be easily torn apart during extractions.

An unexpected and very useful observation made in this study relates to the sensitivity of sorption to the preparation of the ATF. In our lab we found that sorption from ATF is very sensitive to the composition and preparation of the fluid. Even seemingly minor differences, such as bovine versus porcine mucin, stir bar size and stirring speed affect sorption in measurable amounts. In view of the importance of artificial tear composition, we published a recommendation that ophthalmological societies and institutions should collaborate in formulating an appropriate ATF that can be easily and reproducibly made and used by various investigators around the world to generate more consistent and directly comparable data [34].

Other elements such as variation in the lens surface treatment, the material composition, and the differences in the lens thickness also contribute to the overall scatter in the data. We are surprised that other publications have not reported such sensitivity in their results to minor experimental variables. Perhaps the high accuracy and precision of our radiolabelled experiments revealed this sensitivity that other methods could not detect.

CHAPTER 8 SOLUBILITY OF COMFORT AGENTS

The fouling of silicone hydrogel (SiHy) lenses by lipid sorption lowers the surface wettability may contribute to dry eye syndromes. My thesis study has shown that lipid deposition to silicone hydrogel (SiHy) lenses appears very difficult to be removed or prevented. An alternative method to maintain wear comfort would be altering the lens materials itself; release of comfort agents from the lens matrix could mitigate the irritation by fouling and sustain consistent high levels of comfort, hopefully resulting in better customer satisfaction. This chapter reports an initial study of which comfort agents might be added to SiHy lenses.

8.1 Estimating the solubility of polymeric comfort agents in various solutions

The potential to deliver polymeric comfort agents to the anterior eye from a SiHy contact lens requires that the comfort agent reside in the lens and not significantly reduce the optical clarity of the lens. The optical clarity of the agent in the monomer or macromer formulation (prior to lens polymerization) is a screening method for potential candidates. Those agents that scatter light before polymerization have a high probability of scattering light after polymerization. Light scattering is usually caused by poor solubility of the comfort agent in the solution – undissolved particles scatter light if their refractive index is different from that of the solvent.

In this research, the optical densities of a series of solutions of comfort agents in potential contact lens macromer formulations were measured. Not only is this a convenient lab technique, but the results pertain directly to the optical density of a macromer formulation, and potentially

the optical density of the polymerized contact lens. Optical density (OD) is defined as – $Log_{10}(I_{in}/I_{out})$, where I_{in} is the intensity of light entering a sample, and I_{out} is the intensity exiting the sample.

There are 2 factors that contribute to optical density. First is absorption. Light may be absorbed by molecules in a solution, even when the mixture forms one phase and no scattering occurs. The second contribution is scattering in which insoluble particles scatter light at various angles depending on their size, shape and refractive index, and thus less light is directed forward along the path to the detector *in vitro* or to the eye retina in vivo. Although a single reading of OD cannot distinguish the proportion of each factor contributing to a single reading of OD, a series of readings at different concentrations can in theory distinguish the two. Light absorption is linear with concentration at low concentrations, and thus a plot of OD vs concentration will go through zero if only absorption occurs. If a material is totally insoluble, its plot of OD vs concentration will also go through zero (and usually with a steep slope), because even the smallest amount of solute causes light scattering. However, if the solute is soluble up to a certain limit, there will be no contribution from light scattering until that concentration limit is reached, and thereafter the OD will increase steeply with solute concentration, as shown schematically in Figure 13.

In this research we measured the optical density of solutions of several comfort agents in a solvent and 3 different proprietary macromer formulations provided by ALCON.

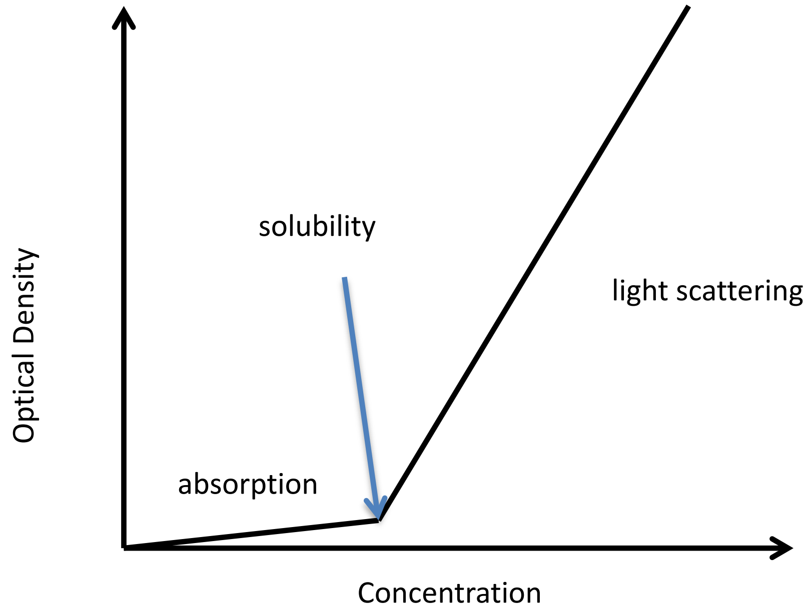

Figure 13: Optical density vs concentration for agents with slight solubility.

8.1a Materials

Dipropylene glycol methyl ether (DPGME) was purchased from Aldrich and stored at room temperature in a solvent cabinet (Figure 14). Macromers A, B, and C were shipped from ALCON in Johns Creek, GA. They were stored at room temperature. The comfort agents were provided by ALCON in powdered form.They are poly(N-vinylpyrrolidone) (pNVP), carboxy methylcellulose (CMC), hydroxypropyl methylcellulose (HPMC), Dextran, Carbopol, poly(ethylene oxide)-poly(butylene oxide) (EOBO), Kollidon (polyvinyl pyrrolidone-polyvinyl acetate copolymer) , poly glycol (PEO), Guar, and sodium hyaluronate (NaHA). Chemical structures of the comfort agents are presented in Figure 15. A small marble mortar and pestle was purchased from Fischer Scientific. UV-vis optical cuvettes were purchased from the BYU Chemistry Stockroom, as were stir bars, spatulas, and other small lab supplies.

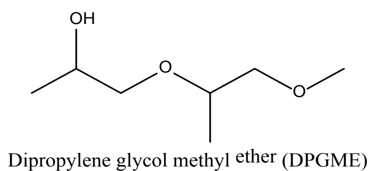

Figure 14: Chemical Structure of dipropylene glycol methyl ether (DPGME).

poly(N-vinylpyrrolidone) (pNVP)

R = H or CH2CO2H

Carboxymethyl cellulose (CMC) or cellulose gum

R = H or CH3 or CH2CH(OH)CH3

Hydroxypropyl methylcellulose (HPMC)

Dextran

Polyethyleneglycol (PEO)

Guar

Carbopol

Hyaluronate (NaHA)

Ethyleneoxide butyleneoxide (EOBO)

polyvinylpyrrolidone-polyvinyl acetate (Kollidon)

Figure 15: Structures of comfort agents.

8.1b Methods

For solubility in DPGME, this solvent was weighed on an analytical balance in a small container, and a small mass of comfort agent powder was weighed and added to the container. It was stirred with a magnetic stir bar for minutes (if it dissolved quickly) to hours. Often the preparation and stirring occurred in an optical cuvette. Sometimes it was prepared in a glass vial, in which case it was transferred to the optical cuvette. The cuvette was spun in a swinging bucket centrifuge for 5 minutes to consolidate the liquid on the bottom and remove any air bubbles that would have scattered light. The cuvette was transferred to a Beckman 550 UV-vis spectrometer for measurement. The reference was pure DPGME. After the OD of a sample was measured, more DPGME was weighed and added to the sample to create a lower concentration of solute. Due to the abundant supply of DPGME, this was repeated several times to generate many data points. Optical densities were recorded and plotted, from which solubilities were estimated graphically.

For solubilities in macromer formulations, we found that the high viscosity of the macromers prevented any magnetic stirring. Therefore we developed a technique in which known masses of macromer and solute was mixed using a mortar and pestle, and then transferred to an optical cuvette, centrifuged to remove air bubbles, and measured in the spectrometer. Because of the limited supply of macromer, we could not make serial dilutions as were done for DPGME. Instead we measured very small amounts of powdered comfort agent on a Mettler microbalance (precision to 0.1 μg). These small amounts were added to previous mixtures, generating a series that increased in concentration of comfort agent. The very small masses of comfort agent, and the difficulty of removing all of the mixture from the mortar surface (with a rubber policeman),

produced more scatter in the measurement with macromers. Optical densities were recorded and plotted, from which solubilities were estimated graphically.

8.2 Results and discussion

This study examined the solubility of 10 polymeric comfort agents in various solutions to determine their potential to dissolve in macromer formulations for contact lenses. The comfort agents were pNVP, Kollidon, EOBO, CMC, HPMC, Dextran, Guar, poly glycol, Carbopol, and sodium hyaluronate. The solutions were DPGME solvent and 3 macromer formulations provided by ALCON. Solubility was assessed by light scattering and optical density of solution with various weight fractions of comfort agents.

Results indicated that pNVP, Kollidon and EOBO are very soluble in the solvent dipropylene glycol methyl ether (DPGME), and Guar, poly glycol and Carbopol are slightly soluble. In Macromer A, pNVP and Kollidon are very soluble, while EOBO and HPMC have slight solubility. pNVP and HPMC are slightly soluble in Macromer B. In Macromer C, only Kollidon is slightly soluble. We did not study the effect of temperature or of macromer concentration on the solubility values.

8.2a Solubility in DPGME

Figure 16 shows the optical density of the comfort agents in DPGME. There are comfort agents that have obviously very high optical density such poly glycol, Dextran, Carbopol and Guar. The most probably causes are low solubility and poor match of refractive index, as evidenced by the steep slope of the plots. Although these agents have high scattering, Guar and poly glycol are slightly soluble with solubility limits of 0.01wt% and 0.02wt% respectively

(Figure 16 and Figure 17). Carbopol appears to have an intermediate solubility of about 0.11% as evidenced by the break in the curve at that point. Even though NaHA appears to be insoluble, it has a fairly low scattering and low overall optical density. Likewise, CMC appears to be insoluble, but has very low light scattering.

Three comfort agents appear to have very high solubility, such that we could not see evidence of insolubility at less than 1% by mass. These are pNVP, Kollidon, and EOBO. They also have very low absorption.

Some general observations about the solubility of these agents in DPGME are as follows. **Poly glycol** took a while to break up and become dispersed in the DPGME. Each dilution was very cloudy and there were particulates that settled very quickly. **CMC** solutions seemed to have distinct particles suspended with no real cloudiness. **HPMC** solution dilutions were cloudy and the particles settled slowly. **Dextran** solutions were cloudy, but became less so with each dilution. The particles stayed suspended for a while, but eventually settled out. The particles seemed to aggregate on the sides of the glass vials. **NaHA** solutions were cloudy and the particles seemed to go to the top of the solution after sitting for a while. **Carbopol** solutions were cloudy, but became less so with each dilution. The last dilution seemed pretty clear. **Guar** was very cloudy and yellow, and also had particles that settled out very quickly. **EOBO** dissolved very slowly, going from a semisolid pellet, to slowly becoming a paste in solution; finally the solution went completely clear. **Kollidon** dissolved very quickly and each solution was completely clear. **pNVP** seemed to be very clear in all concentrations with almost no visible particles suspended. The dilutions didn't seem to become clearer by visual inspection, but the spectrophotometer showed decreasing absorbance.

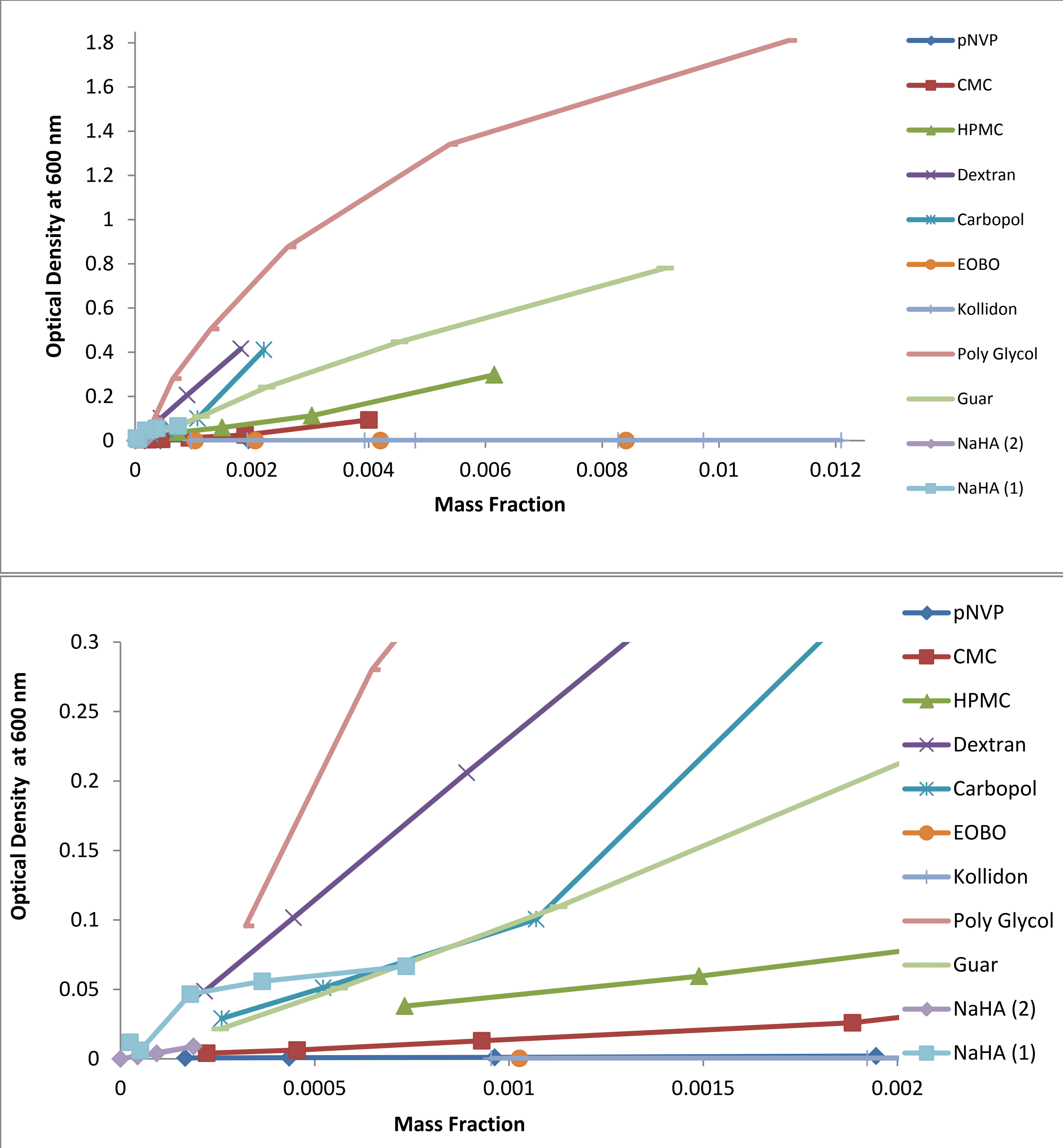

Figure 16: Overlay plots of optical density of comfort agentsin DPGME over the entire range (top), at low mass fraction (bottom).

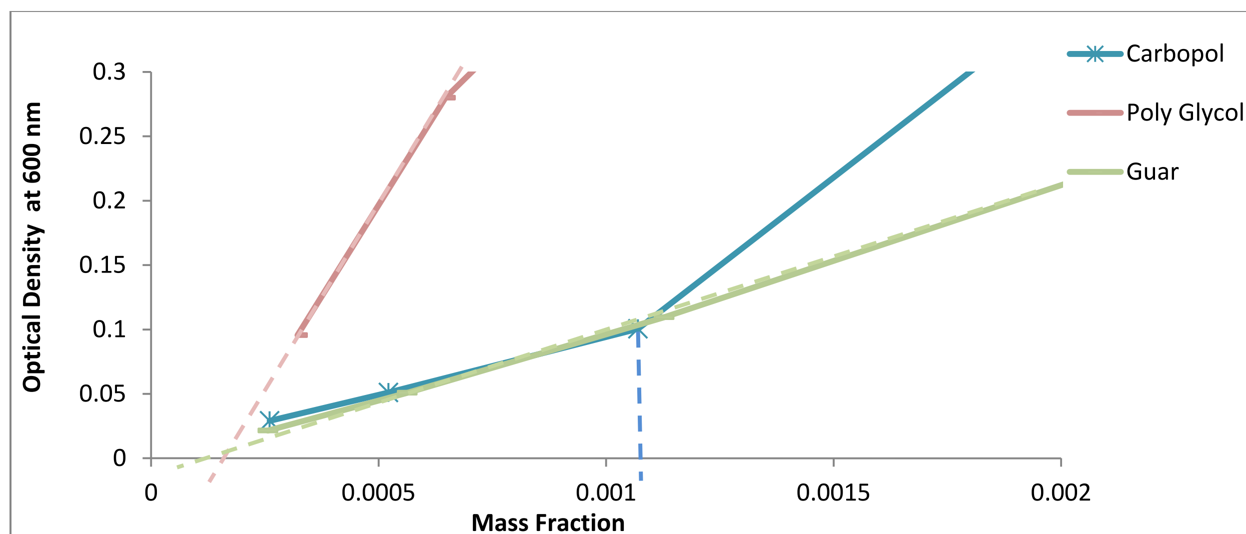

Figure 17: Detail of carbopol, poly glycol and guar solubilities in DPGME. The dotted lines help evaluating the solubilities.

8.2b Solubility in Macromer A

We received from ALCON two batches of Macromer A. The first was used in preliminary experiments to develop the experimental protocol. The data in the thesis was done on material from the second batch of Macromer A in DPGME.

The comfort agents produced much more scattering in Macromer A than in the solvent. The data are presented in Figure 18. The macromer itself had small floating particles in it that we could not centrifuge out or remove. It had an optical density of 0.1 compared to the DPGME reference. Dextran, Carbopol, CMC, Guar and NaHA (data not shown) were still completely insoluble and had very high optical density. Poly glycol, which was marginally soluble in DPGME, was insoluble in Macromer A. EOBO, which was soluble in DPGME, became marginally soluble in Macromer A, with an estimated solubility limit of 0.06%.(Figure 18 and Figure 19) Interestingly, HPMC, which appeared insoluble in the DPGME solvent, was slightly soluble in Macromer A, with a limit of about 0.027%. pNVP and Kollidon remained totally soluble to the limit investigated (about 1%).

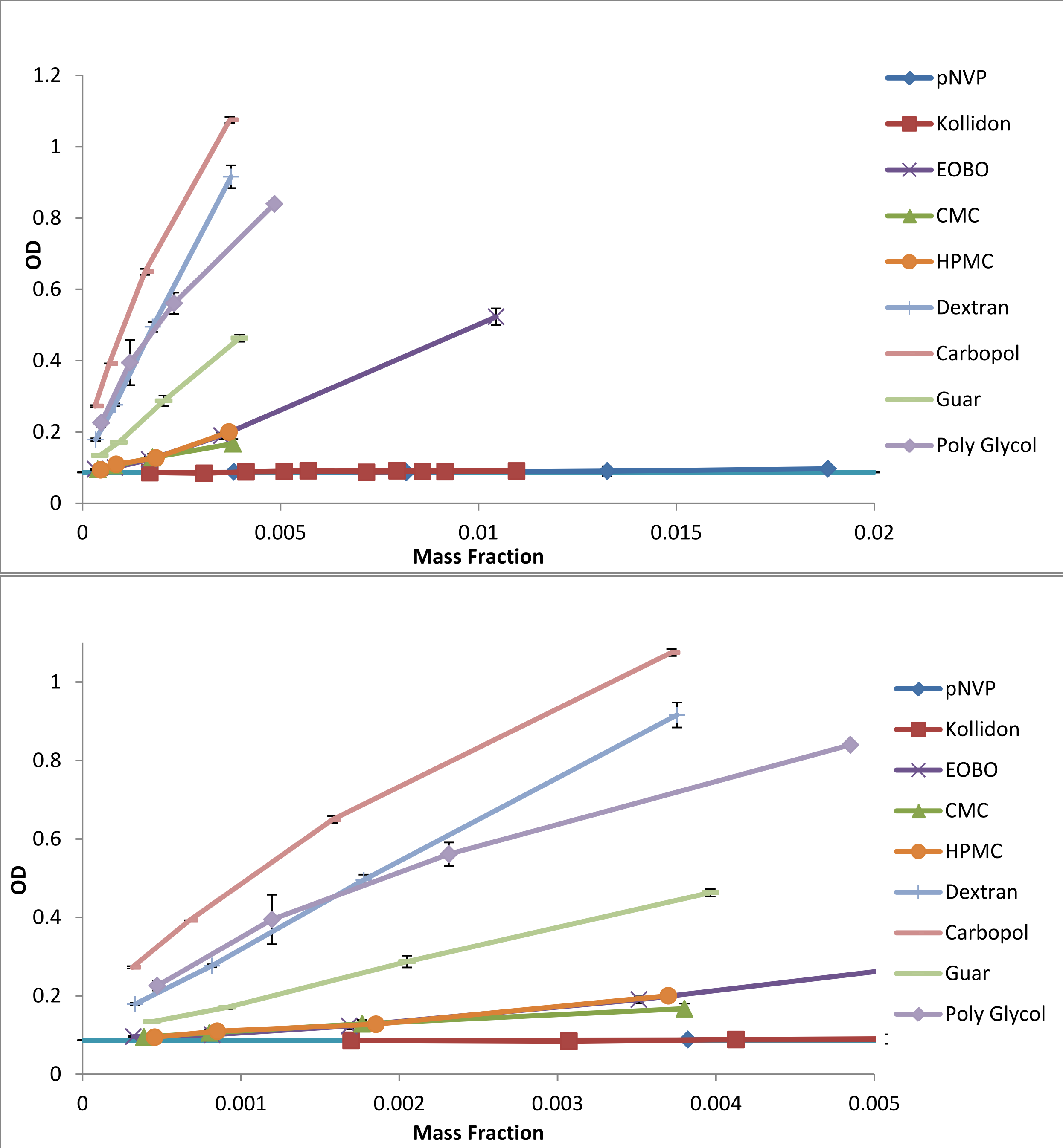

Figure 18: Overlay plots of optical density of comfort agents in Macromer A at high mass fractions (top) and at low mass fractions (bottom). Error bars show standard deviations of repeat measurements.

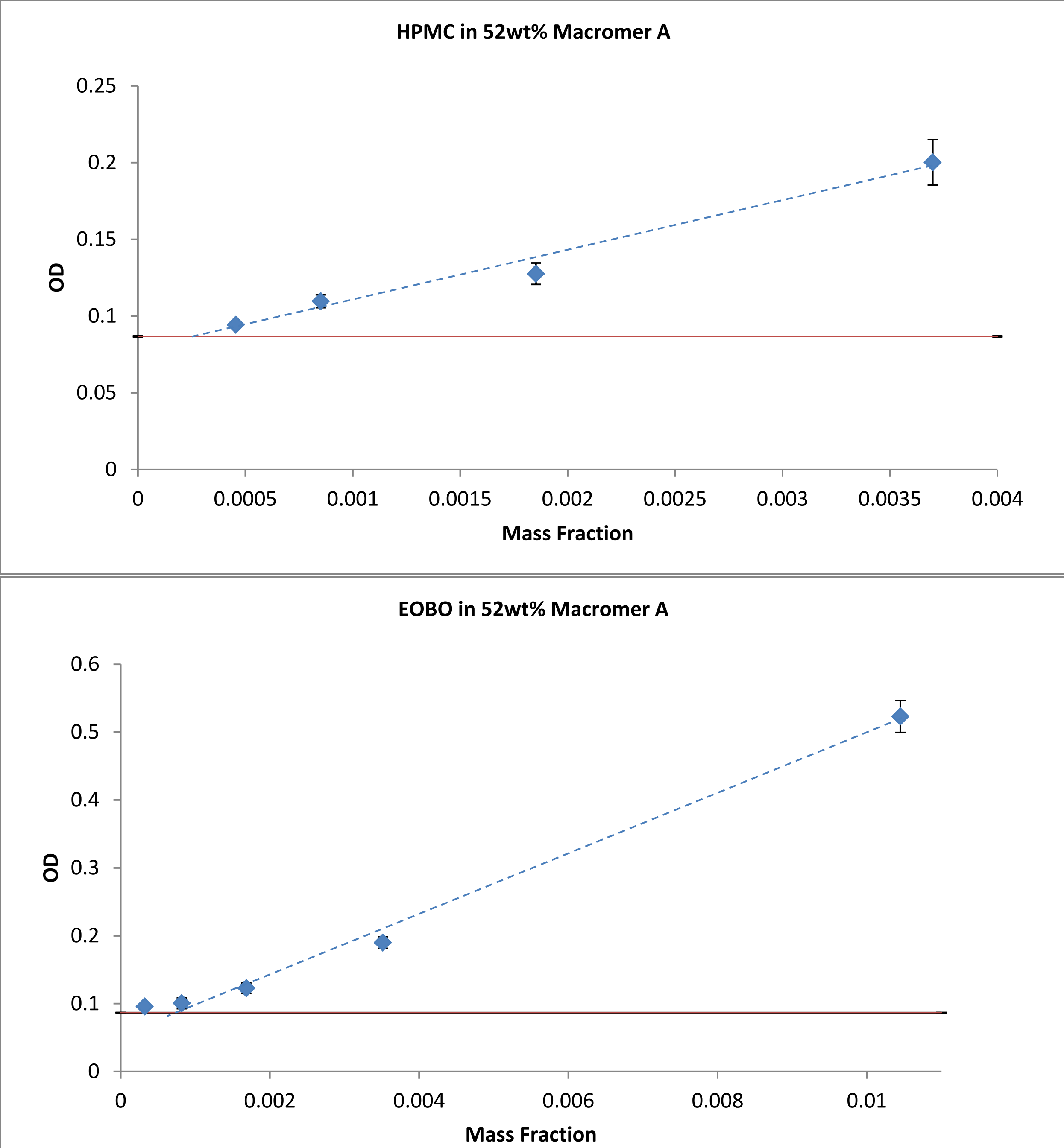

Figure 19: Detail of HPMC (top) and EOBO (bottom) solubilities in Macromer A. The red line indicates the OD of the pure Macromer A. Error bars show standard deviations of repeat measurements. The dotted lines are generated from linear regression of the data to evaluate the solubilities.

8.2c Solubility in Macromer B

We received from ALCON sample Macromer B in DPGME.This macromer did not have suspended particles. It had an optical density of 0.03 compared to the DPGME reference. All of the comfort agents appeared to be insoluble in Macromer B with the exception of pNVP and HPMC. Even Kollidon appeared to be insoluble, in that the plot of optical density vs concentration intersected the y-axis above the OD of the Macromer B (See Figure 20 and Figure 21). However, the OD of Kollidon remained very low, even at high concentrations. HPMC, with an estimated solubility limit of 0.006% had somewhat high OD beyond the solubility limit. pNVP had a solubility limit of 0.2% (Figure 21) .

The solubility of NaHA was not measured in this macromer by instruction of ALCON personnel who indicated that since it was not soluble in the DPGME or the 60% Macromer A, we did not need to measure the solubility in the other macromers.

8.2d Solubility in Macromer C

We received from ALCON sample Macromer C in DPGME. This macromer did not have suspended particles. It had a low optical density of 0.0126 compared to the DPGME reference. All of the comfort agents appeared to be insoluble in Macromer C with the exception of Kollidon (See Figure 22 and Figure 23). Even pNVP (Figure 23) and HPMC were not soluble in this macromer. Although Kollidon was slightly soluble, beyond the solubility limit of about 0.00026 mass fraction, the OD of Kollidon increased very rapidly, suggesting a poor match in refractive index (Figure 23). The solubility of NaHA was not measured in this macromer by instruction of ALCON personnel who indicated that since it was not soluble in the DPGME or the 60% Macromer A, we did not need to measure the solubility in the other macromers.

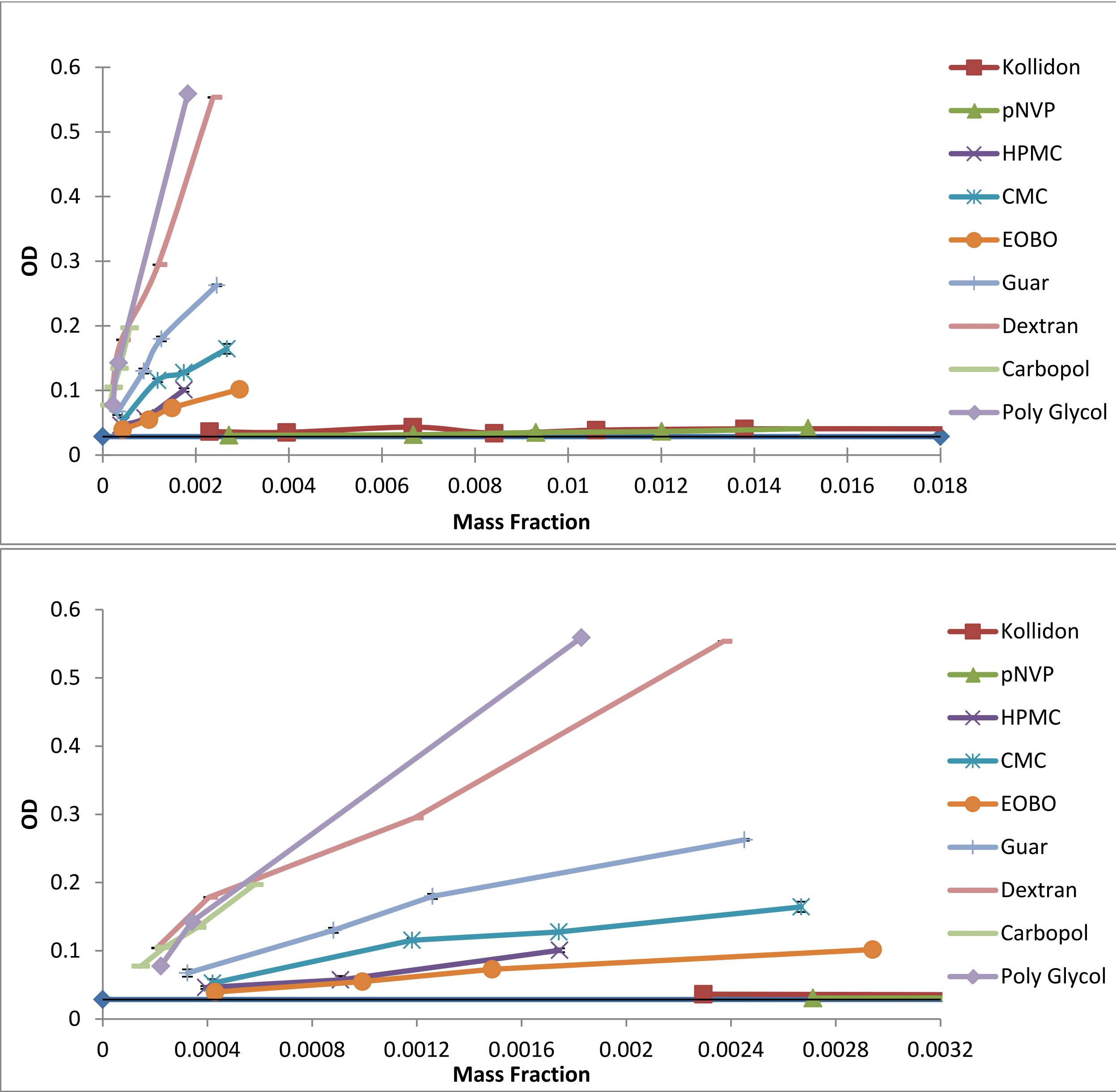

Figure 20: Overlay plots of optical density of comfort agents in Macromer B over the entire range (top) and at low mass fractions and (bottom). Error bars show standard deviations of repeat measurements.

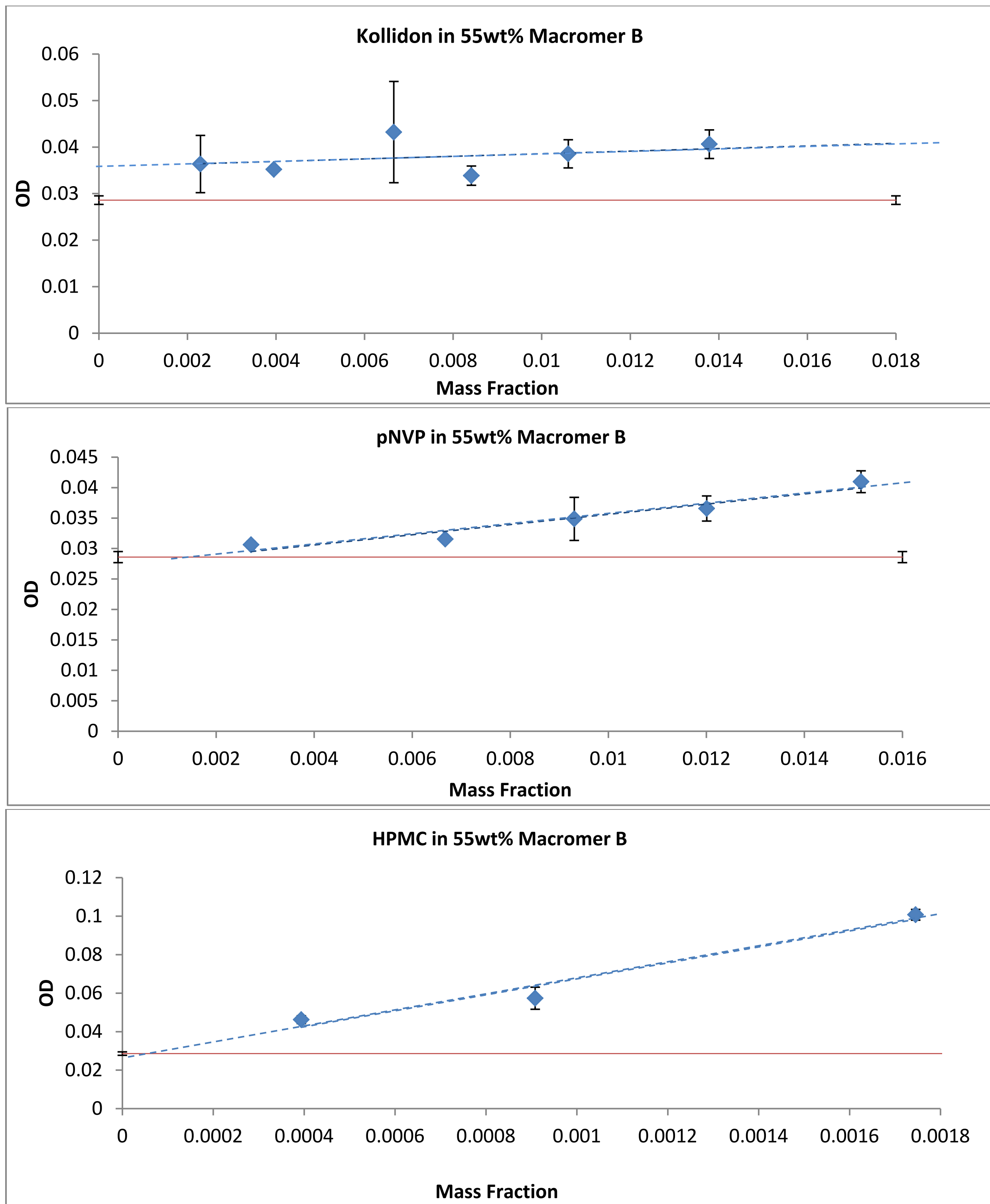

Figure 21: Detail of Kollidon (top), pNVP (middle) and HPM (bottom) solubilities in Macromer B. The red line indicates the OD of the pure Macromer B. Error bars show standard deviations of repeat measurements. Error bars show standard deviations of repeat measurements. The dotted lines are generated from linear regression of the data to evaluate the solubilities.

A summary of the estimated solubilities are given in Table8. It appears that pNVP, Kollidon, HPMC and perhaps HPMC may be candidates for agents in SiHy contact lenses. While pNVP and Kollidon both contain polyvinylpyrrolidone chains, Kollidon is a copolymer with additional polyvinylacetate units, it is not surprised that they behave similarly, and the slight difference in solubility between them probably due to the acetate groups.

The next step of this research would be to determine if the polymerization process reduces the solubility of these agents.

Table 8: Summary of the estimated solubilities of comfort agents in DPGME and macromer formulations. An infinite solubility indicates no significantincrease in OD compared with the control.

Comfort Agent	DPGME	Mac A	Mac B	Mac C
pNVP	Infinite	Infinite	0.17%	Insoluble
Kollidon	Infinite	Infinite	Insoluble	0.030%
EOBO	Infinite	0.060%	Insoluble	Insoluble
CMC	Insoluble	Insoluble	Insoluble	Insoluble
HPMC	Insoluble	0.027%	0.006%	Insoluble
Dextran	Insoluble	Insoluble	Insoluble	Insoluble
Guar	0.010%	Insoluble	Insoluble	Insoluble
Poly glycol	0.020%	Insoluble	Insoluble	Insoluble
Carbopol	0.11%	Insoluble	Insoluble	Insoluble
HA	Insoluble	Insoluble	not measured	not measured

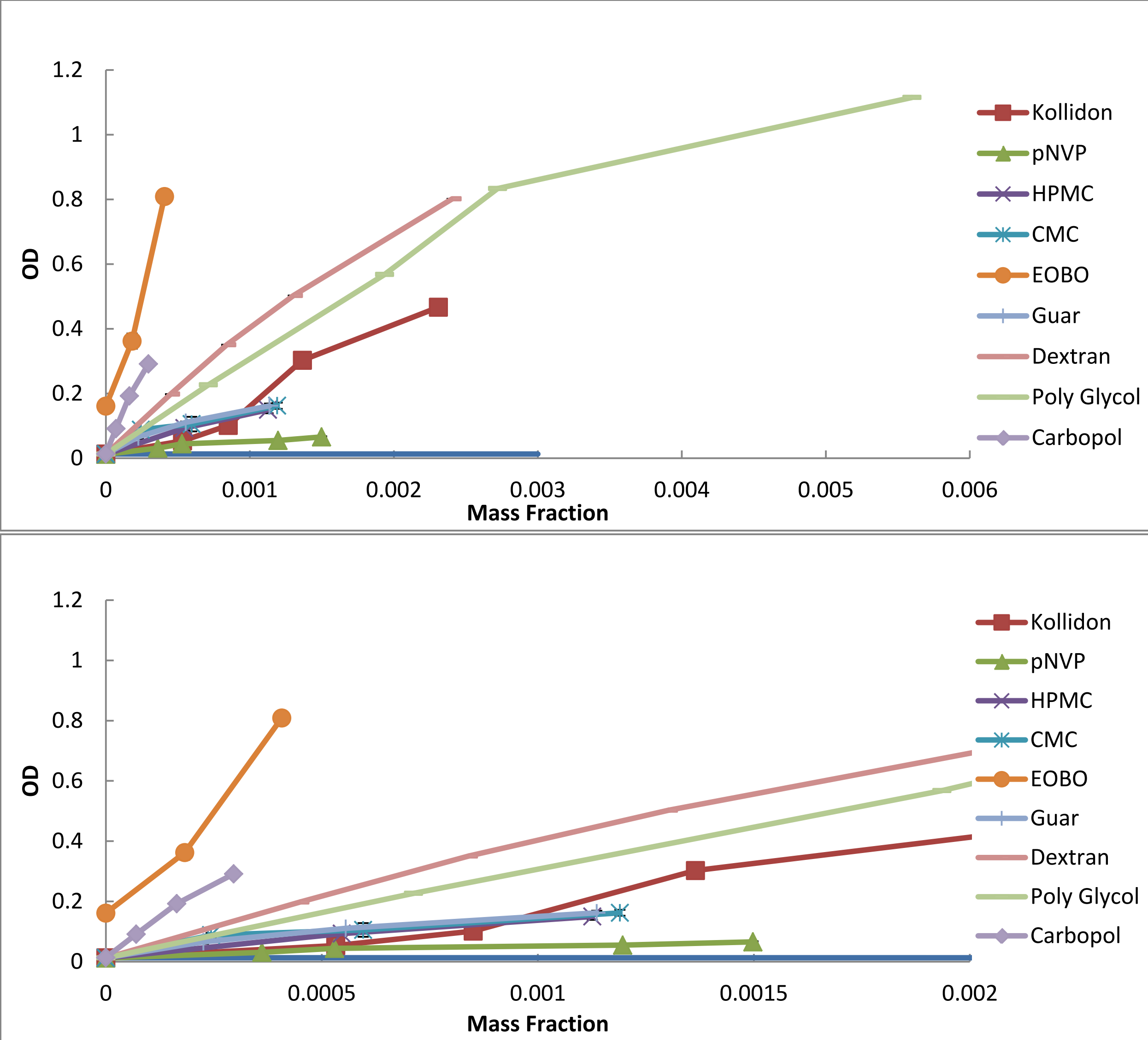

Figure 22: Overlay plots of optical density of comfort agents in Macromer C over the entire range (top) and at low mass fraction (bottom). Error bars show standard deviations of repeat measurements and are sometimes smaller than the data symbols.

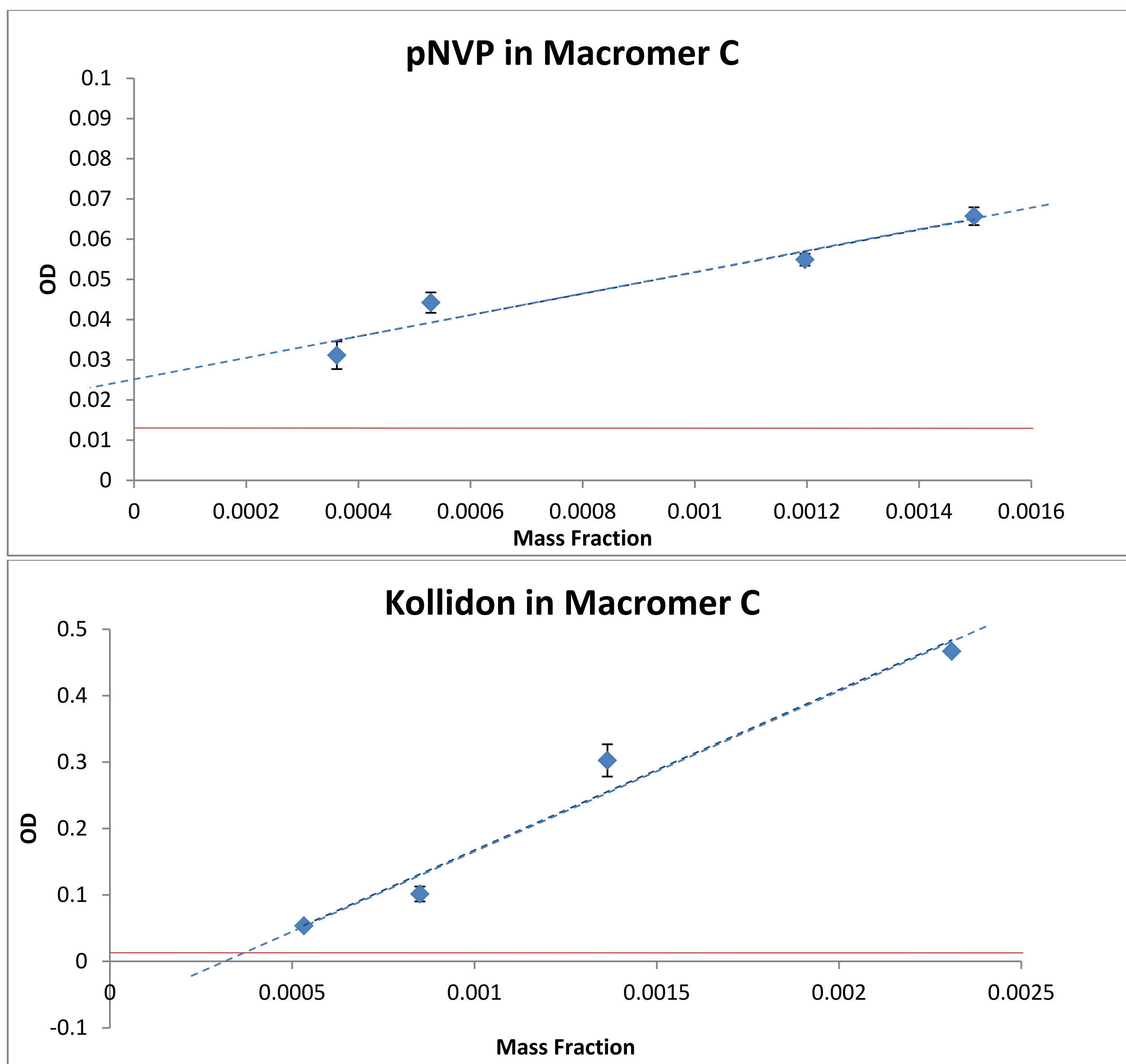

Figure 23: Detail of pNVP (top) and Kollidon (bottom) solubilities in Macromer C. The red line indicates the OD of the pure Macromer C. Error bars show standard deviations of repeat measurements. Error bars show standard deviations of repeat measurements. The dotted lines are generated from linear regression of the data to evaluate the solubilities.

CHAPTER 9 CONCLUSIONS AND RECOMMENDATIONS

In this research, multi-purpose solutions (MPSs) for lens care were not found capable of significantly preventing or removing lipid deposition from artificial tear fluid to silicone hydrogel (SiHy) contact lenses, and their performances were similar or were not statistically different when comparing with a simple boric buffered solution (BBS). Also an experimental peroxide-based solution performed similarly to other MPSs in its ability to remove lipids. An additional rubbing step while using MPSs improved the lipid removal slightly. However, the overall removal percentages are still very low (< 1% of cholesterol sorption were removed from senofilcon A lenses with PureMoist or SEEDO). In addition, the benefits from rubbing were independent of solution types, and the effects in general are not different than rubbing lenses with BBS ($p>0.05$). The disappointing result suggests that the surfactants in the MPSs are not hydrophobic enough to remove lipids from SiHy lenses during a simulated overnight soak.

Compared to proteins, lipids are much smaller in size, so the majority of the lipids might be absorbed into the matrix rather than adsorbed on the surface. The surface wettability of a lens mainly depends on its surface energy. Lipids that absorb deep inside a lens may affect the lens clarity but may have much less influence on the surface wettability and comfort felt by the lens wearer. Although the MPSs that were investigated in this research did not remove significant amounts of lipid from the SiHy lenses as hoped, a lens could remain highly wettable if MPSs were only removing the lipid adsorption on the lens surface. A future study could focus on determining the lipid concentration profile through the lens thickness. A radiolabel technique can

be employed to detect the profile of the cross section of a lens. Another study could evaluate the surface wettability of the lenses before and after soaking in MPSs with or without rubbing. Then the relationship between lipid deposition and comfort could be correlated to aid the development of contact lens products. Captive bubble techniques are commonly applied to evaluate surface wettability of polymers and could be used in such a study on contact lenses.

There is a lot of potential in developing SiHy lenses with controlled release of comfort agents. In this research (Chapter 8), some agents were identified to be compatible with several SiHy macromer formulations. Unfortunately ALCON chose not to reveal the chemistry of their formulations so it is difficult to relate the data to chemical principles. Likewise it is difficult to suggest further studies. It would be exciting to apply molecular imprinting techniques to enhance loading capacity and to manipulate the release kinetics, so specific release profiles could be developed to suit different types of wear. Future researches in such area could identify potential functional monomers to create specific imprinted sites for comfort agents in SiHy polymers, and optimize the concentrations of comfort agents and functional monomers to achieve the desired performances.

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APPENDIX

Protocol of preparing artificial tear fluid

Materials Needed: 100 mL Volumetric Flask, 500mL Volumetric Flask, Hot/Stir Plate, 1.5cm Stir Bar, Sharpie Marker, Double Distilled Water (500 mL), Boric Acid (5 g), Sodium Borate (0.6 g), Sodium Chloride (2 g), Cholesterol Hot (0.54 mL ~0.03wt% Chol in Ethanol), DPPC Hot (0.108 mL ~0.00025wt% DPPC in Ethanol), Cholesterol Cold (0.19mL of 16mg/mL in chloroform), DPPC Cold (0.2mL of 16mg/mL in chloroform), Methyl-β-cyclodextrin (0.2mL of 40mg/mL in chloroform), Cholesteryl Linoleate (0.2mL of 33mg/mL in chloroform), Oleic Acid (0.2mL of 2mg/mL in chloroform), Methyl Oleate (0.2mL of 44mg/mL in chloroform), Triolein (0.2mL of 5mg/mL in chloroform), Lysozyme (2.68 mg), Lactoferrin (3.58 mg), Albumin (8.7 mg), Mucin (10 mg)

Purpose: To prepare artificial tear solution containing radiolabeled Cholesterol (^{14}C) and DPPC (^{3}H).

Preparation of borate buffered saline

1. Prepare 1 volumetric flask by thoroughly cleaning out the flask. Fill flask to 500 mL with deionized water and add a large stir bar. Mark the level on the glass using a sharpie marker. Empty out the flasks and clean thoroughly before placing stir bar back in the flask. Autoclave both flasks for 10 minutes at 121°C. Autoclave tape can be used to ensure proper autoclaving.
2. Fill flask with 500 mL double distilled water. Carefully measure out 5 grams Boric Acid, 0.6 gm Sodium Borate and 2 grams Sodium Chloride and add to flask while stirring. Stir until the chemicals are completely dissolved. Test pH of the water and add NaOH or HCl until the pH is 7.3. Place mixture in the fridge with a lid on top.

Preparation of materials for tear solution (if not already prepared)

Cold cholesterol preparation (16mg/mL)

1. Pipette 10 mL of Chloroform into Scintillation Vial
2. Carefully weight out 160 mg Cold Cholesterol and add to vial

3. Label with black marker and store in freezer

Cold DPPC preparation (16mg/mL)

1. Pipette 10 mL of Chloroform into Scintillation Vial
2. Carefully weight out 160 mg Cold DPPC and add to vial
3. Label with black marker and store in freezer

Methyl-β-cyclodextrin preparation (40mg/mL)

1. Pipette 10 mL Chloroform into Scintillation vial
2. Weigh out 400 mg Methyl-β-cyclodextrin and add to vial
3. Label with black marker and store in freezer

Cholesteryl oleate esters preparation (33mg/mL)

1. Pipette 10 mL Chloroform into Scintillation vial
2. Weigh out 330 mg Cholesteryl Linoleate and add to vial
3. Label with black marker and store in freezer

Oleic acid preparation (2mg/mL)

1. Pipette 10 mL Chloroform into Scintillation vial
2. Weigh out 20 mg Oleic Acid and add to vial
3. Label with black marker and store in freezer

Methyl oleate preparation (44mg/mL)

1. Pipette 10mL Chloroform into Scintillation vial
2. Weigh out 440 mg Methyl Oleate and add to vial
3. Label with black marker and store in freezer

Triolein preparation (5mg/mL)

1. Pipette 10mL Chloroform into Scintillation vial
2. Weigh out 50 mg Triolein and add to vial
3. Label with black marker and store in freezer

(Cold cholesterol, methyl-β-cyclodextrin, cholesteryl oleate esters, oleic acid, methyl oleate, triolein in fridge, DPPC in freezer. Hot Cholesterol in fridge)

Preparation of artificial tear fluid

Lipids addition

1. Mark the liquid level on a 100mL volumetric flask with 100 ml of water and a 1.5 cm stir bar.
2. Wash the volumetric flask with the 1.5cm stir bar with chloroform 3 to 4 times, and dry the flask completely with nitrogen.
3. ln the volumetric flask with no stir bar, pipette 0.54 mL from the Hot Cholesterol and 0.108 mL from the Hot DPPC Vial
4. Pipette 0.2 mL of the Cold DPPC, Methyl-β-cyclodextrin, Cholesteryl oleate Esters, Oleic Acid, Methyl Oleate, Triolein solutions into vial.
5. Pipette 0.19 mL Cold Cholesterol solutions into vial.
6. Rinse the vial with 1 mL chloroform
7. Place in hood under nonreactive gas to evaporate for 2 hours
8. Vacuum until dry for 2 hours
9. Add 50 mL BBS and the stir bar to rehydrate lipids for at least 5-6 hours at 750 rpm

Proteins addition

1. Add sequentially 2.68 mg Lysozyme, 3.58 mg Lactoferrin, 8.7 mg Albumin, 10 mg Mucin to solution in vial. (The scale takes time to weight such small substances, so add the proteins in small portions and each time close the scale window and wait for 20 seconds to show the actual weight.)
2. Stir at the same speed until completely dissolve. (Overnight, at least 8 hrs.)
3. Now add rest of BBS until you reach the new 100 mL mark
4. Label Volumetric Flask “Tear Solution” and keep stirring until the use of the soltuion

Protocol of the experiment of reduction of lipid deposition in chapter 4

*First Follow "Protocol to Prepare ATF" to prepare BBS and ATF solutions

Materials Needed: Borate Buffered Saline, 100 mL of Radiolabeled Tear Solution, Experimental Solutions, Contact Lenses, 5 mL vials (24 + 1 for each contact lens that will be resting in solution), Pipette and Tips, Kim Wipes, Well Plates (2), Forceps, Sharpie Markers (Red/Black), Deionized Distillated H2O

Purpose: To measure Effect of Solutions on Cholesterol and Phospholipid uptake on hydrogel lenses

Soaking in solution

1. Label 2 sets of 5 mL vials with the contact brand and number
2. Pipette 2 mL of solution (A,B,C, or D) into each vial keeping track of which solution is in each vial
3. Gently blot lens on kimwipe
4. Place contact in one of the first set of vials corresponding to its number and brand
5. Repeat placement for each lens (Steps 3-6)
6. Once complete for all lenses, let lenses sit for 16 hours at room temperature
7. Label an additional set of vials for a set of lenses that will not soak (serve as control)

Sorption of lipids

1. Pipette 2mL of Tear solution into second set of labeled vials
2. Remove lens that have been soaking for 16 hours from first set of vials and rinse with BBS
3. Dab dry with Kimwipe.
4. Place lens in corresponding vial that contains Tear Solution.
5. Remove set of "control" (no soak) lenses from blister pack and rinse with BBS, blot dry with kimwipe, place lens in tear solution
6. Place all vials in shaker at body temperature (37°C) for 16 hours
7. Prepare hot standards ([100µL TS]×3, [100µL TS + 3mL n-propanol] ×3)
8. Prepare background standards ([10mL SL] ×2, [3mL npropanol+10mL SL] ×2).

Extraction of lipids

1. Prepare 10 mL scintillation vials containing 2 mL n-propanol for each lens
2. Remove vials from shaker after 16 hours has expired
3. Fill 2 sets of 24 count wells with DDH20
4. Remove contact lens from tear solution vial, rinse with DDH2O into rinse cup and place in first well
5. Repeat until all the contacts are resting in wells
6. Take the contact lenses out of the first set of wells, rinse again, and place in second set of wells
7. Remove lenses from second set of well, rinse again, and blot dry with kimwipe
8. Place lenses into appropriate n-propanol vials to begin 3 n-propanol extractions on lenses (follow standard procedure)
9. Count the cholesterol and tritium adsorption on each lens

N-propanol extraction

1. Prepare vials by pipetting 2 mL n-Propanol in three extraction vials for each lens
2. Place Lenses into first vial
3. Allow to shake in incubator for 1 hour
4. Rinse each lens with 1mL n-Propanol into the first extraction vial before transferring to the second extraction vial
5. Repeat for the second extraction vial before placing into last extraction vial
6. Similarly, rinse each lens with 1mL n-Propanol before placing into direct counting vial of 10 mL Scintillation Fluid
7. Add 10mL scintillation fluid to the extraction vials
8. Count all vials twice to ensure that machine is counting correctly

Protocol of the experiment of removal of sorbed lipid in chapter 5

*First Follow "Protocol to Prepare TS" to prepare BBS and TS solutions

Materials Needed: Borate Buffered Saline, Radiolabeled Tear Solution (100 mL), Experimental Solutions, Contact Lenses, 5 mL vials (2 for each contact lens), Pipette and Tips, Kim Wipes, Well Plates (2), Forceps, Sharpie Markers (Red/Black), Double Distilled H2O

Purpose: To measure the effect of cleaning solution on removal of Cholesterol and Phospholipid

Sorption of lipids

1. Label 2 sets of 5 mL vials with the contact brand and number
2. Pipette 2 mL of radiolabeled Tear solution into each vial
3. Remove lenses from blister packs and gently blot on Kimwipe
4. Place lens in corresponding vial that contains Tear Solution
5. Place all vials in shaker at body temperature (37°C) for 16 hours
6. Prepare hot standards ([100µL TS]×2, [100µL TS + 100µL BBS]×2, [100µL TS + 3mL n-propanol] ×2)
7. Prepare background standards ([100µL BBS] ×2, [200BBS] ×2, [3mL n-propanol] ×2).

Soaking in solution

1. Pipette 2mL of solution (A,B,C, or D) into each vial keeping track of which solution is in each vial with one control of just BBS
2. Remove vials from shaker after 16 hours has expired
3. Fill 2 sets of 24 count wells with DDH20
4. Remove contact lens from tear solution vial, rinse with DDH2O into rinse cup and place in first well
5. Repeat until all the contacts are resting in wells
6. Take the contact lenses out of the first set of wells, rinse again, and place in second set of wells
7. Remove lenses from second set of well, rinse again, and blot dry with kimwipe
8. Place contact in one of the first set of vials corresponding to its number and brand

9. Take 0.2 mL samples of the lens in solution
10. Repeat rinse and placement for each lens (Steps 3-6)
11. Take 0.2 mL samples of the lens in solution at times 1,2 and 4 hrs
12. At 8 hrs soaking, remove lens and place into 2 mL n-propanol for standard extraction removal.
13. Take 0.2 mL sample from the left over lens solution.
14. Count the cholesterol and tritium adsorption on each lens.

Protocol of the experiment of rubbing and peroxide-based solution in chapter 6

*First Follow "Protocol to Prepare TS" to prepare BBS and TS solutions

Materials Needed: Borate Buffered Saline (for rinsing), Radiolabeled Tear Solution (2 per mL), Experimental Solutions, Contact Lenses, 5 mL vials (2 for each contact lens), Pipette and Tips, Kim Wipes, Well Plates (2), Forceps, Sharpie Markers (Red/Black), Double Distilled H2O, BBS

Purpose: To measure the effect of rubbing on removal of Cholesterol and Phospholipid

Sorption of lipids

1. Label 2 sets of 5 mL vials with the contact brand and number
2. Pipette 2 mL of radiolabeled Tear solution into each vial
3. Remove lenses from blister packs and gently blot on Kimwipe
4. Place lens in corresponding vial that contains Tear Solution
5. Place all vials in shaker at body temperature (37°C) for 16 hours
6. Prepare hot standards ([100µL TS]×2, [100µL TS + 100µL BBS]×2, [100µL TS + 3mL n-propanol] ×2, [100µL TS + 3mL heptanol])
7. Prepare background standards ([100µL BBS] ×2, [200BBS] ×2, [3mL n-propanol] ×2, [3mL heptanol] ×2).

Removal of sorbed lipids with rubbing step

1. Pipette 2 mL of solutions into each vial (use neutralization vials when using peroxide-based solution) keeping track of which solution is in each vial.
2. For lenses that does not required rubbing, skip step 2 to 11
3. Transfer 400 µL of MPS into zipper bags
4. Remove vials from shaker after 16 hours has expired
5. Fill 2 sets of 24 count wells with DDH20
6. Remove contact lens from tear solution vial, rinse with DDH2O into rinse cup and place in first well
7. Repeat until all the contacts are resting in wells
8. Take the contact lenses out of the first set of wells, rinse again, and place in second set of wells

9. Remove lenses from second set of well, rinse again, and blot dry with kimwipe
10. Place lenses into the center of the bags and close the bags. Place the bag on the left palm, use the right thumb to rub the lens 20 times in circular motion, focus on the peripheral region.
11. Take 200 μL of MPS sample after rubbing to scintillation vials filled with 10 mL of scintillation fluid
12. Rinse the lens with DDH20 and blotted dry with kimwipe
13. Place contacts in one of the first set of vials corresponding to its number and brand
14. Take 0.2 mL samples of the lens in solution
15. Repeat rinse and placement for each lens (Steps 3-6)
16. Take 0.2 mL samples of the lens in solution at times 1,2 and 4hrs
17. At 8hrs soaking, transfer lens into 2 mL n-propanol for standard extraction removal.
18. Take 0.2 mL sample from the left over lens solution.

Extraction of bags

1. Rinse the bags with DDH2O three times and transfer 2 mL of heptanol to the bags
2. Close the bag and keep the bag horizontal and let it sit at room temperature for one hour
3. Transfer the heptanol in bags to scintillation vials containing 10 mL of scintillation fluid
4. Rinse the bags with additional 1mL of heptanol to the scintillation vials
5. Repeat the extraction procedure by transfer another 2 mL of heptanol to the bags and wait for an hour, then use 1 mL of heptanol to rinse the bags into the same vial
6. Extract the bags three times in total
7. Place 10 mL of Scintillation fluid in the bags and place them in scintillation vials (Direct Counting)
8. Submit all generated vials to liquid scintillation counter, use program 1 that count both 3H and 14C to quantify the amount of DPPC and CH in each vial.

Protocol of the experiment of solubility of comfort agents in Chapter 8

Materials Needed: Comfort agents, DPGME,

Devices: Beckman 550UV-Vis spectrometer, Mettler microbalance, centrifuge extractor, optical cuvette (UV-light compatible), a motar and pestle

Purpose: To measure the optical densities of a series of solutions of comfort agents in potential contact lens macromers.

Solubility in DPGME

1. Weigh the solvent on an analytical balance in a cuvette
2. Weigh small amount of comfor agent powder and add to the cuvette to achieve desired wt%
3. Stir the solution with a megnetic stir bar until completely dissolved
4. Centrifuge the cuvette for 5 minutes to remove any gas bubble that would scatter light
5. Prepare the reference OD with pure DPGME
6. Meausre the optical density of the cuvette containing the solution at 600nm
7. Weight additional DPGME and add to the cuvette to create a lower wt% of comfort agent
8. Recorded the wt% and repeat step 3 to 6
9. Obtain enough data point and plot them to estimate the solubility limits.

Solubility in macromer fomulations

1. Transfer known masses of macromer to a mortar
2. Weight small amount of comfort agent on a small piece of aluminum foil with a microbalance, transfer comfort agent to the martor
3. Meausre the foil again to obtain the excat transferred weight (same as the macromer)
4. Mixed the solution with the pestle and transfer to an optical cuvette
5. Centriguge the cuvette to remove air bublles
6. Meausre the OD of the solution at 600nm
7. Repeat step 2 to 6 to obtain data point at higher wt% of the comfort agent
8. Plot the OD and estimate the solubility of the comfort agent graphicaly