Lipid Deposition on Hydrogel Contact Lenses

By

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I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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ABSTRACT

The primary objective of this study was to quantify and characterise lipid deposition on soft (hydrogel) contact lenses, particularly those containing siloxane components. Studies involving a variety of *in vitro* doping and *in vivo* worn contact lenses were undertaken, in which lipid deposition was analyzed by either TLC or HPLC. Specific experiments were completed to optimize a method to extract the lipid from the lens materials, to compare the total lipid deposition on nine different hydrogel lenses and to analyze the effect that lipid deposition had on wettability. A method for extracting lipid from contact lenses using 2:1 chloroform: methanol was developed. This study also showed that siloxane-containing contact lens materials differ in the degree to which they deposit lipid, which is dependent upon their chemical composition. Small differences in lipid deposition that occur due to using variations in cleaning regimens were not identifiable through TLC, and required more sophisticated analysis using HPLC. Contact lens material wettability was found to be influenced by *in vitro* lipid deposition. Specifically, conventional hydrogels and plasma surface-treated silicone-hydrogel materials experienced enhanced wettability with lipid deposition. Reverse-phase HPLC techniques were able to quantify lipid deposits with increased sensitivity and accuracy. From the HPLC studies it was found that contact lens material, concentration of the lipid doping solution, and the composition of the lipid doping solution in *in vitro* deposition studies influenced the ultimate amount and composition of lipid deposits. *In vivo* HPLC studies showed that the final lipid deposition pattern was influenced by the interaction between the composition of the tear film and the various silicone hydrogel contact lens materials. In conclusion,

HPLC analysis methods were more sensitive and quantitative than TLC. Lipid deposition was ultimately influenced by the concentration and composition of the lipid in the tear film and the contact lens material. Contact lens wettability was influenced by the presence and deposition of lipid onto the contact lens surfaces. Finally, this reverse-phase HPLC lipid analysis protocol was not the most sensitive, robust, or accurate. In the future, other methods of analysis should be explored.

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DEDICATION

This thesis is dedicated to my loving husband Nicholas and my beautiful daughter Gabrielle. Thank you for all of your encouragement and understanding. You never lose faith in me, especially during the difficult times, and you are always completely supportive of everything that I do or aspire to do. This is a journey that is bound to have an exciting ending and better more fulfilling future. I am so glad that I have both of you to share in this journey with me. I love you both. You are my family.

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TABLE OF SYMBOLS

θ	Angle
Å	Angstroms
cm	Centi-meters
^{o}	Degrees
^{o}C	Degrees centigrade
>	Greater than
hr(s)	Hour(s)
<	Less than
µg	Micro-grams
µL	Micro-liters
µm	Micro-meters
mg	Milli-grams
mL	Milli-liters
mm	Milli-meters
min	Minutes
mV	Milli-Volts
nm	Nano-meters

TABLE OF ABBREVIATIONS

AA	Acuvue Advance
ACN	Acetonitrile
ANOVA	Analysis of Variance
AL	Alphafilcon A (Soflens 66)
ATS	Artificial Tear Solution
AV	Acuvue 2
BA	Balafilcon A (PureVision)
C	Cholesterol
CA	Contact Angle
CCLR	Centre for Contact Lens Research
CH	Conventional Hydrogels
CL	Contact Lens
CLASH	Contact Lens and Silicone Hydrogels
CW	Continuous Wear
DMA	N, N-dimethyl acrylamide
ET	Etafilcon A (Acuvue 2)
EW	Extended Wear
FDA	Food and Drug Administration
FND	Focus Night & Day
GA	Galyfilcon A (Acuvue Advance)
GC	Gas Chromatography
HEMA	2-hydroxyethyl methacrylate
HPLC	High-Performance Liquid Chromatography
LASIK	Laser-Assisted In Situ Keratomileusis
LDS	Lipid Doping Solution
LOA	Lotrafilcon A (Focus Night & Day)
LOB	Lotrafilcon B (O_2 Optix)

MS	Mass Spectrometry
NMR	Nuclear Magnetic Resonance
NVP	N-vinyl pyrrolidone
O_2	O_2 Optix
OA	Oleic Acid
OAME	Oleic Acid Methyl Ester
OASYS	Acuvue OASYS
OCA	Optical Contact Analyzer
OM	Omafilcon A (Proclear)
PBS	Phosphate Buffer Solution
PC	Proclear
PMMA	Polymethylmethacrylate
PO	Polymacon (Soflens 38)
PV	PureVision
PVA	Polyvinyl alcohol
PVP	Polyvinylpyrrolidone
Rf	Retention Factor
RGP	Rigid Gas Permeable
S38	Soflens 38
S66	Soflens 66
SE	Senofilcon A (Acuvue OASYS)
SEM	Scanning Electron Microscopy
SH	Silicone Hydrogel
SPA	Sustained Performance and Adaptation of Silicone Hydrogel Contact Lenses During Daily wear
TLC	Thin Layer Chromatography
TRIS	Trimethylsiloxy Siloxane
USAN	United States Adopted Names Council

1 INTRODUCTION

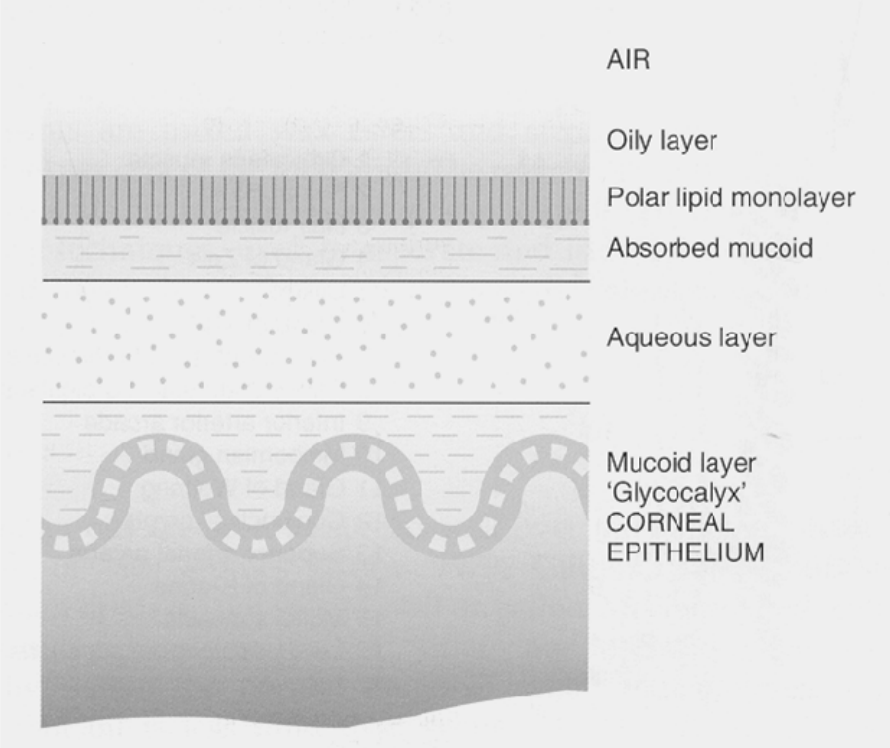
1.1 Structure of the tear film

The tear film is a highly structured film that lies on top of the conjunctiva and the cornea, which provides many specialized functions and therefore needs to be strictly maintained in terms of composition. The tear film is broadly described as having five main functions: it allows for a smooth optical surface by alleviating any small imperfections in the corneal epithelium; it protects the cornea from debris and foreign materials by forcing them away from the central cornea upon blinking; it provides oxygen and nutrition to the underlying corneal epithelium; it keeps the bulbar and palpebral conjunctiva moist and lubricated; and finally, the tear film contains various antibacterial and immunological agents to protect against ocular infection.[1]

Historically, the tear film was believed to be a fairly rigidly structured film, consisting of 3-layers, with an outermost lipid layer, middle aqueous layer and a mucin layer that lies closest to the ocular surface.[2, 3] Most studies suggest that the outermost lipid layer accounts for approximately 1-1.5% of the total thickness of the tear film (0.1 μm), while the aqueous layer makes up 98% (7 μm) and the mucin layer 0.5% (0.02-0.05 μm).[2-4] This model has been revised to reflect a more complex structure of the tear film, which suggests that the mucin layer is a more complex "gel-like" structure and that the lipid layer is far more complex than previously proposed. This updated tear film model proposes that the tear film has many more "layers", comprised of a superficial oily layer

against the air interface, polar lipid layer, absorbed mucoid, an aqueous layer and mucoid layer 'glycocalyx' on top of the corneal epithelium.[5-7] A diagrammatic view of this tear film model can be seen in Figure 1. Tiffany's arrangement of the tear film was proposed in 1988, now almost twenty years ago. Since then, the arrangement of the tear film model has not changed, but much research has been completed looking at the thickness of the tear film and all of the layers[8-11] as well as the specific components of each layer.[12-16]

Figure 1: Diagram of the 6-layer tear film model as proposed by Tiffany[5]



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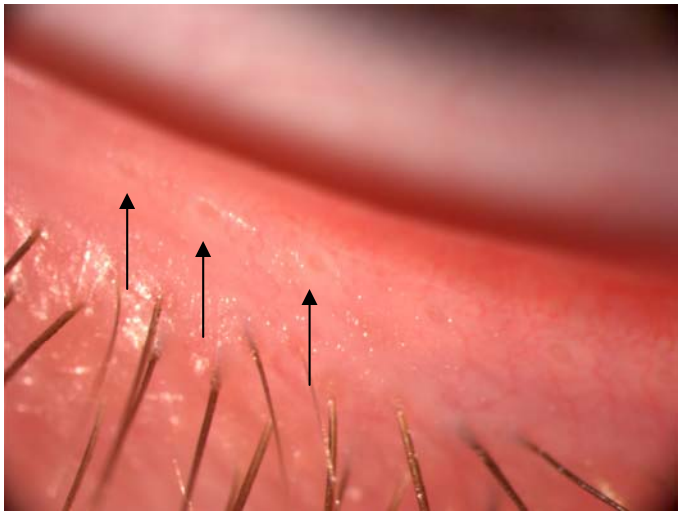
1.2 The meibomian glands: structure and function

Meibomian glands are holocrine glands that secrete lipids onto the ocular surface through an opening in the eyelid margin (Figure 2). The actual glands are found to be withdrawn into the tarsal plate, with 20–25 individual glands in the lower lid and 30-40 glands in the upper lid.[17-20] Individual glands are composed of acini that are attached together via a central duct running through the entire gland.[20] These acini produce both non-polar and polar lipids.[17-20] It is the individual cells in the acini that produce the lipid components and then release them into the central duct. This process is called acinar cell degeneration.[20] The lipids secreted give protection by providing a hydrophobic barrier to reduce the chance of tear overflow onto the lid margin. The lipids also function to form a seal while the eye is closed during sleep and to reduce evaporation while the eye is open.[21] The lipids function as a lubricant while the eye blinks, and may provide a protective layer against bacterial infection.[20, 21]

Figure 2: Typical appearance of healthy meibomian gland orifices within the lower-lid.



The exact mechanism controlling the secretion of lipids from the meibomian glands is incomplete, but it is thought that the glands respond to neuronal, hormonal, and/or vascular controls.[20, 22] Lipid secretion is thought to be partially controlled via neuronal control, due to the fact that the meibomian glands are surrounded with vessels that are richly innervated.[23, 24] This regulation may be direct, through innervations of the acini, or indirect through the vasculature.[22] In addition, many of the lacrimal glands, including the meibomian gland, gland of Moll, and gland of Zeis, have vasoactive intestinal polypeptide (VIP) innervation.[25] Due to this innervation found in the lacrimal gland and the meibomian gland, the two glands may form a unit where secretion is controlled by the same neurotransmitter.[24] Hormonal control is also suspected, as the meibomian gland acini express both estrogen and androgen receptors.[26-28]

Considerable research has been completed examining androgen influence on meibomian glands in animal and human tissues.[29-34] This research has shown that androgens regulate meibomian gland function and affect the pattern of lipids that are expressed.[33] Androgen influence on the meibomian glands does not appear to influence the structure of the meibomian glands, however, the meibomian glands are an androgen target organ.[26, 27, 33] Lipid secretion patterns from the meibomian glands are changed when the amount of androgens in a system decreases.[29] This commonly occurs in women when they are going through menopause. During menopause, there is a drastic decrease in overall sex hormone levels, and therefore androgens.[35] Post-menopausal women are therefore more likely to experience changes in lipid expression, which appear to contribute to symptoms of dry eye. Specifically, differences in neutral and polar meibum lipid profiles seem to be linked to dry eye.[29]

By whatever means the glands are actually controlled and activated, the process to expel the lipid from the gland remains the same. Two excretory methods exist, which are termed “active” and “passive”. The active method occurs when the lipid is forced out of the meibomian gland orifice during a blink. The passive method occurs during sleep when the lid closure’s contractile force raises the intraductal pressure and causes the meibomian oil to exit the gland.[20, 22] During sleep there is a lack of blinking so the secretion is based on high intraductal pressure. The normal rate of excretion is 6.7µl/hr per gland, or approximately 333µl/hr per eye.[20, 21] When the lid opens and closes, the meibomian oil moves along the entire ocular surface in a wave pattern.[20, 21]

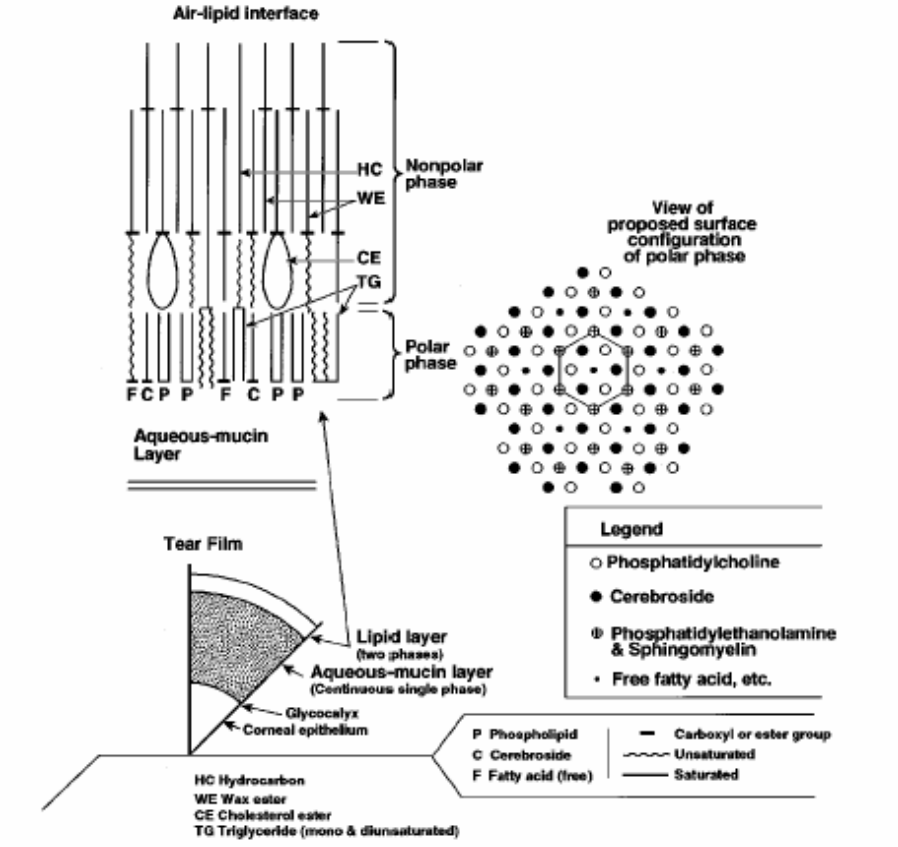
1.3 Formation of the lipid layer of the tear film

The meibomian gland lipids tend to have a low melting point, which allows for smooth delivery of the lipids through the ducts and orifices to reach the tear film. The melting point ranges from 19-23^{o}C.[20] The integrity of the tear film, and therefore the overall function of the tear film, is dependent on the specific composition of lipids released from the meibomian glands. Specifically, the configurations of fatty acids in the meibomian secretions are very important. If more unsaturated and more branched fatty acids are found in the meibomian lipid then the melting point will decrease.[20]

The role that the meibomian gland lipids play in the tear film is complicated and not entirely understood. Lipids are found in several locations in the tear film, including the base of the tear film adjacent to the outermost corneal epithelium.[36] The corneal epithelium has microvilli protruding outwards and a unique covering drapes over the microvilli.[36] This covering is made up of a polar glycocalyx and carbohydrates that separates the epithelium and the aqueous layer and anchors the aqueous-mucin layer.[6]

On the outermost surface of the tear film there is a lipid layer which contains two different lipid phases. The interior layer is believed to be a polar-surfactant phase and the outermost phase is a non-polar phase. Each phase of the lipid layer of the tear film has unique characteristics and provides differing, yet crucial, functions.[36] Figure 3 shows a diagrammatic view of the lipid layer of the tear film.[6]

Figure 3: A diagrammatic view of the lipid layer of the tear film[6]



This Figure was taken from McCulley JP, Shine W. A compositional based model for the tear film lipid layer. *Trans Am Ophthalmol Soc* 1997; **95**: 79-88, Figure 1. Permission was granted to reproduce this figure from Dr. McCulley and *Trans Am Ophthalmol Soc.*

Before the polar and non-polar phases of the lipid tear film can be discussed in full there are a few terms that will be defined, including polarity, saturated and unsaturated fatty acids, and the concept of stability.

Polar molecules, those are molecules which have a dipole or an uneven distribution of electrons that result in one "end" of the molecule becoming more negatively charged and the other "end" becoming more positively charged.[37] An example of a polar lipid is phosphatidylethanolamine, from the phospholipid group of molecules. These molecules

have a hydrophobic hydrocarbon "tail" that has a positive charge and a hydrophilic "head" that has more of a negative charge, due to the phosphates. Non-polar or apolar lipids are those lipids that do not have the uneven distribution of electrons and therefore do not have a dipole. An example of a relatively nonpolar lipid is triolein, a triglyceride[37]. Polarity is on a continuum and therefore there are molecules which are only slightly polar or non-polar.

Short-chain saturated fatty acids are fatty acids chains of a carbon length of 12-18,[6] that do not contain any double bonds and are therefore full of hydrogen molecules. Unsaturated fatty acids are molecules which have a least one double bond. The more double bonds an unsaturated fatty acid has the lower the melting point and the more fluid the surrounding structure will be.[6, 38] This is caused by the lack of hydrogen molecules. The lack of hydrogen molecules within the fatty acid causes a decrease in strength of the molecule's intermolecular bonds and therefore reduces the melting point.[38]

For the purposes of this paper the stability of the tear film and its component layers is defined as: the ability for the tear film to be resistant to change and to continue to perform its functions despite influences on its composition or structure.[39]

1.3.1 Polar Phase

The polar phase of the lipid layer of the tear film is abundant in short-chain saturated fatty acids, which provide it with enhanced stability due to strong intermolecular forces between the hydrogen atoms.[6] There are also a number of different types of polar lipids found in this phase, especially phosphatidylethanolamine, phosphatidylcholine, sphingomyelin, ceramides, cerebrosides plus many other specific phospholipids.[6, 40, 41] The polar layer of the lipid tear film contains an estimated 3-5% of triglycerides.[42] If there is a shortage of triglycerides it is possible that wax esters can supplement the triglycerides, but cannot replace them totally.[6] This conclusion is based on the fatty acid composition of these lipid types. The wax esters and triglycerides are not always strictly found in the polar phase but can bridge over to the non-polar phase. Not all "normal" patients contain cholesterol esters in their tear film, but if they are present, and they bridge between the two lipid phases, the stability of the layers can be compromised.[43] Additionally, free fatty acids, short-chain fatty alcohols, monoglycerides and diglycerides with short fatty acid chains are common in the polar phase.[44] Some short-chained hydrocarbons may be found in the polar phase and these hydrocarbons function to stabilize this phase.[6]

The overall stability of the polar lipid phase of the lipid tear film depends on three main factors: the balance of polar lipids in the layer, the presence of ions, and the pH.[6] Increases in pH, possibly due to calcium, can affect the nature of internal phospholipid bonding and therefore jeopardize the stability of the polar phase of the tear film. The

polar layer acts as a surfactant and a base for the more superficially located non-polar phase.[6]

1.3.2 Non-Polar Phase

The non-polar phase contains a large amount of non-polar lipids, including wax esters, cholesterol esters, triglycerides, and hydrocarbons. The non-polar phase is larger than the polar phase and, therefore, it is these lipids that are found in the greatest quantities.[6] The hydrocarbons found in the non-polar phase are much longer and therefore decrease the water vapor transmission rate of the lipid layer.[45] The function of the non-polar phase is that it regulates the transmission rate of water vapor, carbon dioxide, oxygen, and ions. Additionally, the non-polar layer is a storage unit for triglycerides, wax esters, and other non-polar lipids.[6]

The stability of the lipid layer relies on the chemical bonds that form between specific lipid types. Hydrogen bonds form between polar molecules, between water molecules and polar molecules, and between other lipid types such as triglycerides, wax esters, and sterol esters. Polar molecules can also bond with ionic bonds. The final type of bond is the van der Waals forces that form between fatty acids and fatty alcohol carbon chains.[22]

1.4 The meibum composition

To determine what specific lipids are found in the meibomian secretions, the glands can be compressed, forcing the lipid to be excreted onto the lid margin, where it can be collected for analysis.[46-48] Subsequent analysis by various forms of chromatography has determined the lipid types and relative amounts (Table 1).[21, 41, 44, 49, 50]

Table 1: The lipid composition of meibomian gland secretions[21, 41, 44, 49, 50]

LIPID	% of Meibomian Gland Secretions
Wax esters	≥ 47
Sterol esters	< 38
Triglycerides	4
Diesters	2
Free Fatty Acids	< 2.5
Free Cholesterol	1.5
Hydrocarbons	3-7
Polar Lipids	6-16

The meibum has been found to contain over 45 types of lipids. [6, 40, 41, 47, 51-53] Although all of these lipids are usually found in meibum, the lipid composition varies greatly in an individual person. [54, 55]

Despite the inconsistency between subjects, the lipid types and the exact lipids found in the meibomian glands remain fairly consistent in healthy eyes. Some of the common

lipids found in the meibum include cholesterol, cholesteryl oleate, cholesteryl linoleate, linalyl acetate, triolein, oleic acid methyl ester, oleic acid propyl ester, dicaproin, and undecylenic acid.[51, 56] As stated previously, there is a mixture of polar lipids found in the meibum, especially phospholipids and sphingolipids. The most common are phosphatidylethanolamine, phosphatidylcholine, sphingomyelin, ceramides, cerebrosides and a high percentage of unknown lipids.[6, 40, 41, 47] Alkylacylphosphatidycholine, dihydrosphingomyelin, dimethylphosphatidylethanolamine, ethanolamine plasmalogen, lysoethanolamine plasmalogen, lysophosphatidylethanolamine, lysophosphatidylserine, phosphatidic acid, phosphatidylinositol, phosphatidylserine, phosphatidylgycerol sphingosylphosphorylcholine, lysophosphatidylcholine, and cardiolipin are other phospholipids that have been identified to reside in the meibum.[41, 52] There are a significant amount of saturated and unsaturated fatty acids. The typical saturated fatty acids include capric, lauric, myristic, palmitic, stearic, arachidic, behenic, lignoceric, and cerotic acid.[21, 44, 55] The typical unsaturated fatty acids are oleic, erucic, linoleic, linolenic, palmitoleic, and arachidonic.[21, 44, 53, 55] These are only a fraction of the fatty acids found in the meibum.

When lipid is excreted from the meibomian glands, in the form of meibum, it mixes with the tears found on the ocular surface of the cornea. Once the lipid is incorporated within the tears, the composition of lipid is thought to remain similar, but may undergo reactions with the other components of the tear film and the environment, thus creating new lipid types or concentrations. As in the meibum, the predominant lipid types found

in the tear film are cholesteryl esters, wax esters, triglycerides, free fatty acids, monoglycerides, diglycerides, fatty sterols, and fatty alcohols.[55, 57-61]

If an individual has an altered concentration of a particular lipid in the tear film, the whole tear film may become unbalanced, resulting in complications such as contact lens intolerance and dry eye.[62] Furthermore, diet, prescription medications, age, gender, environment, work atmosphere and the presence of contact lenses can also alter the final composition of the lipid tear film.[63-66]

1.5 Stability of the tear film

Research on the stability of the tear film in "healthy" individuals has been consistent throughout the years, showing that the tear film itself is considerably stable between blinks, even in the presence of particles and bubbles.[3, 67] When there is stability of the tear film between blinks, this is confirmation that the lipid layers are functioning to their full potential, with compression of the layer during blinking. During the down stage of a blink, if stability exists in the lipid layer, then the lipid layer will fold and the lipid will experience little mixing between layers.[68] The stability of the entire tear film is therefore tied directly to the composition and integrity of the lipid layers and therefore the meibomian composition.

In the past ten years many studies have been completed to directly link the lipid layer of the tear film to the evaporation rate and tear film stability. Specifically, it is known that

when the lipid layer is absent, not structurally sound, or thinned there is a dramatic increase in evaporation and the film itself becomes unstable.[69-72]

There are several reasons why the lipid layer of the tear film may not be fully functional. There may be a blockage of some or all of the meibomian excretion ducts, unusual arrangement of the gland orifices at the margin, contamination of non-ocular lipids, the occurrence of blepharitis, and/or contamination by mucous.[69] Along with these reasons, changes in the actual lipid composition can also affect the function of the tear film. For example, an excess of fatty acids or triglycerides can result in instability of the tear film.[69] In addition, an altered ratio of polar to non-polar lipids can have the same effect.[69]

It has been shown that an unstable tear film can damage the ocular surface and cause symptoms of ocular discomfort or dry eye. The two main causes of instability of the tear film are a decrease in the quantity of tears and a decrease in the quality of tears. The causes of dry eye are directly linked to the types of dry eye: aqueous tear deficiency and tear evaporative dry eye. [73] Tear deficient dry eye occurs when the lacrimal gland does not function to produce an adequate tear flow or volume.[73] In evaporative dry eye, the lacrimal gland functions normally, but the tears are evaporating quickly from the ocular surface, which can be caused by numerous specific ocular disorders.[73-75] These disorders include meibomian gland disease, lid/globe apposition, ocular surface disorders, blink disorders, lid aperture disorders, blepharitis, and tear film disorders.[73]

In evaporative dry eye, the lipid or mucin layer of the tear film has been altered. Specifically, the symptoms of dry eye can be increased by altered concentrations of lipids in the tear film, especially phospholipids.[52] Specifically, lower concentrations of neutral and anionic phospholipids have been found in dry eye sufferers.[76, 77] There are several theories as to what components of the lipids lead to stability. Some view that it is the hydroxyl groups that increase hydrogen bonding between the lipids and therefore lead to stability.[77] Others believe it is the neutral phospholipids that stabilize the tear film by lowering the surface tension.[52, 78] Throughout the history of *in vitro* experiments it can be seen that isolating the lipid layer of the tear film does not give an accurate view of the overall function of the tear film and its corresponding stability. The tear film does not act alone to form a stable tear film, but interacts with protein and mucin in the underlying layers.[52]

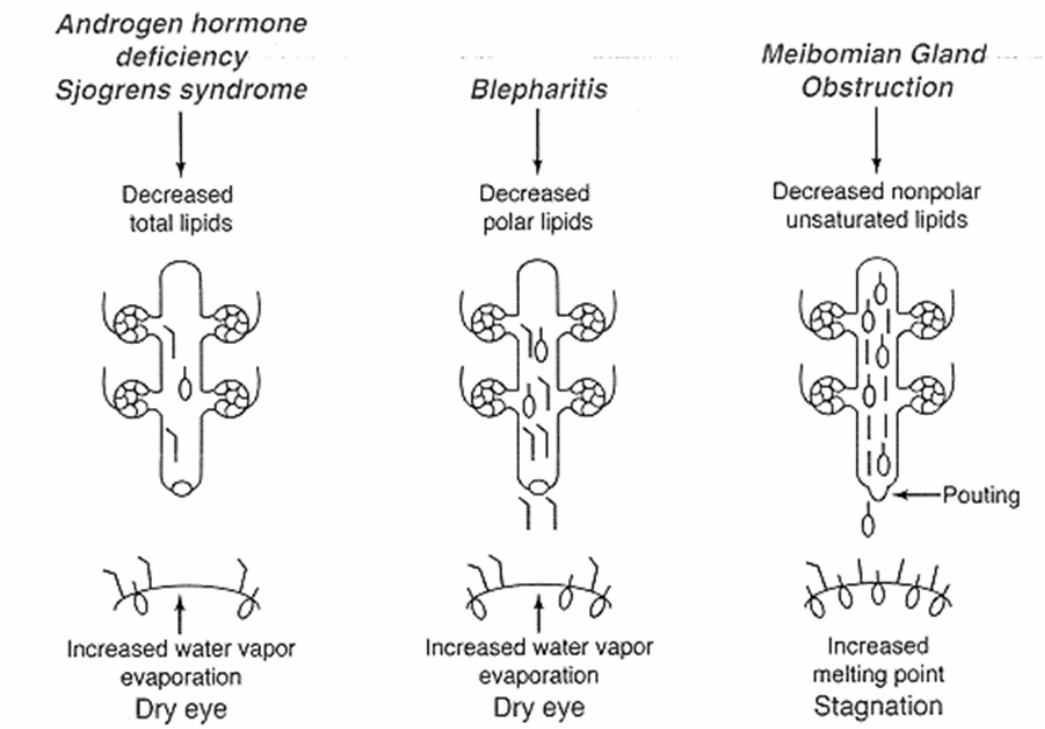
1.6 Blepharitis

Blepharitis is an ocular disease which affects the meibomian glands and their function. Often blepharitis is diagnosed due to alterations to the meibomian glands. In a normal patient, the individual grape-like acini can be seen forming the meibomian glands in the tarsal plate.[22] In contrast, a person with blepharitis has marked changes to the structure of their meibomian glands.[22] In particular, they could have an absence of the glands, dilation of the ducts or other physical changes.[79, 80] In addition to the changes to the glands themselves, the meibum excreted is often altered itself, commonly in viscosity and colour.[81] The changes in the meibomian glands and the meibum cause an increase in

tear film osmolarity[81] and an increase in tear evaporation.[71] These symptoms indicate the presence of an unstable tear film.[71]

Specific meibomian gland diseases and problems have been linked to changes in specific lipids. For example, subjects with obstructive meibomian gland dysfunction are seen to have decreased amounts of triglycerides[50] and monounsaturated fatty acids like oleic acid.[46] There are differences in the cause and consequences of blepharitis, meibomian gland obstruction, and androgen hormone deficiency. These differences are shown in Figure 4.

Figure 4: A diagram demonstrating the consequences of blepharitis, meibomian gland obstruction, and androgen hormone deficiency. [22]



This Figure was taken from McCulley J, and Shine, W. Meibomian gland function and the tear lipid layer. *The Ocular Surface* 2003; **1**: 97-106. Figure 4. Permission was granted to reproduce this figure from Dr. McCulley and *The Ocular Surface.*

1.7 Tear film lipid and contact lens wear

Contact lens wear causes changes in the structure of the tear film, particularly within the lipid layer.[1] Contact lenses lie within the aqueous layer of the tear film and therefore create a much thinner aqueous layer for the lipid layer to cover. The presence of a contact lens also eliminates the smooth ocular surface over which the eyelid moves during a blink and therefore it is more difficult to reconstruct the tear film.[82] Due to these factors, there is only a thin tear film layer on the outer surface of a soft hydrogel lens and no tear film layer covering a rigid lens. [83] With little or no lipid layer present, the tear film can easily become unstable.[84, 85] In order for a contact lens to remain totally biocompatible while being worn, the lens must form an overlying tear film that is structured similarly to that seen with no lens in place. This remains the ultimate goal in contact lens material research, but due to the hydrophobicity of the lens materials currently available this is currently not possible. A person with a thin or unstable tear film when there is no lens in place may not be able to wear a contact lens successfully for extended periods of time, without jeopardizing the tear film stability even further.[84] This manifests itself as either reduced wearing time, discomfort or, ultimately, discontinuation of wear.

As was seen previously with tear film stability, any changes in lipid composition can affect the success of contact lens wear. Specifically, higher concentrations of lipocalin,

a lipid binding protein, and secretory phospholipase, a lipolytic enzyme, promote lipid breakdown and may cause intolerance to contact lens wear.[62]

Furthermore, when a contact lens is placed on the corneal surface, the tear film (including the lipid layer) covers the anterior and posterior surface of the contact lens.[65] The lipids in the tear film may interact and dissolve into the lens material itself, causing changes to the chemical structure of the lens.[86] Examples of lipid deposition on the contact lens surface can be seen in Figures 5 and 6.

Figure 5: Lens calculi (jelly-bump) deposited upon a high water content soft contact lens after 18 months of wear.

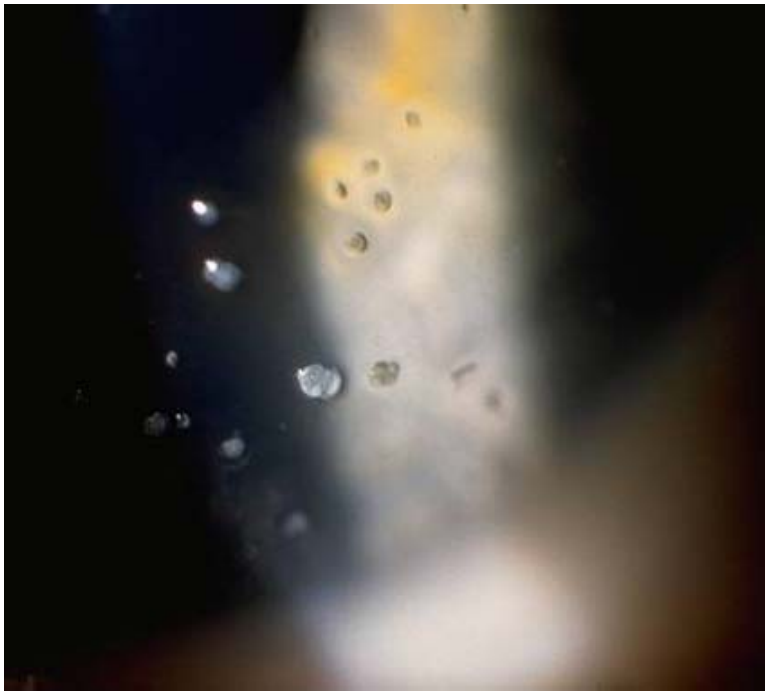
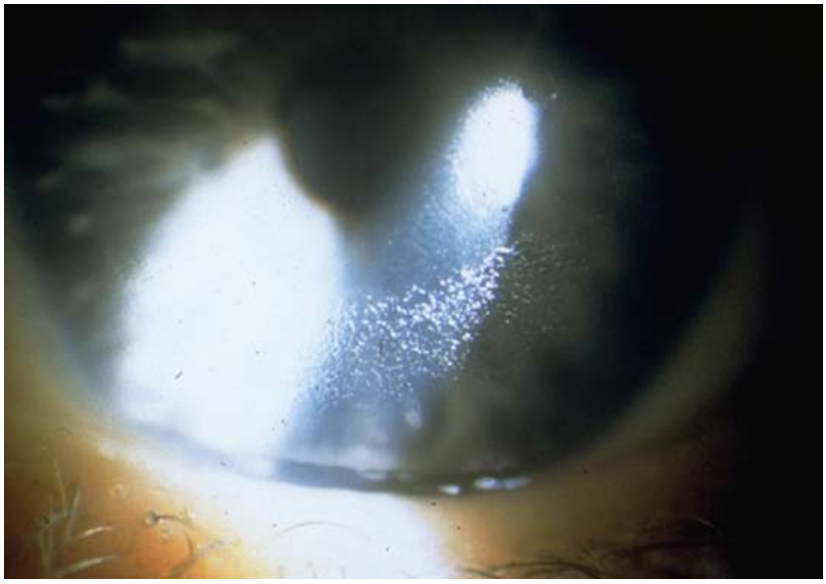


Figure 6: Heavy lipid film deposited on a low water content soft contact lens in a patient with meibomian gland dysfunction.



1.8 Lipid deposition on contact lens materials

Data on protein deposition on contact lens materials and its subsequent impact has been widely published. However, there is a relative dearth of information on the interaction of lipid with contact lenses, with only a handful of researchers publishing data on this topic.

Some of the earliest observations of the interaction of lipid with hydrogel contact lenses was that of Hart and co-workers,[66, 87, 88] who examined lenses from both daily and overnight wearers. In one study, [87] Hart reported that 15% of hydrophilic extended wear

contact lens wearers needed to replace their lenses due to deposition. The rate of deposition ranged from a few weeks to a few months and was highly subject-dependent. The deposition pattern commonly seen was a central deposition of "oily bumps", which Hart termed "jelly-bumps", "mulberry spots" or "lens calculi".[87] Hart demonstrated by various forms of microscopy and histochemical staining analysis that lipid was present in all deposits and was the prime component, with the principal lipid type being cholesteryl esters.[87]

Scanning and scanning transmission electron microscopy found small amounts of calcium within the deposits, at much lower levels than the lipid. This was an important finding, as previously calcium was considered to be a major component of these nodular deposits,[89-95] which often are white in appearance. Hart also found that lipid deposits formed in an *in vitro* model were morphologically and histochemically similar to those formed *in vivo*.

In a later study, [88] Hart determined that the jelly bump deposits had a fairly consistent composition of long and intermediate sized cholesteryl esters, triglycerides, and waxy esters. This composition is similar to the composition of lipids found in meibomian gland secretions. It was also found that individuals with higher deposition rates may have a lipid-rich tear film and a decreased tear flow. These lipids are insoluble in aqueous mediums and therefore showed some resistance to cleaning products.[88] Hart also examined lifestyle choices and their effect on lipid deposition of contact lenses. [66] Individuals who consumed larger amounts of alcohol, protein and fat exhibited increased

lipid deposition on their lenses. Patients with diabetes who were medicated with diuretics, anticholinergic or sympathomimetic drugs were found to have lower potassium levels in the tear film and this correlated with increased lipid deposition.[66] This was one of the first times that attention was drawn to the marked inter-subject variability in lipid deposition patterns.

Hart proposed that the reason such nodular deposits occurred were due to localised spots of drying, resulting in hydrophobic areas that attracted lipids, which then soaked into the lens material.[87] This area then acted as a larger non-wetting area, which acted as a nidus for more lipid deposition. This continuous cycle of dewetting and lipid deposition resulted in a lipid-based nodule forming.

The work by Hart and colleagues in the US was closely mirrored by that of Tighe and Bowers in the UK, at around the same time. Bowers and Tighe focussed on analyzing the gross morphology, chemical composition, and arrangement of "white spot deposits" that form on different contact lens materials.[96, 97] In Bower's first experiment, he examined the occurrence, location, and gross morphology of elevated white spot deposition which formed on contact lenses taken from a controlled contact lens trial and randomly from a clinical setting.[97] Deposit morphological assessments were analyzed using several microscopy techniques including phase contrast, light, dark-field, and scanning electron microscopy (SEM). Additionally, stereo-microscopy was used to examine deposit occurrence. These experimental techniques found that there are three interactive sub-layers to the morphology of an elevated white spot deposit and that

differences in lens material and wearing protocol do not affect this morphology. In contrast, the rate of deposition was markedly influenced by the lens materials and patient variability.[97]

The second experiment completed by Bowers and Tighe continued their previous white spot deposit analysis, to examine their chemical composition and geological arrangement.[96] Light microscopy, scanning electron microscopy, energy dispersive X-ray analysis, and compound specific-histological staining were used to analyze elevated white spot deposits which formed on patient worn lenses described above. These experimental techniques found these deposits to have a well-formed tri-layer structure of lipid, where the primary or basal layer was composed of unsaturated lipids, while the secondary and tertiary layers were predominantly cholesterol and their esters.[96] Other tear components, like proteins, were present in the deposits, but were not found to play a role in the morphology of the deposits. The wearing schedule, lens material chemistry and individual differences in tear film structure did not influence the composition or location of these deposits.[96] Bowers and Tighe hypothesized from these results that it was the primary layer or unsaturated lipid deposition that altered the biological surface of the hydrogel lens materials and thus cause decreased biocompatibility with the surrounding ocular environment.[96]

Throughout the 1990's, Franklin, Tighe, and colleagues set out to further their exploration into lipid deposition on contact lenses. They published a series of papers examining the influence that calcium, lens materials, and surfactant cleaners has on lipid

deposition.[98-101] In their first paper, Bowers, Franklin and Tighe examined the formation of white surface films and the importance of the role of calcium.[101] Various contact lenses were collected, from a controlled clinical study and other clinical settings. During the controlled clinical study, the care solutions used were modified to increase the calcium concentration in the lens material in order to see the influence calcium has on deposit formation. Through light microscopy, SEM, energy dispersive X-ray analysis, histological staining, HPLC and fluorescence spectroscopy Franklin, Bowers, and Tighe determined that these white surface films are morphologically different than elevated white spots, as these films have a heterogeneous structure where the lipid components are easily separated from the calcium portion. The lipid components were mainly cholesterol and cholesterol esters. The lens materials that were subjected to artificially raised calcium levels did not exhibit increased formation of elevated white spots. These results suggest that calcium may only have a secondary role in stabilizing lipids that have already been immobilized.[101]

In 1991, Franklin and Tighe examined lipid and protein deposition on human worn lenses after one week and studied the effect of surfactant cleaning on these deposits. [100] Lipid and protein deposition was assessed using fluorescence spectroscopy at their respective wavelengths of emission and optimal excitation. This technique revealed that lipid deposition was largely influenced by an individual's life style, tear film composition and surrounding environment, whereas protein deposition was driven by the composition, charge and water content of the contact lens material. Individual tear film chemistry also influences the effectiveness of surfactant cleaners on lipid deposition,

making them only moderately helpful, especially within the first week of lens wear.[100] Other studies have indicated that some surfactant cleaners are more efficient at removing lipid and protein deposits than others, and that these cleaners are important in reducing reactive lipids that can accumulate further along in the deposition process.[102]

During a controlled clinical study in 1991, Franklin and colleagues examined the deposition of lipids onto a contact lens surface and the subsequent penetration into the lens matrix.[99] This experiment showed that there is a dramatic range of lipid types that deposit on lenses, from polar, poorly polar, to non-polar and that this deposition is highly patient dependent, but over time lipid deposition was found to be influenced by lens material composition. Surfactant cleaners are relatively helpful in minimizing lipid deposition and autoxidation of the lipids, but this is only temporary, as the lipid layer of the tear film is being constantly replenished.[99]

A further study examined the different types of cleaners available on the market and their efficiency at removing *in vitro* doped lipid from the surface of a contact lens.[98] Soft contact lens surfactant cleaning solutions were compared with traditional chlorine-based and peroxide-based disinfectant systems. Surfactant cleaning solutions were found to vary widely in their ability to remove lipid from lens surfaces and disinfectant systems were found to remove virtually no lipid.[98]

In the early 1990's, Mirejovsky reported on an *in vitro* artificial tear solution that contained proteins, mucin and lipids.[51] This was a significant advance over previous

doping solutions, which were almost exclusively based on proteins dissolved in buffer. Her work looked at both *in vitro* doped lenses and also investigated the ability of two histochemical stains (Nile Red and Oil-Red-O) to stain lipids. Mirejovsky showed that the Nile Red stain was far superior at detecting lipids and that the *in vitro* model solution produced a lipid deposition pattern that was similar to that obtained from human worn lenses. She also demonstrated that lipids could deposit to the hydrogel lenses either in isolation or bound to tear film proteins.[51]

Some of the most widely cited data on the interaction of lipids with hydrogel lenses was that undertaken during the mid 80's and 90's by Rapp and colleagues, who completed a series of experiments examining lipid deposits on a wide variety of contact lens types.[103-107] In Rapp's first experiment, patient worn soft contact lenses were examined for lipid deposition and analyzed for various lipid types using thin layer chromatography (TLC), high pressure liquid chromatography (HPLC) and gas chromatography (GC). Using these techniques, Rapp showed that wax esters, fatty sterols, fatty alcohols, free fatty acids, and diglycerides were all detectable on hydrophilic lenses, whereas cholesterol, cholesteryl esters and triglycerides were not detectable. He concluded that the more polar lipids will deposit preferentially on hydrophilic lenses when compared to non-polar lipids and that not all available lipids present in the tear film appear to deposit on hydrogel lenses.[107]

Subsequent studies revealed that all lipid types interact with contact lens materials, but that the interaction is driven by both the lipid types and the chemical composition of the

lens material.[65, 103-106, 108] The United States Food and Drug Administration (FDA) classification system for hydrogel contact lens materials is shown in Table 2. Lenses with water content above 50% are classified as being "High Water", and materials with >0.2% ionic monomers (typically methacrylic acid) are classified as being "Ionic", having a net negative surface charge.

Table 2: FDA Materials Classification System for Hydrogel Lenses

Group	Water Content	Ionic Character
I	Low Water	Non-ionic
II	High Water	Non-ionic
III	Low Water	Ionic
IV	High Water	Ionic

Rapp's work with Bontempo[103, 105, 106] was crucial in indicating that FDA group II lenses deposited the most lipid, and that group III materials deposited the least. They also reported that non-ionic materials deposited more lipid than ionic materials, and that high water lenses deposited more lipid than low water materials.[103] This data led to the development of the "pull/push" theory of lipid deposition, in which the "pull" represents the polymer lens material adhering the lipid and the "push" represents the water in the lens material driving the lipid into the matrix.[53, 103] Further research has been undertaken to find the differences between monomeric compositions within the same FDA group,[109] which show that FDA classification alone is insufficient to accurately describe the pattern of lipid deposition that can occur.

Rigid gas permeable (RGP) lenses were also examined by Rapp,[103] and this work indicated that these materials generally deposit more lipid than many soft lens materials, probably due to the hydrophobicity of the lens. RGP lenses contain low amounts of water, and therefore the high lipid adherence is tied to the individual characteristics of the polymer. For instance, silicone-based RGP lenses deposit more lipid than fluorine-containing RGP lenses, because the silicone addition increases the hydrophobicity of the lens, but the fluorine addition decreases the hydrophobicity and thus decreases lipid deposition.[103]

Bontempo and Rapp also analyzed the interactions between proteins and lipid on the surface of hydrophilic and rigid gas-permeable contact lenses *in vitro*.[104, 105] They reported specific interactions that occur on a hydrophilic contact lens surface when lipids and proteins are present concurrently. When a group IV lens undergoes protein deposition, the surface of the lens becomes less hydrophilic and therefore attracts lipid deposition. For group II lenses, the proteins compete with more polar lipid deposited on the lens surface and displace them.[105] When RGP lenses were examined for lipid and protein interactions on the lens surface, different interactions were found. The surface of an RGP lens is hydrophobic and thus attracts more lipids than proteins. The polarity of some lipid molecules allow for binding with the matrix and attraction toward the aqueous. When lipids bind to the contact lens, the surface becomes less hydrophobic and this allows for subsequent protein deposition.[104] In their final experiment, Bontempo and Rapp continued their protein and lipid interaction research by studying these interactions on group I and group IV lenses *in vivo*.[106] They found that lysozyme was preferentially

deposited on group IV lenses due to the available negative charges attracting the strongly positively charged protein. Group IV lenses showed deposition for both protein and lipids, but the specific deposition composition depended on the individual. [106]

Some of the more recent work on conventional hydrogel deposition with lipid was undertaken by Tighe and colleagues.[108-113] In the first of these, an *in vivo* study was conducted to evaluate the deposition of protein and lipid on FDA group II lenses worn for various lengths of time.[111] This was the first work to demonstrate that degree of deposition was influenced by frequency of replacement, with significantly increased deposition being noted for lenses worn for three months as opposed to one month. Overall lipid deposition increased with longer replacement schedules and 44% less lipid was detected for the shorter replacement time, with individual lipid deposition being shown to vary greatly.[111] In a subsequent study,[109] protein deposition was shown to be related to the degree of ionicity of the contact lens material, being greater in FDA group IV materials, whereas lipid deposition was strongly related to the monomeric composition, with increased lipid deposition being encountered in FDA group II materials, particularly those containing N-vinyl pyrrolidone (NVP). Group II lenses containing polyvinyl alcohol (PVA) exhibited much less lipid deposition. Lipid deposition was also found to be dependent on the individual.[109]

Another study completed by Tighe and co-workers examined both the effects of lens material and individual subject differences in lens deposition. [113] This controlled clinical study involved clinical and analytical techniques to analyze the deposition of tear film

components on group II and IV lenses. This experiment implemented a specialized technique using sterile, plastic-wrapped tweezers to collect the lenses from subject's eyes to reduce skin lipid transfer. Lipid analysis completed using fluorescence spectrophotofluorimetry determined that contact lenses containing NVP have the highest lipid deposition compared to all other lens materials and lipid deposition is greatly affected by patient-to-patient variations.[113]

In a further study,[112] the progressive deposition of lipids was examined over a one-month period in both group II and group IV lenses. Lipid deposition was found to be a cumulative process that does not plateau in a similar manner to that found in protein deposition on FDA group IV lenses. Once again, significant differences in individual lipid deposition were observed.[112] A related study by Tighe, Maissa and colleagues[108] found corroborating evidence that increased lipid deposition was detected on contact lenses that contained NVP and that lipid deposition was found to slowly imbed itself into the polymer matrix.[108] To summarize, the major findings of the studies conducted by Tighe and co-workers during the 1990's was that lipid deposition was more prominent on FDA group II lenses compared with group IV lenses, that group II lenses containing the relatively hydrophobic monomer NVP were particularly likely to deposit lipid and that large inter-subject variations in lipid deposition commonly occur.[110]

The most recent work on lipid deposition on hydrogel lenses is that of Jones and Senchyna, [114] who examined the deposition of both protein and lipid on highly oxygen permeable silicone hydrogel contact lenses. Their work clearly showed that while the

deposition of proteins on silicone hydrogel materials was less than that seen with conventional hydrogels, the more hydrophobic surfaces of the siloxane-based lenses resulted in substantially greater amounts of lipid deposition being seen. PureVision or Balafilcon lens materials deposited up to 600 µg of lipid per lens, while the Acuvue 2 or Etafilcon lenses deposited 20 µg of lipid per lens.[114] This clearly shows the dramatic increase in lipid deposition on silicone hydrogel lenses.

All of the research described above has contributed to our current model of understanding the processes involved in the deposition of lipid on contact lenses. However, many of these studies do not provide a complete picture of the topic, as the focus of the research is on one individual piece. For example, *in vitro* studies do not take into account the fact that lens surface drying between blinks and the constant replenishment of the lipid within the surrounding fluid will both influence the results obtained. Such studies need to be complemented and confirmed by *in vivo* or *ex vivo* studies to ensure that the results are comparable to what is occurring in human subjects. However, these studies are complicated by variations between subject tear films, compliance with cleaning and replacement schedules and environmental influences.

Other topics that have been inadequately examined are the degree to which lipid penetrates into the matrix of lens materials, the influence of various care regimens, the interaction between lipids and various constituents of the tear film, such as mucins, and the symptoms associated with lipid deposition. Lipid deposition patterns on the lens surface also need to be examined. This includes the kinetics of deposition, the

arrangement of lipid types, and the location of deposition on the surface of the lens. In addition, to-date very little research has examined the interaction of lipids with the new silicone-containing hydrogels, which are rapidly gaining in popularity for both daily and overnight wear. Finally, the study of the influence of the lipid composition of individuals' tear films on the lipid deposition that occurs remains an untapped area of research.

It is clear from the literature review above that there are many factors that can affect the deposition process and dictate the ultimate amount of lipid on the contact lens. Just as individuals show large variations in lipid composition, the corresponding deposition on various contact lens materials can vary greatly, possibly up to 55%.[110] The replacement frequency of the contact lenses has been found to affect the amount of lipid deposition, [111] with longer replacement times being associated with more lipid uptake. This work has also shown that lipid does not plateau, but continues attaching to the lens surface.[111] Therefore, it is apparent that lipid deposition is dependent upon material composition, replacement interval and individual patient variability.[112]

The effects that lipid deposition has on contact lens comfort, visual acuity, and the possibility of injection or infection has not been well studied. However, general deposition of tear film components can cause unpleasant symptoms for the lens wearers, primarily relating to reports of discomfort, [115-117] probably due to reduced lens wettability.[118] As deposition occurs on the lens surface, the contact lens becomes progressively dewetted, resulting in poor wettability and subsequent sensations of

dryness and discomfort. Poor vision is another negative effect of deposition, as the amount of deposition on the surface of a contact lens increases the corresponding vision through that lens decreases.[119-121] Occasionally, these symptoms can lead to discontinuation of lens wear.[115, 117] The more uncomfortable and irritating a contact lens becomes, the more likely the individual will remove the lens.[117]

1.9 Silicone hydrogel lenses

In the past 30 years, contact lens wear has increased from 10 million to over 100 million wearers. The reason for this drastic increase is due to patient's desire for safe, convenient, long term vision correction options to spectacles. Some patients turn toward laser refractive surgery, in particular LASIK (Laser-Assisted In Situ Keratomileusis), but many would rather wear a safe and comfortable contact lens on an overnight or extended wear (EW) basis. This has resulted in the contact lens industry developing a new range of contact lens materials based upon silicone, which are termed "silicone hydrogel" (SH) contact lenses.[122]

Silicone hydrogel contact lens materials utilise silicone groups, from silicone rubber, combined with conventional hydrogel monomers. The silicone addition to the lens significantly increases the material's oxygen transmission, whereas the hydrogel component allows for fluid transport and lens movement. The combination of these components allows for safe, extended wearing times compared with previous lens material polymers.[123]

From a historical perspective, water-containing hydrogel materials intended for EW were initially developed in the late 1970's. Those lenses were commercially unsuccessful due to their inability to supply adequate oxygen to the cornea. Chronic deficiencies in oxygen can lead to permanent corneal damage due to the development of a number of hypoxic complications, including epithelial microcysts, epithelial thinning, loss of hemidesmosomes, changes in epithelial cell size and slower cell development.[124] During the same time frame, silicone-based silicone-elastomeric materials were introduced as contact lenses, but were only used for special therapeutic cases or as lenses for paediatric aphakia following cataract surgery.[125] These types of lenses had increased oxygen transmission compared with conventional hydrogel materials, which was highly beneficial to the cornea, but it was found that such lens materials rapidly deposited lipid from the tears and bound to the cornea during overnight wear, which severely limited their success.[126, 127]

Polymers are based on the ability of atoms to bond together to form a long complex stable structure. Carbon's ability to bond with other carbon atoms, as well as various other atoms, including oxygen, hydrogen, nitrogen, and chlorine is the basis for polymer structure and function. Silicon is placed directly below carbon on the periodic table of elements and therefore behaves very similarly in its ability to bond with oxygen and hydrogen. Silicone-based polymers, siloxane polymers or silicones are ideal for contact lenses, as silicon-oxygen bonds are longer, flatter, and require less energy to rotate than

carbon-oxygen or carbon-carbon bonds, thus allowing a contact lens based on silicone to be more flexible and less affected by temperature, but they are very hydrophobic. [128-130]

The contact lens industry has strived for over 20 years to develop materials with the comfort and clinical performance of hydrogel lenses, and the oxygen transmission performance of silicone-elastomers. Through considerable financial investment, the release of such a group of materials – termed "silicone hydrogels" (SH) – became a reality in 1999.[128] Currently there are five silicone hydrogel contact lenses available on the North American market. All five lens materials are unique in polymer structure, surface treatment, modulus, oxygen transmissibility, patient fit, comfort, and deposition. The unique characteristics of each lens are outlined in Table 3.

Table 3: Material specifics of the five commercially available silicone hydrogel contact lenses

Proprietary name	Focus Night & Day	O_2 OPTIX	PureVision	Acuvue OASYS	Acuvue Advance
United States adopted name	lotrafilcon A	lotrafilcon B	balafilcon A	senofilcon A	galyfilcon A
Manufacturer	CIBA Vision	CIBA Vision	Bausch & Lomb	Johnson & Johnson	Johnson & Johnson
Water content	24%	33%	36%	38%	47%
Oxygen permeability ($\times 10^{-11}$)	140	110	91	103	60
Oxygen transmissibility ($\times 10^{-9}$)	175	138	101	147	86
Surface treatment	25 nm plasma coating with high refractive index	25 nm plasma coating with high refractive index	Plasma oxidation process	No surface treatment. Internal wetting agent (PVP) throughout the matrix that also coats the surface	No surface treatment. Internal wetting agent (PVP) throughout the matrix that also coats the surface
FDA group	I	I	III	I	I
Principal monomers	DMA + TRIS + siloxane macromer	DMA + TRIS + siloxane macromer	NVP + TPVC + NCVE + PBVC	mPDMS + DMA + HEMA + siloxane macromer + TEGDMA + PVP	mPDMS + DMA + EGDMA + HEMA + siloxane macromer + PVP

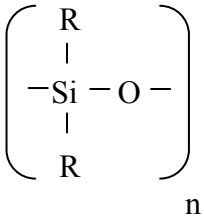
DMA (*N*,*N*-dimethylacrylamide); EGDMA (ethyleneglycol dimethacrylate); HEMA (poly-2-hydroxyethyl methacrylate); mPDMS (monofunctional polydimethylsiloxane) NVP (*N*-vinyl pyrrolidone); TEGDMA (tetraethyleneglycol dimethacrylate); TPVC (tris-(trimethylsiloxysilyl) propylvinyl carbamate); TRIS (trimethylsiloxy silane); NCVE (*N*-carboxyvinyl ester); PBVC (poly[dimethysiloxy] di [silylbutanol] bis[vinyl carbamate]); PVP (polyvinyl pyrrolidone).

The Focus Night & Day (FND) and the O_2 Optix (O_2) lenses have a biphasic, inter-penetrating network-like or two-channelled molecular structure, where the fluorosiloxane phase (silicone phase) facilitates the majority of oxygen transmission and storage and the hydrogel phase transmits water and a small amount of oxygen for lens movement.[131, 132] These two phases work together for smooth transportation of oxygen and water. The exact materials used for this lens are a fluoroether macromer co-polymerised with the monomers trimethylsiloxy siloxane (TRIS) and N,N-dimethyl acrylamide (DMA).[132] Purevision (PV) lenses are a homogenous combination of the silicone-containing monomer polydimethylsiloxane (a vinyl carbamate derivative of TRIS) co-polymerized with the hydrophobic hydrogen monomer N-vinyl pyrrolidone (NVP).[129, 132-134] To date, no specific details of the structure of the Acuvue Advance (AA) or Acuvue OASYS lens materials have been released. However, important differences between these lenses and the others described above is that these materials have an internal wetting agent composed of PVP (polyvinyl pyrrolidone) to aid surface wettability.[135]

The increased oxygen transport property of siloxane-based lens materials relates to the fact that oxygen is far more soluble in silicone rubber than it is in water or in polymethylmethacrylate (PMMA) contact lenses. This is because of the silicon-oxygen and silicon-carbon bonds that help make up the basis of silicone rubber.[128] The siloxane groups incorporated into these contact lenses have the molecular structure displayed in Figure 7. [123] In conventional soft contact lens materials, oxygen dissolves in the water phase and is transported via the water components. In such materials, increased oxygen

transmission is obtained by increasing the water content. However, the oxygen transport characteristics of water are significantly inferior to those seen in silicone.[123]

Figure 7: The siloxane group arrangement when found incorporated into a contact lens.



Si = the silicon, O= oxygen, R = the linking groups.[136]

As mentioned above, initial attempts to use silicone within hydrogel lenses in the silicone elastomers released in the 1970's and early 1980's failed due to lipid deposition, increased lens binding to the cornea and decreased in-eye wettability of the lens, due to the exposure of hydrophobic silicone on the surface of the lens material.[123] To minimize this problem a process to convert the hydrophobic surface to a more hydrophilic lens surface is required.[137] Other factors also need to be taken into account when developing a successful surface treatment. Ideally, the treatment needs to maintain a stable tear film layer, provide low bacterial adherence, minimise deposition of substances from the tears, and be non-irritating.[138]

The FND and PV lenses have different methods of creating this surface treatment. The FND lenses are permanently surfaced in a gas plasma reactive chamber to give the lens a thin, high refractive index, homogenous hydrophilic surface. In contrast, the PV lenses are also treated in a gas plasma reactive chamber, but this chamber alters the silicone to

give the surface of the lens hydrophilic glassy islands to mask the underlying hydrophobic material.[123] The process involved in the gas plasma reactive chamber includes many complex steps including etching, ablation, oxidation, and polymerization. The steps are controlled by several factors, and the success of the coating depends heavily on controlling the specific parameters required.[136]

Both the FND and PV lens surface treatments are a fundamental part of the lens and are not just surface modifications that can easily be removed during the cleaning and disinfection process.[139] The AA and OASYS lens materials have a proprietary internal wetting agent (HydraClear™) which is based upon polyvinyl pyrrolidone (PVP) and provides increased lens wettability and makes them the only SH materials currently available that do not require a surface treatment.

1.10 Wettability

The wettability of a contact lens is described as the ability of the tear film to cover the surface of a contact lens.[140] Conventional hydrogel contact lenses have high water contents with a hydrophilic surface. Therefore, these lenses when fresh out of the original packaging have no issues with wettability. However, once these lenses have been inserted into the eye, changes to the lens occur which can decrease their wettability. [141]

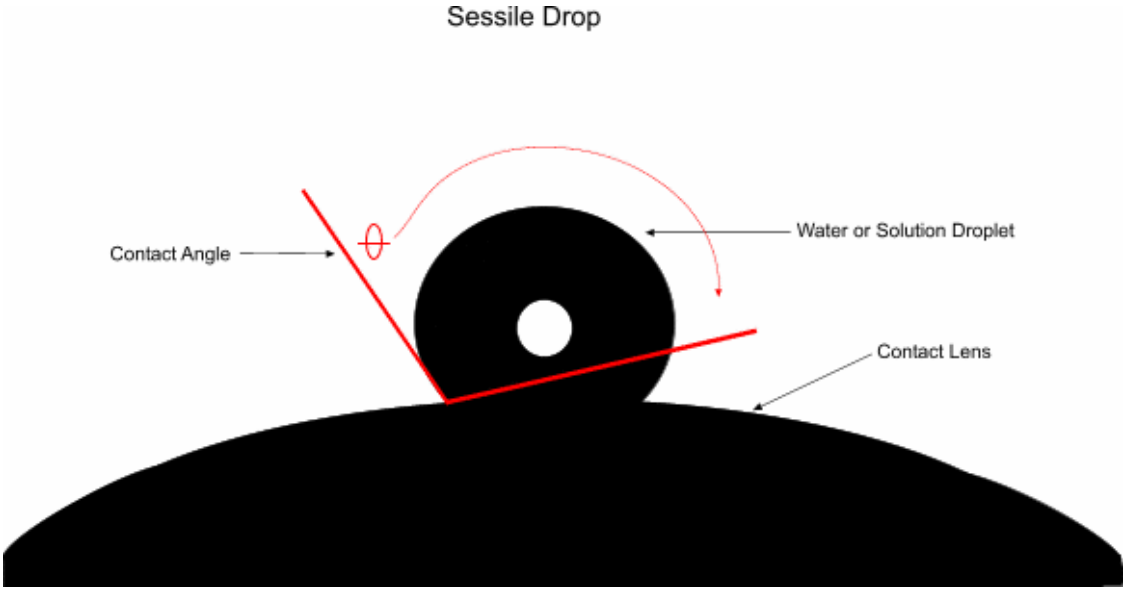
There are two factors that can lead to a lens becoming decreasingly wettable. When the lens is placed on the cornea, water from the lens will be lost to the tear film and into the environment. The amount of water lost will depend on the environmental conditions the lens is exposed to. This process is progressive and therefore occurs over an extended period of time. The second factor is a dynamic process that occurs during blinking. Every time a contact lens wearer blinks the surface of the contact lens and the tear film changes. When the contact lens is exposed to the tear film and other aqueous substances the lenses hydrophilic groups "flip" or reorientate so that they are exposed at the surface. In contrast, when the lens is exposed to hydrophobic environments, such as air, the hydrophilic groups "flip" to expose the hydrophobic groups of the lens. This process is called chain rotation. This progressive evaporation of water contributes to the discomfort felt by lens wearers at the end of the day.[141]

Historically, wettability is measured by the contact angle that forms between the hydrogel lens material and a water interface. There are three methods of measuring the contact angle of a contact lens material: sessile drop, captive bubble, and Wilhelmy plate.

In the sessile drop method, a contact lens has any excessive surface fluid removed using lens paper and is placed posterior side down on a convex shaped mantle. A 5 µl drop of water or saline is dispensed from a syringe on to the apex of the contact lens. The drop is allowed to settle for 2-3 seconds and the contact angle can be measured between the

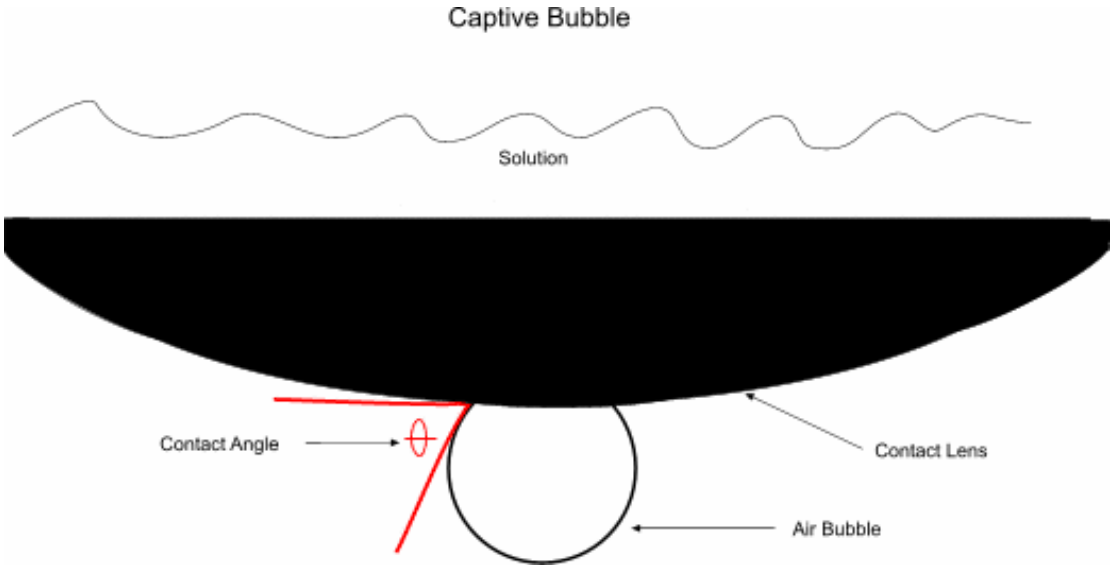
drop of water and the contact lens surface, as seen in Figure 8. The sessile drop method measures the advancing contact angle.

Figure 8: The sessile drop method used to measure the contact angle and ultimately the wettability of a contact lens.



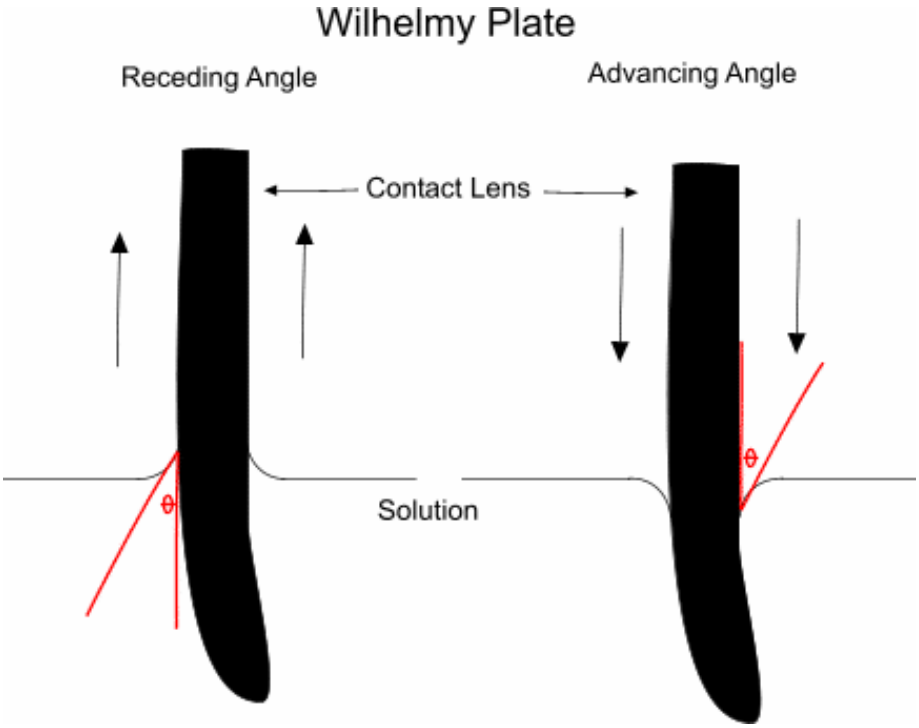
In the captive bubble method the contact lens is immersed in a chamber that contains saline solution or water with the contact lens oriented anterior side down. An air bubble is placed on the apex of the contact lens surface using a syringe. The contact angle between the contact lens surface and the air bubble is then measured. This technique can be seen in Figure 9. [142,143] This technique is analogous to a receding contact angle.

Figure 9: The captive bubble technique to measure the contact angle and ultimately the wettability of a contact lens.



The Wilhelmy plate method can be used to measure both the advancing and receding contact angles of the same contact lens. In this method, a contact lens is mechanically lowered into a beaker containing water or a saline solution. Advancing contact angles are measured as the contact lens is being lowered into the solution. Then, receding contact angles are measured as the contact lens is slowly being removed from the solution. The specific angles that are being measured in each case can be seen in Figure 10.[142,143]

Figure 10: The Wilhelmy plate technique to measure the contact angle and ultimately the wettability of a contact lens.



Each method of measuring contact angles has its advantages and disadvantages and each method delivers different final measurement values.[142, 144, 145] However the contact angle is measured, better contact lens material wettability corresponds with smaller contact angles. Therefore, the ideal contact angle is zero, which would denote a completely wettable contact lens. Unfortunately, the newer silicone hydrogel contact lens materials are more hydrophobic and thus have the distinct characteristic of being unwettable in nature. This is the main reason for surface treatments and internal wetting agents. [145]

Contact angle methods are based on the contact angle or Young-Dupree equation as seen below:[146]

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos \theta_e$$

Where:

γ_{SV} = solid/vapour interfacial tension

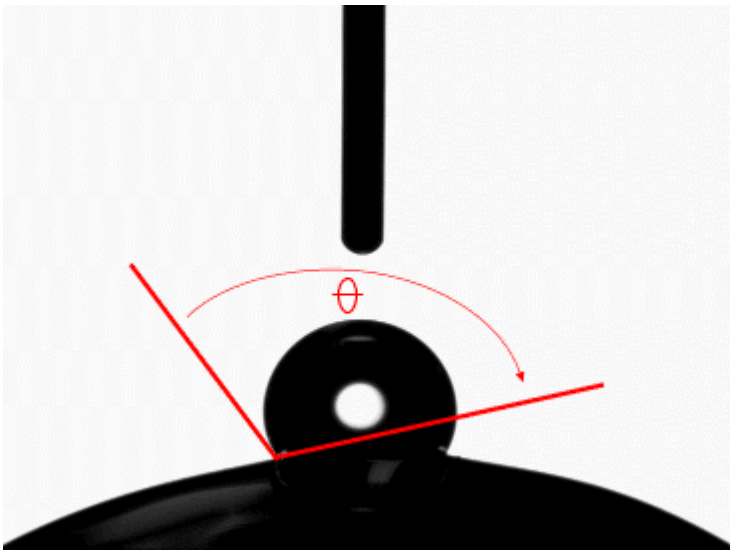
γ_{SL} = solid/liquid interfacial tension

γ_{LV} = liquid/vapour interfacial tension

$\cos \theta_e$ = equilibrium contact angle

In order to obtain a visual representation of what an unwettable and wettable lens looks like, two figures using the sessile drop method are provided (Figures 11 and 12). In the sessile drop method, a contact lens material that is deemed unwettable will have a high contact angle of $>90^o$ and will have the water drop sitting entirely on the surface of the contact lens, without any spreading of the drop over the surface. This can be seen in Figure 11.

Figure 11: A sessile drop contact angle image demonstrating an unwettable contact lens.



In contrast, a sessile drop image demonstrating a contact lens material that is deemed completely wettable will have a contact angle close to 0^{o} and the water droplet will be virtually indistinguishable from the contact lens surface. This can be seen in Figure 12.

Figure 12: A sessile drop contact angle image demonstrating a totally wettable contact lens. No drop of fluid can be seen as it has spread completely across the surface.

In the 1980's, rigid gas permeable (RGP) or hard contact lenses were dominant in the contact lens market.[142, 147] These lenses replaced polymethyl methacrylate (PMMA) lenses, which provided no oxygen transmission to the cornea, resulting in very unhealthy corneas with many signs of hypoxia.[142, 147, 148] These newer RGP lenses were siloxane/acrylate polymers or fluoro-silicone polymers. The silicone component allowed for increased oxygen transmission, but made the lens much more hydrophobic. Therefore, when choosing and analyzing these lenses researchers found that the wettability of the lens was an important measure to take into consideration.[142, 149, 150] Several researchers studied wettability and found that different polymers, contact lens cleaners, wetting solutions, and lens wear schedules altered the wettability of each individual contact lens.[142, 149-151]

In the 1990's, soft hydrogel contact lenses took over the contact lens market. These lenses were softer, more comfortable, allowed for more oxygen transportation, had increased water content, and increased wettability due to the absence of silicone in the polymers.[152] In this era, wettability did not influence lens comfort to the same extent. Unfortunately, these lenses were associated with high deposition rates of lipids,[103] proteins[153] and mucins.[154] These deposits in turn, affect comfort,[115, 116] inflammatory effects,[155-157] vision,[120, 158] and, as a consequence, wettability can be compromised.

Recently, Ketelson examined Acuvue 2 contact lens materials to see if lysozyme influenced the wetting properties of these lenses, using the sessile drop method.[159] They found that lysozyme deposited on the surface of pHEMA-MAA materials did not alter the wettability of the lenses, however, polymer surfactants did play a major role in determining their wettability. [159]

In 2001, Tighe and colleagues recognized that the problems associated with dry-eye symptoms in soft contact lenses wearers were significant.[160] They conducted a study using the Wilhemy plate contact angle method to test whether Etafilcon A *ex vivo* wettability improved with a surfactant pre-treatment. It was concluded that a 12 hour pre-soaking in a contact lens solution containing a surfactant (ReNu Multipurpose) significantly increased the comfort of lens wear throughout the first 8 hours of lens wear.[160] In 2006, this experiment was replicated by testing the *ex vivo* wettability of Etafilcon A lenses in three different commercially available care regimes, Opti-Free Express, ReNu MoistureLoc, and SoloCare Aqua.[161] In this study, using a sessile drop

methodology, it was found that the Opti-Free Express solution had significantly lower contact angles throughout 8 hours of lens wear when compared to the other two care solutions, thus making the contact lens more wettable.[161]

Novel silicone hydrogel materials are, as described previously, less wettable than conventional hydrogels and thus the issue of contact lens wettability has become a topic of great interest again.

Since the release of these silicone hydrogel materials there have been only a few researchers that have examined their wettability and even less who have examined the effect that lipid has on wettability.

In 2005 and 2006, researchers at the Centre for Contact Lens Research (CCLR), presented studies examining the wettability of silicone hydrogel contact lenses using the sessile drop method.[162, 163] The 2005 study presented by Rogers[163] determined that silicone hydrogel lens materials are less wettable than pHEMA-based hydrogel materials. Despite this, pre-soaking in various care regimes can improve the wettability of these silicone hydrogel lens materials.[163] The 2006 study presented by Keir [162] compared the first generation SH lens PureVision and the second generation SH lens Biofinity (comfilcon A; CooperVision; not yet available commercially). *Ex vivo* wettability was measured using the sessile drop measurement and found that the second generation lens, that did not have a surface treatment, provided better wettability and comfort for contact lens wearers throughout the month.[162]

In the last couple of years, Radke and colleagues have examined the wettability of silicone hydrogel contact lens materials.[164, 165] They have assessed the influence that various tear film components have on wettability using the captive bubble method. From these experiments, they concluded that the captive bubble technique is comparable to on-eye lens wear and that both advancing and receding contact angles are important to examine in order to fully diagnose the wettabililty of a contact lens material. [164] Tear film components including lysozyme, mucins, and proteins, influence the contact angles of the lens materials when they deposit on the surface of and tend to lower contact angles thus making lenses more wettable.[164, 165]

In 2006, Maldonado-Codina and Morgan completed an *in vitro* comparison study where they tested and compared both the sessile drop and captive bubble method on all five commercially available silicone hydrogel contact lens materials.[144] They concluded from this study that the contact angle values obtained were dependant on the method used. It was also determined that the surface active ingredients or surfactants added to the blister pack, when manufactured and shipped, tend to lower the contact angle measurements. The one interesting result is that the lenses had the tendency to group together based on their polymers, surface treatments, or internal wetting agents. The Acuvue Advance and Acuvue OASYS had similar results and the O_2 Optix and Focus Night & Day behaved alike. This is thought to occur due to increased surface chain mobility of the polymers in the Acuvue lenses and PureVision lens. As for the CIBA Vision lenses, there is decreased mobility due to the high refractive index, tightly-knit surface coatings.[144]

1.11 Lipid collection methods

To study the lipid in either the tear film or that which is deposited on contact lenses requires initial collection and then analysis of the lipid. When analyzing lipid on contact lenses, the lenses are exposed to either an *in vitro* lipid doping solution[51, 103] or removed from the eye after a pre-designated period of time.[106, 107] The lenses are then exposed to an extraction solvent, which is commonly based on methanol and chloroform in various ratios,[103] and the extract then analyzed by a number of various analytical methods, as described below.

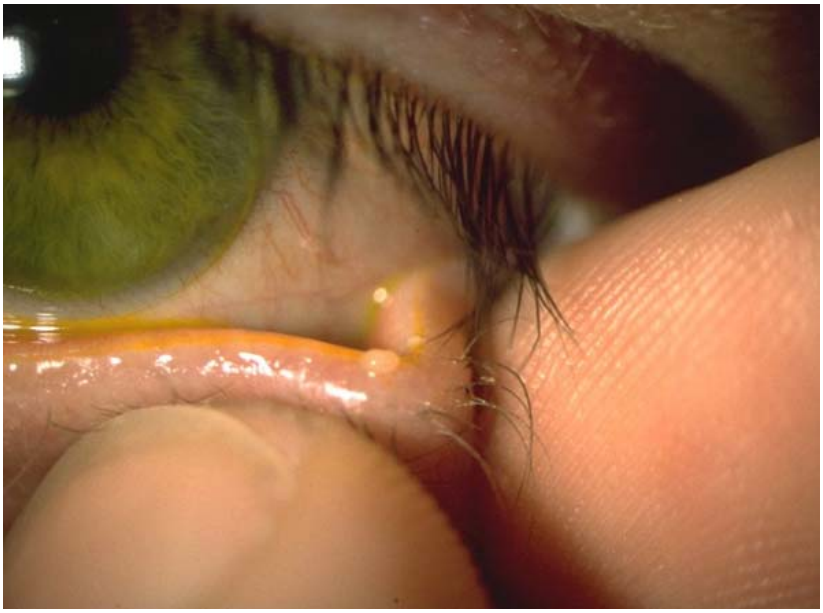
There are different procedures that are commonly used to collect lipid from various areas in the eye. The methods used to obtain meibomian gland secretions and samples of tear film will be briefly discussed in this section.

1.11.1 Meibomian gland fluid

The principal method used to collect lipid from the meibomian glands involves wiping the lid clean with a sterile swap, compressing the eyelid to gently squeeze out the lipids, and collecting the lipid.[166] The lid can be compressed between a lid conformer and a swab [44, 46, 47, 166] or between the clinician's fingers (Figure 13), [21, 48, 55, 70] with or without

the use of an anaesthetic. The meibomian gland secretions can then be collected using a spatula [44, 46, 47, 166] or a curette. [48, 55]

Figure 13: "Squeezing" the meibomian glands of the lower lid to express meibomian gland fluid, in a patient with frank meibomian gland dysfunction.



1.11.2 Tear Film

There are two commonly used methods to collect tear film samples for the analysis of lipid content: schirmer strips[88] and microcapillary tubes. [62, 66, 78, 167] Schirmer strips are filter paper strips which are commonly used to help diagnose dry eye syndrome, but can also be used to collect tear film samples. Schirmer strips are positioned to contact only the bulbar conjunctiva of the eye and to absorb tear fluid.[88] The tear film lipids can then be extracted from the strips and analyzed. This procedure is usually completed without an anaesthetic.

The most popular method to collect lipid tear film samples involves using microcapillary tubes. These are tiny glass tubes that draw in fluid via capillary action when the tube is placed in contact with the tear film. The microcapillary tube is gently placed in the eye to collect tears from the lower tear pool that forms above the lower lid (Figure 14).[78] In some cases, experimenters purposely collect stimulated tears[78], but usually unstimulated tears are preferred.[62] Once a tear film sample is collected in the microcapillary tube the fluid is then removed, extracted and analyzed. The popularity of this method can be seen in the number of studies that have used this technique for the collection and analysis of lipids in the tear film.[62, 66, 78, 167]

Figure 14: Tear film collection using a glass microcapillary tube.

1.12 Lipid analysis methods

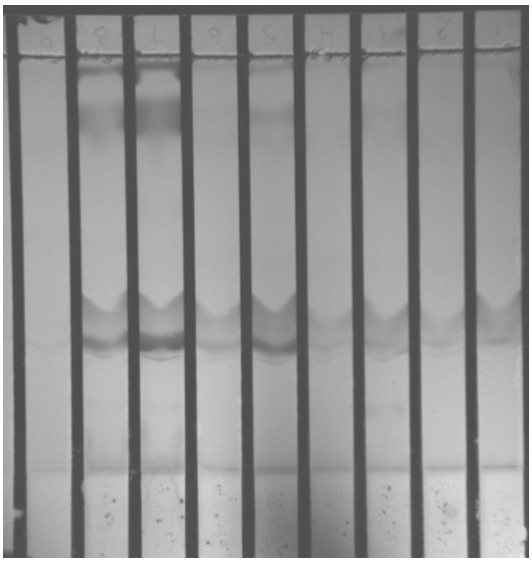
In the early days of research investigating lipid deposition on lens materials, qualitative techniques based on histochemical staining were used, primarily to determine the presence or absence of lipids only.[51] Light microscopy and electron microscopy were typically used in conjunction with these staining techniques to determine differences in deposition patterns.[87, 88] Radiochemical methods are occasionally used to analyze the uptake of certain lipids on to the surface of lenses.[168] By inserting radiolabelled lipids such as ^{3}H-cholesteryl oleate and ^{14}C-dioleoyl phosphatidylcholine into complex artificial doping solutions and incubating the lenses for 24 hrs at 37^{o}C, Prager and colleagues[168] were able to quantitatively analyze the radiolabelled lipid using scintillation counting.

More recently, quantitative or semi-quantitative methods have been employed, typically based around the use of chromatographic methods. The three common chromatographic techniques used to analyze lipids are thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography (GC).

1.12.1 Thin Layer Chromatography (TLC)

In every TLC procedure a plate made of glass, metal or plastic is coated with a thin layer of solid adsorbent material, usually alumina or silica. The sample is applied to the bottom of the plate, which is then placed in an enclosed chamber with a shallow pool of solvent. The liquid in the chamber is known as the mobile phase and is drawn up the plate via capillary action. The components in the sample solution separate on the plate according to their differing solubilities, polarity and their strength of adsorption. If the sample solution contains many different types of substances to be separated, then more than one solvent can be used. The types of substances separated dictate the method that will be used to view the plate. For ink separation, the bands can be seen by the naked eye. If the components separated are colourless, then the compounds can be viewed under UV light. In the case of lipids, the plate can be charred by a fine mist spray of sulphuric acid and is then baked. This blackens the resolved bands so they can be viewed without special equipment.[37] A TLC plate used to separate and identify lipids found on contact lenses can be seen in Figure 15. TLC is a fundamental qualitative method to determine the individual lipids which are present in a sample and can be used for quantitative means with limited accuracy. Despite this, many researchers have used this method to detect and analyze lipids from various locations in the eye.[52, 55, 88]

Figure 15: A sample TLC plate charred with sulphuric acid to visualize lipid banding patterns



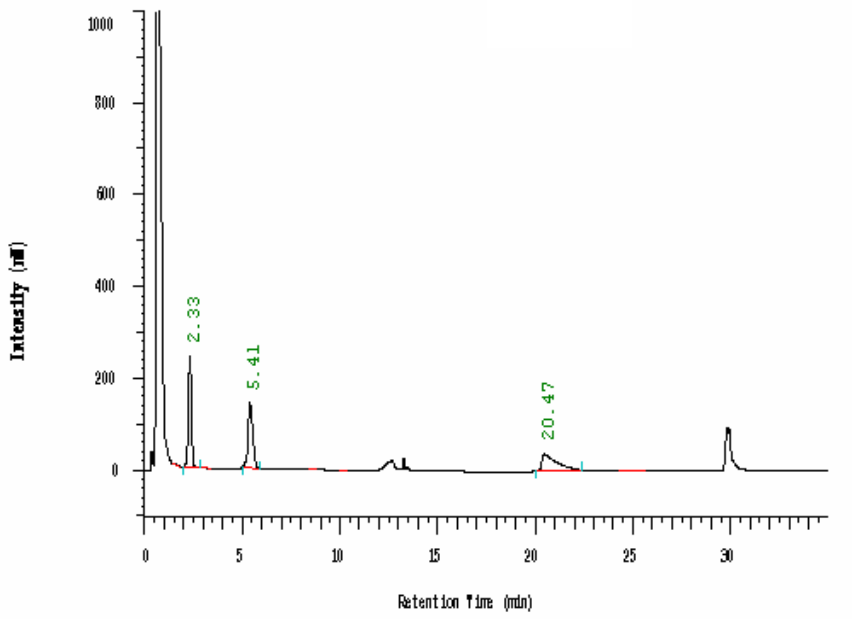
A sample TLC plate charred with sulphuric acid to visualize lipid banding patterns following lipid removed from an experiment in which 5 model lipids were deposited onto silicone hydrogel contact lenses using an *in vitro* model and were then extracted and separated using TLC. Each lane represents one extract from one contact lens. The various bands that are visible are due to the presence of one or more of the lipid extracted.

1.12.2 High Pressure Liquid Chromatography (HPLC)

HPLC is a significantly more technically advanced chromatography method of separation, which is relatively easy to use and is not limited by the volatility or stability of the sample compound. The separation techniques involve mass transfer between the stationary and mobile phases. Like its name, HPLC uses a liquid mobile phase to separate the components of a substance. The first step to separate a mixture is to dissolve it in a solvent and then to force it through a chromatographic column under high pressure, where the mixture is separated into its individual components.[37]

The type of compounds being separated dictates the types of solvents, columns, and detectors used to analyze the sample. Frequently, different HPLC procedures are used to analyze polar and non-polar lipids.[53] Once separated, the lipids of interest are studied by using specific detectors such as UV absorption, fluorescence, infrared, flame ionization, radioactive or mass spectrometry. Due to the variety of solvents, columns, and detectors available, HPLC has proven to be a very powerful tool in lipid analysis which is seen in the number of studies that have used this technique.[21, 63, 107, 169] An HPLC lipid chromatogram, used to identify and quantify lipid on contact lenses can be seen in Figure 16.

Figure 16: A typical reverse-phase lipid chromatogram analyzed using UV-LC

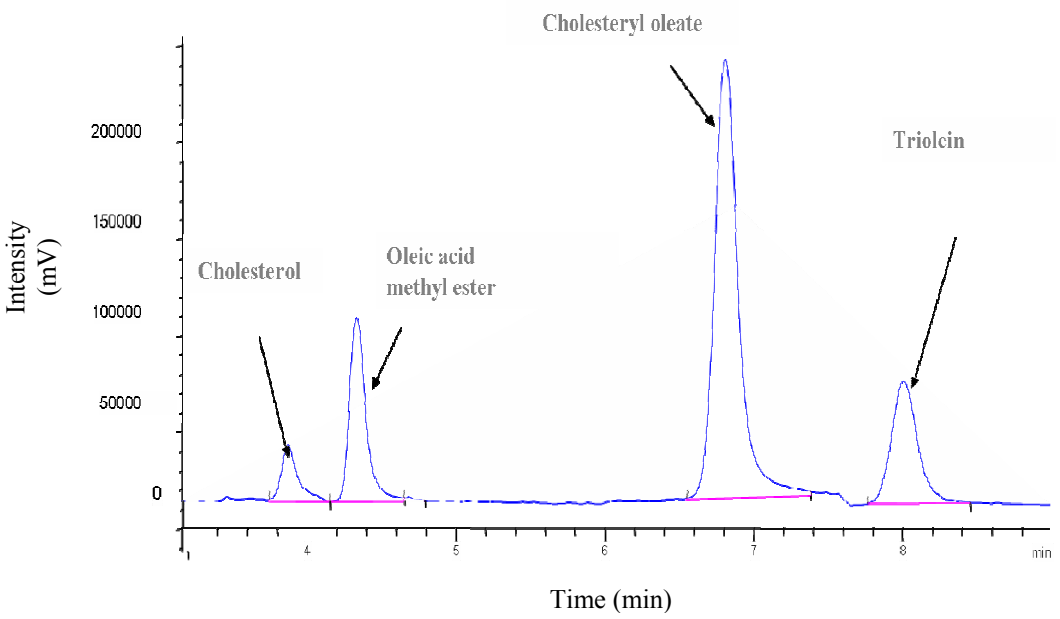


A typical reverse-phase lipid chromatogram analyzed using UV-LC for an experiment in which 5 model lipids were deposited onto silicone hydrogel contact lenses using an *in vitro* model and were then extracted and separated using HPLC. Each annotated peak represents one specific lipid identified at a wavelength of 205 nm.

1.12.3 Mass Spectrometry

Mass spectrometry is very commonly used as a detector due to its ability to reveal structural detail about the sample under investigation and quantify its components, but it is a costly technique. The process of mass spectrometry involves bombarding the sample with high-energy electrons that creates ions that are separated in a magnetic or electric field according to their mass-to-charge ratio. The resulting output is a spectrum of peaks corresponding to the molecular fragments and ionized molecules.[37] Specifically, mass spectrometry detection methods have been used to determine lipid content in meibomian gland secretions [6, 30, 55] and contact lenses.[107] An example of a mass spectrometer chromatogram used to identify the lipids in tears can be seen in Figure 17. Mass spectrometry detection methods are often used in eye-related lipid research, but it is not the only detection method, as UV absorption and fluorescence are also used.[6]

Figure 17: A sample mass spectrometry lipid standard chromatogram.



A sample mass spectrometry lipid standard chromatogram. Four lipid standards at a concentration of 2 ppm were analyzed using LC MS in ESI SIM mode with a mobile phase of chloroform, methanol and 10mM NH_4OAc. Each annotated peak represents one specific lipid.
Figure courtesy of Yu Gu.

1.12.4 Gas Chromatography (GC)

Gas chromatography (GC) induces separation of a compound using a gaseous mobile phase. The main component of the GC system is the separation column. Since the sample is carried through the column within the gaseous phase, the sample must be volatile. Samples of low volatility can be separated at high temperatures that allow for a high vapour pressure. However, samples separated at temperatures that are too high can cause unwanted decomposition of the sample and its components. The specific compound classes that can be analyzed via GC are dictated by their thermal stability. Therefore, large polar molecules are not usually separated using GC. The separated

compounds can be identified by their various retention times[170] and there have been many studies that have utilized GC techniques to analyze lipid. [27,44,38]

Just like HPLC, GC techniques can be linked to various types of detectors including mass spectrometers, infrared, UV absorption, and flame ionization. These detectors produce a chromatogram that is analyzed to identify each component. The type of detector used depends on the class of compound.[170]

1.12.5 Combined Techniques

Many studies have used TLC, HPLC, or GC or a combination of these to quantify lipid deposits from contact lenses, meibomian gland, and tear film samples. Often one chromatographic technique is not sufficient to analyze all lipid types found in the eye due to the wide range of polar and non-polar lipids. Each method has its own strengths with certain lipid types. TLC is usually used as a general separation technique where many different lipid types are separated from a complex unknown sample.[37] Following TLC separation, the broad lipid bands, that represent different groups of lipids, are removed and analyzed using other techniques like HPLC and GC. HPLC is often used for the separation of polar lipids, like cholesterols, and GC for the separation of non-polar lipids, like fatty acid methyl esters.[37] TLC has been used to quantify lipid content from contact lens depositions,[52, 88, 103-107] from tear samples,[78, 88] and from meibomian gland secretions.[21, 44, 47, 49, 55, 64, 166, 171] The quantification of lipids within these samples allow for a comprehensive understanding of the lipoidal role in the eye and what and

how external factors affect lipid content. HPLC has also been commonly used to analyze lipid content. Jones *et al.*[114, 139] utilized HPLC to quantify *in vitro* and *in vivo* lipid content deposited on silicone hydrogel lenses. These are the only published works to date outlining lipid on these lens materials. In contrast, HPLC has been used to analyze lipid content from conventional contact lens materials,[53, 63, 101, 107] tears[63, 169] and meibomian gland secretions.[6, 17, 29, 30, 32, 46-48] GC is most often used for meibomian gland secretions[17, 21, 32, 44, 46, 47, 49, 55, 171] and occasionally tears.[78] The majority of these studies have used at least two chromatographic techniques.

Other techniques are available for the quantification of lipids taken from tissues and contact lens surfaces. One technique involves a fluorescence assay for contact lens deposits. [109, 111, 112] Fluorescence techniques can be used to analyze lipid deposition due to the fluorescence signal emitted from lipids themselves. In this technique, lenses are placed in distilled water in a quartz cell. The sample is excited with an incident beam measured at a wavelength of 360 nm and the emission peak is monitored at a wavelength of 440 nm. The height of the emission peak is correlated with the amount of lipid deposition on the lens. Lipid depositions on conventional hydrogel lenses were analyzed using this technique to discover the deposition patterns on group II and group IV lenses that were previously discussed. This method is accurate for determining relative total lipid content, but not applicable for individual lipid concentrations.[109, 111, 112]

A second fluorescence technique has been used in the past. This technique involves staining the lenses with Nile Red, a fluorescence probe. [172] The lenses are then mounted

on silica plates and loaded into a fluorescence cell and imaged using customized equipment. From this technique, differences in lipid deposition between different contact lens materials could be seen. This method of quantification is an imaging technique, which is not applicable for individual lipoidal species quantification.[172]

The final technique of note, ^{31}P nuclear magnetic resonance (NMR) spectroscopy, has been used specifically for the analysis of phospholipids in meibomian gland secretions.[40, 41] NMR methods have allowed for the successful identification of seventeen distinct phospholipids.[40, 41]

It is clear from reviewing the literature on lipid analysis that this is a very technically challenging area, whether the lipid under investigation is from the tear film, meibomian glands, or contact lens materials. Since the eye contains such a large range of lipid types, there is no one direct or correct method to analyze all the lipids present. Therefore, more research on methods of lipid analysis must be undertaken, particularly given the interest in these areas relating to the role of lipids in dry eye and the deposition of lipids on hydrophobic silicone hydrogel materials.

2 OBJECTIVES AND IMPORTANCE

To date, there has been very little research focussing on lipid deposition on silicone hydrogel contact lenses, with only two publications suggesting that lipid does deposit more on silicone hydrogels than ionic conventional hydrogel materials.[114, 139] Clearly, additional studies are required on a class of materials that, due to their increased oxygen transmission, will dominate the development of new materials during the forthcoming 10 years.

The overall objectives of this thesis were to determine the best method of lipid extraction and to compare the total lipid deposited on silicone hydrogel lenses and conventional hydrogel lenses. The sensitivity, accuracy and ability of TLC and HPLC were tested for lipid analysis. Analysis of the effect that *in vitro* lipid deposition has on contact lens material wettability via contact angle measurement was also examined. These experiments should provide an insight into the process of lipid deposition on contact lenses, to establish a model and make connections between *in vivo* and *in vitro* conditions.

3 METHODS

A variety of studies were completed, to achieve my previously stated major objectives.

3.1 General Procedures

3.1.1 Lipid Stock Solution

The development of the lipid stock solution involved combining the desired lipids together in specific concentrations and dissolving them in the solvent ether or hexane. All individual lipids were purchased from Sigma (St. Louis, MO, USA). These specific lipids were chosen as they match the most common lipids found in the tear film, and therefore are the most likely to deposit onto a contact lens.[51, 56, 173] The five lipids were triolein, cholesterol, oleic acid, oleic acid methyl ester, and cholesteryl oleate. The characteristics of these lipids can be found in Table 4. The final concentration of lipids needed was quite small, so a 300X or 200X stock solution was made, and then the solution was diluted.

All measurements of lipid were taken on an analytical balance or using a pipettor. The lipid stock solution was placed in an amber vial, covered with aluminium foil, and stored at -20ºC to prevent oxidation and degradation of the lipids.

Table 4: Molecular and experimental details of the specific lipids used for all lipid doping solutions[51, 167, 174]

	Triolein	Cholesterol	Oleic acid	Oleic acid methyl ester	Cholesteryl oleate
Lipid type	Triglyceride	Sterol	Fatty acid	Fatty ester	Cholesteryl ester
Formula	$C_{57}H_{104}O_6$	$C_{27}H_{46}O$	$C_{18}H_{34}O_2$	$C_{19}H_{36}O_2$	$C_{45}H_{78}O_2$
Molecular Weight (g/mol)	885.5	386.7	282.5	296.5	651.0
Human Concentration mg/mL	0.016	0.056	0.008	0.024	0.075
Doping Concentration mg/mL (30X)	0.48	0.17	0.24	0.72	0.23

3.1.2 Lipid LDS

To make a lipid doping solution (LDS), the lipid stock was removed from the freezer and allowed to thaw in a dark place at room temperature. The required amount of saline PBS (phosphate buffer solution) was heated up to 37ºC in a culture tube in a water bath. Each lens was doped with 1.5 mL of LDS and the required amount of lipid stock was pipetted into the PBS under a culture fume hood, to maintain sterility of the solutions. The lid of the culture tube was left off to allow the hexane/ether to evaporate off. Once the solution was cool, the culture tube was reheated to 37ºC. The evaporation and reheating stage was completed 2-3 times to ensure maximal evaporation of the solvent. Once all the hexane/ether had evaporated, the solution was stored at –20ºC. When the LDS was ready to be used, it was taken out of the freezer and sonicated for 30 minutes before use, to ensure a homogenous solution.

The specific 30X lipid doping concentration used compared with human concentrations is found in Table 4. It should be noted that the 30X lipid doping solution contains 30X the human concentration of oleic acid, oleic acid methyl ester and triolein, but only 3X the human concentration of cholesterol and cholesteryl oleate. Cholesterol does not easily go into solution at high concentrations as it tends to form micelles.[175] A micelle is a mass of surfactant molecules that aggregate together so that the each molecule's hydrophilic head is in contact with the surrounding aqueous and the hydrophobic tail is "hidden" in the centre of the mass.[176] The critical micelle concentration or the concentration in which cholesterol forms micelles rather than going into solution is quite low, as the micelles are stabilized by intermolecular forces and encouraged to form due to repulsive forces from the solvent.[175]

3.1.3 Lipid Doping

To dope the unworn contact lenses, the sonicated LDS, doping vials and the contact lenses were placed in the culture fume hood. 1.5 mL of LDS was placed in a doping vial and one contact lens was placed in each vial as per Mirejovsky *et al*[51] The vial was then placed in a shaking water bath for the required amount of time at 37ºC.

3.1.4 Lipid Extraction

After sitting for 24 hours in 1.5 mL 2:1 chloroform: methanol at room temperature, lipid was extracted for 3 hours at 37ºC with constant stirring in the same solution. At this stage, the lens was removed and placed in a new vial and the lens and extract were frozen at –80ºC. A few days later the lens was extracted using the same protocol two more times.[30]

3.1.5 Evaporation

To condense the 1.5 mL extracts they were either lyophilized (Thermo Electron (Savant) speed vacuum SPD101B system with an RVT400 refrigeration vapour trap and a VLP80 pump) (Figure 18) to dryness and then re-suspended in 20 μL of 2:1 chloroform:methanol or they were evaporated under nitrogen (Organomation Association N-Evap® Model 111) (Figure 19) in a fume hood to dryness and re-suspended in 20 μL of 2:1 chloroform:methanol due to malfunction of the lyophilizer.

Figure 18: The refrigeration unit, pump, solvent trap, and centrifuge that make up the lyophilization system.

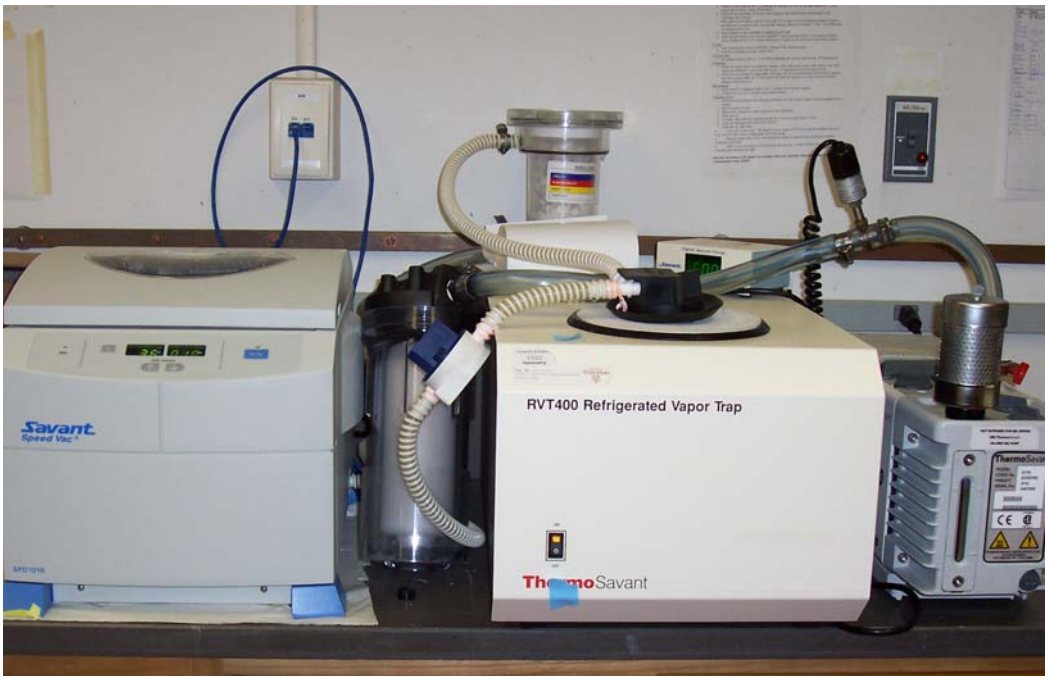
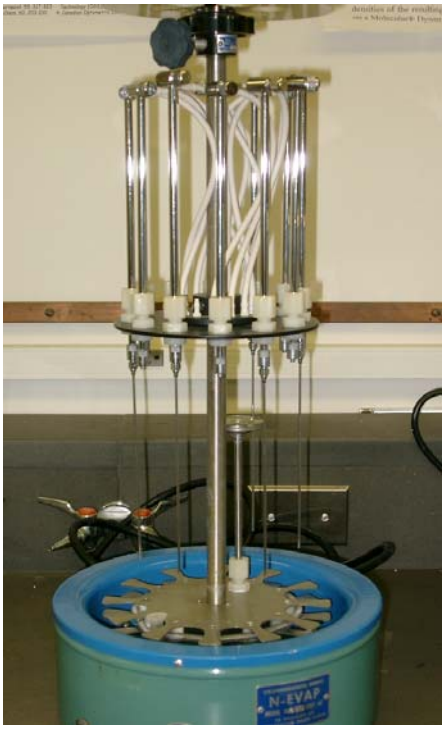


Figure 19: The nitrogen evaporation unit that is able to evaporate 12 samples simultaneously.



3.2 TLC Procedure

3.2.1 TLC Development

All TLC development took place in the fume hood. A ditch was scribed 0.5 cm from the top of the plate using a sharp pencil and care was taken to avoid getting dust or silica on the rest of the gel.[103, 105] The re-suspended samples were applied to the pre-absorbent strip of the plate using an eppendorf pipettor. Each sample was plated onto its own column of a 10x10 cm channelled, high resolution, silica gel, thin layer chromatographic plate (Whatman) and allowed to dry. Each vial was washed five times with 20 μL of 1:1 chloroform:methanol and each was carefully plated over the original spot and each spot was allowed to dry before the application of a new spot.[103, 105] A piece of filter paper was placed around the tank to equilibrate the vapour. (Figure 20) The plate was then exposed to four successive solvent systems:

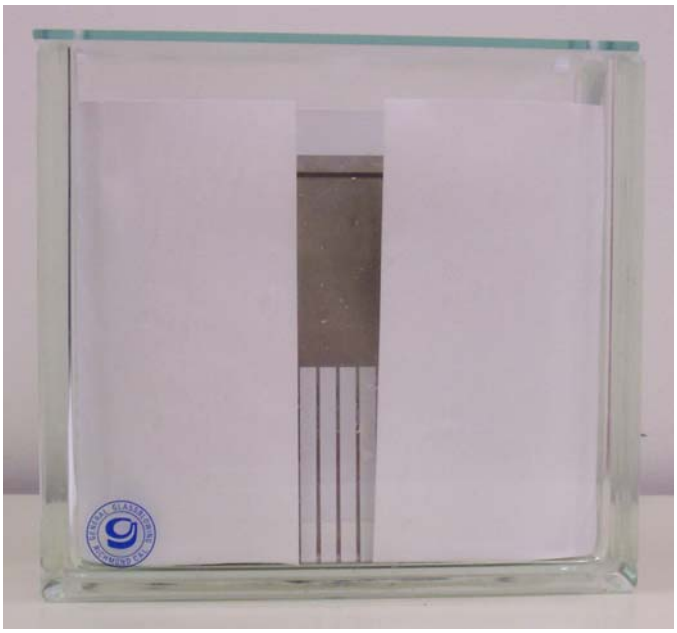
a) 30 mL of hexane

b) 30 mL of benzene

c) A mix of 30 mL of hexane, 20 mL of ether, and 0.5 mL of acetic acid

d) A repeat of (c), but allowing the solvent level to migrate only half way up the active silica layer rather than all the way to the ditch.[103, 105]

Figure 20: The TLC development chamber with the filter paper and TLC plate developing



3.2.2 Charring

The plate was placed in a Pyrex® rectangular dish (23 cm x 38 cm) in the fume hood, sprayed with a fine mist of 50% sulphuric acid using a 50 mL Pyrex® chromatographic sprayer (No. 4980) (Figure 21), and then placed in an oven (Memmert; UM400) at 115°C for 1 hour. After being removed from the oven the plate was placed in a dessicator (20 cm in diameter) to cool. [37]

Figure 21: The specialized glass chromatographic sprayer that is used to spray 50% sulphuric acid onto the developed TLC plates.

3.2.3 Quantification

After the plates had cooled they were imaged in white light using the Syngene Gene Genius Gel Documentation System™ and associated software to give relative amounts of lipid using densitometry. The quantification of lipid was only in relative amounts, as it is difficult to make comparisons between plates without different software and standards of all lipids on each plate.

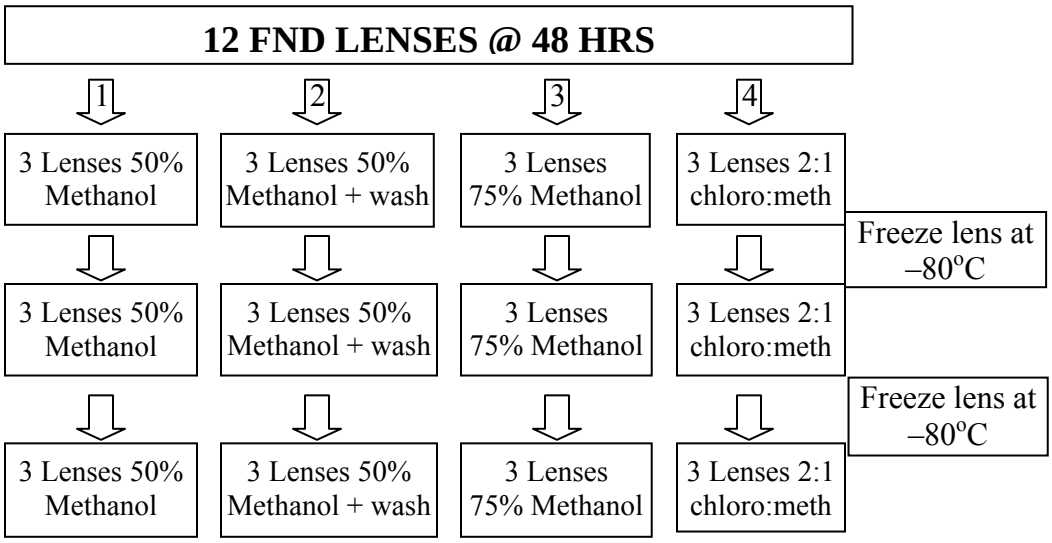
3.3 TLC Experiments

3.3.1 Extraction Efficiency Study

The first experiment undertaken was designed to optimize the extraction of lipids from silicone hydrogel contact lenses. This was an *in vitro* doping study which involved the synthesis of a lipid stock solution, development of a 30X lipid doping solution (LDS), development of a lipid-doping protocol for doping the lenses, and development of a protocol for using TLC plates to examine the lipids of interest.

To determine which extraction procedure was the best, four different extraction methods were developed and compared. Two lens types, Focus Night & Day and PureVision, and two time periods of doping, 48 hrs and 96 hrs, were also tested. The flow chart outlining this method is displayed in Figure 22.

Figure 22: Extraction efficiency experimental procedure



FND = Focus Night&Day

This same procedure was completed for FND lenses doped for 96 hrs and PV lenses for both time periods with n=3

The detailed extraction procedures were:

a) Soaking a lens in 1.5 mL of 50% methanol for 3 hours at 37ºC with constant stirring. The lens was then extracted a second and third time using 50% methanol. [103, 105]

b) Soaking the lens in 1.5 mL of 50% methanol for 3 hours at 37ºC with constant stirring. After 3 hours the lens was removed and 200 µL of chloroform: methanol (1:1) was used to wash the lens. The methanol extraction and the chloroform/methanol wash were pooled together. The lens was then extracted a second and third time using the same protocol.[103, 105]

c) Similar to (a), except that 1.5 mL of 75% methanol was used. Three extracts in total were completed.[103, 105]

d) After sitting for 24 hours in 1.5 mL 2:1 chloroform: methanol at room temperature, lipid was extracted for 3 hours at 37ºC with constant stirring in the same solution. At this stage, the lens was removed and placed in a new vial and the lens and extract were frozen at –80ºC. A few days later the lens was extracted using the same protocol two more times.[30]

All of the lenses were removed from the extract and were placed in a clean vial. All of the extracts and the lenses were frozen at -80ºC.

All extracts were brought to room temperature, evaporated and analyzed using the general TLC procedure detailed previously.

3.3.2 CLASH Study (Contact Lenses and Silicone Hydrogels)

This second completed experiment consisted of the laboratory assessment of lenses from an *in vivo* study, during which 30 subjects wore FND SH lenses on an overnight basis for two consecutive one-month periods. During the first month, patients used a saline rewetting drop four times per day to improve lens comfort, and for the second month they used a surfactant-containing rewetting drop (Alcon CLENS100™) in an identical manner. At the end of each month lenses were collected in saline and then immediately extracted using the 2:1 chloroform: methanol extraction procedure. After extraction, TLC analysis of the elute was completed, as previously described.

3.4 Contact Angle Experiment

3.4.1 Lipid Deposition and its Effect on Lens Wettability

Nine different lens materials in total were used for this experiment. 5 silicone-hydrogels: balafilcon A (PureVision, Bausch&Lomb), lotrafilcon A (Focus Night&Day, CIBA), galyfilcon A (Acuvue Advance, Johnson & Johnson), lotrafilcon B (O_2 Optix, CIBA), and senofilcon A (OASYS, Johnson & Johnson). The 4 conventional hydrogel lenses

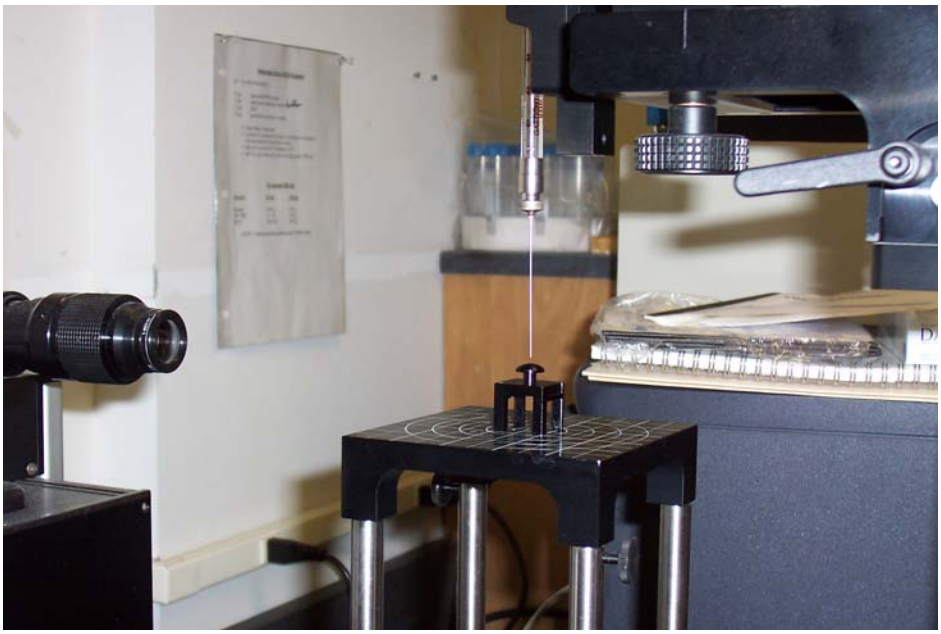
were: etafilcon A (Acuvue 2, Johnson & Johnson), omafilcon A (Proclear, CooperVision), alphafilcon A (Soflens 66, Bausch & Lomb), and polymacon (Soflens 38, Bausch & Lomb). Individual lenses were doped with two different lipid doping solutions (LDS) containing cholesterol, oleic acid, and oleic acid methyl ester at 37 ^{o}C with constant shaking. The concentration of the lipids in the LDS was approximately 2.5x and 30x that seen typically in the eye. Lenses were soaked in the two LDS types for 2 or 5 days and compared with lenses soaked in Phosphate Buffered Saline (PBS) only. All experiments were completed in triplicate.

The contact lens was removed from the doping solution using silicone tipped forceps and placed anterior side down on a piece of lens paper for thirty (30) seconds in order to wick off any excess fluid that may affect the result. After the time elapsed, silicone forceps were used to pick up the lens and place it posterior side down on a custom convex mantle that matches lens curvature. The mantle was then centred beneath the syringe of the Optical Contact Analyzer (OCA) (Dataphysics OCA 20L) and a high speed camera was focused on both lens and syringe, as seen in Figure 23 and Figure 24. Sixty (60) seconds after the lens was removed from the paper and placed onto the mantle, a 5 μl drop of HPLC grade water was dispensed from the syringe via a computer controlled mechanism. The drop was allowed to stabilize for a few seconds, and then the mantle was manually raised up to the drop until contact was made. Allowing 2-3 seconds for the drop to settle, an image of the drop on the lens was taken and saved to the computer hard drive. Due to the curved profile of the surface, a curved baseline profile-detection fitting algorithm resident in the software was used to determine the

angle that formed between the drop and the lens surface. (SCA 20 software, Version 2.04, Build 4).

Figure 23: The optical contact analyzer used to analyze contact angles.

Figure 24: The optical contact analyzer showing the syringe, stage, custom convex mantle, and digital camera.

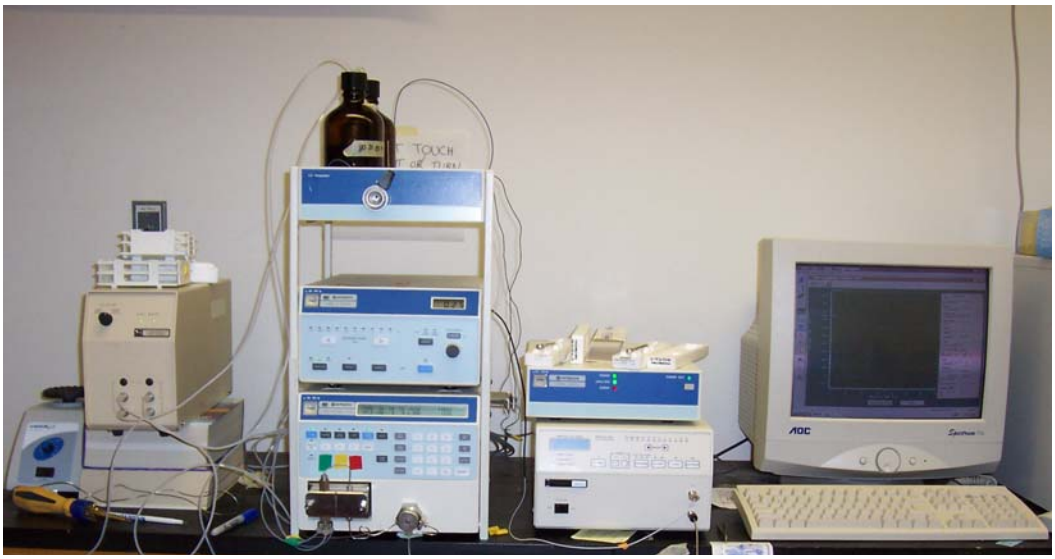


3.5 HPLC Procedures

All lipid stock solutions, artificial lipid solutions, doping, extracting and evaporating were completed as previously described. Following evaporation, samples were re-suspended in 50µL of a 1 mg/mL reference standard dissolved in 2:1 acetonitrile:MilliQ water. Samples were then processed using reverse-phase HPLC.

A Hitachi HPLC system (pump L6200, UV detector L4000) (Figure 25), was used to analyze the lipid deposits.

Figure 25: The Hitachi HPLC system including the pump, UV detector, and degasser.



3.5.1 Reverse Phase Procedure

Chromatographic conditions:[114]

Column:	C18 nucleosil 5µ 125 x 3mm
Mobile phase A:	HPLC grade acetonitrile
B:	80:20 (HPLC grade acetonitrile:Millipore water)
Flow:	0-10 min => 1.5 mL/min mobile phase B
	10.5 – 12 min => 1.5 mL/min mobile phase A
	12 - 27 min => 2.0 mL/min mobile phase A
	27.5 – 35 min => 1.5 mL/min mobile phase B
Detection wavelength:	205 nm
Syringe fill:	20 µL
Injection volume:	10 µL

Before samples were injected into the HPLC machine, a calibration curve was run to determine the R^2 value for each lipid component and assess the linear range. The R^2 value should be 0.98 or above. Once a calibration curve was established for each of the detectable lipid, samples could be run. The first and last injections were a reference standard and then every 5-8 sample injections were bracketed by reference standards. [114]

The lipid deposit mass could then be calculated using the average standard area count of the two standards bracketing the given samples. The equation is as follows: Deposit mass (μg) = [(sample area count * standard concentration / average standard area count) – standard concentration] * 0.05 * 1000.[114]

3.6 HPLC Experiments

3.6.1 In vitro Lipid Doping Experiment

This study compared total lipid deposition on a variety of lens materials using the *in vitro* methodology previously described. For this experiment, only the most efficient extraction method was used, 2:1 chloroform:methanol. In this experiment five silicone hydrogel materials (FND, PV, AA, O_2 Optix, and OASYS) and four conventional hydrogels (Soflens 66, Acuvue, Soflens 38, and Proclear) were artificially incubated for 5 days in a 30X and 2.5X lipid doping solution containing five lipids, oleic acid, oleic methyl ester, cholesterol, cholesteryl oleate, and triolein (n=4 for each condition).

Following incubation and extraction, lenses were analyzed using the reverse-phase HPLC method described previously.

Even though the LDS contained five different lipids, the reverse phase HPLC procedure used is only able to detect three lipids, oleic acid, oleic acid methyl ester, and cholesterol. This procedure was optimized for these three specific lipids. Cholesteryl oleate and triolein do not separate using the specific mobile phases and step-wise gradient characteristic of this method. However, a LDS containing all five lipids was still used for the HPLC experiments so that the results could be compared with the TLC and wettability results, completed previously.

3.6.2 SPA (Sustained Performance and Adaptation of Silicone Hydrogel Contact Lenses During Daily wear)

This final experiment consisted of the laboratory assessment of lenses from an *in vivo* clinical study, during which 55 subjects wore all five commercially available silicone hydrogel lenses for two cycles of two weeks on a daily-wear basis. Eight subjects were randomly chosen and the five contact lens materials analyzed, Acuvue OASYS, Acuvue Advance, Focus Night & Day, O_2 Optix and PureVision. All subjects used the same cleaning care regime, Clear Care. After each two week cycle, lenses were collected and analyzed for several experimental laboratory based parameters, including lipid deposition. Lenses were immediately extracted upon collection using the previously

described 2:1 chloroform:methanol procedure and then evaporated and analyzed using the reverse-phase HPLC protocol.

4 RESULTS AND DISCUSSION

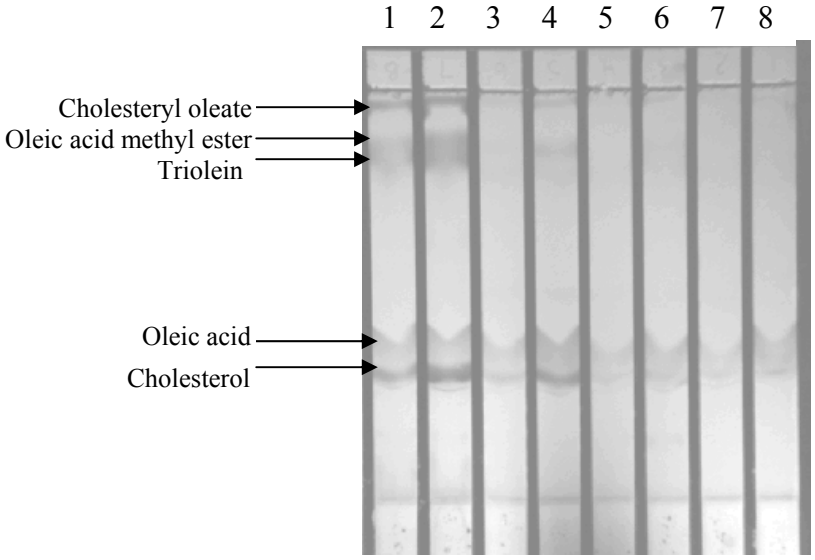
4.1 TLC Experiments

4.1.1 Extraction Efficiency

The extraction efficiency of four different extraction solutions were examined when lipid was deposited on two different silicone hydrogel lens types, as described in section 3.1.1.

Typical first, second, and third extraction plates are displayed in Figures 26, 27, and 28 to compare the lipid recovered in each extraction phase. Please refer to the legend below to determine what samples were plated in each lane.

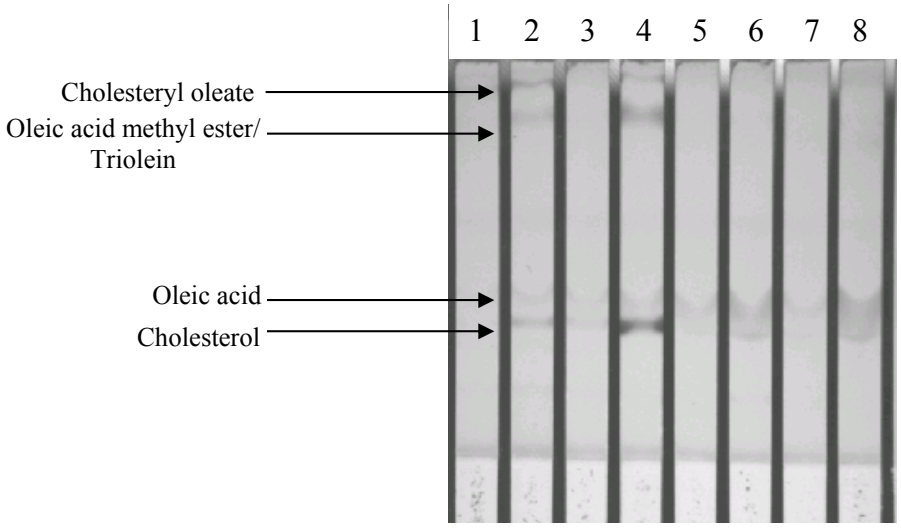
Figure 26: Typical first extract plate for 96 hrs of doping.



All five lipids were detected at their highest concentrations, as they had the darkest banding patterns, using the 2:1 chloroform:methanol extraction method (lanes 1 and 2). The other extraction procedures did not display all five lipid bands, in fact, most only displayed 3 distinct bands. PV lenses tend to display darker banding patterns than FND.

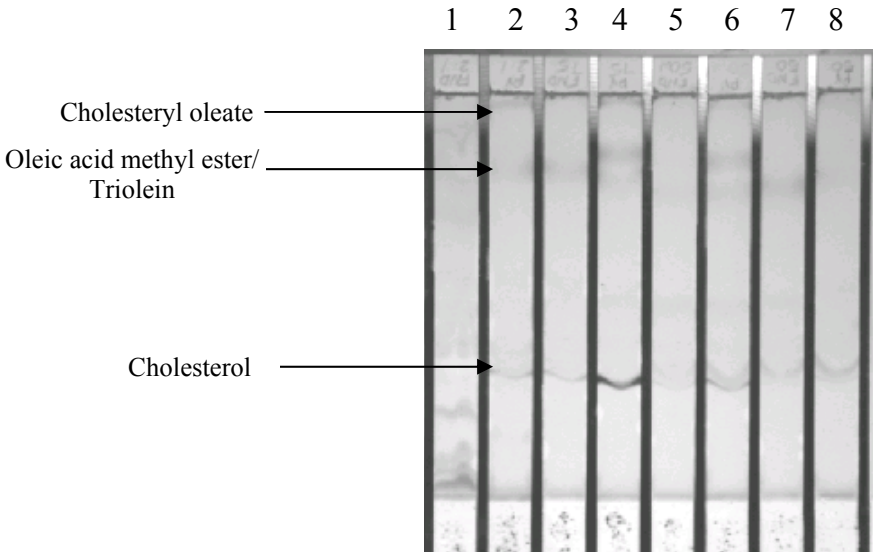
Legend:
Lane 1 – FND + 2:1 chloroform:methanol
Lane 2 – PV + 2:1 chloroform:methanol,
Lane 3 – FND + 75% methanol,
Lane 4 – PV + 75% methanol,
Lane 5 – FND + 50% methanol + wash
Lane 6 – PV + 50% methanol + wash
Lane 7 – FND + 50% methanol
Lane 8 – PV + 50% methanol

Figure 27: Typical second extract plate for 96 hrs of doping.



Four lipids were detected using the chloroform:methanol method. Varying amounts of lipid were extracted depending on the extraction method and lens type. Much more lipid was extracted from the PV lenses (lanes 2, 4, 6, 8) as opposed to the FND lenses (lanes 1, 3, 5, 7).

Figure 28: Typical third extract plate for 96 hrs of doping.



Two to three lipids were detected using chloroform:methanol method. Faint banding and therefore small concentrations of lipid was extracted using chloroform:methanol (lanes 1 and 2). The other three extraction methods (lanes 3-8) have higher concentrations of lipid and therefore a darker banding pattern. No lipid was found on the FND lens using chloroform:methanol extraction (lane 1).

The statistical summary for the four-way repeated measures ANOVA is presented in Table 5 and the summary graph demonstrating the statistical significance found in the lens type and extraction method can be seen in Figure 29**.**

Table 5: Four-way repeated measures ANOVA summary table.

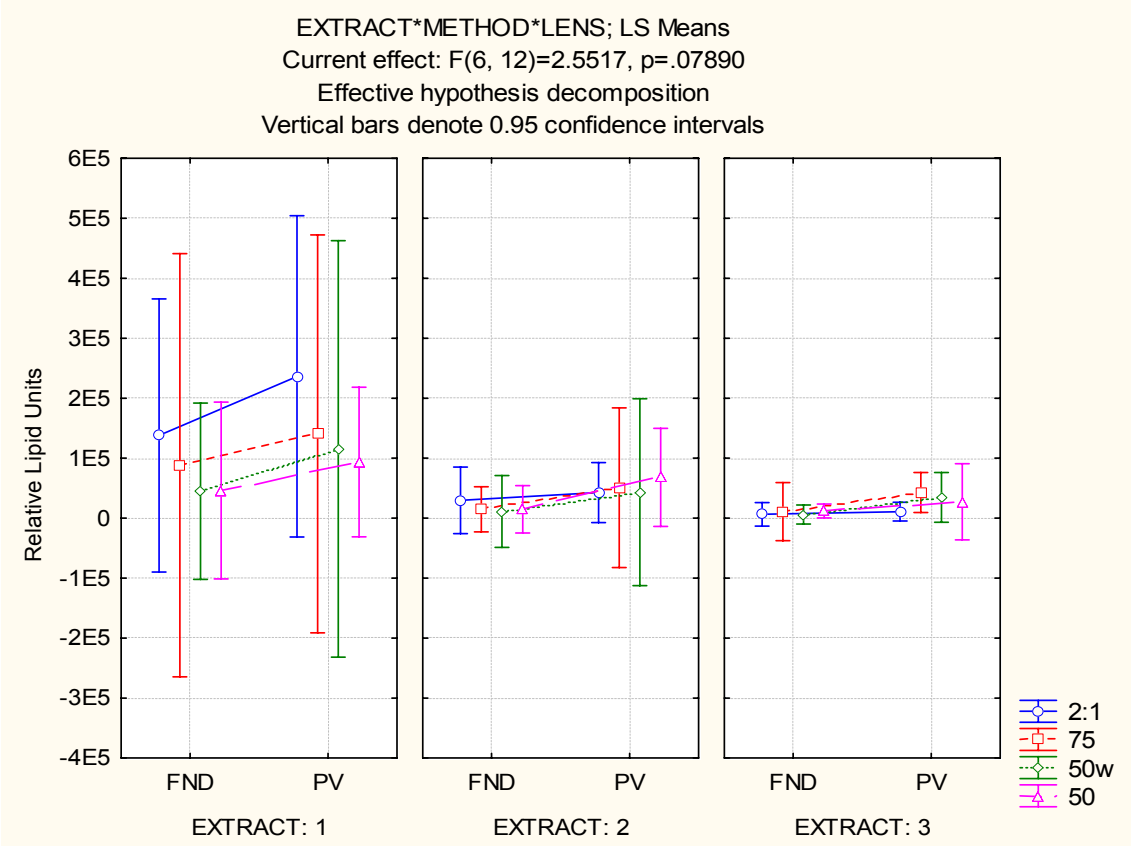
Four-Way Repeated Measures Analysis of Variance ANOVA				
	Variables	**Degrees of Freedom**	**F**	**p**
Time	Length of doping (48, 96 hrs)	1	0.48214	0.559269
Extract	Extraction Number (1^{st}, 2^{nd}, 3^{rd})	2	4.07602	0.108348
Method	Method of extraction (50% meth, 50% meth + wash, 75% meth, or 2:1 chloro:meth)	3	10.68803	0.008044
Lens	Lens Type (PV or FND)	1	81.50546	0.012048
Time*Extract		2	0.72159	0.540028
Time*Method		3	2.64862	0.143111
Extract*Method	Interaction between method of extraction and number of extractions performed	6	11.12586	0.000265
Time*Lens		1	2.69797	0.242185
Extract*Lens		2	13.12407	0.017487
Extract*Method* Lens		6	2.55172	0.078900
Time*Extract* Method*Lens		6	1.05815	0.437380

P values displayed in red are the variables that were statistically significant ($p < 0.05$).

Statistical analysis showed statistical significance for lens type, with PV lenses depositing more than FND ($p<0.05$). Significance was also found for the method of extraction alone ($p<0.05$), and an interaction was found between the number of extractions performed and the method of extraction ($p<0.05$). In other words, some methods of extraction consistently remove moderate amounts of lipid after each extraction, where others remove the most after the first extraction and decreasing amounts after that. Significant differences were not found between the two doping times, 48 and 96 hours ($p>0.05$). Significant differences were not found for the 3 way

interaction between the method of extraction, lens type, and number of extractions (p=0.079)

Figure 29: Extraction efficiency summary graph.



PV =PureVision, FND = Focus Night&Day

This graph demonstrates that PV lenses had greater lipid deposition and that the most efficient extraction method for both lens types is 2:1 chloroform:methanol. This graph also demonstrates that the bulk of lipid was extracted in the first extraction.

The results of the extraction efficiency experiment show that PV lenses deposit significantly more lipid than FND lenses and the 2:1 chloroform:methanol extraction

method was the most efficient of the four methods tested. Not only did 2:1 chloroform:methanol extract more lipid over the three sets of extractions, but it also extracted over 95% on the first two extractions. It must be kept in mind that these lenses were doped with a lipid solution 30X the concentration normally found in the eye, therefore causing excessive amounts of lipid to deposit onto the lenses.

The results from this experiment demonstrate the ability for this TLC method to adequately separate and detect differences in lipid deposition between two different silicone hydrogel contact lens materials. However, TLC was not able to adequately quantify the various lipid deposits, therefore, another analysis method is required.

Historically, the primary use for TLC was separation of many different lipid types using a multiple solvent system, just as we have demonstrated. Further quantification could be attained by scraping off individual lipid class bands from the plate and analyzing them separately, using specific analytical and chromatographic techniques for the specified lipid group. This cannot be done if the lipids were charred using sulphuric acid, as our procedure described.[37] Additional analysis of each lipid group is time consuming, expensive, but much more accurate and sensitive. If one does not have the time or resources to analyze each lipid group individually, the ultimate protocol would be to separate and quantify main groups of lipid with one novel method. Quantification can be done with TLC by densitometry after charring, but it requires the constant addition of all lipid standards on each plate, making this process unrealistic.

The combination of chloroform and methanol as an extraction solvent is one of the most common in lipid analysis.[177] It was first developed by Folch and colleagues in 1957.[178] The Folch method, involves extracting lipid contents from a tissue in 2:1 chloroform:methanol. The water within the tissue mixes with the chloroform:methanol and then separates into two phases. The upper phase is predominately water and methanol which contains most of the non-lipid contaminants, leaving the bottom chloroform and methanol phase containing all of the lipids. This can be repeated several times to attain a pure lipid solution.[178] This is thought to be the most efficient and accurate method, when compared to the other commonly used, Bligh and Dyer extraction method.[177] The Bligh and Dyer method involves the addition of water in the original extraction solution, which is removed later.[179] This method was developed to reduce costs when extracting lipid from fish which specifically contain a very small amount of lipid in their muscle.[179]

The use of chloroform and methanol as an extraction solution, with or without the addition of water, is used in many different areas of lipid research, including vision research. This technique for lipid extraction is very efficient as all lipid groups are soluble in either chloroform or methanol. Many lipid groups can be extracted using chloroform:methanol, including phospholipids,[180-186] sterols,[182, 185, 186] [30]fatty acids,[33, 180, 184-187] sphingolipids.[186, 188], and triglycerides[185, 186] This has become the universal extraction solvent for lipids.

The increase in lipid deposition seen in PV lenses when compared to FND lenses can be explained by their corresponding surface treatments and their polymer composition. PV lenses are treated in a gas plasma reactive chamber, and this chamber alters the silicone to give the surface of the lens hydrophilic glassy islands to mask the underlying hydrophobic material.[123] This surface modification is not homogenous and thus causes large amounts of hydrophobic silicon to be exposed to the tear film, thus encouraging lipid deposition on the lens surface.[123]

As explained previously, Purevision (PV) lens materials are a homogenous combination of the silicone-containing monomer polydimethylsiloxane (a vinyl carbamate derivative of TRIS) co-polymerized with the hydrogen monomer N-vinyl pyrrolidone (NVP).[129, 132-134] Previous research has shown that NVP containing contact lens materials tend to deposit more lipid, as NVP is a hydrophobic monomer.[108-110, 113] In contrast, FND lenses do not contain NVP, but have a fluorosiloxane phase (silicone phase).[131, 132] The addition of fluorine is known to decrease the hydrophobicity of the lens material and thus decreases lipid deposition.[103]

It is know that lipid deposition occurs very quickly upon insertion of the contact lens onto the cornea, with lipid deposition being noted within the first couple of hours of wear. This deposition continues rapidly within the first week and continues, without a plateau, for the entire duration of wear or until the lens is replaced.[189] Therefore, the doping times of 2 days and 4 or 5 days are chosen to examine lipid deposition within the

first week. It is suggested that future studies should look at lipid deposition kinetics throughout the month of wear.

To this point, this is the only research analyzing lipid deposits on silicone hydrogel contact lenses via TLC. This technique has been used previously to separate lipids deposited on conventional hydrogel lenses and gas permeable contact lens materials.[103-105, 107]

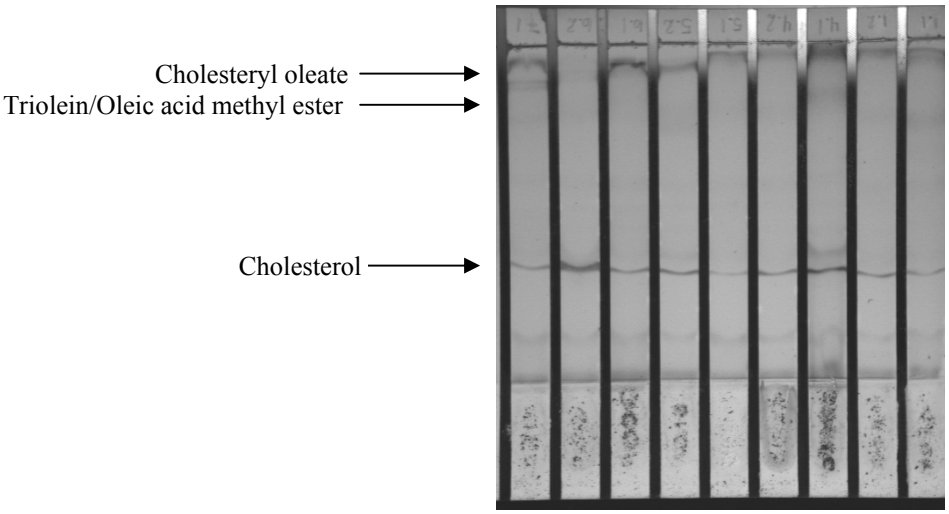
4.1.2 CLASH Study

This study examined FND lenses from a clinical study where two drop-types are compared, as previously described in section 3.3.2.

The first extracts recovered a modest amount of lipid, but not all five common lipids were always found. The five common lipids that we being analyzed were cholesterol, cholesteryl esters, oleic acid, oleic acid methyl esters, and triolein. Figure 30 shows a typical first extract plate. A second extract was completed on all lenses to ensure the removal of all lipids from the lenses. The second extract revealed a unique banding pattern consistent with unworn lens extracts. This led to the conclusion that all of the lipids had been removed during the first extraction, and that the banding seen in the second extraction was a result of interaction with the lens polymer. This confirms the ability of 2:1 chloroform:methanol to extract the lipid, and increased the efficiency rate

of the method for human worn lenses. In this case, 100% of the lipid was removed with the first extraction.

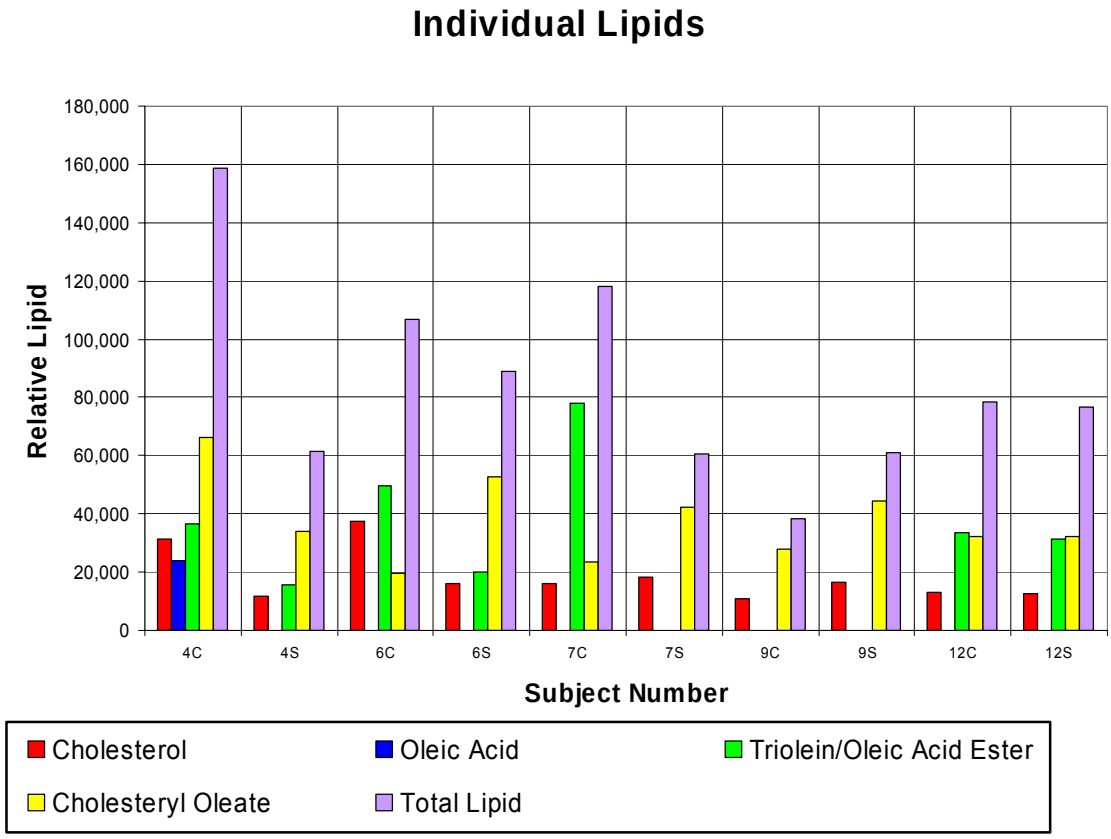
Figure 30: Typical TLC plate for first extraction of CLASH lenses



Only three to four lipids were commonly identified. Each lane represents the first extraction for one subject's contact lens, which was used with one of the rewetting drops. The second drop regime was plated in the next lane.

Figure 31 shows the range of individual lipid types found deposited on contact lenses treated with both the saline and CLENS100. In this graph, the lipid types can be examined and general trends in individual lipid deposition could be seen. Cholesteryl oleate and cholesterol are the lipids that were seen to deposit on every lens from every subject. Cholesteryl oleate deposited the highest concentration compared to the other four lipids. This dominating presence of cholesterol esters and cholesterol was also seen by Maissa and colleagues in tear samples taken from contact lens wearing subjects.[63]

Figure 31: This graph demonstrates the common lipid types that deposited.



On the x-axis the subject numbers are described as follows:
4C= subject # 4 treated with Clens100 drops. 4S= subject # 4 treated with saline drops.
This graph shows only a portion of the total subjects.

Oleic acid methyl ester and triolein were not extracted from every lens, but when they were, their combined concentrations were comparable to cholesteryl oleate. Triolein and oleic acid methyl ester were grouped together for densitometric and statistical analysis as the two bands had a tendency to smear together. Oleic acid only deposited on a few of the lenses. These are similar lipids that have been found in the tear film and deposited on contact lenses;[51, 56, 173] however, concentrations are difficult to compare, as individual variations in lipid concentration vary so greatly.[65, 110] Another study completed by Rapp, was able to examine *in vivo* worn conventional hydrogel contact lenses using TLC

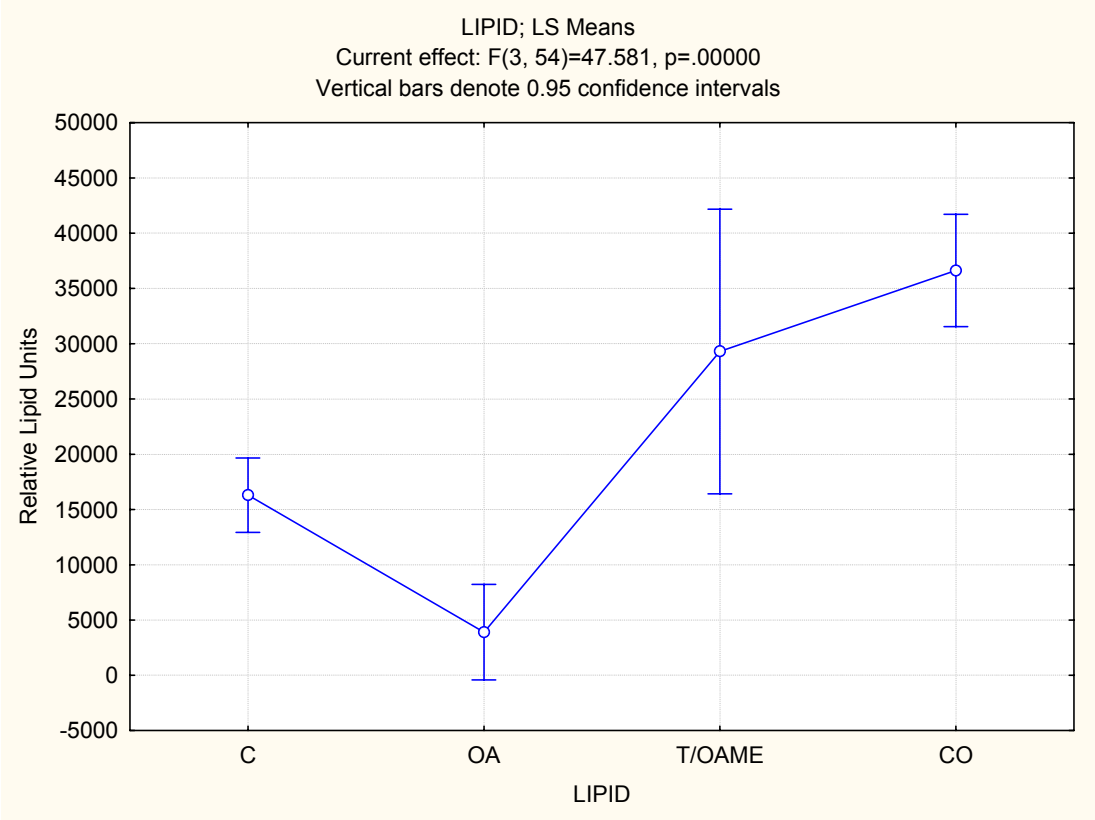
and could qualitatively identify several classes of lipids including, wax esters, monoglycerides, diglycerides, free fatty acids, fatty alcohols, and fatty sterols. Cholesterol, cholesteryl esters, and triglycerides were absent in these experiments.[107] Once again, this demonstrated the variation in lipid deposition results.

Table 6 and Figure 32 provide statistical analysis of the data. Statistical analysis (ANOVA) revealed that there is no statistical significance in the amount of lipid that deposited on contact lenses used with either CLENS100 or the saline drops. There was a statistically significant difference in the lipid types that deposited. The interaction between the drops and the various lipid types, did not reach statistical significance.

Table 6: The repeated measures analysis of variance summary chart analyzing the lipid types and solution drops used.

Repeated Measures Analysis of Variance					
	Sum of Squares	**Degrees of Freedom**	**Mean Squares**	**F statistic**	**p**
Drop Type	5.564131E+08	1	5.564131E+08	3.1327	0.093674
Lipid Type	2.381163E+10	3	7.937209E+09	47.5811	0.000000
Drop Type*Lipid Type	6.829184E+08	3	2.276395E+08	2.0471	0.118143
Error	6.004962E+09	54	1.112030E+08		

Figure 32: The relative lipid types deposited



C = cholesterol, OA= Oleic acid, T/OAME = Triolein/Oleic acid methyl ester, CO = Cholesteryl oleate

It is clear from these results that there was no meaningful difference in total or individual lipid deposition when comparing the saline and CLENS100 regimes. If we examined the lipid types that deposited more closely, we could make a few conclusions. Each lipid type or group deposited consistently among subjects irrespective of the drop regime and the relative average concentrations between the lipid types that deposited were distinctly different.

The results from this experiment did not provide concrete answers regarding the value of using a surfactant-containing drop like CLENS100 to affect the amount of lipid

deposition, due to the high degree of variability in the data accumulated. If the CLENS100 drop is meant to reduce lipid deposition, then the results would show decreases in lipid deposition when that drop was used. However, most drops are used to reduce protein and not lipid deposition, and CLENS100 is no different.[190] Therefore, since CLENS100 does not affect the lipid deposition, then contact lens wearers with normally high lipid deposition should have shown the same lipid deposition with CLENS100 use, but once again total lipid deposition varied. Therefore, it was possible to analyze relative lipid amounts using TLC, but the technique was too insensitive to measure small differences.

4.1.3 TLC Experimental Conclusions

The overall conclusions that can be drawn from these TLC experiments were that an extremely efficient extraction method was developed using 2:1 chloroform:methanol. This extraction solution was able to sufficiently extract all lipid types found in the human tear film and therefore deposited on contact lens surfaces. This extraction method can be used to extract lipid from silicone hydrogel lens materials, which are known to deposit greater quantities of lipid when compared with conventional hydrogel lens materials.

TLC could adequately separate a lens extract into distinct banding patterns that represent various lipid types. By charring these bands with sulphuric acid and imaging the banding pattern, relative lipid amounts could be analyzed for comparison with other samples.

This technique was sensitive enough to detect broad differences in materials, but was not sensitive enough to detect subtle differences in human-worn lenses that were subjected to differing care regimes. Therefore, HPLC may provide a more robust analysis procedure for contact lens lipid deposition analysis where lipid deposited can be quantified.

The final conclusion that can be drawn from these experiments was that PV contact lens materials do deposit more lipid than the FND. These were the first generation silicone hydrogel contact lens materials. Further investigation is required to analyze the newer silicone hydrogel lens materials and conventional polyHEMA-based hydrogel materials.

4.2 Wettability

4.2.1 Lipid Deposition and its Effect on Lens Wettability

The influence of lipid deposition on advancing contact angles for various lens types was examined, as described in section 3.4.1.

When all variables and data points were analyzed using an ANOVA statistical test, statistical significance was found as seen in Table 7.

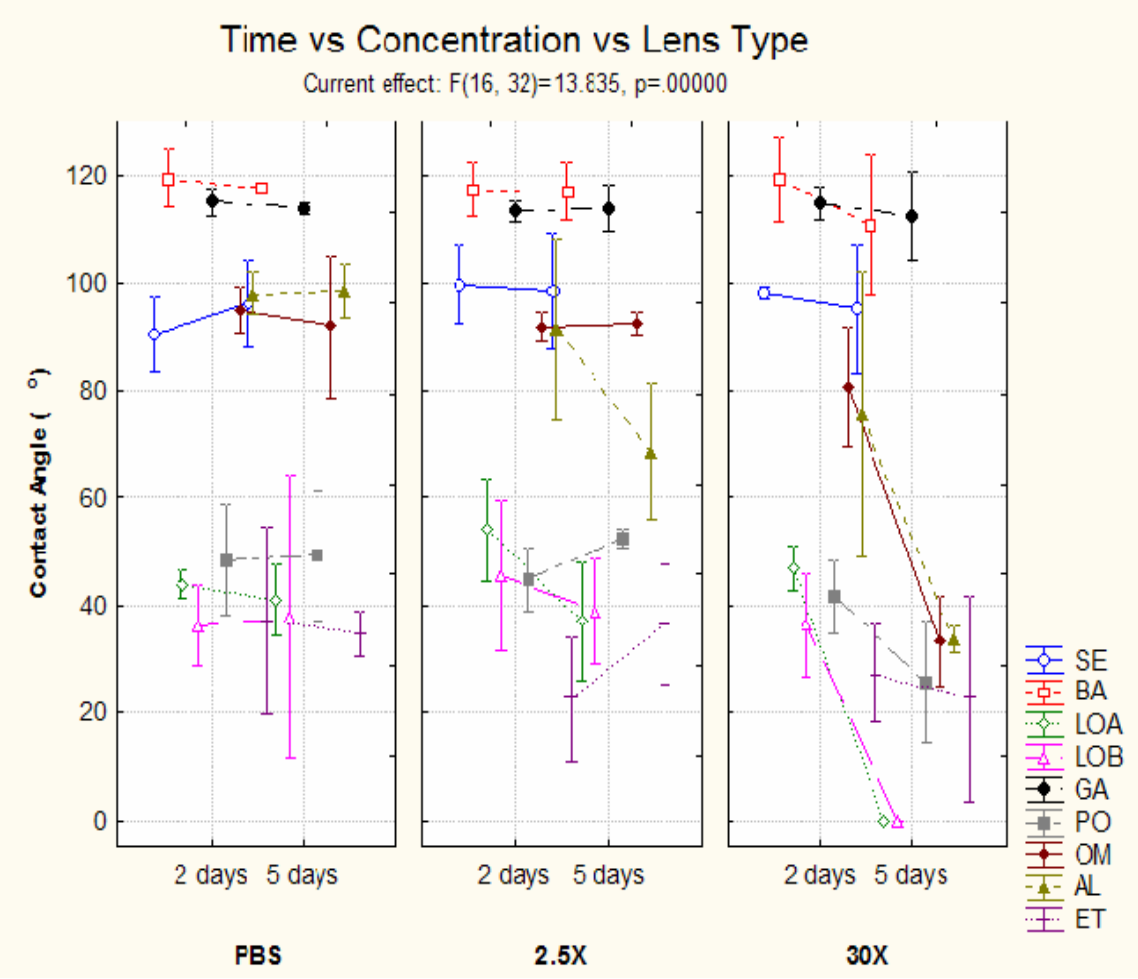
Table 7: A three way repeated measures ANOVA summary table outlining the statistically significant variables

Repeated Measures Analysis of Variance					
	Sum of Squares	**Degrees of Freedom**	**Mean Squares**	**F statistic**	**p**
Time	3028.1	1	3028.1	330.84	0.003009
Concentration	8525.0	2	4262.5	310.23	0.000041
Lens Type	176004.4	8	22000.6	2025.98	0.000000
Time*Concentration	4174.9	2	2087.4	1254.23	0.000003
Time*Lens Type	3414.2	8	426.8	32.49	0.000000
Concentration*Lens Type	7453.2	16	465.8	20.29	0.000000
Time*Conc*Lens Type	2895.0	16	180.9	13.83	0.000000
Error	418.5	32	13.1		

From the three-way repeated measures ANOVA summary table it can be seen that all of the individual variables, incubation time, lens material, and concentration of the LDS,

and all of the interactions between these variables, were statistically significant ($p<0.05$). In order to better understand the interactions between these variables see Figure 33, which shows the three way interaction.

Figure 33: The three-way interaction between time, concentration, and lens type when wettability is assessed for in vitro lipid doped lenses.



SE= senofilcon (Acuvue OASYS), BA = balafilcon (PureVision),
LOA = lotrafilcon A (Focus Night&Day), LOB = lotrafilcon B (O_2 Optix),
GA = galyfilcon (Acuvue Advance), PO = polymacon (Soflens 38), OM = omafilcon (Proclear),
AL = Alphafilcon (Soflens 66), ET = etafilcon (Acuvue2)

From this graph, results can be broken down into three distinct groups based on contact lens materials and their changes in wettability when comparing PBS against 30X lipid doping, via sessile drop contact angle measurements.

Group 1: Silicone Hydrogel Wettability

Compared with PBS, CAs for BA, GA and SE, three of the five silicone hydrogel lenses tested, were unaffected by soaking in the LDS, with typical CA values of >95° ($p>0.05$). These results were echoed in the sessile drop images taken from the contact angle analyzer below in Figures 34 and 35.

Figure 34: The sessile drop image demonstrating the contact angle/wettability of an Acuvue OASYS lens after 5 days of incubation in PBS.

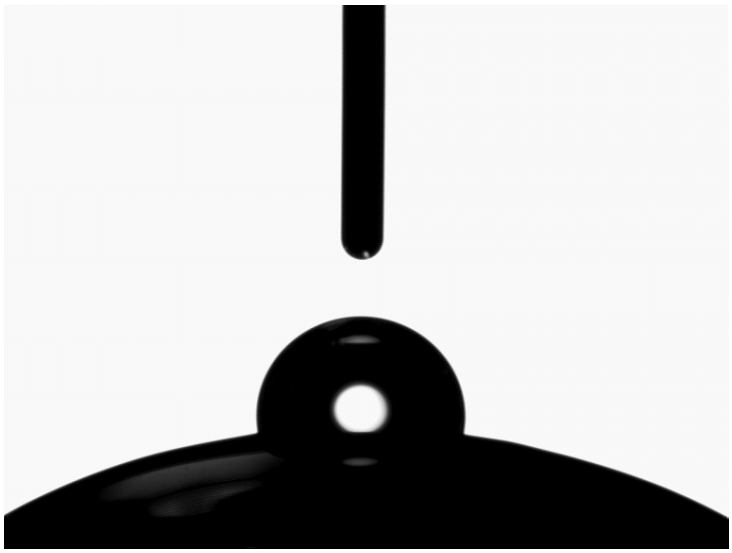
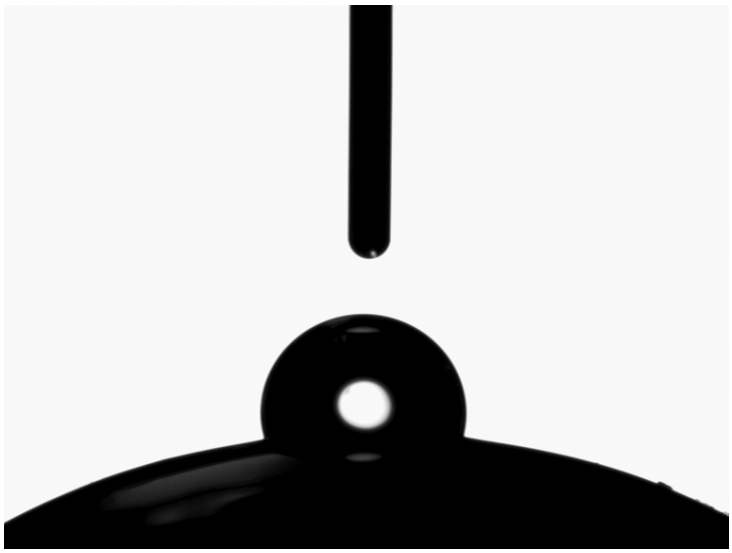


Figure 35: The sessile drop image demonstrating the contact angle/wettability of an Acuvue OASYS lens after 5 days of incubation in a 30X lipid doping solution.



Group 2: Conventional Hydrogel Wettability

The CH materials all exhibited lower CAs after soaking, with values typically decreasing to 35°, which was significantly lower than that seen with PBS ($p<0.01$). This result can be seen when comparing conventional lenses incubated in PBS and the 30X LDS over 5 days, as seen in Figures 36 and 37.

Figure 36: The sessile drop image demonstrating the contact angle/wettability of a Soflens 66 lens after 5 days of incubation in PBS.

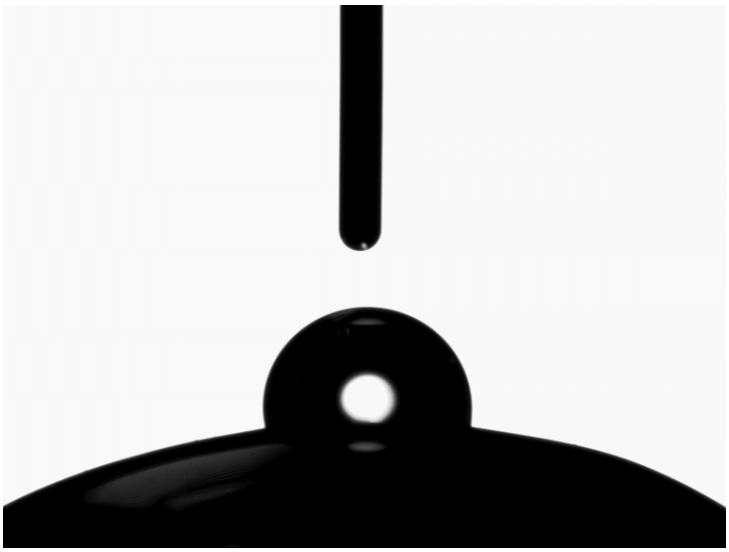


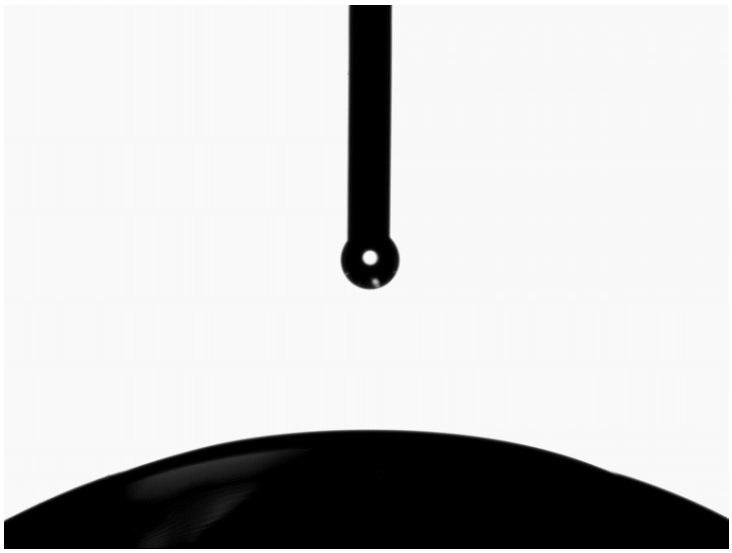
Figure 37: The sessile drop image demonstrating the contact angle/wettability of a Soflens 66 lens after 5 days of incubation in a 30X lipid doping solution.

Group 3: Plasma Coated Silicone Hydrogel Wettability

The plasma coated SH materials (LOA; LOB) both exhibited markedly reduced CAs after lipid exposure, with the 30x LDS reducing the CA to <5° ($p<0.01$). Images demonstrating the increase in wettability when exposed to 30X LDS can be seen in Figures 38 and 39. BA is also a surface modified SH material, but it is surface treated through a plasma oxidation process that leaves hydrophobic glassy islands. LOA and LOB have a unique plasma coating that give the contact lens a homogenous surface. BA is therefore grouped with the other silicone hydrogel materials as it behaves similarly to non-surface treated SH materials. LOA and LOB are grouped independently, as they behave differently from this group.

Figure 38: The sessile drop image demonstrating the contact angle of an O_2 Optix lens after 5 days of incubation in PBS.

Figure 39: The sessile drop image demonstrating the contact angle of an O_2 Optix lens after 5 days of incubation in a 30X lipid doping solution.



The lenses in this experiment seemed to behave according to their broad lens material classifications, irrespective of their relative affinity to lipid deposition. For example, it is known that group II conventional hydrogel lenses deposit lipid at higher quantities when compared to group I or IV lenses.[103, 104, 106, 109, 111] Despite this, all conventional hydrogels, no matter what their initial contact angles were, all had a contact angle of approximately 35^{o} when they were subjected to a 30X LDS for 5 days. All conventional hydrogels ended up with the same degree of wettability despite their individual differences in material. This decrease in contact angle seen for Acuvue 2 lenses, when lipid was deposited, was also seen in the captive bubble technique by Copley.[165]

The silicone hydrogel contact lenses divide into two distinct groups: those that are plasma coated and those that are not. All silicone hydrogels, except for the CIBA Vision lenses, behaved similarly despite their differences in material and manufacturing

processes. The CIBA Vision lenses behaved extremely uniquely throughout this experiment when compared to all other lenses. This grouping of surface treated and non-surface treated materials has also been found in wettability research by Maldonado-Codina and Morgan.[144] The plasma coated materials began with a reasonably low contact angle and became completely wettable with lipid deposition. These lenses must have a unique quality to substantiate these results; such as their polymer or plasma surface coating. Similar increases in wettability or decreases in contact angles were found using the captive bubble method by Copley when *in vitro* lipid deposited Focus Night & Day lenses were analyzed.[165]

It is hypothesized that the degree of wettability of these contact lenses, when lipid is involved, is highly dependent on the degree of penetration of lipid into the matrix. The contact lens materials that encourage lipid to penetrate deep into the matrix do not interrupt the surface chemistry of the lenses and therefore there is no change in wettability. This explains the results seen for the non-plasma coated silicone hydrogel materials.

The materials where lipid only slightly penetrates into the matrix with some lipid also remaining at the surface produce a moderate improvement in surface wettability. This explains the conventional hydrogel lens results.

The plasma-coated silicone hydrogel lenses do not allow any lipid to penetrate into the matrix and therefore the lipid is forced to remain on the surface of the lens, causing a

significant increase the wettability. In this case, the lipid deposition must alter the surface chemistry is such a way that decreases the surface tension between the tear film and the contact lens, therefore making it easier for the tear film to spread over the contact lens surface. The exact mechanism and cause for the changes in wettability is not known. It is possible that one or a couple of the specific lipids may induce these surface changes. More research is required to look at the degree of penetration of specific lipids into the matrix, possibly using fluorescently labeled lipids analyzed with confocal microscopy.

These results may help to explain why some contact lens wearers report an increase in comfort in the first few hours or days of wear,[86] but the time comes when lipid deposition stops being advantageous to contact lens wear and starts being disadvantageous.

Not only was there a material effect, there was also a very pronounced concentration effect. Increased concentrations of lipid available in the LDS, and therefore most likely depositing, produced more wettable contact lens surfaces. Does this concentration effect have any relevance to *in* vivo data especially when a 30X lipid doping solution was used? Due to this question, a short explanation is required for the choice for lipid doping solution utilized in this experiment. A 2X and a 30X lipid doping solution were chosen to incubate these lenses in, as the 2X lipid doping solution is similar to the concentration that is found in the tear film at any given time, thus it mimics a stationary human model.[51, 167, 174] The 30X lipid doping solution was used for two reasons. The first is to mimic

the constant renewal of lipid that is cycled in the human tear film. Lipids are being continuously released from the meibomian glands, during open-eye and closed-eye times, throughout any day. [20, 21] Secondly, some contact lens wearers have certain conditions that produce drastically increased secretion of lipids into the tear film on a continuous basis. One of the conditions that can cause this overload in lipid secretion, and therefore presence in the tear film to readily deposit on the surface of contact lenses, is hypersecretory meibomian gland dysfunction.[75]

The last variable that influenced the final wettability of the contact lenses was the length of time in which the lenses were incubated in the LDS. The longer the contact lenses were incubated in the lipid doping solution the more the contact angles decreased. This is thought to occur due to accumulating lipid on the surface of the contact lens. Within the first week of wear, lipid continually deposits on the surface. In fact, lipid deposition does not reach a plateau throughout one month of wearing time, as occurs with protein deposition.[111] These contact lenses were incubated for a maximum of 5 days; therefore, lipid is sure to continue depositing each and every day. A longer length of incubation in the LDS would be required to determine if wettability continually increases along with the length of doping.

These experiments were completed with HPLC grade water and not an artificial tear solution (ATS) or saline. The use of HPLC water as the liquid to dispense from the syringe on the OCA, make contact with the contact lens surface, and therefore measure the contact angle was chosen so that no matter what experimental parameters were used

comparisons between studies could be examined. There is no doubt that the use of an artificial tear solution (ATS) would alter the contact angles for the contact lenses tested. The problem arises when choosing what composition the ATS should have. What proteins should be present? Mucins? Lipids? Salts? In what concentrations?

Despite the difficulty in choosing an appropriate ATS for each experiment, the utilization of an ATS, instead of water, would provide a more accurate representation of what is occurring when a contact lens is on the cornea. The ultimate goal for all *in vitro* experiments is build an *in vitro* model of what is happening in the human eye. This obviously could not be achieved when the parameters of a study do not mimic closely what is occurring in a human worn contact lens.

4.2.2 Wettability Experimental Conclusions

Overall, we could make several conclusions based on this experiment. Lipid deposition played a significant role in overall wettability and therefore comfort of a contact lens. This is true, especially for those with increased lipid secretion and who were wearing their contact lenses for long periods of time without cleaning. Specifically, lipid deposition tends to increase wettability with a decrease in contact angle measurements for conventional hydrogel lens materials and plasma coated silicone hydrogel lens materials. This may help to explain why certain SH materials improve in comfort after the first few hours or days of wear.

It is clear that this experiment introduced more questions than it answered; therefore more research needs to be completed to determine when lipid deposition becomes deleterious, the degree in which lipid penetrates the contact lens material, and what results when a more complex artificial tear solution is used containing proteins and mucins.

4.3 HPLC

4.3.1 In vitro lipid doping experiment

This experiment was designed to test the reverse-phase HPLC protocol and to quantify the amount of lipid that is depositing on silicone hydrogel and conventional hydrogel contact lens materials when they are incubated in two different LDS of 2.5X and 30X that of normal concentrations.

HPLC chromatograms demonstrating the vast difference in lipid deposition between the 30X and 2.5X lipid doping solution are found in Figures 40 and 41. These chromatograms are from PureVision contact lens materials. The earliest peak is oleic acid, next is oleic acid methyl ester, and the last peak is cholesterol. These samples were processed on different columns and therefore have slightly different retention times. Table 8 shows the respective peak areas taken from the two chromatograms displayed. Once again, only three out of the five lipids in the LDS could be quantified and identified using this specific reverse-phase HPLC procedure.

Figure 40: HPLC chromatogram displaying the lipid peaks from a PV lens doped with 2.5X LDS

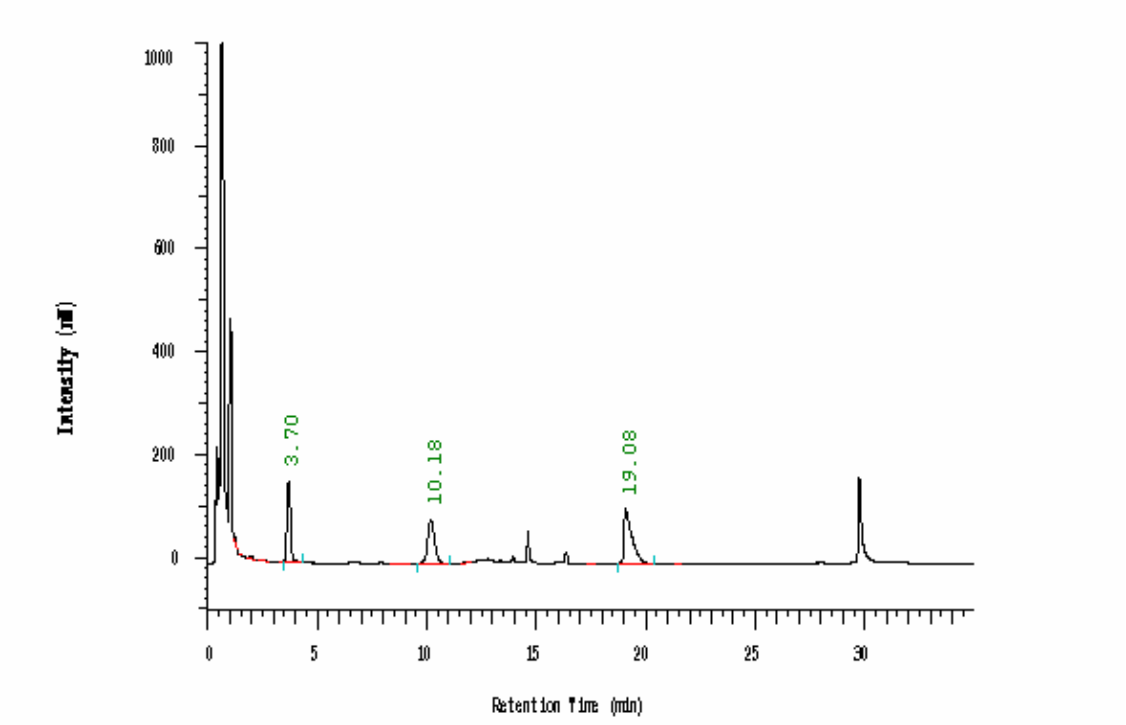


Figure 41: HPLC chromatogram showing the lipid peaks from a PV lens doped in a 30X LDS

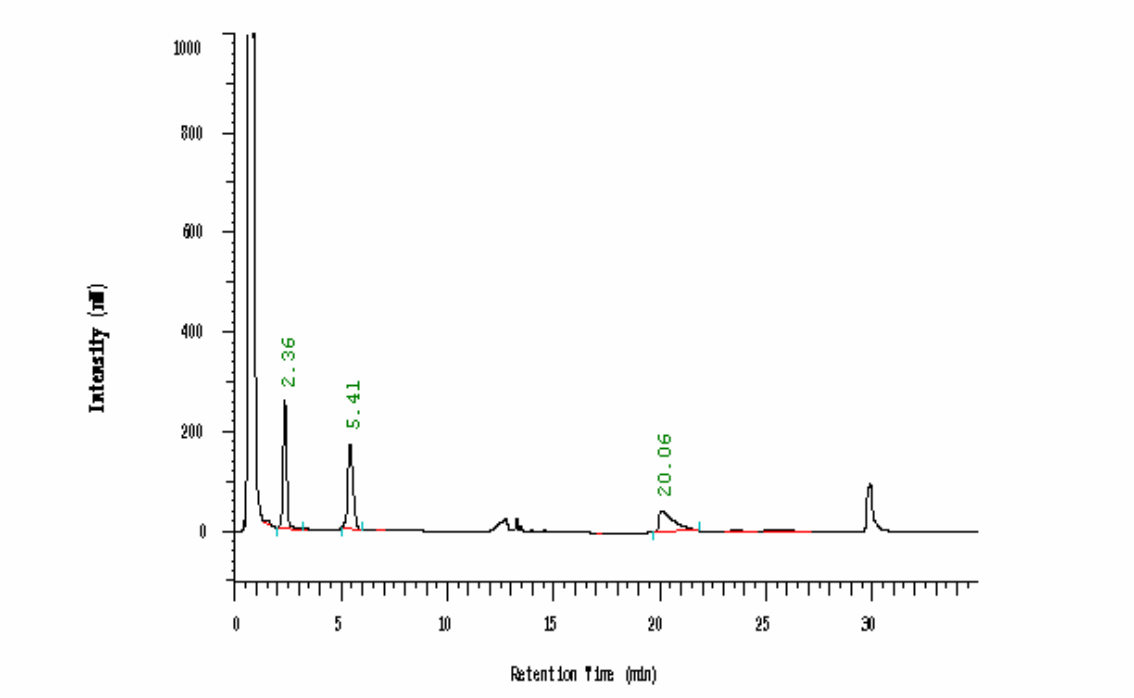


Table 8: The corresponding peak areas for 2x5 and 30x5 PV lenses shown in the preceding chromatograms

Chromatogram HPLC Areas			
Peak Number	**Lipid**	**PV - 2x5**	**PV - 30x5**
Peak 1	Oleic acid	1909415	2784353
Peak 2	Oleic acid methyl ester	1821971	2860204
Peak 3	Cholesterol	2566437	2030706

2X5 = lenses doped in a 2X lipid doping solution and incubated for five days
30X5 = lenses doped in a 30X lipid doping solution incubated for five days

Table 9 and Figure 42 show the average lipid deposits found on each of the lens types for both LDS concentrations. The individual lipids are also shown.

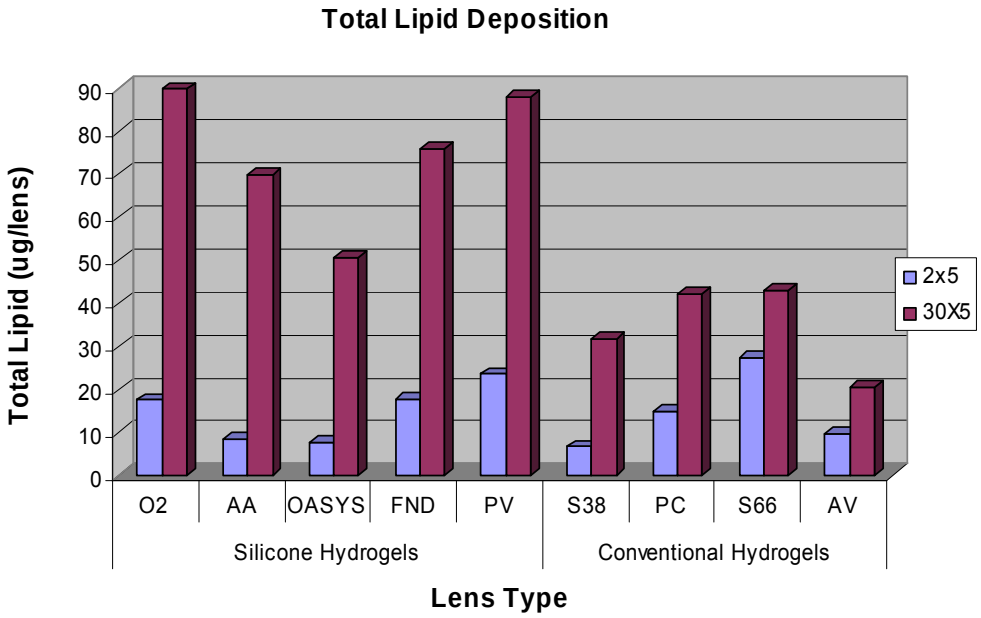
Table 9: The average lipid deposits found on silicone hydrogel and conventional hydrogel contact lens materials

	Average Lipid Deposits (µg/lens)							
	2x5				**30x5**			
	OA	**OAME**	**C**	**Total**	**OA**	**OAME**	**C**	**Total**
O_2	0.0	8.4	9.0	**17.4**	57.9	29.3	2.6	**89.8**
AA	0.8	1.9	5.6	**8.4**	39.7	25.9	4.3	**69.9**
OASYS	0.0	3.6	4.0	**7.5**	29.5	19.6	1.5	**50.6**
FND	5.4	4.7	7.5	**17.6**	42.3	29.3	4.4	**76.1**
PV	4.0	6.1	13.3	**23.5**	35.2	45.5	7.3	**88.0**
S38	0.0	2.7	3.9	**6.6**	13.6	12.6	5.4	**31.6**
PC	5.8	3.6	5.3	**14.7**	17.3	16.4	8.5	**42.3**
S66	6.4	9.7	11.2	**27.2**	20.7	15.5	6.8	**43.1**
AV	0.0	4.6	5.0	**9.6**	5.5	9.8	5.1	**20.5**

OA = oleic acid, OAME = oleic acid methyl ester, C = cholesterol
O2 = O2 Optix, AA = Acuvue Advance, OASYS = Acuvue OASYS, FND = Focus Night&Day, PV = PureVision, S38 = Soflens 38, PC = Proclear, S66 = Soflens 66, AV = Acuvue2

In Table 8 and 9, it can be seen that the 30X LDS deposits lower amounts of cholesterol than the 2.5X LDS. As previously described, because cholesterol does not stay in solution when it is found in high concentrations, the 30X LDS only contains 3X the cholesterol found in the basic LDS. However, despite this, significantly less cholesterol deposits with the 3X than the 2.5X. This may be due to competition between cholesterol and the other lipids which are much higher in concentration, and are therefore preferentially depositing.

Figure 42: Total lipid deposition on all lens types using both lipid doping solutions



O2 = O2 Optix, AA = Acuvue Advance, OASYS = Acuvue OASYS, FND = Focus Night&Day, PV = PureVision, S38 = Soflens 38, PC = Proclear, S66 = Soflens 66, AV = Acuvue2

More lipid was clearly deposited on the silicone hydrogel lens materials than the conventional hydrogels. It is also evident that a 30X lipid doping solution concentration deposits more lipid onto these lens materials over a five day time period than a 2.5X solution does. For the SH materials, PureVision and O_2 Optix have the most lipid deposited, with deposition >85 μg/lens and Acuvue OASYS has the least lipid deposition pattern with 50 μg/lens. Among the conventional hydrogel lenses, Soflens 66 and Proclear tend to deposit more lipid, with ~42 μg/lens, than the Soflens 38 and Acuvue2 lenses, < 32 μg/lens.

The clinical relevance of these results require further investigation and, to-date, no study in which *ex vivo* worn lenses have been harvested in a clinically controlled study has been conducted with this combination of lenses.

A three-way repeated measures ANOVA was used to analyze all variables in this experiment. From the statistical summary seen in Table 10, it can be seen that all variables individually or tested as an interaction were found to be statistically significant. Specifically, the lipid type, lens material, and LDS concentration were all statistically significant, as was the three way interaction.

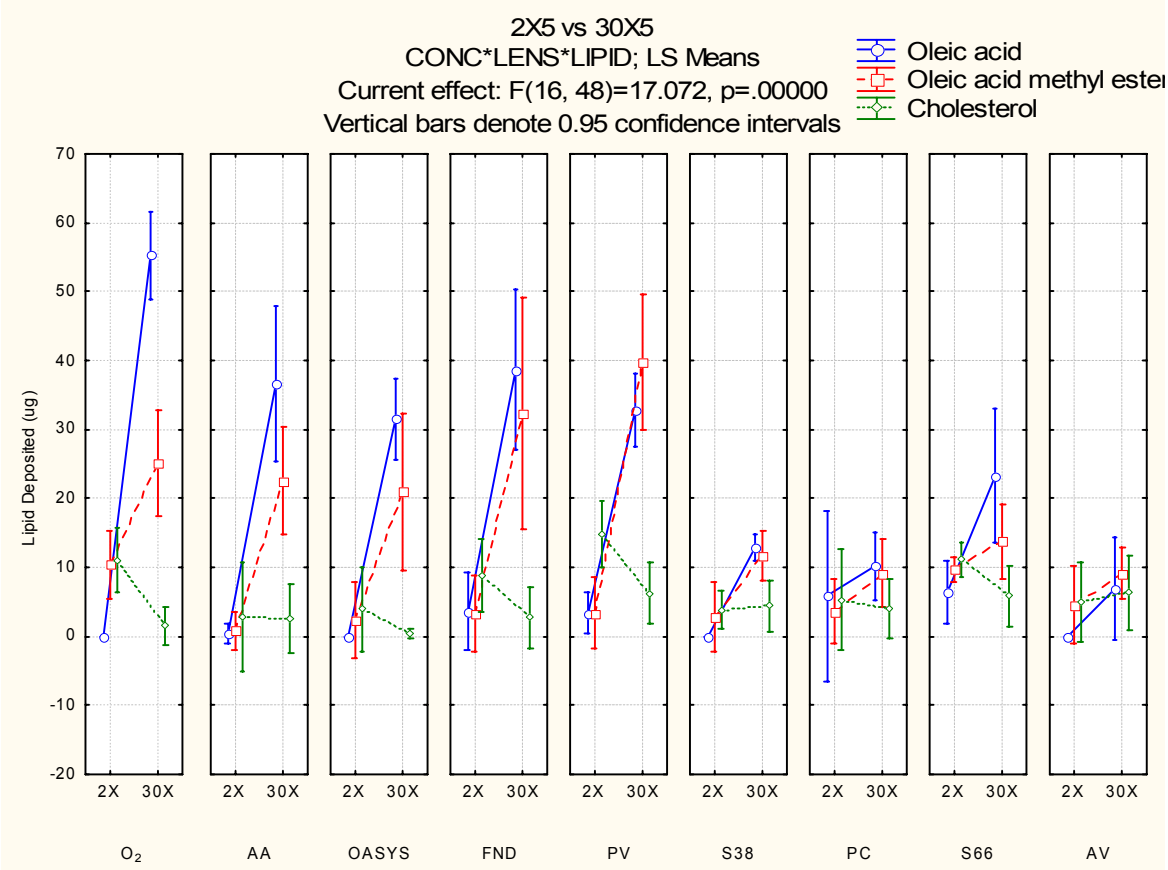
Table 10: Repeated measures analysis of variance statistical significance summary chart comparing the 2X 5 day results with the 30X 5 day results.

Repeated Measures Analysis of Variance (2x5 vs 30x5)					
	Sum of Squares	**Degrees of Freedom**	**Mean Squares**	**F statistic**	**p**
Concentration	8538.40	1	8538.40	1694.455	0.000032
Lipid Type	1120.16	2	560.08	29.110	0.000816
Lens Material	5498.60	8	687.32	35.914	0.000000
Concentration*Lipid	2005.18	2	1002.59	60.769	0.000104
Concentration*Lens	8945.75	8	1118.22	65.136	0.000000
Lipid*Lens	2863.44	16	178.96	14.286	0.000000
Concentration*Lipid *Lens	3648.68	16	228.04	15.525	0.000000
Error	705.05	48	14.69		

The significance of these results is better seen in the three-way interaction graph labeled as Figure 43. From this graph it is seen that silicone hydrogels deposit more lipid than conventional hydrogels.

Significant differences were also seen in the lipid deposited, with the 2.5X LDS when compared to the 30X LDS. The 30X LDS showed remarkable increases in deposition, especially for silicone hydrogel lenses.

Figure 43: Three-way statistical interaction graph showing the significance that lipid type, LDS concentration and lens type have on lipid deposition.



O2 = O2 Optix, AA = Acuvue Advance, OASYS = Acuvue OASYS, FND = Focus Night&Day, PV = PureVision, S38 = Soflens 38, PC = Proclear, S66 = Soflens 66, AV = Acuvue2

In this experiment it was found that lipid deposition was influenced by many different factors, including the contact lens material, the composition of the lipid doping solution and the concentration of lipid doping solution. Many researchers in the past have found that differences in polymer structure can dictate the ultimate amount of lipid that deposits. If we focus on polymer structure and its lipid deposition, silicone hydrogels deposit more lipid than conventional hydrogels.[114] This is due to the increase in hydrophobicity that is caused by the silicone incorporation into the lens material.

Within the silicone hydrogel lenses, the surface-treated lenses, PV, FND, and O_2 Optix tend to deposit the most lipid and the PVP internal wetting agent inherent in OASYS and Acuvue Advance provide for a decrease in lipid deposition. The increase in lipid deposition seen for surface treated lenses is caused by the inability to totally mask the hydrophobic "arms", using the specially designed plasma coatings. The inclusion of the internal wetting agent, HydraClear, composed of PVP, along with a reduction in silicon, allow for a less hydrophobic surface and therefore lipid does not deposit as readily.

The conventional hydrogel lenses materials tend to deposit lipid according to their FDA classification. Group II lenses, the high water non-ionic lenses, ProClear and Soflens 66, tend to deposit the most lipid. The reason for this increase in deposition has previously been described by the "pull/push" theory of lipid deposition. The "pull" represents the polymer lens material adhering the lipid and the "push" represents the water in the lens material driving the lipid into the matrix.[53, 103]

In addition to the contact lens material, the composition of the artificial doping solution or tear film will ultimately dictate the amount and specific lipids that will deposit on the lens surface. There are significant inter-patient differences in lipid deposition, due to the vast range of lipids found in the tear film.[110, 113] The make-up of an individuals' tear film will effect the deposition on the lens material. There is such variability in individual tear films that it is difficult to predict what will deposit and why. From this experiment, it was seen that oleic acid deposited more preferentially than oleic acid methyl ester,

which was greater than cholesterol. Previous *in vivo* experiments have reported increased cholesterol deposition compared to oleic acid and its methyl ester.[63] Bontempo and Rapp's hydrophilic *in vitro* lipid doping experiment found that oleic acid deposited in the highest concentration, followed by cholesterol, with very little oleic acid methyl ester.[103]

The presence of proteins and mucins will also affect the amount of lipid that deposits on the contact lens surface. This has been examined by Bontempo and Rapp with conventional hydrogel lenses.[105] They reported specific interactions that occur on a hydrophilic contact lens surface when lipids and proteins are present concurrently. When a group IV lens undergoes protein deposition, the surface of the lens becomes less hydrophilic and therefore attracts lipid deposition. For group II lenses, the proteins compete with more polar lipid deposited on the lens surface and displace them.[105] Bontempo and Rapp continued their protein and lipid interaction research by studying these interactions on group I and group IV lenses *in vivo*.[106] They found that lysozyme was preferentially deposited on Group IV lenses due to the available negative charges attracting the strongly positively charged protein. Group IV lenses showed deposition for both protein and lipids, but the specific deposition composition depended on the individual. [106]

Protein-lipid interactions, such as these, have not been examined in silicone hydrogel contact lenses. Despite this, if specific lipid types or groups are absent in the tear film, artificial or real, the subsequent deposition pattern will change.

If the composition of the artificial lipid doping solution affects the ultimate lipid deposition, then the concentration of lipids in the lipid doping solution will also influence the deposition pattern. Individuals vary in the concentration of lipids in their tear film due to differences in secretory release from the meibomian glands. The meibomian glands are constantly releasing more and more lipid into the tear film.[20, 21] The rate at which this lipid is released varies from one person to another and is highly correlated to the ocular health or the presence of meibomian gland dysfunction or blepharitis. [75] These diseases can alter the excretion of specific lipids and the volume of lipid, thus changing the deposition that will occur on a contact lens surface.[22, 46, 50]

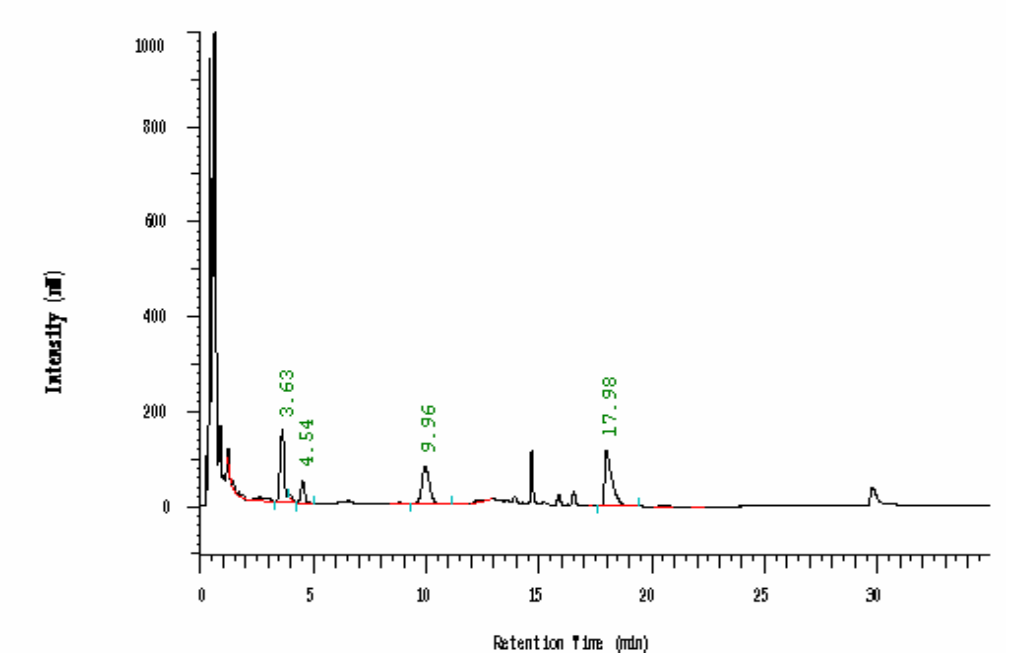
The specific reverse-phase HPLC protocol used was able to separate and analyze three out of the five lipids used in the lipid doping solution, with moderate accuracy. However, this method is not time effective, highly sensitive, and does not allow for the analysis of all five lipids with the current mobile phases and step-wise gradient. The robust nature of this technique will be discussed in a following section.

4.3.2 SPA Study

Five silicone hydrogel lens types, which were worn on a daily-wear basis for a two week period, were examined from a clinical study. This study was described in section 3.6.2.

A sample HPLC chromatogram from subject #8 can be seen in Figure 44. From this chromatogram, it can be seen that a number of other lipid peaks were detected, but were not identifiable. These peaks are most likely other free fatty acids and derivatives of cholesterol, due to their retention times. Once again, only oleic acid, oleic acid methyl ester, and cholesterol can be quantified using this HPLC procedure.

Figure 44: SPA chromatogram showing subject #8 lipid depositions on a PV lens



Oleic Acid = RT of 3.63, Oleic Acid Methyl Ester = RT of 9.96, and Cholesterol = RT of 17.98

A repeated measures ANOVA was used to analyze the statistical significance of the results from this study. There were two variables in this study, the five different silicone hydrogel lens types and the three lipid types that were analyzed. The statistical summary table can be seen in Table 11.

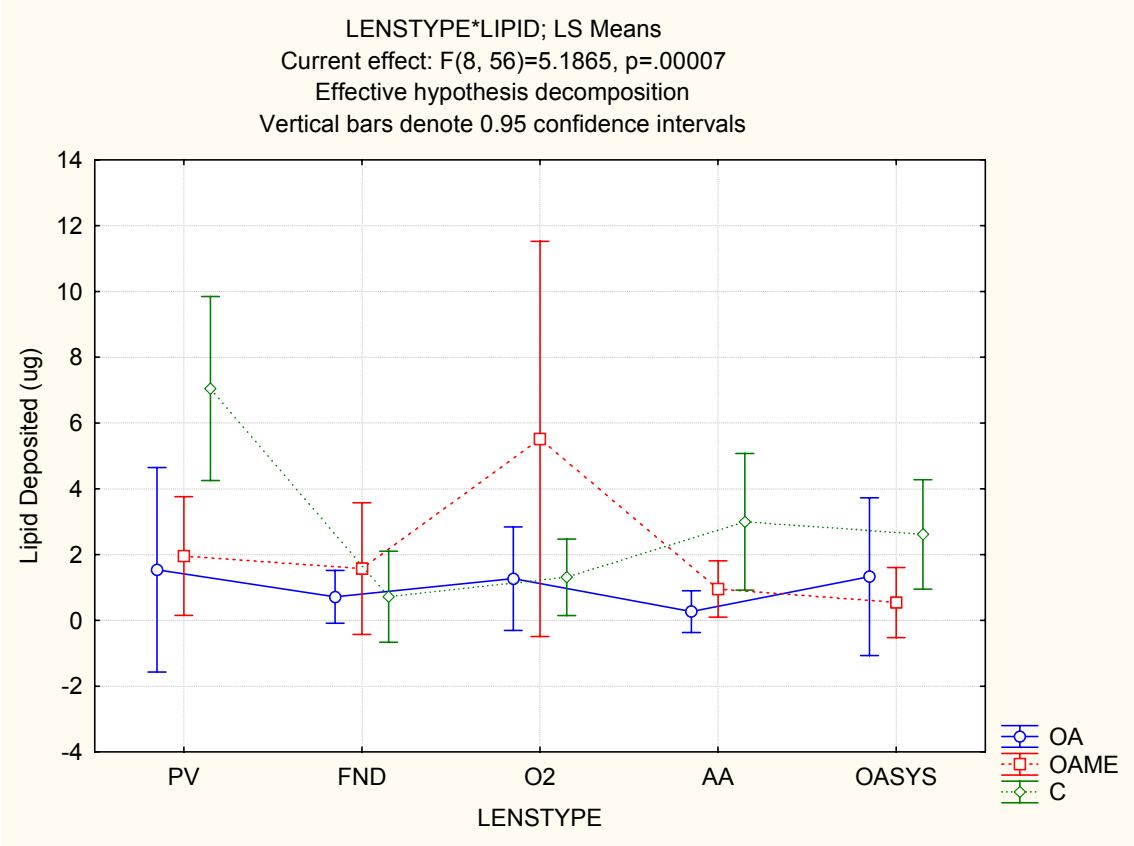
Table 11: Statistical summary table for SPA samples

Repeated Measures Analysis of Variance					
	Sum of Squares	**Degrees of Freedom**	**Mean Squares**	**F statistic**	**p**
Lens Type	105.1470	4	26.2867	1.98226	0.124639
Lipid	73.6928	2	36.8464	4.70645	0.027334
Lens type*Lipid	225.9505	8	28.2438	5.18655	0.000072
Error	304.9532	56	5.4456		

From this summary table above, it can be seen that there was no significant difference seen between the lens types tested in this study, with $p>0.05$. However, there was a significant difference in the lipid types and in the interaction between the lens type and lipid ($p<0.05$).

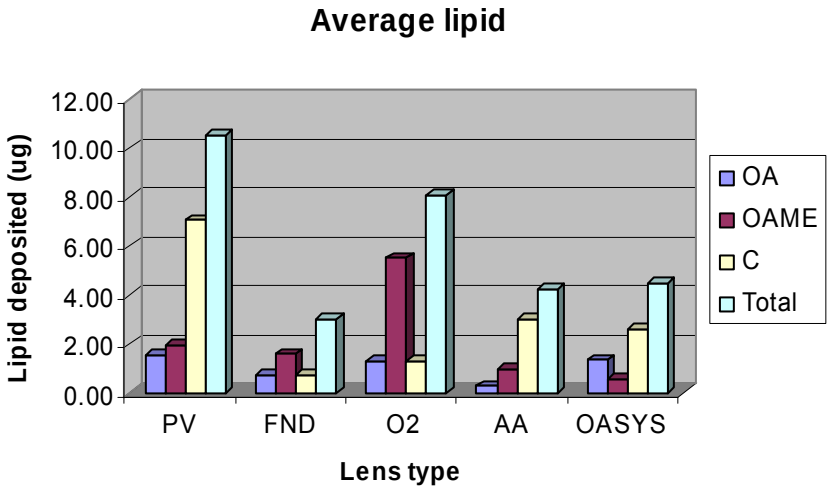
The two-way interaction between the lens type and the lipids analyzed can be seen in Figure 45 and the average lipid deposition, including total lipid, can be seen in Figure 46. From these graphs it can be seen that PureVision lenses deposited the most lipid, with cholesterol having the highest concentrations, followed by oleic acid methyl ester, then oleic acid. The average total lipid deposition seen on PV lenses was 10 µg. O_2 Optix had the second highest total deposition and had a uniquely high concentration of oleic acid methyl ester when compared to other lens types. The average total lipid deposition for O_2 Optix was 8 µg. Focus Night and Day, Acuvue Advance, and Acuvue OASYS all had relatively low concentrations of all three lipid types, with their average total lipid measuring <4.5 µg.

Figure 45: The two-way interaction between lens type and lipid



OA = Oleic Acid, OAME= Oleic Acid Methyl Ester, C = Cholesterol
PV=PureVision, FND = Focus Night and Day, O2 = O_2 Optix, AA = Acuvue Advance, OASYS = Acuvue OASYS

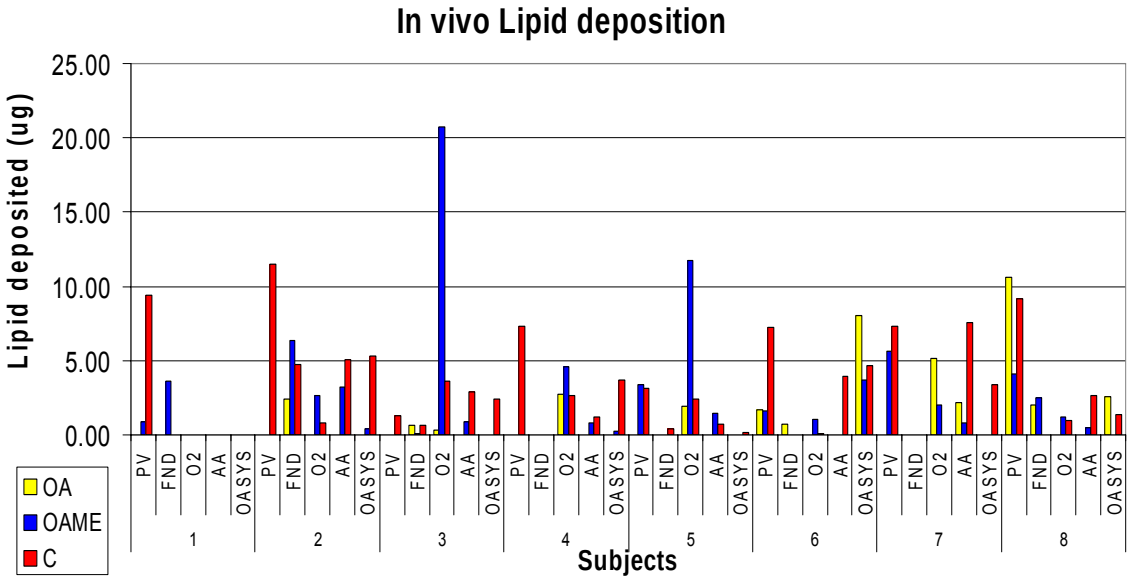
Figure 46: Average individual and total lipid deposition on five lipid types



PV=PureVision, FND = Focus Night and Day, O2 = O_2 Optix, AA = Acuvue Advance, OASYS = Acuvue OASYS

This was an *in vivo* study, where contact lenses were worn for two weeks on a daily wear basis. Figure 47 shows the individual lipid deposition from each subject and each lens type. From this graph, the individual variation in lipid deposition can be seen. Some subjects, such as subject 1, deposited very little lipid on all lens types, whereas Subject 8 deposited at least two lipid types on all five contact lens materials.

Figure 47: Individual subject lipid deposition on all contact lens types



C= cholesterol, OAME = oleic acid methyl ester, OA = oleic acid
PV=PureVision, FND = Focus Night and Day, O2 = O_2 Optix, AA = Acuvue Advance, OASYS = Acuvue OASYS

If the individual lipids are examined for each subject and contact lens material, it was found that oleic acid deposits the least. Out of all the contact lens materials, FND and O_2 Optix deposited oleic acid most frequently, with deposition seen on four out of the eight subjects. PV, AA, and OASYS only had one or two subjects deposit oleic acid.

Oleic acid methyl ester (OAME) was seen to deposit more frequently than oleic acid, although there was a definite difference in materials that showed this deposition. OAME was deposited upon O_2 Optix materials most frequently, with seven out of the eight subjects showing deposition. When the other contact lens materials are examined, the deposition pattern of OAME is as follows: AA was seen with six subjects, PV with five, FND with four and OASYS was seen in three subjects.

Cholesterol was the last lipid that was examined. It was deposited most frequently out of all three lipid types. Cholesterol was detected on PV lenses with all eight subjects, followed by AA and OASYS with seven subjects. O_2 Optix deposited cholesterol on six patients and FND only had detected cholesterol in three individuals.

The results of this experiment show that *in vivo* lipid deposition is influenced by the interaction between the various contact lens materials and the lipids that are present in the tear film. Once again, it is seen that individual variances in tear film composition affect the overall lipid deposition seen. This is a continuing trend seen in lipid deposition research in conventional and silicone hydrogel contact lens materials.[110, 113]

To date, there has been very little research examining the lipid deposition on silicone hydrogel contact lens materials. The original paper from Jones et al.[114], in which the same protocol was used, detected lipid concentrations that were significantly higher than that detected in this experiment. The original study found total lipid deposition for PV and FND lenses to exceed 500 μg of lipid per lens.[114] In this study, total human lipid deposition ranged from 0 μg to 25 μg, for a single lens material. It must be reminded that in this study contact lenses were only worn for a two-week period and on a daily wear basis that involved cleaning on a nightly basis. Therefore, some lipid may have been removed with cleaning and rubbing, or at least lipid may have been discouraged from continually depositing. It is also a fair assumption that if the lenses had been worn for one month, there would have been more lipid deposition. The differences in wearing and

cleaning schedules may be the cause of the large differences seen in lipid deposition between this and the Jones experiment, [114] as the lenses that were studied in the original Jones paper were worn for 30 days continuously without removal.[114]

If the results from this experiment are compared to the *in vitro* lipid deposition experiment performed earlier, a number of factors can be noted. The total lipid deposition found in the *in vivo* experiments were approximately half the total lipid deposited on the *in vitro* 2.5X lipid doping solution. In both experiments, cholesterol deposited the most frequently, followed by oleic acid methyl ester and then oleic acid. The last similarity is that in both the *in vitro* and *in vivo* experiments, PV and O_2 Optix contact lens materials deposited the most lipid.

When the oleic acid and cholesterol results of SPA are compared to Bontempo and Rapp's *in* vivo hydrophilic contact lens experiment,[106] where they had eight subjects wear group I and IV conventional hydrogel contact lenses for 13 hours then analyzed the results via TLC, similarities are seen. In that experiment, the average OA deposited was around 2 μg and for C was 1.75 μg for both lens types.[106] This was similar to the deposition that was found in SPA, where the average of all the silicone hydrogel lens types deposited approximately 1μg of OA and 3 μg of C.

The reverse phase HPLC lipid analysis method used in this study was able to detect, separate, analyze, and quantify lipid deposited on human worn contact lens materials,

but far smaller quantities were calculated than what was expected. Therefore, in the future, a different HPLC or HPLC/GC procedure may be more accurate and sensitive.

4.3.3 HPLC Experimental Conclusions

The overall conclusions that can be drawn from the *in vitro* and *in vivo* HPLC experiments are that lipid deposition is influenced by the composition of the lipid tear film, the concentration of the lipid tear film, and the polymeric composition of the contact lens materials under investigation. Cholesterol tends to deposit preferentially over oleic acid methyl ester and oleic acid. Tear films, artificial or real, with higher concentrations of lipid, tend to deposit lipid in higher quantities. Silicone hydrogel contact lens materials deposit more lipid than conventional hydrogel lens materials. Within the silicone hydrogel materials, PureVision deposits the most lipid, followed by O_2 Optix, then FND, Acuvue Advance, and OASYS, with the last three lens types depositing similar lipid deposit amounts.

4.4 Critical Analysis of Reverse-Phase HPLC for Lipid Analysis

It has been seen from the previous experiments that this reverse-phase HPLC protocol can be used for lipid analysis with relative ease and accuracy, but how good is this procedure? The following sections will critically analyze the reverse-phase HPLC procedure used, its requirements for filtering and cleaning, the details of why each component was chosen, the advantages and disadvantages of the procedure, and the limitations of the instruments. This analysis will aid future researchers in lipid analysis when considering using reverse-phase HPLC.

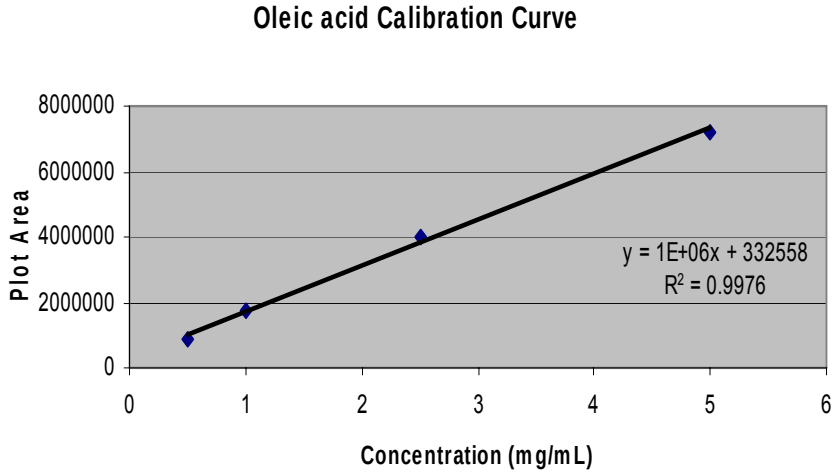
4.4.1 In-depth reverse-phase HPLC protocol

Contact lenses are extracted, at least twice, in 2:1 chloroform:methanol and the extractions are combined and evaporated to dryness using nitrogen. The dry extract samples are then frozen at -80^{o}C until the day of processing. On that day, extracts are removed and allowed to reach room temperature and are re-suspended in 50 µL of a 1mg/mL reference standard. This reference standard contains 1 mg/mL of oleic acid, oleic acid methyl ester, and cholesterol dissolved in 2:1 acetonitrile:chloroform. A reference stock is made in the concentration of 200X and is frozen at -20^{o}C and is then diluted for use for the reference standard.

Prior to any standard or sample injections into the HPLC machine, especially with a new column, when beginning a new experiment, or after cleaning the system and column, the machine is allowed to equilibrate with the initial or dominant mobile phase. This can take anywhere from 20 minutes to many hours. The equilibration status can be monitored by testing the noise/drift ratio. Once the noise level is less than 100 and the drift is less than 500, the system can be considered equilibrated so standard or sample injections can proceed. If these conditions are not left to equilibrate then there will be interference which will lead to drifting and noisy baselines. Noise is seen by short baseline peaks that are caused by lamp instability, temperature changes and other environmental and experimental conditions. Drift is the deviation from the horizontal baseline that occurs as the detector is heating up.

Before samples are injected into the HPLC machine, a calibration curve is run to determine the R^2 value for each lipid component and assess the linear range. The R^2 value should be 0.98 or above. Once a calibration curve has been established for each of the detectable lipids, samples can be run. Each calibration curve should include at least 4 different concentrations of standard lipid samples. Figure 48 demonstrates a calibration curve conducted for oleic acid.

Figure 48: Sample calibration curve for oleic acid



After the calibration curve has been completed, the sample and reference standard injections can commence. The first and last injections should be a reference standard and then every 5-8 sample injections are bracketed by reference standards.[114]

Chromatographic conditions:[114]

Column:	C18 nucleosil 5µm 125 mm x 3mm
Mobile phase A:	HPLC grade acetonitrile
B:	80:20 (HPLC grade acetonitrile:Millipore)
Flow:	0-10 min => 1.5 mL/min mobile phase B
	10.5 – 12 min => 1.5 mL/min mobile phase A
	12 - 27 min => 2.0 mL/min mobile phase A
	27.5 – 35 min => 1.5 mL/min mobile phase B
Detection wavelength:	205 nm
Syringe fill:	20 µL
Injection volume:	10 µL
HPLC machine	Hitachi HPLC system (pump L6200, UV detector L4000, interface D-6000)

The lipid deposit mass can then be calculated using the average standard area count of the two standards bracketing the given samples. The equation is as follows: Deposit mass (μg) = [(sample area count * standard concentration / average standard area count) – standard concentration] * 0.05 * 1000.[114]

4.4.2 Filtering and Cleaning

With the chromatographic and analytical protocol used, proper cleaning and filtering of all components is a necessity. This reverse-phase protocol uses acetonitrile and water as the mobile phase to separate and quantify various lipid types. Lipids are not all easily soluble in acetonitrile and water; because of this the lipids may precipitate out of the solution and clog the HPLC machine. Due to this, everything needs to be filtered and cleaned on a regular basis.

In terms of filtering, all solvents and samples are to be filtered prior to injection or use in the HPLC machine. It is preferable that 0.2μm filters are used to prevent any large molecules, or molecules that are not fully dissolved into the solution, from getting into the machinery. Due to the differences in volume, different sized syringes and filters are used for each application.

In addition to the pre-filtering of solvents and samples, it is necessary to have a pre-column filter or guard column installed in the system. This will prevent large particles

from entering the column and clogging it. The pre-column filter frits need to be changed on a regular basis or else it can become congested and cause pressure increases. When installing the pre-column filter or guard, it is important to check that it does not leak when subjected to a normal flow-rate. If so, the filter casing may be installed incorrectly or not tightened enough.

Even with all of these filters, lipid does start to accumulate on the column and through the system. Lipid accumulation on the column is caused by the inability of the mobile phase to remove it from the hydrophobic column and thus causes decreasing retention times. This can be visible after about 30 injections, depending on the concentration of lipid in the samples. When the retention times start decreasing, the system and column can be cleaned with a solution of methanol with 10% chloroform. This should remove the lipid from the column and thus return retention times. This methanol:chloroform solution should be allowed to run through the system for a least a couple of hours or overnight. Following cleaning, the system is equilibrated using the acetonitrile:water mobile phase.

Clogging of the column is not the only problem. Even with all of the filtering, lipid can still precipitate out of solution and cause blockages in the injector, tubing, or in the needle syringe used for injection. Therefore, consistent cleaning rituals need to be maintained to reduce the risk of blockages from occurring. Once a blockage starts to form or occurs, the pressure in the system will spike and it could be difficult to resolve. For these reasons, the methanol:chloroform cleaning is a necessity. As for the syringe,

after every injection it is imperative that the syringe be cleaned with chloroform and/or methanol at least 2-3 times. If this is not completed it too can become clogged. This can be seen in the syringe by the build up of white crystals. When this happens it is extremely difficult to fix.

4.4.3 Explanation of Procedure

Every part of an HPLC protocol is specifically designed, based on the substances to be separated and quantified, including the mobile phase, solvents, columns, and detector.

Choosing a suitable column can be quite a daunting task as there are so many variables to take into consideration, including the type of column, particle size, length, internal diameter, and pore size. The first step when trying to choose a column is to decide the type of column that is best for the experiment and budget. There are two main types of column hardware: the standard HPLC column and the cartridge column that requires interchangeable end fittings.[191] These cartridge columns tend to be more inexpensive than the standard HPLC column, but are especially good if multiple columns are used. After the column hardware is chosen, the choice needs to be made between a reverse phase and normal phase column type. Normal phase columns have highly polar stationary phases and a mobile phase that is non-polar, such as hexane. Reverse phase column, have a non-polar or hydrophobic stationary phase and a polar mobile phase. Reverse phase columns have been widely used for lipid analysis, but usually within a single lipid class. The most common reverse phase stationary phase is the C_{18} or

octadecylsilyl groups. [191] The column used for our protocols is a C_{18} reverse phase cartridge column.

After the type of column is chosen, the column internal diameter, particle size, pore size, and column length have to be considered. The smaller the particle size the better the resolution and the faster the runs, but back pressure increases. Narrow particle size will increase column efficiency. The most common particle size is 5 μm, but 10μm and 3μm are also available.[191] The internal diameter of the column will have an influence on the optimum flow rate and can affect the sensitivity, efficiency and resolution. Common internal diameters are 4.0-5.0 mm, but narrowbore columns (2.0-3.0 mm) and microbore columns are available. Smaller internal diameters require a slower flow rate and can improve sensitivity.[37] The pore size is chosen according to the size of the molecules that will be examined. Generally, the smaller the pore size the greater the surface area for carbon loading. Large molecules such as proteins are usually analyzed using pore sizes over 300Å and smaller molecules are analyzed using pore sizes 80-150 Å. The column length can also affect efficiency and resolution, with longer columns (25cm) providing better results, but increased pressure and retention times. Shorter columns (<5 cm) allow for high speed analysis.[191] The column that is used in our HPLC analysis is a reverse phase 125 mm column made of nucleosil with an internal diameter of 3.0 mm, particle size of 5μm, and pore size of 100 Å. This specific column was chosen to optimize sensitivity and efficiency and to reduce backpressure.

The mobile phases chosen are based on the substance to be separated and the type of chromatographic analysis chosen.[191] Not only are the mobile phase solvents an important decision, but the solvents in which the samples are dissolved in also need be considered. Acetoniltrile (ACN) has been a popular choice for reverse-phase HPLC in the analysis of lipids.[191] There are many other options for lipid HPLC solvents, but mixtures of solvents have to be fully miscible with each other to create a clean homogenous solution and not interrupt the UV detection.[191] The polarity of the solvent is also important. Due to the differences in polarity and the extended use of acetonitrile in lipid HPLC, a combination of ACN:water is the chosen mobile phase. ACN:chloroform is the solvent in which the samples are resuspended in, so that the lipid will go into solution and the so that the phase are miscible. The type of water used as a solvent can also have an effect on the sensitivity and the ability of the detection method. Water itself, or even HPLC water, is not pure enough, as it tends to absorb at 205nm. Therefore, for all HPLC experiments MilliQ (Millipore) water is used.[192]

All solvents must be HPLC grade solvents and should be filtered using a 0.2 μm filter.[191] The filter must be HPLC grade so that the filter materials do not interfere with the solvents used in HPLC analysis. It is also imperative that the filters be compatible with the solvents being filtered, especially for solvents like chloroform. Of course, the correct size filter and syringe must be chosen, based on the volume to be filtered. For the reasons described above, the PALL Life Sciences GHP Acrodisc syringe filters with a pore size of 0.2 μm and a diameter of 25mm or the Naglene nylon 0.2 μm 4 mm diameter filters are used for most sample filtering.

After the correct solvents have been chosen and filtered for HPLC analysis, the mobile phases have to be degassed. All solvents have air and other gases, from the surroundings, dissolved into them.[191] These dissolved gases have increased solubility at high pressure and can cause pressure fluctuations and decrease the sensitivity of the column. Air within the solvents can also cause autoxidation of the lipids.[191] For these reasons it is necessary to remove all dissolved gases. There are several ways in which the solvents can be degassed, including using a filter in a vacuum, purging the solvents with helium gas, or to use a vacuum chamber that degas the solvents in line. The vacuum filter only partially degasses the solvents and air will continually continue to dissolve back into solution, therefore it is not the most desired method. Purging with helium is simple and effective. The vacuum pump chamber is a separate machine that the mobile solvents will travel through before heading to the column. Therefore the solvents are degassed in line. This is even simpler and also effective. The method of degassing chosen is the vacuum pump chamber method and therefore the mobile phase is degassed in line with the Erma (Tokyo) ERC-322 degasser.

The next step is to determine the flow rate and whether a gradient or isocratic solvent system should be used. The desired result should provide distinct individual peaks for each desired lipid, hopefully in the least amount of time and using the least amount of solvent. Several isocratic and gradient solvent systems were tested until the described protocol was chosen. This specific sequence of flow rates and mobile phases were

chosen so that the lipids would peak within the run time, separately, and not within the change of phases with increased sensitivity.[192]

There are several options to choose from when trying to pick a detection system. The number of detection methods and the type of methods that can be used are based on the substance to be identified, the chromatographic conditions, and the equipment available. The most common detection methods are wavelength detectors as they have high sensitivity and are not as affected by changes in phase or environmental condiditons.[37, 191] UV spectrophotometric detectors are the most widely used as they appropriate for isocratic or gradient. Most lipids have absorbance characteristics between 200-210nm.[191] Fluorescence detectors are not as robust, as only a few lipids exhibit fluorescence naturally, but with the use of fluorescent derivatives, these detectors are 2 to 3 fold higher in sensitivity than UV.[37, 191] Refractive index detectors are usually less sensitive than UV detectors and are sensitive to changes in temperature and gradient solvent systems, but are commonly used for triglycerides and fatty acids.[37] These are the most common lipid HPLC detectors, but flame ionization detectors, mass detectors and infrared spectrophotometric detectors are also an option, but can be expensive. UV detection was chosen for these experiments due to its ability to detect a broad range of lipids without being affected by the environment. 210 nm was tested as a possible wavelength of detection, but 205 nm provided increased sensitivity.[192]

The final consideration relates to the needle syringe used to inject the sample. There are many variables to consider when choosing a needle syringe, including the type of

needle, gauge, length, point type, and volume. The gauge of the needle and the length are based on the size of the tubing and injector of the HPLC machine. The syringe volume should be optimized to the volume injected for normal samples and the point type is dependent on whether a septum needs to be punctured. The type of needle is usually based on personal preference. There are choices of removable needles or cemented needles and gastight plungers. The syringe chosen for these experiments based on cost, availability, and the criteria above is the Hamilton cemented needle, 22 gauge, 2", point style #3, 50 μL, model number 705SN.

4.4.4 Advantages and Disadvantages with this Protocol

This protocol has been found to have various advantages and disadvantages when it comes to lipid analysis.

As mentioned previously, this protocol lends itself to accumulating lipid in many different parts of the HPLC machine, including the tubing, injector, column, and syringe. Due to this accumulation of lipid, pressure spikes are a normal occurrence. This can become frustrating and will interrupt the normal flow of sample analysis. In order to prevent clogs or the accumulation of lipid build up, normal cleaning protocols need to be maintained. The experimenter should be conscious of the normal pressure levels when there is no column in place, with a column, and during the experimental runs. Under these chromatographic conditions the step-wise gradient contains changes in solvent and flow rate, and therefore will induce changes in pressure throughout the run. An outline

of the normal pressure readings are found in Table 12 and 13. With the use of this table, it is easy to determine if there is a clog in the system and where it may be.

Table 12: Normal pressure readings in the system

Condition	Flow-Rate	Normal Pressure (psi)
Column attached with complete circuit	1.5 mL/min	~1700
Column attached but post-column tubing not attached	1.5 mL/min	~1600
Pre-column attached w/o column	1.5 mL/min	<100 psi
Inlet and outlet tubing only	1.5 mL/min	<100 psi
Inlet tubing only attached	1.5 mL/min	<100 psi
Inlet tubing not attached	1.5 mL/min	<100 psi

Table 13: Normal pressure readings throughout an HPLC run

Time (min)	Phase and Rate Changes	Pressure (psi)
0	no changes	~1700
0-10	no changes	~1700
10-11	As mobile phase changes	~1800
11-12	As flow rate changes	up to 2100
12-13	no changes	decrease to 1550
13-27	no changes	~1550
27-30	Flow rate and mobile phase change back	decrease to 1150
30-35	no changes	return to 1700

One major disadvantage of this protocol is that the mobile system and column are not sensitive enough by themselves and therefore a reference standard is added to every sample. For this reason, plain reference standards need to be injected as the first and last injection, and depending on the number of total samples, also in the middle. If there are any more than 7 samples, a reference standard needs to be completed in the middle. This will confirm the accurate calculation of total lipid in the sample when the reference standard is removed.

There are several problems linked to using a protocol that requires a reference standard, just as this. The first problem is that there can be variation in the reference standard injection peaks. If the reference standards are not consistent between runs, one might even have to run more, in order to give an accurate read-out. The use of a reference standard can drastically increase the length of time that a set of runs will take and therefore decrease the amount of samples that can be processed in one day. For every reference standard that is processed, an extra 35 minutes is added on to the total time, and since this particular HPLC machine is not equipped with an auto-sampler or auto-injector, this leads to long processing days. Along with the extra time, there is also the extra consumption of solvents. This protocol has a flow rate of 1.5mL/min and 2.0 mL/min, therefore a normal eight hour working day will consume approximately one liter of solvent and only approximately 6-7 samples can be processed. Not only does this protocol require more time and solvent, but also the use of lipid. Every reference standard requires more lipid in the reference standard injections and also in every

sample. This can become costly, and can lead to increasing accumulation of lipid in the system, as previously discussed.

The one advantage to using a 50 µL resuspension in the reference standard, is that our protocol only requires 10µL volume and a 20 µL syringe fill. Therefore, upon the time that a system error occurs or the need to double check a sample arises, there is enough sample left for a second injection. Despite this, at times 50µL can produce a viscous solution, especially if a lot of lipid was present in the sample prior to resuspension.

The step-wise gradient used can also create some problems. First, at times, the changes in flow rate and solvents can change the pressure of the system and can disrupt the baseline. However, a wash-out period is incorporated into this protocol and therefore another sample can be injected directly following the completion of the previous sample. When using a step-wise gradient such as this the UV detector is the only choice for detection on the Hitachi HPLC system (pump L6200, UV detector L4000, interface D-6000). A refractive index detector (Shodex RI-71) is attached and available with the system, but step-wise gradients will produce an unstable baseline and it would be difficult to identify and calculate a peak. Refractive index (RI) detectors are much more suited to isocratic protocols.

Since the RI detector is unsuitable for this protocol an ultraviolet (UV) detector is the primary detection system. The wavelength chosen is 205 nm. This specific wavelength has advantages, as there are many different substances that will absorb at 205nm. This is

great for *in vitro* experiments when it is known what will be in the sample, but for *in vivo* experiments, 205 nm will also detect many other substances, even some which might be undesired. Unfortunately, this wavelength is not specific to lipids. The wavelength 205 nm does not react to acetonitrile or water, the mobile phases, but it does limit the other solvent systems that can be tried. Chloroform, for example, would be a great mobile phase for lipid identification, but it also absorbs at 205 nm, and therefore makes it unsuitable.[191]

Another observation that has been noted with respect to this specific protocol is that the first run of almost every set tends to be an outlier. For this reason, at least two blanks are injected into the system to try and saturate the loop, but the problem still persists. Therefore, for every set of runs, especially when a new column is used, an extra reference standard should be injected first. This also consumes time, solvents, and lipids.[192]

One last disadvantage to this protocol is that it is only able to separate and identify a set amount of lipids. All of the lipid doping solutions contain five lipids and human tear films contain many more than that, but this protocol has only been designed to look at three of these lipids. This major disadvantage is that this only allows us to look at a part of the lipid deposition "story".

Even with all of these disadvantages, this protocol produces clean chromatograms with consistent baselines and no interference with the contact lens polymers that may be

extracted along with the lipid. The results are consistent and many of the experiments do reach statistical significance. There is no doubt that another procedure needs to be developed to separate and identify more lipid types with more sensitivity, but this protocol does provide useful information with one-step processing.

4.4.5 Limitations with the Hitachi HPLC machine

Along with the limitations of the protocol there are also limitations of the HPLC machine itself. The first limitation is that it is not attached to an auto-sampler or auto-injector, therefore a technician needs to be present to operate the machine for every injection, for all of the runs. This is time consuming and limits the other activities that can be completed at the same time.

Attached to the Hitachi HPLC machine there are only two available detectors; RI and UV. Therefore, these are the only two detection systems that are available to use, and RI can only be used in specific conditions. This limits the sensitivity and the productivity of this HPLC system. More detection systems provides for increased sensitivity and detection. Not all substances separate and therefore are detected to the same degree with every detector. Some substances may react better with fluorescence over UV. More detectors can increase the power of the HPLC system, especially for identification of a diverse sample group like lipids.

4.4.6 Reverse Phase HPLC Method Conclusions

The HPLC reverse-phase lipid analysis protocol is not the ideal protocol for *in vitro* or *in vivo* analysis. Lipid is retained on the column and throughout the system tubing, thus decreasing the sensitivity of the protocol and decreasing the amount of lipid that can be detected. This protocol is only optimized to detect and quantify three specific lipid types and is not easily adapted to identify or quantify other lipid types, specifically those other lipid peaks that were detected in the *in vivo* contact lens materials. For further, lipid analysis a normal phase HPLC procedure, possibly joint with gas chromatography analysis would optimize the sensitivity and the range of lipid types and number of lipids that could be detected and quantified.

5 OVERALL CONCLUSIONS

A method has been optimised for extracting lipid deposited on conventional hydrogel and silicone hydrogel contact lens materials using a chloroform:methanol extraction protocol. This is a very common and efficient procedure that will extract all lipid types from the contact lens surface.

A TLC procedure has been developed to separate and identify the five lipids that have been included in our lipid doping solution and that are commonly found within the human tear film, including oleic acid, oleic acid methyl ester, cholesterol, cholesteryl oleate, and triolein. Using this technique, I was able to detect and quantify these lipids in both *in vivo* and *in vitro* studies. It has been determined that PureVision lenses deposit significantly more lipid than Focus Night & Day lenses. However, it was determined that TLC is not sensitive enough to detect the subtle differences in lipid deposition when two different care regimes are being utilized. TLC can detect broad differences in materials, but is not sensitive enough to detect subtle differences such as those seen in human-worn lenses.

The lipid deposition of various conventional hydrogel and silicone hydrogel contact lens materials and its effect on wettability has been examined using contact angle measurements using an optical contact analyzer. Material wettability was found to increase with lipid deposition on conventional hydrogel and plasma surface treated

silicone hydrogel lens materials. No difference was seen on the newer non-surface treated silicone hydrogel materials. This effect is therefore material specific and may be linked to the penetration of lipid into the matrix. The concentration of lipid doping solution also affects wettability. A more lipid-rich doping solution was found to produce increased wettability for conventional and surface treated contact lenses. The length of time that the contact lenses are incubated and the concentration of the lipid doping solution are also variables that affect the final wettability.

Decreases in contact angle may help to explain why lipid deposition may produce an increase in comfort for contact lens wearers within the first couple hours or days of wear, but it is not known when this deposition will become disadvantageous to comfort and vision.

A reverse-phase HPLC procedure has been developed and optimized to identify and quantify three specific lipids, oleic acid, oleic acid methyl ester, and cholesterol. Using this procedure *in vitro* and *in vivo* contact lens materials were extracted and analyzed to determine the mass of lipid that was deposited on the surfaces. From these experiments it was found that there are three main variables that affect the ultimate amount of lipid that deposit on contact lens surfaces: the contact lens material, the composition of the lipid doping solution or tear film, and the concentration of the lipid doping solution or tear film. Specifically, it was found that silicone hydrogel lenses deposit more lipid than conventional hydrogel lens materials.

The reverse-phase HPLC lipid analysis procedure that has been utilized has been extensively assessed to determine its versatility and some changes could be implemented. The protocol encourages lipid to stick to the column and the HPLC tubing, causing increasing pressure in the system, decreasing retention times, and decreasing sensitivity. Therefore, new HPLC and GC procedures need to be developed to better separate, analyze and quantify lipid that is extracted from various contact lens materials.

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