In vitro analysis of wettability and physical properties of blister pack solutions of hydrogel contact lenses

by

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AUTHOR'S DECLARATION

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.

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

Abstract

Contact lens success is primarily driven by comfort of the lens in eye. Over the years, many modifications have been made to the lens surface and bulk material to improve comfort of the lens, however 50% of contact lens wearers still report dry eye symptoms while wearing their lenses.

Wettability of the lens material plays a large role in lens comfort, primarily due to its influence in tear film stability. In vitro wettability of contact lenses has typically been assessed by measuring the water contact angle on the lens surface. Currently there are three techniques to measure the in vitro wettability of contact lenses, the sessile drop technique, captive bubble technique, and the Wilhelmy balance method. To date, there is much published on assessing wettability using the sessile drop and captive bubble technique, however there is no data published looking at the in vitro wettability of hydrogel contact lenses measured by the Wilhelmy balance method.

Accumulation and deposition of tear components on the lens surface can also affect lens performance, by altering the wettability of the lens surface and causing lens spoilage. The majority of in vitro studies looking at deposition of tear components on the lens surface dope the lenses in tear solutions for a set period of time. None of these studies have investigated the impact of exposing the lenses to tear solutions, then exposing them to the air and then back into the tear solution, which mimics the process during blinking.

In Chapter 2, an evaluation of the influence of lens preparation on the wettability of contact lenses measured by the sessile drop technique was conducted. The wettability of 6 silicone hydrogel and one conventional lens material was assessed. Lenses were blot dried on either a microfiber cloth or lens paper for different drying periods and contact angles were

measured using the sessile drop technique. There were large variations in results using the microfiber cloth after all drying periods, but there was little variation in results after lenses were blot dried on lens paper for approximately 20 seconds. Thus, it was determined that for future contact angle analysis using the sessile drop technique that lenses should be blot dried for roughly 20 seconds on lens paper. This method was used consistently for the rest of the experiments in which the sessile drop technique was used to measure contact angles. The remainder of Chapter 2 compared the contact angles of different lens materials measured by the sessile drop technique and Wilhelmy balance method. The wettability of five different silicone hydrogel lens materials was assessed directly out-of-blister and after a 48 hour soak in saline. There were significant differences in contact angles for the lens materials between the two techniques. There were also significant differences in contact angles directly out-of-blister and after the 48 hour soak. Results from this study suggested that different methods of measuring wettability can produce different results and that blister pack solutions can alter the wettability of lens materials.

Chapter 3 measured the physical properties of blister pack solutions of silicone hydrogel lenses. The pH, osmolality, surface tension, and viscosity of the blister solutions for 9 silicone hydrogel lenses, 2 conventional lenses, and 2 saline solutions were measured. The osmolality of the blister solutions followed a trend, in that blister solutions manufactured by the same company had the same osmolality. Products produced by Johnson & Johnson had the highest osmolality. Blister solutions that contained additional wetting agents had higher viscosities compared to blister solutions without added wetting agents. The main conclusion from this study was that adding wetting agents to blister solutions could alter the physical properties of the blister solutions.

The purpose of Chapter 4 was to measure the physical properties of the blister pack solutions of daily disposable lenses and to evaluate the wettability of the lens materials and substantivity of the blister solutions, using a method in which lenses were cycled through 5 minute soaks in saline to mimic blinking. Five daily disposable lens materials were evaluated, one of which was shipped in a blister solution with added surfactants and wetting agents. The wettability of the lenses was assessed using the sessile drop technique and Wilhelmy balance method. The lens with the modified blister solution had a lower surface tension and higher viscosity compared to all the other blister solutions. The same trend in osmolalties as those reported in Chapter 3, were found with blister solutions made by the same manufacturer having the same osmolality. The wettability varied across lens materials. Overall, the lens material with the added components to the blister solution had the lowest contact angle.

Chapter 5 investigated the deposition of tear components onto the surface of conventional and silicone hydrogel lens materials and looked at the impact of this on changes in wettability. Three lens materials used in Chapter 4 were exposed to a saline solution, lysozyme solution, and a complex tear solution for 5 minutes, 1 hour, 4 hours, and 8 hours. The wettability was assessed after each time point using the sessile drop and Wilhelmy balance methods. There was little to no deposition on the lens materials that had the highest in vitro CAs in Chapter 4, exemplified by no change in wettability after being soaked in the lysozyme and complex tear solutions. There was deposition on the lens materials with the lowest CAs in Chapter 4, exemplified by a significant increase in wettability after being soaked in the lysozyme and complex tear solutions. Results indicate that there is some deposition onto one lens material, as shown by the change in wettability of the lens surface. These results were further used to validate a method used in Chapter 6.

The experiment conducted in Chapter 6 was similar to the experiment in Chapter 5, except that the lenses were not soaked in the three solutions but rather exposed to the solutions in a "model blink cell". The model blink cell moves lenses in and out of solution at a set time interval, in an attempt to mimic blinking. The interval was set so the lenses would be placed for 1 second in solution and 5 seconds exposed to the air. The same lens materials used in Chapter 5 were used in for this experiment. The lenses were exposed to a saline solution, lysozyme solution and complex tear solution for 5 minutes, 1 hour, 4 hours, and 8 hours. Much like in Chapter 5, deposition on the lens materials was determined by a change in the lens wettability. There were differences in the results of this chapter and that of Chapter 5, with deposition occurring on two of the lens materials rather than just one. This result indicates that the drying of the lens surface for 5 seconds out of solution has an effect on the deposition of tear components on certain lens materials. Thus, the model blink cell may be a useful tool for future deposition studies.

Overall this thesis demonstrated that preparation of the lens material can cause variation in contact angles. Different methods of measuring in vitro wettability of contact lenses can produce different results and thus the method used to assess wettability should always be stated. The physical properties of blister pack solutions can change with added wetting agents and surfactants, and components from blister solutions can alter the initial wettability of contact lenses. In vitro deposition of proteins onto the lens surface can vary with techniques, and finally, deposition of tear components onto the surface of contact lenses can alter the lens wettability.

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1. Chapter 1: Introduction

Contact lens success has been primarily driven by comfort of the lens in eye. Comfort is a trivial qualitative component to assess in terms of contact lens wear, as comfort can be influenced by oxygen permeability of the lens material, lens design, modulus, wettability, and accumulation of deposits on the lens surface.[1-6] Over the years, many modifications have been made to the lens surface and bulk material to improve comfort of the lens (discussed in detail later) however 50% of contact lens wearers still report dry eye symptoms while wearing their lenses.[7, 8]

Wettability of the lens material plays a large role in lens comfort, primarily due to its influence on tear film stability. In addition, deposition of tear components on the lens surface can also affect lens performance by altering the wettability of the lens surface and producing lens spoilage

1.1 Biomaterial Wettability

The term "wettability" refers to the ease with which a fluid spreads across a solid surface, or more specifically how the fluid adheres to the solid surface.[9-11] There are two general terms used to describe a solid's surface wettability: hydrophilic and hydrophobic.[12] A surface that is hydrophilic is a "fluid- loving" surface and will have the tendency to pull the fluid over its surface. A surface that is hydrophobic is "fluid-fearing" and will have the tendency to push the fluid away from the surface to minimize contact with the fluid.

Wettability of a solid substrate is influenced by three forces: the surface tension of the solid, the surface tension of the liquid, and the interfacial tension. At the interior of the liquid

there are more neighbouring molecules, than at the liquid surface. The attractive forces between neighbouring molecules in the interior region are the same in every direction, generating a lower energy state. However, at the surface of the liquid there are only inward attractive forces generating a higher energy state at the surface of the liquid (Figure 1-1).

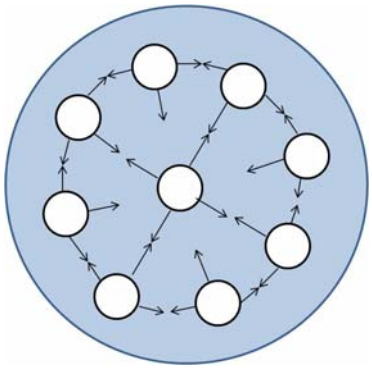


Figure 1-1: Attractive forces of molecules within a liquid drop.

Molecules have a tendency to remain at low energy states, thus molecules try to remain in the bulk of the liquid. This creates a tendency for liquids to maintain a minimum surface area by remaining as droplets, particularly on a hydrophobic surface.[13] Liquids that have strong attractive forces in the bulk of the fluid have high surface tensions. Conversely, lowering the surface tension of a liquid will cause the liquid to spread more evenly over the surface of the solid.

The surface tension of the solid is similar to that of a liquid; however, the intermolecular bonding of molecules in the solid is tighter and the inward pull is not enough to change the shape of the solid. A solid with a high surface tension acts to pull the liquid over the surface of the dry solid in an attempt to reduce the surface tension.[14]

The surface tension of the solid is countered by a force at the solid-liquid interface which pulls the liquid away from the surface of the dry solid. This force is known as the “interfacial tension”. The force of the interfacial tension can be increased or lessened depending on the

attractive forces between the molecules in the liquid and the solid. The greater the attraction between the liquid and the solid, the lower the interfacial tension will be, and the more spreading of the liquid over the surface of the solid.

1.1.1 Why is Wettability Important?

Biomaterials, including tissue engineering substrates, blood-contacting medical devices, artificial joint replacements, dental impressions, breast implants, ocular implants, and contact lenses, are compounds of natural or artificial origin that can mimic, store, or come into close contact with living biological cells or fluids.[12].

The performance of a biomaterial is generally evaluated by its biocompatibility. Biocompatibility refers to the measured success of the interaction between the biomaterial and the biological cells for a specific biomedical task.[12, 15] If the biomaterial retards or affects the natural biological process for which it is intended to assist, the biomaterial would be considered incompatible.[16] For example, in tissue engineering, if a substratum is used which does not promote the growth of a smooth monolayer of cells on its surface, it would not be deemed biocompatible, as its "desired outcome" is to allow the growth of a tissue on its surface. If a contact lens is unable to support a stable tear film, it would not be deemed as being biocompatible.

The surface of the biomaterial is the first component of the implant that comes into contact with the biological cells or fluids. Thus, biocompatibility will be influenced primarily by the surface characteristics of the biomaterial, particularly the surface chemistry of the exposed atoms, surface energy, surface topography, and the surface wettability.[17, 18]

Much work has been undertaken on measuring and reporting the wettability of a variety of biomaterials. It has long been assumed that enhanced wettability will result in improved biocompatibility.[19, 20] For example, the material used for dental impressions is highly important, in that it needs to be able to form readily around the teeth and gums and be void of any bubbles or defects. The environment inside the mouth is moist due to the excretion of saliva, and therefore the material used for dental impressions must be hydrophilic to ensure compatibility with the environment inside the mouth.[21] It is also important that the material is hydrophilic both before and after setting. The material should be hydrophilic before setting so that it can easily form around the teeth and gums, and needs to remain hydrophilic after setting so that no air bubbles are entrapped when the gypsum products are poured. If a surface is hydrophobic, water, saliva or any other fluid on the surface of the teeth or gums would create a small droplet and hence create a small void in the impression material or gypsum.[22, 23] This was confirmed by Michalakis et al,[21] who looked at the impact of wettability on void formation. The six materials examined were polyether, four poly(vinyl siloxanes) and one condensed silicone impression material. The results showed that the material with the lowest wettability, polyether, exhibited the fewest voids before setting.

However, improved wettability does not necessarily lead to improved biocompatibility for all biomaterials. Much research has been undertaken investigating the biocompatibility of the different materials used to make intraocular lenses (IOLs) and with differing surface wettabilities. Biocompatibility of IOLs is based on the proliferation of anterior lens epithelial cells (LECs) onto the surface of the IOL, anterior capsule opacification (ACO) and posterior capsule opacification (PCO). ACO is the opacification or clouding of the anterior portion of the

capsule which holds the lens of the eye. PCO is the opacification of the posterior portion of the capsule. ACO and PCO generally occurs after cataract surgery.

In 2003, Tognetto et al[24] compared the biocompatibility of a foldable hydrophobic acrylic IOL and a heparin surface-modified PMMA IOL. Biocompatibility was based on postoperative cell adhesion to the anterior surface of the IOL and ACO.[24] Postoperative analysis showed that the mean cell density on the IOLs were lower for the acrylic IOL compared to the heparin surfaced-modified IOL, throughout the entire follow-up period. There was also little to no anterior capsule opacification on the acrylic IOL compared to the heparin surface-modified IOL.[24] The researchers suggested that the results were due to a greater adhesion between the anterior surface of the acrylic IOL and the capsular margins, preventing cell migration, attachment, and proliferation.[24] Thus, the results indicated that the more hydrophobic IOL had a greater biocompatibility than the more hydrophilic IOL (heparin surface-modified).

In the contact lens literature, wettability in-eye is typically assessed by determining the ease with which the tear film spreads on the contact lens surface and how stable the tear film remains adherent to that surface. This is usually achieved by visible inspection of the lens at the slit lamp,[25] measuring the non-invasive tear break-up time,[1, 26-28] or determining the tear film thickness and stability using interference fringes.[28-30] However, in the context of general biomedical materials the surface wettability is usually assessed by determining water contact angles at the material surface.

1.1.2 Contact Angles

Measuring wettability of biomaterials in vitro is evaluated by measuring the contact angle (CA) at the liquid-solid interface. CAs are usually calculated using the Young- Dupré equation:

$\cos\theta = (\gamma_{SV} - \gamma_{SL})/ \gamma_{LV}$, where γ is the interfacial tension between the solid (S), liquid (L), and vapour (V) phase.[31] A high CA or low adhesion of the fluid to the solid indicates low wettability or a hydrophobic solid surface (Figure 1-2a).[32] A low CA, in which there is a smooth, continuous fluid film over the solid surface, signifies high wettability or a hydrophilic surface (Figure 1-2b). A surface that is completely wettable will have a CA of 0°.

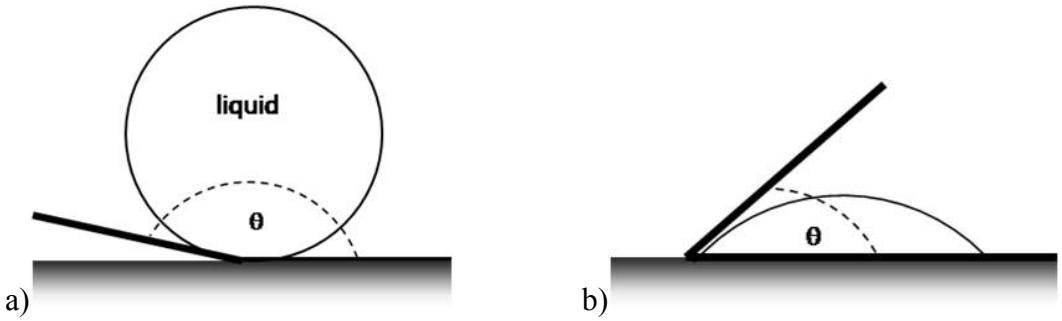


Figure 1-2: a) Schematic diagram of a hydrophobic surface with a high CA and b) schematic diagram of a hydrophilic surface with a low CA.

There are two different CAs that can be measured: advancing CA and receding CA. The advancing CA is the angle at which the liquid spreads across the surface of the dry solid at first contact. The receding CA is measured when the drop of liquid is withdrawn from the surface of the solid. The receding angle is usually smaller than the advancing angle as it is measuring a liquid being withdrawn over a surface that is already moist.

The difference between the advancing and receding CAs is known as the "hysteresis".[9] Although hysteresis occurs because of withdrawing the droplet over an already wet surface, it is also thought to be caused by possible polymer reorientation at the material surface.[33] Polymeric

surfaces are very mobile and the orientation of the molecules can change depending on the surrounding environment. When the materials are exposed to air or other hydrophobic environments, the hydrophobic groups within the polymers will migrate towards the surface of the material, making the surface less wettable. For example, when poly[2-hydroxyethyl methacrylate] is exposed to air, the methyl groups rotate towards the hydrophobic interface by chain rotation.[33] This is a more favourable energetic state, thereby lowering the surface free energy. However, on exposure to polar liquids, the polymers will rotate so that the hydrophilic groups are pointing towards the polar phase (Figure 1-3). This increases the wettability of the solid surface.

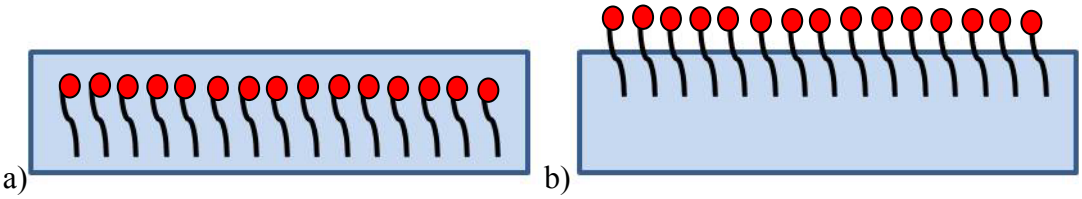


Figure 1-3: Schematic drawing of a) polymers within the bulk of a material due to hydrophobic outside environment and b) polymers rotating to outside of bulk material due to outside polar environment.

There are two types of CA hysteresis: thermodynamic hysteresis and kinetic hysteresis. Thermodynamic hysteresis on a clean surface is due to surface roughness, surface heterogeneity, and possibly surface deformation.[34] Kinetic hysteresis shows changes in a hysteresis loop as a function of time. In other words, kinetic hysteresis is usually caused by swelling of the material, liquid penetration into the surface of the material, and reorientation of the functional groups at the surface of the material.[9, 35, 36]

1.1.3 Techniques for Measuring Wettability In Vitro

Currently there are three techniques for measuring in vitro wettability: sessile drop, captive bubble, and the Wilhelmy balance method,[14] with sessile drop being the most commonly used technique.[37-44]

The sessile drop technique involves placing a drop of liquid from a syringe onto the surface of the test material. After the drop is placed on the material the advancing CA can be measured directly using a goniometer. A goniometer captures an image of the drop on the test material and through data analysis the advancing angle at the liquid-solid interface is measured.

The receding angle is measured by withdrawing the drop of liquid off the test material surface with the syringe and once again an image is taken as the drop is being withdrawn and the angle is measured between the liquid and the solid surface.[39, 45] To analyze the image, five points are placed along the curved surface of the lens image. A solid line then appears which can be manipulated to sit perfectly along the curved surface of the lens. Similarly, five points are placed along the curved surface of the drop image and a hollow sphere appears which can be manipulated to fall along the surface of the drop. An algorithm is used by the respective CA analysis software, which determines the angle between the two intersecting lines on either side of the water drop. The two angles are averaged, and the averaged CA which is used for further analysis of the results. A syringe with a needle diameter that is small in proportion to the drop size needs to be used when measuring the CAs. If the diameter of the syringe exceeds a few tens of microns, the drop shape will be altered, thus inducing errors when measuring the CAs.[46]

Although the sessile drop method is widely used in measuring wettability, there are two major problems with the method, namely evaporation of the liquid and dehydration of the solid surface. A small drop has a large surface area compared to the volume of the liquid and

evaporation will cause the drop to retreat on the surface of the solid and affect the measured CAs.[14] Dehydration of the solid surface can also lead to altered CAs. As mentioned above, polymers may reorient themselves at the surface in respect to the surrounding environment. If the polymer is left in a dry environment, (e.g. air) the hydrophobic groups within the polymer will reorient themselves to the surface of the material, making the material less wettable. Both of these problems can be solved by placing the solid sample and liquid drop in a vapour-tight chamber with clear windows, so that the image can still be captured and the CAs measured.[14]

The captive bubble technique eliminates the problem of the solid surface becoming dehydrated as in this technique the solid material is immersed in the probe liquid. A capillary is placed underneath the solid and an air bubble is dispensed from the capillary tip so that the bubble just touches the surface of the solid (Figure 1-4).[47] As the air bubble becomes larger, it pushes the liquid away from the solid surface and at this point the angle between the solid and liquid is the receding angle. The air bubble is then retracted back into the syringe and the liquid spreads back onto the surface of the lens. This angle would be considered the advancing angle.[39]

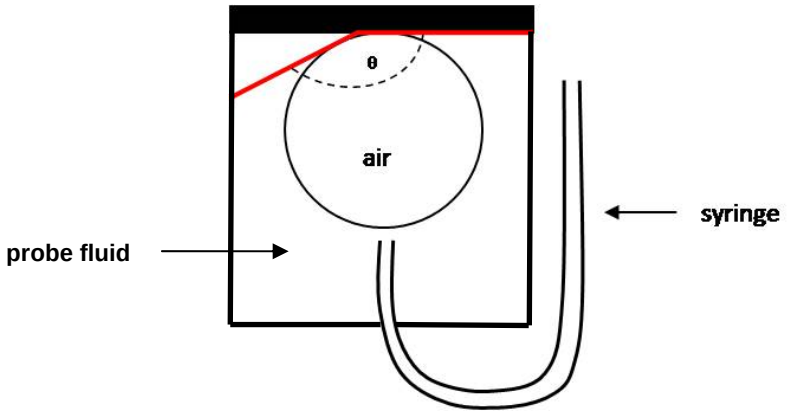


Figure 1-4: Schematic representation of the captive bubble technique. Syringe dispenses an air bubble onto the surface of the solid substrate creating a contact angle θ.

The captive bubble technique also has some drawbacks. Firstly, because the sample is immersed in liquid it may retain an absorbed layer of fluid on its surface making it appear more wettable than it truly is in the natural environment. Secondly, the air bubble is a medium of low refractive index but is observed in a medium of high refractive index. This creates a light path that is from air to water, to air to water, and then back to air. This path makes it difficult to see where the bubble actually contacts the solid surface.[14, 33] Thirdly, the amount of probe liquid required makes the captive bubble technique expensive to perform.

The Wilhelmy balance method uses a plate or sample of test medium which is hung from a microbalance and slowly submerged and removed vertically from a test liquid. The advancing CA is the angle between the solid and the meniscus of the test liquid as the solid is dropped into the liquid (Figure 1-5a) and the receding angle is the angle between the solid and the liquid as the plate is moved out of the liquid (Figure 1-5b).

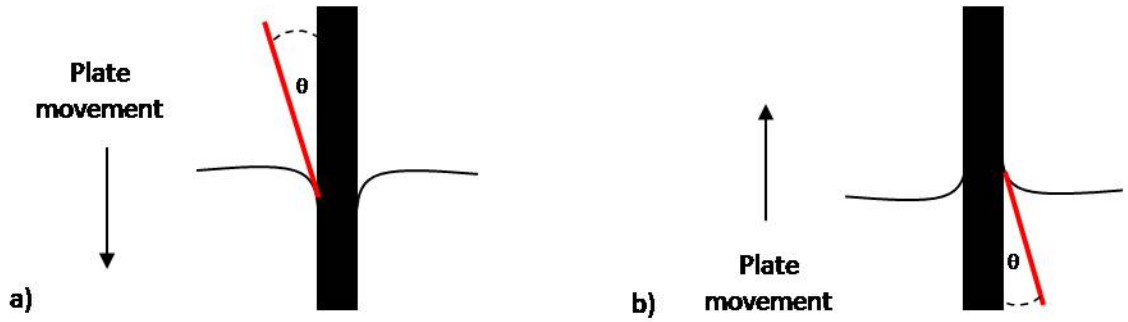


Figure 1-5: Wilhelmy balance method measuring a) advancing CA θ when lens strip is immersed in the probe fluid and b) receding CA θ when lens is removed from the probe fluid.

The main disadvantage of this technique is the cost of the equipment and the time it takes to prepare the test sample for immersion.[35, 46] The cost of lenses is also significant as cutting a strip out of the center of each test lens eliminates further use of that particular lens.

The sessile drop technique, captive bubble, and Wilhelmy balance method have all been used to assess contact lens wettability as well as changes to lens wettability from deposition of tear components. As mentioned previously, deposition on lenses can cause complications such as lens spoilage and eye infections. To date there have been a number of studies reporting related eye complications due to deposition.

1.2 Contact Lens Deposition

1.2.1 The Tear Film

The tear film is a physiological barrier between the anterior surface of the eye and the external environment. It has many functions including: lubricating the ocular surface and eyelids,[48-51] supplying nutrients to the ocular surface,[50] removing foreign material from the cornea and conjunctiva,[52] supplying the cornea with nutrients,[48] protecting the ocular surface from pathogens and bacterial contamination,[48, 49] and promoting tissue maintenance and healing.[48, 53, 54] Initially the composition of the tear film was described as being in three distinct layers: a mucin layer which was in direct contact with the epithelial cells of the cornea, an aqueous layer containing proteins, immunoglobulins, and electrolytes, and an outer lipid layer.[55, 56] However, further examination of the tear film indicated that the mucin layer may exist as a network within the aqueous layer forming what is now known as the mucin-aqueous layer.

The mucin-aqueous layer is 98.2% water and 1.8% of solids, which are primarily proteins. The solids in the aqueous layer are dissolved mucin, lactoferrin, lysozyme, lipocalin, secretory immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), albumin, transferring, ceruloplasmin, glycoproteins, inorganic salts, and glucose.[48, 54] There are also other substances such as magnesium, amino acids, bicarbonate, calcium, urea, and oxygen.

The lipid layer is a bilayer with an inner polar layer consisting of mainly phospholipids spreading over the aqueous layer and an outer nonpolar layer comprised mainly of cholesterol, and wax.[55, 57-60]

1.2.2 In Vivo Contact Lens Deposition

Deposition of tear film components is influenced by material surface charge, wettability, water content, wear time, and tear film composition.[61-63] It has been found that the majority of hydrogel lenses accumulate a layer of deposits on the surface when worn for an extended period of time,[64] and are the major cause of patients seeking contact lens aftercare in 30% of visits.[65]

A study conducted by Leahy et al[66] investigated the in vivo protein deposition on FDA group I (low water content, non-ionic) and group IV (high water content, ionic) lenses. Volunteers in the study wore the lenses contralaterally, with an FDA group I lens in one eye and an FDA group IV lens in the other eye. The lenses were worn for time periods of 1 minute, 15 minutes, 1 hour and 8 hours after which they were removed and protein analysis conducted. SDS-Page analysis was conducted to differentiate between proteins deposited on the lens surfaces. Six groups of different proteins, including lysozyme, albumin, lactoferrin, immunoglobulins, and two unidentified proteins were detected. Total protein analysis at each time point indicated that the FDA group IV lens accumulated significantly more protein than the FDA group I lens material. When looking specifically at the deposition of lysozyme on the surface of the lens material, there was a significantly higher amount of lysozyme deposited on the FDA group IV material at all time points compared to the deposited lysozyme on the FDA group 1 material. It was suggested by the authors that the higher deposition of total protein and lysozyme on the surface of the FDA group IV lens material was due to the ionic-binding

capability at the lens surface.[66] This work supported earlier findings by Sack et al,[67] who also found that protein deposition was highly dependent on the ionic nature of the lens surface.[67]

Lipid deposits are also detected on hydrogel contact lenses particularly FDA group II lenses (high water content, non-ionic).[68, 69] Jones et al[68] investigated the amount of lipid and protein deposition on FDA group II lenses after one group of patients wore the lenses for 3-months and the other group wore the lenses for three 1-month periods. Results indicated a significant increase in visible deposits on the lenses that were worn for 3 months, as compared to deposits on lenses that were worn for the three 1-month periods. Protein deposits were also significantly higher on the lenses worn for 3 months. This was also the trend for lipid deposition, however, some patients showed little difference in lipid deposition when comparing wearing regimes. There was also an observed decrease in tear break-up time (reduced wettability) indicating tear instability from deposits on the lens surface. Overall, this study indicated that deposition on FDA group II lens materials increased over a longer wear time, which could subsequently lead to tear film instability and lens spoilage.[68]

Studies looking at deposition on silicone hydrogel (SH) lenses indicate that deposition is considerably less than conventional lens materials, particularly FDA group IV lens materials. However, studies have also shown that deposits, more specifically lysozyme, on the SH lens materials is denatured. In a study by Boone et al[70], the total lysozyme and lysozyme activity on 5 different SH lens materials was measured. Participants were dispensed lotrafilcon A, lotrafilcon B, balafilcon A, and galyfilcon A lenses to wear for a 2-week period. Senofilcon A was dispensed as a control lens. Balafilcon A had the most deposited lysozyme, which was attributed to the ionic nature of the lens surface. Both lotrafilcon lens materials had the least amount of lysozyme deposited on the surface, however a large percentage of the lysozyme deposited was

denatured. A small percentage of lysozyme deposited on the balaficon A lens material was denatured.[70] Early studies looking at complications in-eye due to deposition vs. complications in-eye due to denatured proteins on the lens surface, indicated that inflammatory responses were more prevalent from denatured proteins on the lens surface rather than the amount of non-denatured protein on the surface of the lens.[71, 72]

1.2.3 In Vitro Contact Lens Deposition

Early in vitro deposition studies focused on the deposition of tear proteins onto the surface of conventional hydrogel lenses. Much like the results above, in vitro data showed that deposition was highly dependent on the ionic nature of the lens surface. In other words, protein deposition (particularly lysozyme) tends to be greater on lenses that have a negatively charged surface.[61, 66, 73-75] Again, there is some degree of deposition of tear proteins onto SH lenses in vitro, however this does not occur to the same extent as that seen on conventional polyHEMA-based hydrogel lenses.[31, 62] Santos et al[62] found that regardless of surface treatment, all SHs absorbed smaller amounts of protein onto the surface than conventional hydrogel lenses. A study conducted by Jones et al[61] looked at the deposition of lysozyme and lipids onto the surface of two SH lens materials (lotrafilcon and balafilcon) and a conventional hydrogel lens material (etafilcon). Results showed that lysozyme deposition was greatest on etafilcon and least on the balafilcon lens material. However, this study also demonstrated that the lysozyme that absorbed onto the surface of the SH lens materials was mostly denatured, particularly on the lotrafilcon lens material. Suggested reasons for protein denaturation were contact time with the substrate, chemical composition of the substrate, protein type, surrounding pH, and temperature.[61] This study also showed that lipid deposition onto SHs was higher than that compared to lipid

deposition onto the etafilcon lens material. It was proposed that this result was due to the attraction of the lipids to the hydrophobic silicone and incorporated N-vinyl pyrrolidone (NVP) monomer (balafilcon) in the lens material.[61]

The results of Jones et al[61] were further supported by two more recent studies that investigated the kinetics of protein and lipid deposition. In 2006, Subbaraman et al[76] investigated the deposition of lysozyme on PMMA, conventional (polymacon, alphafilcon A, omafilcon A, vifilcon A, and etafilcon A) and SH (lotrafilcon A, lotrafilcon B, balafilcon A, galyfilcon A, and senofilcon A) lenses as a function of time. Lenses were incubated in a lysozyme solution with the amount of deposition in μg/lens measured at specific time points over 28 days. As expected, etafilcon A had the highest amount of lysozyme deposition over all time points compared to all the other lenses with a plateau in deposition at the 14 day mark. Due to etafilcon A having a negative charge on the surface, the plateau at day 14 was an indication of a charge reversal on the lens surface. FDA Group I (polymacon) and II (alphafilcon A and omafilcon A) conventional lenses showed a gradual increase in lysozyme deposition over all time points, with no plateau as seen with etafilcon A. These results were suggested to be due to the neutral charge and zwitterionic properties of the surface of the Goup I and Group II lenses. SH lenses deposited significantly lower amounts of lysozyme compared to the conventional lenses at all time points. Over a 7 day period there was little lysozyme deposited on the surface of the lens, however after the 7 day mark there was a dramatic increase in the lysozyme deposited. Both lotrafilcon lens materials exhibited the lowest amount of deposited lysozyme. The lotrafilcon lens materials have a plasma surface coating which is impenetrable to lysozyme,[77] resulting in the lower amounts of deposition. Galyfilcon A and senofilcon A do not have any surface treatment,[78] thus lysozyme was able to penetrate into the pores of the lens materials.[76, 79] Balafilcon A had the highest

amount of deposited lysozyme out of all the SH lenses. This result could be explained by the bulk properties of the lens material. Balafilcon A has a "glassy island" surface which causes the surface to be less wettable that the other SH materials.[80] The hydrophobic glassy islands may attract more protein compared to the hydrophilic surfaces of the other lens materials. Balafilcon A also has larger pores, which could allow more lysozyme to penetrate the lens material and subsequently result in increased levels of protein deposition.[79, 80]

A study conducted by Carney et al[81] investigated the kinetics of lipid deposition, particularly, the deposition of a polar and nonpolar lipid on five SH lens materials and one conventional lens material. The polar lipid used was phosphatidylethanolamine (PE) and the nonpolar lipid used in the experiment was cholesterol (CH). Each lens type was exposed to the individual lipid solution for 20 days, with each day a reading for the amount of lipid deposited on each lens taken. There was significantly more CH adsorbed onto the lens surfaces as compared to the amount of PE adsorbed onto the lens surfaces. Both lotrafilcon lens materials exhibited lower amounts of lipid affinity over the 20 days for both PE and CH. The conventional lens material also exhibited a reduced amount of lipid adsorption for both lipids however, CH initially showed a higher degree of affinity than all lens types until day 8, at which that point, galyfilcon A, senofilcon A, and balafilcon A all had significantly higher amounts of CH on the surface. These differences in adsorption of the lipids between the SH materials was thought to be due to the water content of the lens materials, with a higher water content promoting a higher degree of lipid adsorption. It was also though that the differing surface and bulk properties of each lens contributed to the varying amounts of lipid adsorption. The smooth coating of the lotrafilcon materials may have acted like a barrier to the lipids, preventing adsorption and penetration as compared to the porous nature of the balafilcon A lens material. The senofilcon A and galyfilcon

A materials, which have no surface treatment at all, expose the bulk lens material to lipid penetration.

Many manufacturers have altered the surface and bulk properties of contact lens materials to improve wettability and reduce deposition on the lens.

1.3 Modifications of Modern Contact Lenses

The ocular environment places huge demands on contact lenses as a biomaterial. The lens material must be able to support a continuous tear film for optimum visual clarity, must not dehydrate, and needs to resist sorption of tear components such as lipids, proteins and mucins, as build-up of deposition can lead to decreased visual clarity and reduced comfort.[73] In addition, the cornea requires oxygen to maintain its clarity, structure, and function, thus the contact lens material must be permeable to oxygen.[82] Contact lenses that transmit an insufficient amount of oxygen can induce hypoxic complications that include corneal swelling, epithelial microcysts, increased myopia, corneal neovascularization, epithelial thinning, and increased bacterial adhesion to corneal epithelial cells.[83-91] It has been suggested that the oxygen transmissibility (Dk/t) of a contact lens worn overnight should exceed 125×10^{-9} (cm · mL O_2)/(sec · ml · mm · Hg) to prevent contact lens-induced corneal swelling.[92-94]

Soft contact lenses were initially commercialised in 1970 and were composed of solely poly-hydroxyethyl methacrylate (pHEMA).[95] These lenses were rapidly accepted by patients and practitioners due to their relative comfort over PMMA lenses and adequate wettability.[96] However, overnight wear of pHEMA-based lenses led to hypoxia and marked neovascularisation.[83] Despite many attempts over the next 30 years to enhance the oxygen transmissibility of pHEMA-based materials, through the addition of various monomers and

changes in design, adequate oxygen transmissibility to support overnight wear was never possible, which led to the development of SH contact lenses.

Silicone is a synthetic polymer with repeating silicon to oxygen bonds known as siloxanes.[97] These siloxanes are usually bonded to an organic group such as a methyl, vinyl, or phenyl group. This structure of organic groups bonded to an inorganic backbone gives silicone unique properties, which allows it to be used for various applications, including breast implants and SH contact lenses. The organic groups have a relatively low surface energy compared to the siloxane groups, which have a high surface energy. Thus the typical conformation of silicone is with the siloxane backbone surrounded by the organic groups. The intermolecular interactions are quite low, which allows for diffusion of gases through the molecule and results in a very high permeability to oxygen.[97]

SH contact lenses became commercially available in 1999. They incorporated silicon as siloxane (-Si$(CH_3)_2$-O-) polymers into the lens material to improve oxygen permeability. The increased oxygen permeability arises from the motility of the siloxane group to move to the surface of the lens material.[98] Although siloxane is highly oxygen permeable, it is also highly hydrophobic. This results in poorly wettable surfaces, and in order for these materials to wet adequately in-eye they require some form of modification to enhance their surface wettability. Initially, companies surface-treated the lenses by either plasma surface treatment or plasma oxidation.[95, 98, 99]

Plasma is a highly reactive gas by activation of an electric field.[16, 98] When plasma is placed in the presence of a contact lens, the surface of the lens and the plasma react. Depending on the gases and compounds that are present during the plasma treatment process, different results will occur after the reaction has taken place. In the case of plasma oxidation, as used by

Bausch & Lomb for the balafilcon A lens, oxygen is present in the plasma, which oxidizes the siloxane groups to silicate. This results in what resembles "islands of glass" on the surface of the lens,[41, 80, 100, 101] which are "clumps" of silicate. These silicate islands do not cover the entire surface of the lens and do not affect the oxygen permeability of the underlying balafilcon A material, however their distribution is such that they increase the wettability of lens and thus support a stable tear film.[80, 102]

The two CIBA Vision SH lenses, lotrafilcon A and lotrafilcon B, have a plasma coating in which volatile organic compounds are present in the plasma.[41, 95] When a reaction occurs, these compounds can behave as monomers and polymerize onto the surface of the lens, creating a polymer film which is more wettable than the underlying lens surface. This polymer layer is ultrathin (25nm),[77] does not affect the oxygen permeability of the underlying material and results in a surface that is smoother than that measured on the balafilcon A lens.[79, 95]

Johnson & Johnson lens materials, galyfilcon A, senofilcon A, and narafilcon A, do not use plasma treatments to improve the wettability of their lenses. Instead they incorporate a high molecular weight wetting agent based on polyvinylpyrrolidone (PVP) into the polymer matrix of the lens. PVP in aqueous environments readily binds to water, retaining moisture for the lens. This treatment also helps to support a stable tear film and does not interfere with the oxygen permeability of the lens.[78, 103]

Asmofilcon A (PremiO) is a relatively new SH material from Menicon. It uses a surface treatment known as Nanogloss™, which combines plasma oxidation and plasma surface treatment, creating a very smooth surface on the lens.[104] To date, there is no published data on what monomers are incorporated into the lens material.

CooperVision manufactures two SH lenses, Biofinity™ and AVAIRA™. Neither of these lenses have any surface treatment or internal wetting agent. Rather, these lenses contain two silicone-based macromers that are incorporated into the lens material with hydrophilic monomers, resulting in a lens with a relatively high degree of wettability.[105]

To date, there has been little correlation between contact lens comfort and wettability assessed in vitro by the sessile drop technique. In a recent study, Cheung et al[106] compared a polyHEMA-based hydrogel (etafilcon A) to that of a SH (galyfilcon A) in terms of comfort, ocular performance and surface deposits. Participants wore the lens materials as a contralateral pair for 8-12 hours a day for 6 consecutive days. The results indicated that the lenses were comparable in comfort. However, our sessile drop CA measurements for galyfilcon A revealed a CA of 102° (internal data), as compared with 51º for etafilcon A (internal data), suggesting that the etafilcon A material should be substantially more comfortable if CA was an important factor affecting lens comfort.

Keir et al[107] conducted a study to determine if there was any correlation between lens comfort and both in vivo wettability (determined via pre-lens non-invasive tear break-up time) and ex vivo wettability (measured using the sessile drop technique). Participants were assigned to wear lotrafilcon B and senofilcon A lenses contralaterally for 14 days. As with the study by Cheung et al,[106] the results showed no correlation between in-eye comfort and either in vivo or ex vivo wettability.[107] In 2002, Morgan and Efron compared the comfort rating of balafilcon A (PureVision) and lotrafilcon A (Focus Night & Day).[108] Subjects in this cross-over study wore a pair of each lens type for 8 weeks. Our sessile drop CA measurements (internal data) for balafilcon A revealed a CA of 84°, as compared with 42º for lotrafilcon A, suggesting that the

lotrafilcon A material should be substantially more comfortable. However, the results showed that there was no difference in comfort rating between the two lens materials.[108]

Other studies have also found little correlation between lens wettability and hydration with comfort of the lens in eye indicating that other factors may influence lens comfort.[8, 109] Recently, manufacturers have added surfactants and wetting agents to the blister solutions in an attempt to improve comfort of the lens in-eye.

1.4 Properties of Blister Pack Solutions

The alterations made to the blister packaging solutions are to aid in preventing the lenses from sticking to the blister pack, enhance lens wettability, and improve initial comfort of the lenses in-eye.

Ideally, the blister packaging solution should be designed to have similar physical properties to that of the human tear film, or could be slightly altered to enhance tear film stability. A variety of physical parameters of the blister pack solutions can be determined, including pH, surface tension, viscosity and osmolality, and these may impact initial comfort when lenses are removed from the packaging products and placed on the eye. The pH of the human tear film ranges between 6.6 and 7.8,[110] and if contact lens solutions, lubricating drops, or blister packaging solutions that contact the eye have a pH outside of this range, the eye may experience sensations of discomfort or stinging.[111, 112]

The surface tension of human tears is approximately 40-46 dynes/cm,[113] and may be higher in dry eye patients, suggesting that surface tension plays an important role in tear film stability.[113, 114] Rewetting drops function to lower the surface tension of tears to aid in enhanced

wetting of the contact lens surface. Adding surfactants to the blister pack solution can also lower the surface tension of tears on initial insertion of a contact lens from a blister pack.

The contact time of solutions on the eye can increase by increasing the solutions' viscosity.[115, 116] However, increasing the viscosity too much may cause a "dragging" effect during blinking and lead to epithelial damage[116] or visual blur.[117] The viscosity of the human tear film is approximately 1.5cP[116, 118] and it is likely that viscosities much higher than this would result in temporary reductions in acuity.

"Osmolality" and "osmolarity" are two terms that are often used interchangeably. The osmolality of a solution is the concentration of particles in dry weight (mmol/kg).[119] The osmolarity of a solution is the amount of pressure the particles in a solution exert on a semi-permeable membrane (mmol/L). The osmolarity of a solution is dependent on the number of particles in the solution.[119] The osmolality of human tears is approximately 305mmol/kg.[120] Fluids that come into contact with the eye that have osmolalities higher than the osmolality of human tears may contribute to discomfort in-eye.[120]

While the recent interest from companies in altering the composition of blister-pack solutions shows that there is some belief that this will result in enhancements in clinical performance, to date there is little published information regarding the polymeric and molecular additions to the blister solutions and no data available on their physical properties. The physical properties of the blister pack solutions for SH and daily disposable lenses will be investigated in this thesis (Chapter 3: Physical Properties of Blister Pack Solutions of SH Contact Lenses).

1.5 Conclusion

The ideal contact lens would be manufactured from a lens material that has excellent oxygen transmissibility, and that currently requires it to be made from a SH material. The lens would have adequate wettability, signified by relatively low advancing and receding CAs to allow the spreading of the tear film over the surface of the lens. The hysteresis of the lens material would also be minimal, as rapid wetting and drying of the lens may cause discomfort in eye. The blister solution which houses the lens material would have surfactants and incorporated wetting agents to improve initial comfort of the lens in-eye, by reducing the surface tension and increasing retention time of the tear film on the surface of the lens. Finally, the lens material would be resistant to tear component deposition to prevent inflammatory reactions in-eye and spoilage of the lens.

In the following chapters, the author investigates the differences in CA analysis between the sessile drop and Wilhelmy balance methods. The analysis of advancing and receding CAs and the hysteresis of different lens materials will be explored. The physical properties of the blister pack solutions for SH and daily disposable lenses will be measured and recorded. Finally, the deposition of tear components onto the surface of three different lens materials after the lenses have been placed in a “model blink cell” will also be investigated.

2. Chapter 2: In Vitro Analysis of the Wettability of Silicone Hydrogel Lenses

To date, there has been no data published on the in vitro wettability of hydrogel and silicone hydrogel contact lenses measured by the Wilhelmy balance method. As mentioned previously, the Wilhelmy balance method measures the force required to move the lens into and out of the probe fluid, which is then converted by the instrument software into a CA measurement. This is an indirect measurement of the CAs on the surface of the contact lens, and as a result this may lead to differences in the CAs measured when comparing different techniques. In addition, no work has been published comparing the CA results obtained with this method to the sessile drop method. In preparing lenses for CA analysis using this latter method, the lenses must be appropriately treated to remove any excess fluid from the material surface. This may impact the results obtained, but to date no work has been published on the most appropriate method to remove this fluid and which method would provide the most repeatable results.

In literature, there has been speculation about the accuracy of CA analysis by the sessile drop technique due to variation in blotting methods. The purpose of this chapter is to firstly investigate the CAs determined using a variety of blotting methods to remove excess fluids when preparing lenses for sessile drop analysis, and secondly to report on the advancing CAs of five different silicone hydrogel lens materials using the sessile drop and Wilhelmy balance methods.

2.1 Comparison of Advancing CA After Various Blotting Methods

The accuracy of the method chosen to quantify CA depends primarily on how efficiently the experimenter prepares the solid sample. A recent experiment determined the repeatability of CA measurements on contact lenses using the captive bubble and sessile drop techniques.[121] The results showed that the CAs measured using the sessile drop technique were less repeatable than those measured using the captive bubble technique. This was thought to be primarily due to variability in the method used to blot dry the contact lens during the preparation of the lens material for analysis using the sessile drop technique.[121]

The purpose of this experiment was to investigate if different methods of preparation of the lens would cause variations in CAs measured by the sessile drop technique.

2.1.1 Materials:

The lens materials used in this study were etafilcon A, galyfilcon A, senofilcon A (Johnson & Johnson), lotrafilcon A, lotrafilcon B (CIBA Vision), balafilcon A (Bausch & Lomb), comfilcon A (CooperVision).

2.1.2 Methods:

2.1.2.1 Sessile Drop Technique

All lenses (n=3) were soaked in 5ml of preservative-free saline (Unisol, Alcon, Fort Worth, Texas) for 24 hours to remove the blister pack solution and prevent variations in CA analysis due to the blister solution on the lens surface. After the 24 hour soak, the lenses were blot dried for 5, 10, 15, 20, 25, and 30 second intervals on lens paper (Figure 2-1a) or a microfiber cloth (Figure 2-1b).

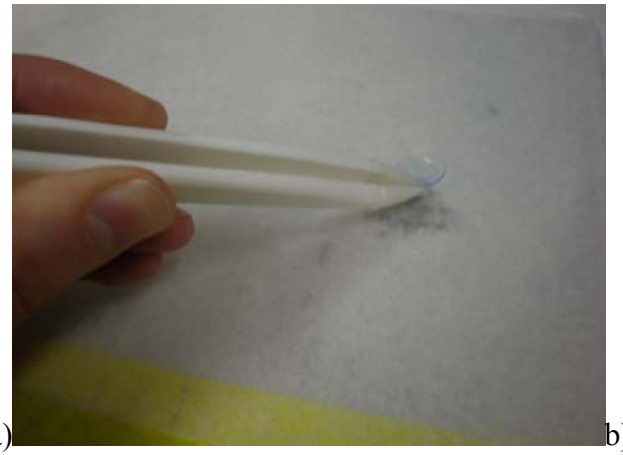
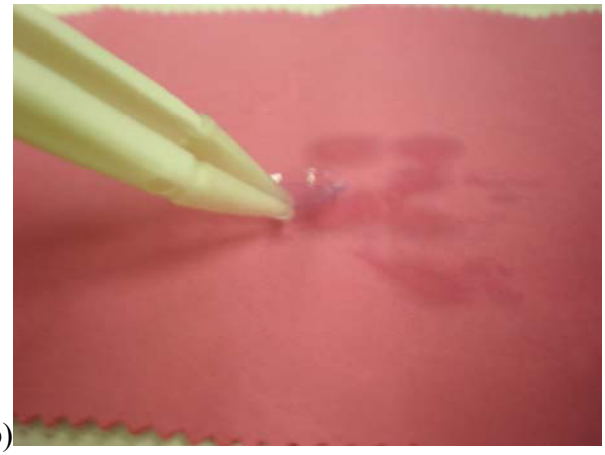
a)  b) 

Figure 2-1: Blot drying contact lens on a) lens paper and b) microfiber cloth.

After blot drying, each lens was placed posterior side down on a custom curved convex mantle and placed directly below the syringe of an Optical Contact Analyzer (OCA - Dataphysics Instruments GmbH, Filderstadt, Germany), as previously described.[41] The lens was centered to make sure that the drop of probe fluid dispensed from the syringe was placed directly on the center of the lens. After the mantle was centered, a 5µl drop of high performance liquid chromatography (HPLC) grade water (EMD Chemicals, Gibbstown, New Jersey) was placed on the lens at a rate of 2µl/second. The drop was allowed to settle for approximately 2-3 seconds, after which a picture image was taken and saved to the computer hardware. Custom software (SCA 20 software, version 2.04, Build 4) was used to analyze the images and determine the advancing CAs for each lens. To analyze the image, the user placed fives points along the curved surface of the lens image. A solid line then appears which can be manipulated to perfectly sit along the curved surface of the lens. Similarly, five points are placed along the curved surface of the drop image and a hollow sphere appears which can be manipulated to fall along the surface of the drop. An algorithm in the SCA software determines the angle between the two intersecting

lines on either side of the water drop. The two angles are averaged, and it is the averaged advancing angle which is used for further analysis of the results (Figure 2-2).

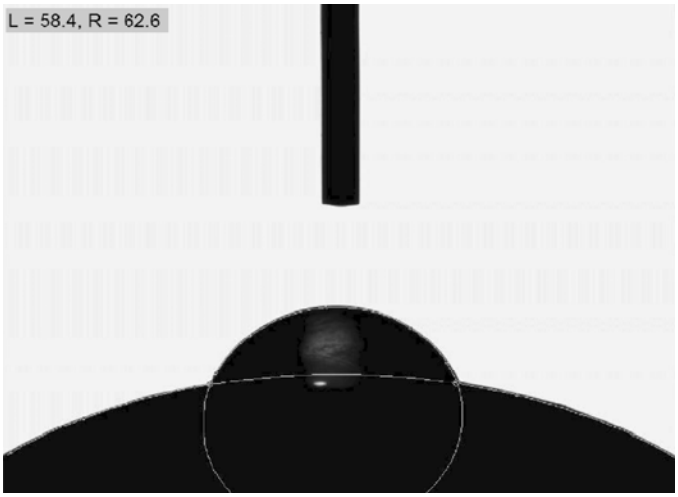


Figure 2-2: Analysis of advancing contact angle (CA) using the sessile drop technique, by the SCA software. The screen capture clearly shows the software analyzing the CA on the left side of the water droplet and the CA on the right side of water droplet, on the top left side of the image. These values are averaged and the mean CA recorded.

2.1.2.2 Statistical Analysis

Analysis of the advancing CAs measured after the lenses were blot dried on microfiber cloth and lens paper were all analyzed independently and compared by repeated measures ANOVA (analysis of equal variance). Further analysis of CAs was undertaken using a Tukey post-hoc test to see all significant or non-significant differences between individual measurements. A p-value of <0.05 was considered significant.

2.1.3 Results

The advancing CAs measured using the sessile drop technique after lenses were blot dried on a microfiber cloth for 5, 10, 15, 20, 25, and 30 seconds are shown below in Figure 2-3. The analysis was undertaken sequentially, in which the lenses were blot dried for 5 secs, then the CA measured, rehydrated then dried for a further 10 secs and the CA measured, etc.

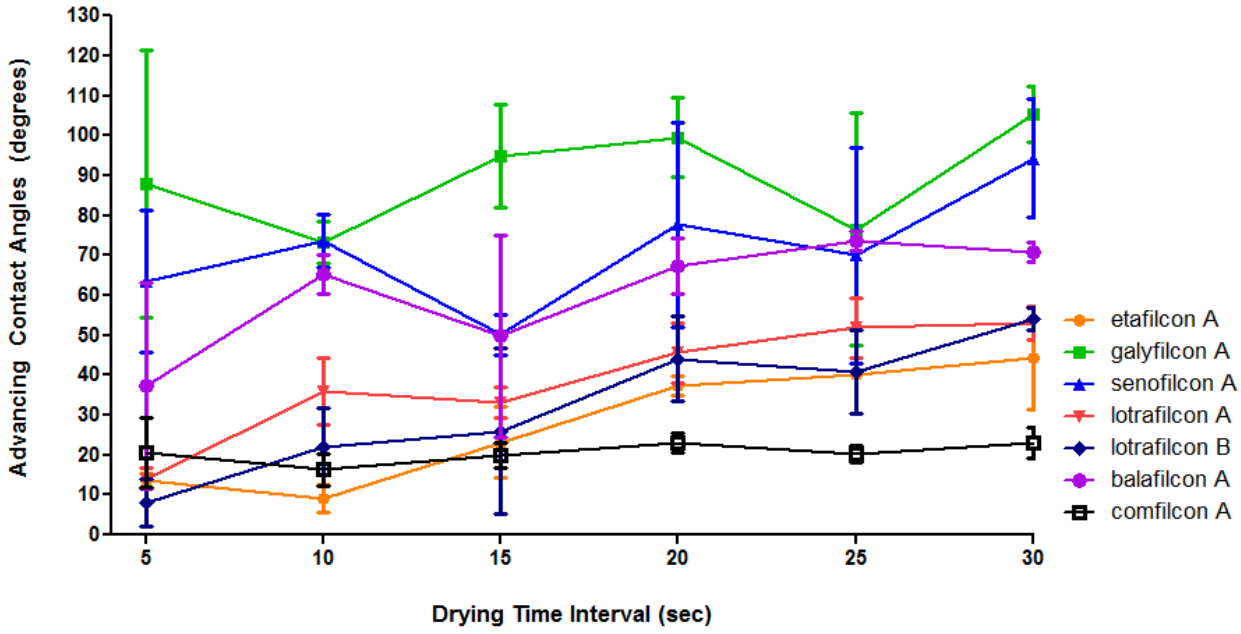


Figure 2-3: Advancing CAs of etafilcon A, galyfilcon A, senofilcon A, lotrafilcon A, lotrafilcon B, balafilcon A, and comfilcon A measured using the sessile drop technique after lenses were blot dried on a microfiber cloth for time intervals of 5, 10, 15, 20, and 30 seconds.

CA analysis showed large variation in results, with substantial standard deviation bars. An overall analysis for each material averaged over all the time points revealed no statistically significant difference between any of the lens materials over time ($p>0.05$). This shows that

while there was a difference between materials, within each material, drying for longer periods of time did not statistically impact the CA measured.

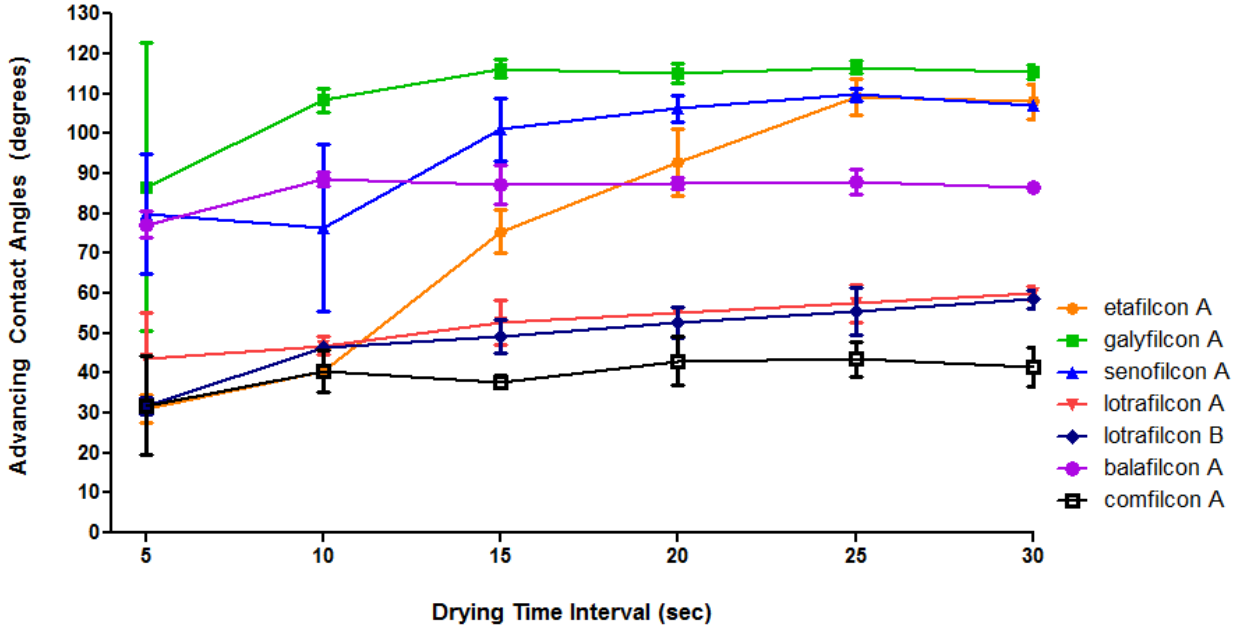


Figure 2-4: Advancing CAs measured using the sessile drop technique after lenses were blot dried on lens paper for time intervals of 5, 10, 15, 20, and 30 seconds.

Figure 2-4 above shows the results of the advancing CAs measured for each lens material after the lenses were blot dried on lens paper for 5, 10, 15, 20, 25, and 30 seconds. Analysis of these results showed that there was no statistical difference between CAs measured after different drying times for the lotrafilcon A, lotrafilcon B, balafilcon A, and comfilcon A lens materials ($p>0.05$). CAs for etafilcon A were significantly lower after being blot dried for 5 and 10 seconds ($p<0.05$) and significantly higher after being blot dried for 25 and 30 seconds ($p<0.05$). There was no statistical difference between CAs for galyfilcon A after being dried for 10 seconds ($p=1.00$). For senofilcon A there was no statistical difference in CAs after the lenses were blot dried for 15 seconds ($p>0.90$).

Comparing CAs measured between the two drying methods, there was statistical difference at all drying times between the two methods for etafilcon A with CAs measured after blot drying on a microfiber cloth being statistically lower ($p<0.05$). The CAs measured after galyfilcon A was blot dried for 25 seconds on a microfiber cloth were statistically lower ($p<0.05$) than the CAs measured after galyfilcon A was blot dried on lens paper for 25 seconds.

Comparing CAs measured between the two methodologies for senofilcon A showed statistical difference when the lenses were blot dried for 15 seconds, with CAs after being blot dried on the microfiber cloth being lower ($p<0.05$). The CAs measured after lotrafilcon A lenses were blot dried on microfiber cloth for 5 seconds were statistically lower than all other CAs measured for either methodology. There was no statistical difference in CAs for lotrafilcon B and balafilcon A after the lenses were blot dried for 20 seconds on either the microfiber cloth or lens paper ($p>0.10$). For comfilcon A, there was statistical difference between CAs when the lenses were blot dried for 10, 20, and 25 seconds with CAs after being blot dried on the microfiber cloth being statistically lower ($p<0.05$).

2.1.4 Discussion

There was no statistical differences in CAs after lenses were blot dried on a microfiber cloth for drying durations of 5, 10, 15, 20, 25, and 30 seconds. However, there were significant standard deviation bars, indicating a large range in CAs. For example, the CAs for senofilcon A ranged from 50-100° after being blot dried for 25 seconds. Similar variations in CAs measured after being blot dried on a microfiber cloth were seen for galyfilcon A, balafilcon A, lotrafilcon B, and etafilcon A. Comfilcon A was the only lens material that had the least amount in variation in CAs over different drying periods.

There was little variation in CAs measured after lenses were blot dried on lens paper after 20 seconds, except for etafilcon A. After blot drying for 20 second on lens paper, CAs for all the lens materials began to plateau, with little variation, as illustrated by the small standard deviation bars. Galyfilcon A and senofilcon A had average CAs of approximately 117° and 109° respectively after being blot dried for 20 seconds on lens paper. Lotrafilcon A and lotrafilcon B had average CAs of 51° and 60° respectively, and balafilcon A and comfilcon A had average CAs of 89° and 41° after being blot dried for 20 seconds. Thus, it would seem from this study that for the sessile drop technique, lenses should be blot dried for 20-30 seconds on lens paper, rather than on a microfiber cloth. Lenses that are blot dried for longer than 30 seconds may dehydrate and skew results.

2.2 Comparison of Advancing CA's Using Two Methods

2.2.1 Materials:

The lens materials used in this study were galyfilcon A (Johnson & Johnson), lotrafilcon B (CIBA Vision), balafilcon A (Bausch & Lomb), asmofilcon A (Menicon), and comfilcon A (CooperVision).

2.2.2 Methods:

2.2.2.1 Sessile Drop Technique

Advancing CAs for each lens material were measured directly out of the blister pack. Each lens (n=4) was removed from the blister pack and blot dried on lens paper for approximately 20 seconds to remove any excess fluid from the blister pack solution, as this may

impact on the initial CA. After blot drying, CA analysis was conducted using the sessile drop technique (see section 2.1.2.1).

After the CA was measured, the lens was immediately soaked in 5ml of preservative-free saline solution (Unisol, Alcon, Fort Worth, Texas) for 48 hours, in order to remove the blister pack components from the lens and to determine the CA of the material without the impact of the blister pack solution. After 48 hours had elapsed, the lens was removed from the saline and the advancing CA was measured exactly as described above.

2.2.2.2 Wilhelmy Balance Method

Advancing CAs for each lens material (n=4) were also measured directly out of the blister pack using the Wilhelmy Balance Method, using a similar method to that previously described.[35] Each lens was removed from the blister pack, with the excess fluid shaken off the lens. A strip was cut from the center of the lens, 3mm in width and 14.0-14.2 mm in length and placed on a low energy surface (Parafilm, Menasha, Wisconsin). One end of the strip was pierced with a small fish hook with an attached weight and the opposite end attached to a micro-crocodile clip. The clip was secured to the arm of the electrobalance of a Cahn Dynamic Angle Analyzer DCA-322 (CAHN Instruments, Madison, Wisconsin).[35]

The lens strip was lowered into the probe liquid (HPLC grade water) until the attached weight was just below the meniscus of the probe liquid. Weights were added to the other end of the electrobalance to balance the weight of the lens strip, fish hook and weight, and micro-crocodile clip. Immersion and emersion depth of the lens strip in and out of the probe liquid was 7mm. Buoyancy effects are negated by the software detecting a “zero depth of immersion” (ZDOI) when the lens strip first touched the surface of the probe liquid. As the lens strip was

immersed into the probe liquid the force required to immerse the lens into the liquid was converted into the advancing CA by the customized software for the CAHN balance.[35] As the lens was removed from the probe liquid, the force required to remove the lens was converted into the receding CA by the software. The CAs of each lens were measured directly out-of-blister and after a 48 hour soak in preservative-free saline (Unisol, Alcon, Fort Worth, Texas).

2.2.2.3 Statistical Analysis

Analysis of the advancing angles measured using the sessile drop technique and Wilhelmy balance method were all analyzed independently and compared between methods by repeated measures ANOVA (analysis of equal variance). Further analysis of the CAs were undertaken using a Tukey post-hoc test, to see all significant or non-significant differences between individual measurements. A p-value of <0.05 was considered significant. Values of statistical significance were indicated with "stars" on the graphs.

2.2.3 Results

The advancing CAs measured for each lens material using the sessile drop technique are shown in Figure 2-5.

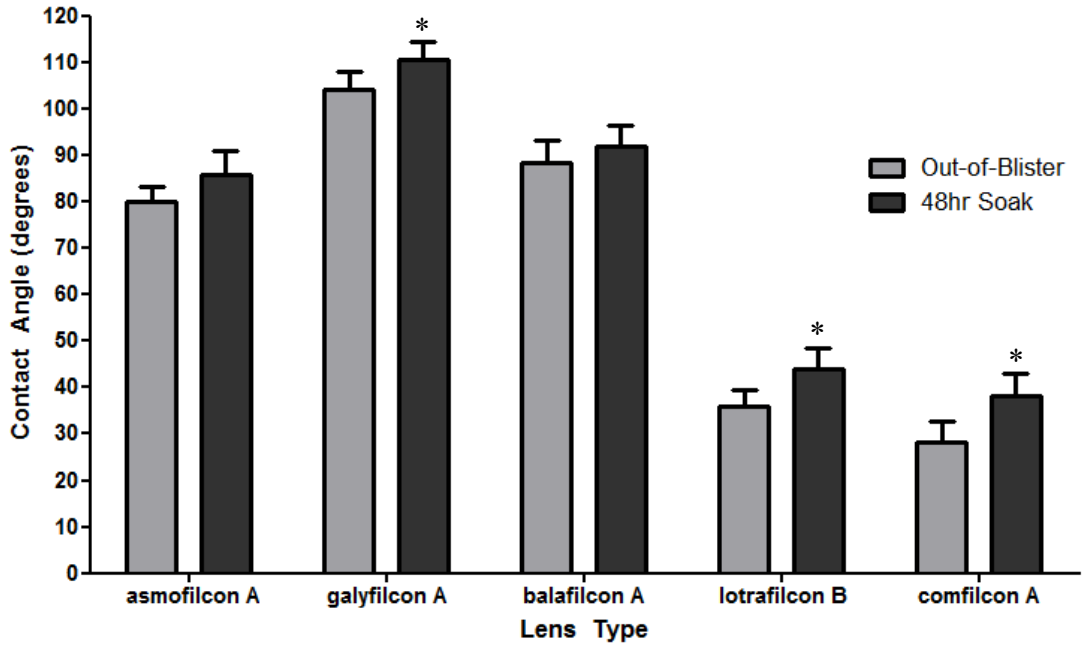


Figure 2-5: Advancing CAs measured using the sessile drop technique directly out-of-blister and after a 48hr soak in preservative-free saline for asmofilcon A, galyfilcon A, balafilcon A, lotrafilcon B, and comfilcon A lens materials.

The advancing CAs for asmofilcon A and balafilcon A out-of-blister and after a 48hr soak were not statistically different from each other ($p>0.05$). The advancing CAs after the 48hr soak for galyfilcon A, lotrafilcon B and comfilcon A were statistically higher ($p<0.03$) than the advancing CAs measured directly out-of-blister.

All of the advancing CAs were statistically different between lenses ($p<0.05$), with the exception of the CAs for asmofilcon A after the 48hr soak and balafilcon A out-of-blister

(p>0.05). The CAs for lotrafilcon B out-of-blister and after 48 hour soak and the CAs for comfilcon A after a 48hr soak were not statistically different from each other(p>0.05).

The advancing CAs measured directly out-of-blister and after a 48hr soak by the Wilhelmy balance method are shown in Figure 2-6.

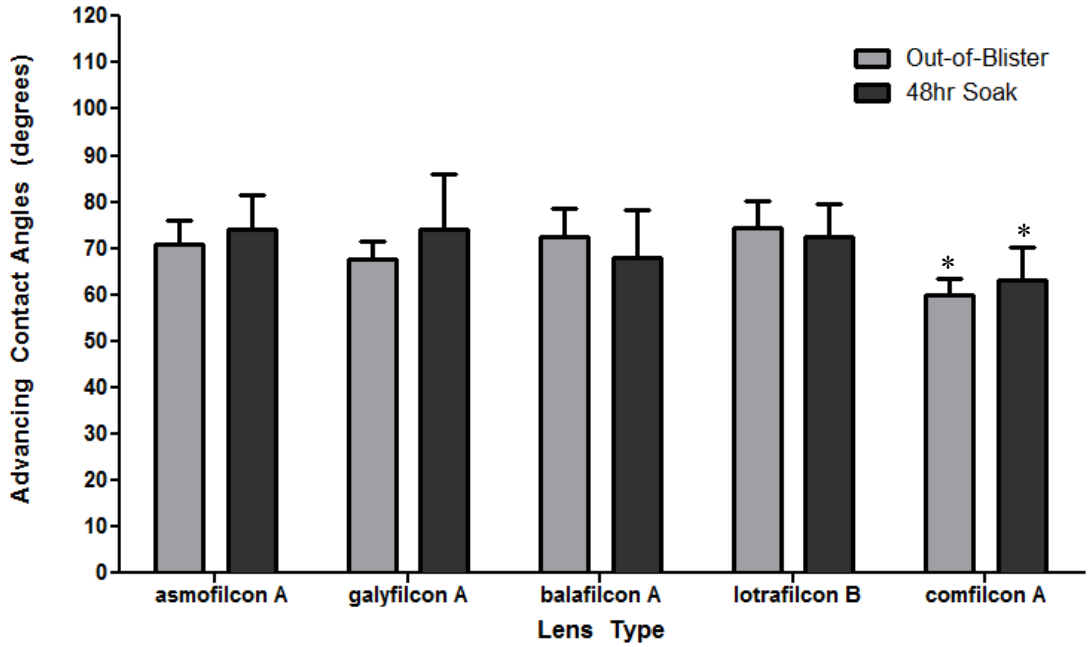


Figure 2-6: Advancing CAs measured using the Wilhelmy balance method directly out-of-blister and after a 48hr soak in preservative-free saline for asmofilcon A, galyfilcon A, balafilcon A, lotrafilcon B, and comfilcon A lens materials.

For all lens types, there was no statistical difference between advancing CAs measured out-of-blister and after a 48hr soak in saline (p>0.10). Comfilcon A CAs were statistically lower than all other CAs for the other lens materials (p<0.03).

When comparing CAs measured between techniques the advancing CAs out-of-blister and after a 48 hour soak in saline for asmofilcon A and balafilcon A lenses were not statistically different from each other for both techniques (p>0.05). The advancing CAs out-of-blister and

after a 48 hour soak for galyfilcon A measured by the Wilhelmy balance method were statistically lower than the CAs measured using the sessile drop technique ($p<0.05$). The CAs out-of-blister and after a 48 hour soak measured using the Wilhelmy balance technique were statistically higher ($p<0.05$) than the CAs out-of-blister and after 48 hour soak measured by the sessile drop technique for the lotrafilcon B and comfilcon A materials.

2.2.4 Discussion

The results of this study indicated that there were differences in CAs measured between the sessile drop technique and the Wilhelmy balance method. Most lens materials, with the exception of lotrafilcon B and comfilcon A, exhibited lower CAs measured by the Wilhelmy balance method as compared to the sessile drop technique. These differences in CAs may have been due to differing methods in preparation of the lenses for both techniques. For the sessile drop technique the lenses are blot dried on lens paper before CA analysis. For the Wilhelmy balance method, the lenses are not blot dried but rather the excess blister or saline solution is shaken off. This is done instead of blot drying to prevent dehydration of the lenses during the process of attaching the lens strip to the microcrocodile clip and balancing the electrobalance. Thus, the lenses for the Wilhelmy balance method are more hydrated than compared to the lenses for the sessile drop technique, which may result in the lower CAs. However, the higher CAs measured by the Wilhelmy balance method for the lotrafilcon B and comfilcon A lens materials, is an indication that preparation of the lens is not the only factor that can be attributed to differences in CAs between the two techniques. Other factors may include surface tension of the lens surface, surface roughness, and surface treatments of the lenses.

Differences in measured CAs by different techniques were also seen in a similar study conducted by Maldonado-Codina and Morgan.[39] In their study, the measurement of CAs on the surface of five different silicone hydrogel lens materials (galyfilcon A, senofilcon A, lotrafilcon A, lotrafilcon B, and balafilcon A) was conducted. The CAs were measured using the sessile drop technique and captive bubble technique. The results showed significant differences in CAs measured for the two techniques for the galyfilcon A, senofilcon A, and balafilcon A lens materials. The difference in CAs measured for both techniques was attributed to the captive bubble technique actually measuring the receding CA rather than the advancing CA.

From the results of this experiment it can also be concluded that the blister pack solution acts to effectively improve the initial wettability of the lens materials when wettability is assessed by the sessile drop technique. This is further supported by the results of Maldonado-Codina and Morgan, [39] which also showed that advancing CAs after the blister solution was washed off the lens surface were higher than CAs measured after the lens was removed from the blister pack solution.

2.3 Overall Conclusions

Overall, it can be concluded that different techniques of measuring contact lens CAs in vitro result in different measured CAs. Due to the differences between the CAs measured by the two techniques, both techniques were continued to be used for further in vitro analysis for the remainder of the experiments described in this thesis. Further, results indicated that lenses should be blot dried for approximately 20 seconds on lens paper to maintain repeatable CA measurements when using the sessile drop technique.

Lastly, blister packaging solutions do alter the initial wettabilities of contact lenses. To date, the physical properties of the blister solutions are unknown. Measurements of the physical properties of the blister pack solutions need to be conducted to investigate any correlations to the physical properties of the blister solutions and the initial wettability of contact lenses directly out-of-blister.

3. Chapter 3: Physical Properties of Blister Pack Solutions of SH Contact Lenses

3.1 Introduction

Silicone hydrogel contact lenses were initially developed to improve oxygen transmissibility to the eye[95, 98] and since then have undergone modifications to the surface and bulk material to improve lens wettability and comfort.[78, 98 80, 105] While the clinical performance of these materials with regards to hypoxia and neovascularization has been noteworthy,[4, 83, 89, 122] some 50% of subjects still complain of end of day dryness and discomfort even with modern lens materials.[8] In an attempt to alleviate these complications a number of manufacturers have begun to incorporate a variety of wetting agents into both the lens material and also the packaging solutions.[2, 78, 123-126] Such changes include incorporation of water soluble polymers, surfactants, and un-named "wetting agents" into the blister solution.[127] The alterations made to the blister packaging solutions are to aid in preventing the lenses from sticking to the blister pack, enhance lens wettability, and improve initial comfort of the lenses in-eye.

As mentioned previously, any solution that comes into contact with the eye, should have similar physical properties compared to the human tear film. To date there is little published information regarding the polymeric and molecular additions to the blister solutions and no data available on their physical properties. The purpose of this study is to measure the osmolality, pH, surface tension, and viscosity of the blister pack solutions for many of the commercially available silicone hydrogel lenses.

3.2 Materials:

The blister pack solutions examined were from lotrafilcon A, lotrafilcon B, and lotrafilcon B with a "modified blister pack solution" (m-lotrafilcon B) (CIBA Vision, Duluth, Georgia), balafilcon A (Bausch & Lomb, Rochester, New York), galyfilcon A, senofilcon A and narafilcon A (Johnson & Johnson, Jacksonville, Florida), and comfilcon A and enfilcon A (CooperVision, Pleasanton, California). The various properties of the SH lenses are described in Table Table 3-1.

Two conventional polyHEMA-based materials - etafilcon A (Johnson & Johnson, Jacksonville, Florida) and omafilcon A (CooperVision, Pleasanton, California) - were also examined to see if there were any systematic differences between the blister pack solutions used for conventional and silicone hydrogel lenses.

Table 3-1: Listing of SH lenses and their individual properties

	Proprietary name								
	AIR OPTIX® NIGHT & DAY® AQUA	AIR OPTIX™ AQUA	AIR OPTIX™	Acuvue® Advance™	Acuvue® OASYS™	1-Day Acuvue® TruEye	Biofinity	AVAIRA™	PureVision™
USAN	Lotrafilcon A	Lotrafilcon B	Lotrafilcon B	Galyfilcon A	Senofilcon A	Narafilcon A	Comfilcon A	Enfilcon A	Balafilcon A
Manufacturer	CIBA Vision	CIBA Vision	CIBA Vision	Johnson & Johnson	Johnson & Johnson	Johnson & Johnson	Cooper Vision	Cooper Vision	Bausch & Lomb
Water Content (%)	24	33	33	47	38	46	38	46	36
Oxygen permeability (Dk)	140	110	110	60	103	100	128	100	99
Center thickness (mm) -3.00D	0.08	0.08	0.08	0.07	0.07	0.085	0.08	0.08	0.09
Oxygen transmissibility (Dk/t)	175	138	138	86	147	118	160	125	110
FDA group	I	I	I	I	I	I	I	I	III
Surface treatment	25-nm plasma coating with high refractive index	25-nm plasma coating with high refractive index	25-nm plasma coating with high refractive index	No surface treatment. Internal wetting agent (PVP) that also coats the surface	No surface treatment. Internal wetting agent (PVP) that also coats the surface	unpublished	No surface treatment.	No surface treatment.	Plasma oxidation
Principal monomers	DMA + TRIS + siloxane monomer	DMA+ TRIS+ siloxane monomer	DMA+ TRIS+ siloxane monomer	mPDMS+ DMA+HEMA+ siloxane macromer+ PVP+EGDMA	mPDMS+ DMA+ HEMA+ siloxane macromer+ PVP+ TEGDMA	unpublished	FM0411M+HOB +IBM+M3U+NV P+TAIC+VMA	unpublished	NVP + TPVC + NVA + PBVC

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

3.3 Methods:

3.3.1 pH

All pH measurements were taken using the VWR Model SB20 pH meter (Thermo Electron Corporation, Beverly, Massachusetts). The pH meter was calibrated using pH 4, 7, and 10 standards. The pH of approximately 5ml samples of each blister pack solution (n=6 samples) were measured and recorded.

3.3.2 Surface Tension

Surface tension (ST) measurements were taken with the Cahn Dynamic Contact Angle Analyzer DCA-322 (CAHN Instruments, Madison, Wisconsin). Calibration was carried out using high performance liquid chromatography grade water, which has a ST of 72 dynes/cm. Blister pack solutions for each contact lens were collected in 10ml glass jars (n=6 samples) and their ST measured.

3.3.3 Osmolality

The osmolality of each blister solution was measured using the Vapro 5520 Vapor Pressure Osmometer (Wescor, Logan, Utah). The osmometer was calibrated using 100, 290 and 1000 mmol/kg standards. The osmolality of 10µl samples for each blister solution (n=6 samples) were measured and recorded.

3.3.4 Viscosity

Viscosity measurements of each blister packaging solution were obtained using the ViscoLab 3000 Viscometer (Cambridge Viscosity, Medford, MA). Calibration was conducted by

measuring the viscosity of high performance liquid chromatography grade water which has a viscosity of 1cP. Samples (1.5ml) of each blister pack solution (n=6 samples) were measured and recorded.

3.3.5 Statistical Analysis

All measurements were taken at room temperature (20.0°C) and analyzed by repeated measures ANOVA (analysis of equal variance). Further analysis was undertaken using a Tukey post-hoc test to determine all significant or non-significant differences between individual measurements. A p-value of <0.05 was considered significant. The researcher conducting the experiment was not masked. However, as all measurements undertaken were automated, they could not be influenced by the researcher. The statistical significance was indicated on the graphs by "star" symbols.

3.4 Results

The averages results of the ST, pH, osmolality, and viscosity of Unisol, Softwear Saline, and the blister pack solutions for etafilcon A, omafilcon A, narafilcon A, senofilcon A, galyfilcon A, comfilcon A, enfilcon A, balafilcon A, lotrafilcon B, m-lotrafilcon B, and lotrafilcon A are reported in Table 3-2. Graphical representations of the results are shown in Figure 3-1-Figure 3-4.

Table 3-2:Physical properties of the blister pack solutions investigated and control solutions.

Saline and Blister Pack Solutions	Surface Tension (dynes/cm)	pH	Osmolality (mmol/kg)	Viscosity (cP)
Unisol	64.05±1.49	7.42±0.01	290.0±1.67	0.94±0.03
Softwear Saline	72.80±0.57	6.99±0.03	292.7±0.82	0.93±0.01
Omafilcon A	60.24±0.37	7.42±0.11	320.3±3.93	0.93±0.01
Etafilcon A	42.03±2.60	7.38±0.09	417.7±8.29	0.94±0.02
Narafilcon A	45.26±0.98	7.36±0.16	425.2±1.94	0.93±0.05
Senofilcon A	58.61±2.46	7.50±0.06	415.7±1.75	0.99±0.01
Galyfilcon A	56.22±5.58	7.57±0.02	420.2±4.07	0.97±0.02
Comfilcon A	58.30±1.71	7.40±0.01	315.5±2.66	0.92±0.04
Enfilcon A	48.11±3.18	7.38±0.02	311.0±1.41	0.90±0.02
Balafilcon A	65.93±1.75	7.11±0.09	325.0±2.61	0.92±0.02
Lotrafilcon B	68.56±1.56	7.19±0.05	306.3±1.21	0.95±0.01
m-Lotrafilcon B	64.06±2.09	7.21±0.07	306.8±2.25	1.30±0.03
Lotrafilcon A	57.62±1.83	7.19±0.02	304.0±1.90	1.26±0.03
p-value	<0.00001	<0.00001	<0.0001	<0.00001

ST results for all blister pack solutions and two saline solutions are reported in Figure 3-1.

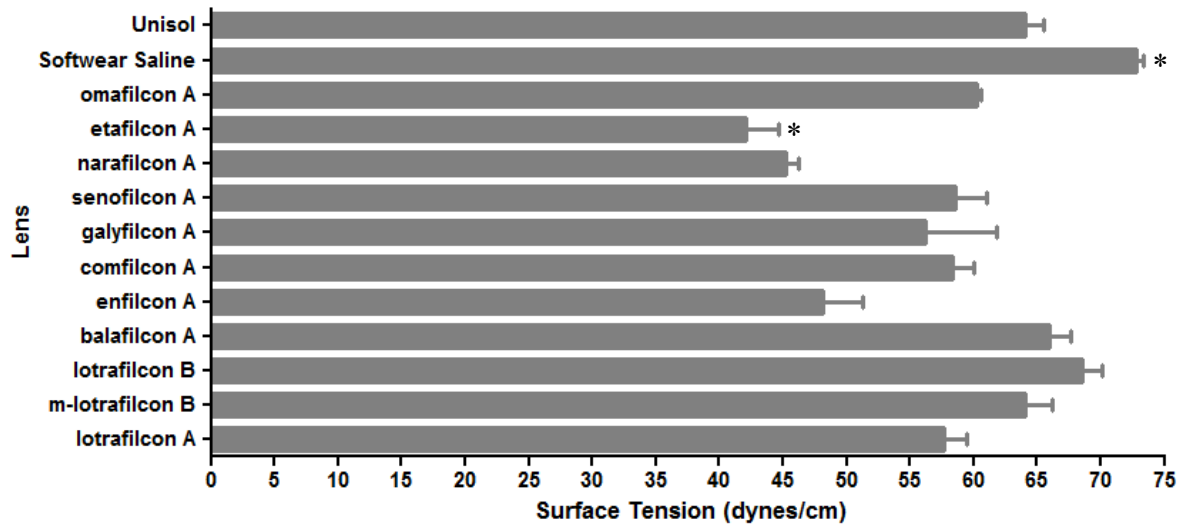


Figure 3-1: ST measurements in units of dynes/cm, for all blister pack solutions for etafilcon A, omafilcon A, narafilcon A, senofilcon A, galyfilcon a, comfilcon A, enfilcon A, balafilcon A, lotrafilcon B, m-lotrafilcon B, and lotrafilcon A, and two saline solutions, Unisol and Softwear Saline.

The ST of Unisol was statistically different from Softwear Saline and all blister pack solutions ($p<0.05$), with the exception of the blister solutions for etafilcon A, lotrafilcon B, m-lotrafilcon B, and balafilcon A ($p>0.10$). The ST of Softwear Saline (72.80dynes/cm) was statistically higher than all other solutions ($p<0.001$) with the exception of the blister solution of lotrafilcon B ($p>0.10$). The lowest ST value was that for etafilcon A (42.03dynes/cm), which was statistically lower than all other solutions ($p<0.01$), with the exception of narafilcon A ($p>0.50$). The ST of omafilcon A was statistically different from the ST of Softwear Saline, and blister solutions of etafilcon A, lotrafilcon B, balafilcon A, enfilcon A, and narafilcon A($p<0.02$). The ST of lotrafilcon B, m-lotrafilcon B, and balafilcon A blister solutions were all statistically higher ($p<0.01$) than all other blister solutions but not statistically higher than the two saline

solutions ($p>0.10$). ST of blister solutions for lotrafilcon A, comfilcon A, galyfilcon A, and senofilcon A were all statistically different from all other blister solutions and the two saline solutions ($p<0.05$) but not statistically different from each other or the blister solution of omafilcon A ($p>0.05$). The ST for the enfilcon A blister solution (48.11dynes/cm) was statistically different from all other solutions, with the exception of narafilcon A.

pH measurements for each blister pack solution are shown in Figure 3-2.

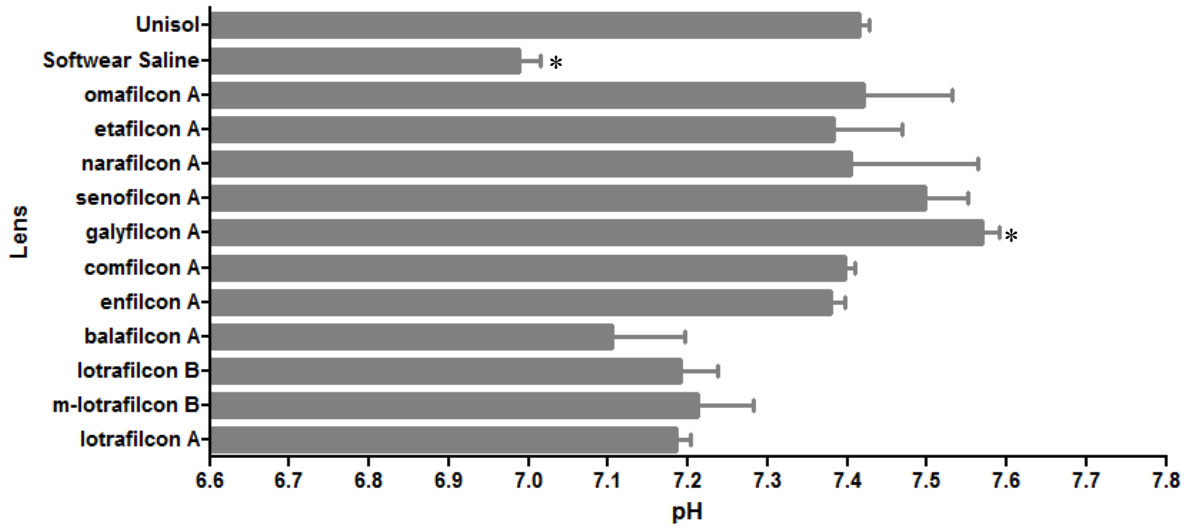


Figure 3-2: pH measurements for all blister pack solutions for etafilcon A, omafilcon A, narafilcon A, senofilcon A, galyfilcon a, comfilcon A, enfilcon A, balafilcon A, lotrafilcon B, m-lotrafilcon B, and lotrafilcon A, and two saline solutions, Unisol and Softwear Saline.

The pH range determined was relatively small, with Softwear Saline being the lowest (6.99) and galyfilcon A being the highest (7.57). The pH of lotrafilcon B, m-lotrafilcon B, lotrafilcon A, and balafilcon A were statistically lower than all other blister solutions ($p<0.002$), but they were not different from each other ($p>0.30$). Unisol, enfilcon A, comfilcon A, senofilcon A, narafilcon A, etafilcon A, and omafilcon A blister solutions had pH measurements

statistically different from all other solutions ($p<0.05$) but not from each other ($p>0.05$). The pH of galyfilcon A blister solution was statistically higher than all other blister solutions ($p<0.05$), except for senofilcon A ($p>0.80$)

Osmolality (as show in Figure 3-3) of the blister solutions for all Johnson & Johnson products (etafilcon A, senofilcon A, galyfilcon A, and narafilcon A) were statistically higher than all other blister solutions ($p<0.001$). The osmolality measurements of the two saline solutions (Unisol and Softwear Saline) were statistically lower than the osmolality of all the blister pack solutions ($p<0.001$), but not statistically lower than each other ($p>0.90$). The osmolality of the blister solutions for all three lotrafilcon lens materials (CIBA Vision) were lower than all other products, but were not statistically different from each other ($p>0.90$). Osmolality of the blister solutions for the three CooperVision products (omafilcon A, comfilcon A, and enfilcon A) were statistically different from all other blister solutions ($p<0.05$), and were not different from each other ($p>0.20$). The osmolality of the blister solution for the single Bausch & Lomb product (balafilcon A) was also statistically different from all other blister solutions ($p<0.01$).

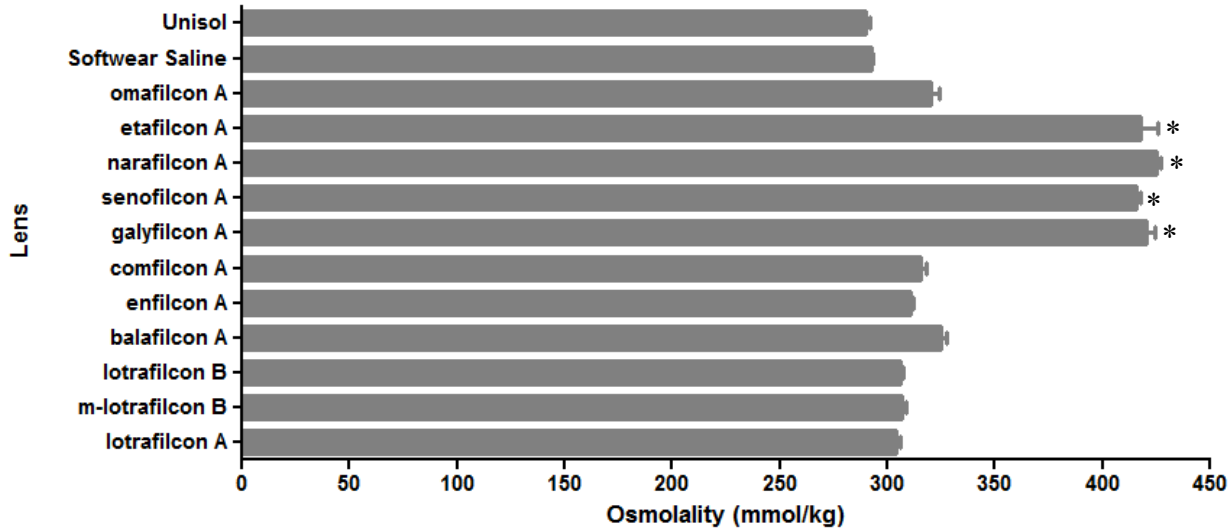


Figure 3-3: Osmolality measurements in units of mmol/kg, for all blister pack solutions for etafilcon A, omafilcon A, narafilcon A, senofilcon A, galyfilcon a, comfilcon A, enfilcon A, balafilcon A, lotrafilcon B, m-lotrafilcon B, and lotrafilcon A, and two saline solutions, Unisol and Softwear Saline.

The viscosity measurements for all the blister solutions of the lens materials tested are shown in Figure 3-4.

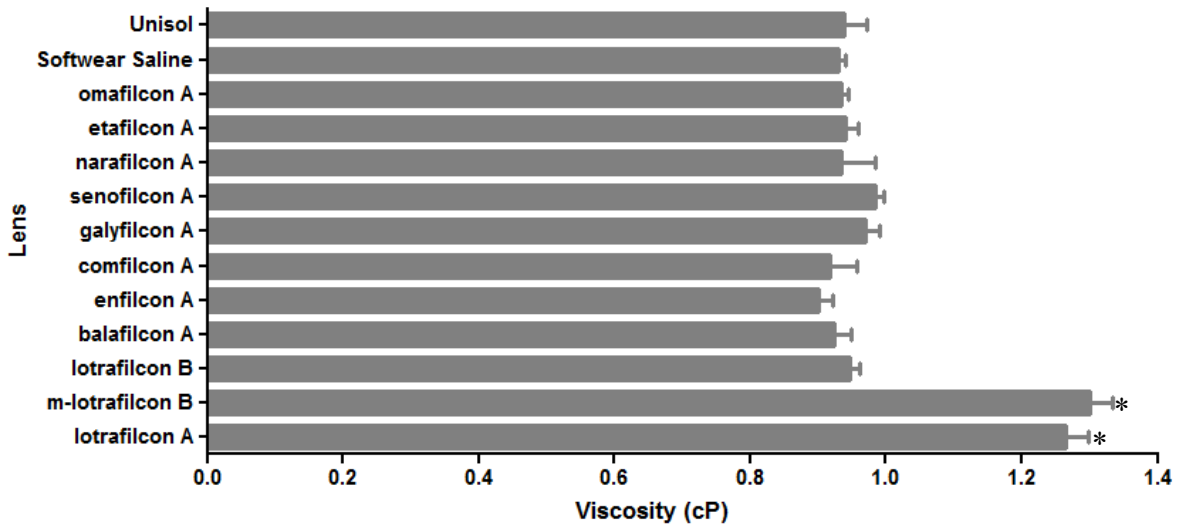


Figure 3-4: Viscosity measurements in units of cP, for all blister pack solutions for etafilcon A, omafilcon A, narafilcon A, senofilcon A, galyfilcon a, comfilcon A, enfilcon A, balafilcon A, lotrafilcon B, m-lotrafilcon B, and lotrafilcon A, and two saline solutions, Unisol and Softwear Saline.

The viscosity of m-lotrafilcon B and lotrafilcon A blister solutions were statistically higher than the viscosity of all other blister solutions ($p<0.001$). The lowest viscosity (0.90cP) was measured for the enfilcon A lens material. The viscosity of the blister solution for senofilcon A was statistically higher than Softwear Saline, balafilcon A, enfilcon A, and comfilcon A ($p<0.05$). Viscosity of Unisol and the blister solutions for etafilcon A, lotrafilcon B, and narafilcon A were statistically different only from the blister solutions of m-lotrafilcon B and lotrafilcon A ($p<0.001$).

3.5 Discussion and Conclusion

Recently, manufacturers have added surfactants and wetting agents to the blister pack solutions of contact lens materials in attempt to improve initial on-eye comfort and tear film

stability. Physical properties of the blister pack solutions should have similar properties compared to that of tears. Adding components to the blister solutions may alter the physical properties and as mentioned previously, could potentially increase comfort in-eye. This experiment was conducted to measure the physical properties of the blister pack solutions, as to date, there has been no data published on their physical properties.

As mentioned in chapter 1, ST is the inward attraction of molecules at the surface of a solid or liquid. Generally, when the ST of a liquid is reduced, it will have the tendency to spread more readily over a solid surface. The surface tension of human tears ranges between 40-46 dynes/cm.[114, 118] The ST of the blister solutions for narafilcon A and etafilcon A remained within the range of the surface tension of tears. Interestingly, the surface tension for the two saline solutions and the remainder of the blister solutions were all higher than the range of tears. The ST of Unisol and the blister solutions for lotrafilcon B, m-lotrafilcon B, and balafilcon A were close to that of water (72 dynes/cm), suggesting that very little surface active agent is added to the blister pack solution. The ST of Softwear Saline (72.8 dynes/cm) was the same as that of water.

None of the solutions tested in this experiment had ST below that of tears and thus would have little impact on reducing the ST of tears to allow more spreading of the tear film over the contact lens surface or cornea.

pH is the measure of acidity or basicity of a solution. A solution which is neutral has a pH of 7. A solution which has a pH of above 7 would be termed as being basic, and a solution which has a pH of less than 7 would be termed as being acidic. The pH of any solution that comes into contact of the human eye is very important. The pH range of the human tear film has been reported to be between 6.6 and 7.8.[110] A solution that comes into contact with the eye with

a pH outside the range of the pH range of the human tear film may cause discomfort or even corneal damage at extremely low pH values.[128] The pH of the solutions tested in this study ranged from 6.99-7.57, with Softwear Saline having the lowest pH and the blister pack solution of galyfilcon A the highest pH. The pH values of the solutions tested in this experiment all fell within the pH range of tears and would not be expected to cause any discomfort following insertion onto the ocular surface.

The average osmolality of human tears is 305mmol/kg.[120] Results indicated a trend in osmolality of solutions, with solutions made by the same manufacturer having similar osmolalities. The osmolalities of the two saline solutions, Unisol and Softwear Saline, were hypo-osmotic to that of tears, having osmolalties of 290mmol/kg and 293mmol/kg respectively. The osmolality of the blister solutions for lotrafilcon materials (CIBA Vision) were very similar to that of tears, ranging from 304-306mmol/kg. The osmolality of the blister solution for balafilcon A (Bausch & Lomb) was 325mmol/kg, residing in the midrange of osmolalities measured. The blister pack solutions for enfilcon A, comfilcon A, and omafilcon A (CooperVision) were 311mmol/kg, 316mmol/kg, and 320mmol/kg respectively. Interestingly, the osmolalities of the blister solutions for all Johnson & Johnson products (etafilcon A, senofilcon A, galyfilcon A, and narafilcon A) were hyper-osmotic to that of tears. The high osmolality of the blister solutions for the Johnson & Johnson products may be used to ensure parameter stability of the lenses, as placing an etafilcon A lens in hypotonic solution causes fluid to enter the lens material, resulting in a 20% increase in lens diameter (data not shown). However, when galyfilcon A, senofilcon A, and narafilcon A are placed in hypotonic solution, there is no significant change in lens parameters thus the reason for the blister solution having a high osmolality for those lenses remains unknown. Theoretically, the high osmolalities of the

blister solutions for etafilcon A, galyfilcon A, senofilcon A, and narafilcon A may cause discomfort upon initial insertion of the lens in-eye. However, the levels of clinical success of these materials and their high levels of initial comfort on immediate insertion demonstrates this to not be the case and demonstrates the ability of the tear film to buffer these high osmolalities.[109]

The viscosity of a solution is the resistance of a fluid to flow. It has long been recognized that increasing the viscosity of a fluid increases its contact time on the surface of the cornea or contact lens. However, solutions with very high viscosities can cause visual blur and epithelial damage.[116, 117, 129] The viscosities of the saline solutions and all the blister solutions except the blister solutions of m-lotrafilcon B and lotrafilcon A, were slightly lower than the viscosity of water (1.00cP) ranging from 0.90-0.99cP. The viscosities of the blister solutions for the m-lotrafilcon B and lotrafilcon A materials were 1.30cP and 1.26cP respectively, which were closer to that of the viscosity of tears (1.5cP).[116] The high viscosities are due to the incorporation of wetting agents polyethylene glycol (PEG) and hydroxypropyl methylcellulose (HPMC)[130] and 1% copolymer 845.[127] The higher viscosities of these two blister pack solutions may contribute to an increase in initial comfort of the lens in-eye by increasing the retention time of the tear film over the surface of the contact lens.

A study by Giles,[131] compared the overall performance of the lotrafilcon B material compared to the m-lotrafilcon B materials. Performance of each lens materials was based on comfort after insertion, end of day comfort, and overall comfort. Results indicated subjective preference for the m-lotrafilcon B materials for initial comfort, end of day comfort and overall comfort. This results were attributed to the modified blister solution of m-lotrafilcon B with added moisturizing agents, and the high wettability of the lens material.

Although this experiment has successfully reported the physical properties of the blister solutions of silicone hydrogel lenses, the substantivity of the blister solutions for these lenses is only effective on the first day of wear, after which the blister solution is removed by storage in a multipurpose lens solution. Thus, the impact of blister solutions is likely more relevant in regards to daily disposable lenses and will be the focus of the next chapter.

4. Chapter 4: In Vitro CA Analysis and Physical Properties of Blister Pack Solutions of Daily Disposable Lenses

4.1 Introduction

In 2005, it was reported that approximately 2.8 million contact lens wearers in the US ceased to wear their lenses due to dryness, discomfort, and general dissatisfaction with their lenses.[132] In an attempt to improve comfort and reduce dryness, many contact lens manufacturers have developed approaches to modify the lens surface or material in an attempt to make the lens more "wettable."[95, 96, 98, 133]

In-eye comfort, particularly initial comfort following insertion, could also be affected by the properties of the packing solution in which the lenses are stored. This is particularly relevant for daily disposable (DD) lenses, which are inserted directly from the blister pack solution each time they are worn. Historically, blister package solutions consisted of merely saline.[134] However, several contact lens companies have recently begun to modify the constituents of the packaging solutions to include a complex array of surfactants and wetting agents to the blister pack solution, in an attempt to improve initial in-eye comfort and support a stable tear film.[124, 135] The previous chapter described in depth the physical properties of the packaging solutions for silicone hydrogel contact lenses.

The purpose of this study was to determine the CAs of five modern DD lenses and also to determine the surface tension, osmolality, viscosity, and pH of their respective blister pack solutions. It is our hypothesis that wetting agents incorporated into the lens material and

packaging solution will reduce the advancing CA and lower the surface tension of the packaging solution.

4.2 Materials

Five DD lenses were examined in this study: omafilcon A (CooperVision, Pleasanton, California), nelfilcon A and modified (m-) nelfilcon A (CIBA Vision, Duluth, Georgia), etafilcon A and narafilcon A (Johnson & Johnson, Jacksonville, Florida). The various properties of these materials are described in Table 4-1.

Table 4-1: Listing of daily disposable lenses and their individual properties

	Proprietary Name				
	Proclear 1-Day	Focus Dailies	Focus Dailies w/ AquaComfortPlus	1-Day Acuvue	Acuvue TruEye
Manufacturer	CooperVision	CIBA Vision	CIBA Vision	Johnson & Johnson	Johnson & Johnson
USAN	Omafilcon A	Nelfilcon A	Nelfilcon A	Etafilcon A	Narafilcon A
Water Content	60.0	69.0	69.0	58.0	46.0
Dk (oxygen permeability)	24	34	34	22	100
Monomers	HEMA + PC	Modified PVA	Modified PVA + various components within packaging solution	HEMA + MA	undisclosed
FDA Group	II	II	II	IV	I

HEMA (2-hydroxyethyl methacrylate); PC (phosphorylcholine); MA (methacrylic acid); PVA (polyvinyl alcohol)

4.3 Methods

4.3.1 Sessile Drop Technique

Advancing CAs for each lens material were measured directly out of the blister pack. Each lens (n=4) was removed from the blister pack and briefly dabbed each side on lens paper (VWR Scientific Products, West Chester, Pennsylvania) for approximately 20 seconds, to remove any excess fluid from the blister pack solution, as this may impact on the initial CA. The lens was not blot dried any longer than 20 seconds, as preliminary data (see section 2.1) showed that blotting for longer than this could cause dehydration of the lens. After blot drying, each lens was placed posterior side down on a custom curved convex mantle and placed directly below the syringe of an Optical Contact Analyzer (OCA - Dataphysics Instruments GmbH, Filderstadt, Germany), and the CA was measured as described in section 2.1.2.1.

After the image of the lens removed from the blister pack was captured, the lens was immediately soaked in 5ml of unpreserved saline solution (Unisol, Alcon, Fort Worth, Texas), in order to remove the blister pack components from the lens and to determine the CA of the material without the impact of the blister pack solution. After a period of 5 minutes, the lens was removed from the saline and the advancing CA was measured exactly as described in section 2.1.2.1. This process was repeated a further 7 times for each lens to determine the substantivity of the blister package solution on the lens surface.

4.3.2 Wilhelmy Balance Method

Advancing and receding CAs for each lens material (n=4) were also measured directly out of the blister pack using the Wilhelmy Balance Method, as described in section 2.2.2.2. Each lens was taken through 8 cycles of being soaked in saline for 5 minutes and the advancing and receding angles measured after each cycle. Hysteresis (the difference between the advancing and receding CA) for each lens was also calculated.

4.3.3 Osmolality

The osmolality of the packaging solutions were measured as previously described (refer to section 3.3.3).

4.3.4 Surface Tension

Surface tension measurements of the packaging solutions were acquired using the same methods as previously described (refer to section 3.3.2).

4.3.5 pH

The pH of each blister pack solution was measured using the method previously described (refer to section 3.3.1).

4.3.6 Viscosity

Viscosity measurements of each blister packaging solution were obtained using the same method previously described (refer to section 3.3.4).

4.3.7 Statistical Analysis

All measurements were taken at room temperature (20.0°C). It was not possible to mask the investigator from the lens types being examined. However, all of the physical measures of the packing solution were automated and determination of the CAs could not be influenced by the investigator, with the exception of sessile drop measurements due to variations in blot drying. However, the investigator followed exactly the same routine for all lenses when undertaking the blotting procedure. Thus, we feel that this lack of masking did not adversely influence the results.

CAs, pH, osmolality, and surface tension measurements were analyzed by repeated measures ANOVA (analysis of equal variance). Analysis of the advancing angles measured using the sessile drop technique, advancing angles measured by Wilhelmy balance, and receding angles measured by Wilhelmy balance were all analyzed independently. Advancing CAs measured by sessile drop and Wilhelmy balance were compared and analyzed. The hysteresis values were also analyzed using repeated measures ANOVA. Further analysis of CAs, osmolality, pH, and surface tension measurements were undertaken using a Tukey post-hoc test to see all significant or non-significant difference between individual measurements. A p-value of <0.05 was considered significant.

4.4 Results

Figure 4-1 shows the advancing CAs measured using the sessile drop technique for all lens types across all cycles, with cycle 0 being immediately out of the blister pack and the remaining cycles being those determined after progressive periods of soaking in saline.

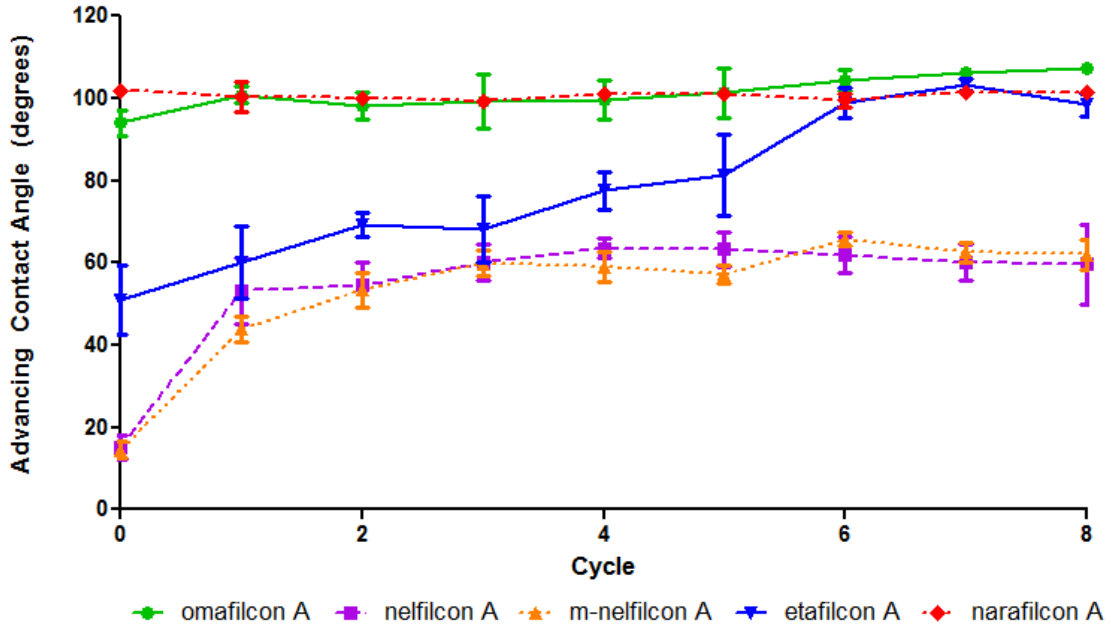
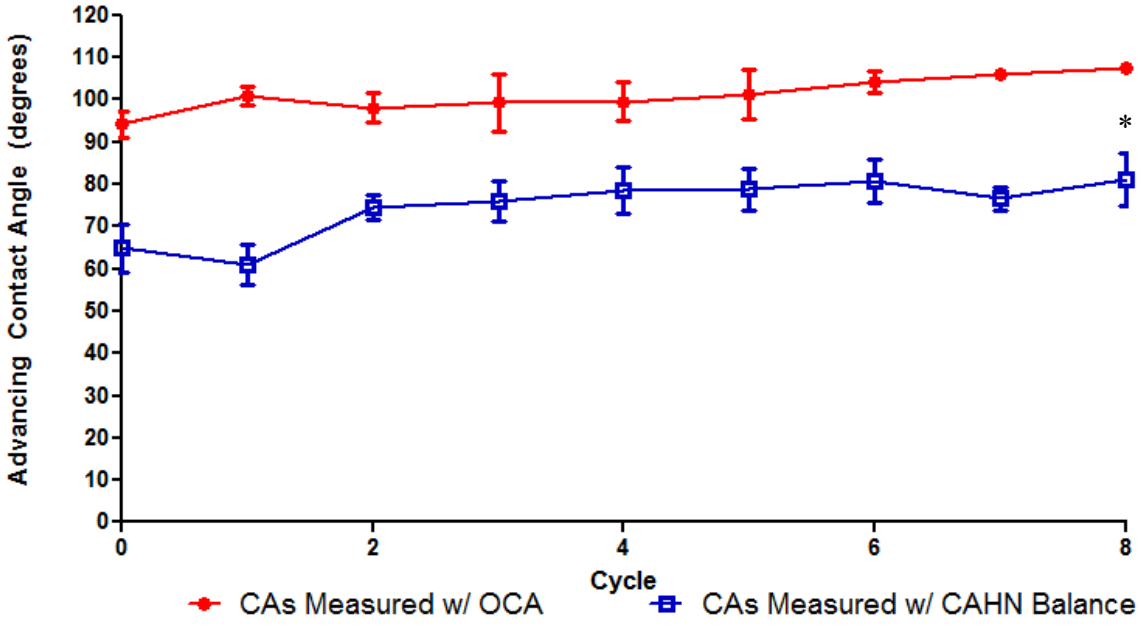


Figure 4-1: The average advancing contact angles (CAs) for all 5 lenses plotted for each cycle, as determined by the sessile drop technique. Cycle 0 reflects the measured CA immediately upon removal from the blister pack and each cycle thereafter is the measured advancing CA after soaking the lens for 5 minutes in unpreserved saline.

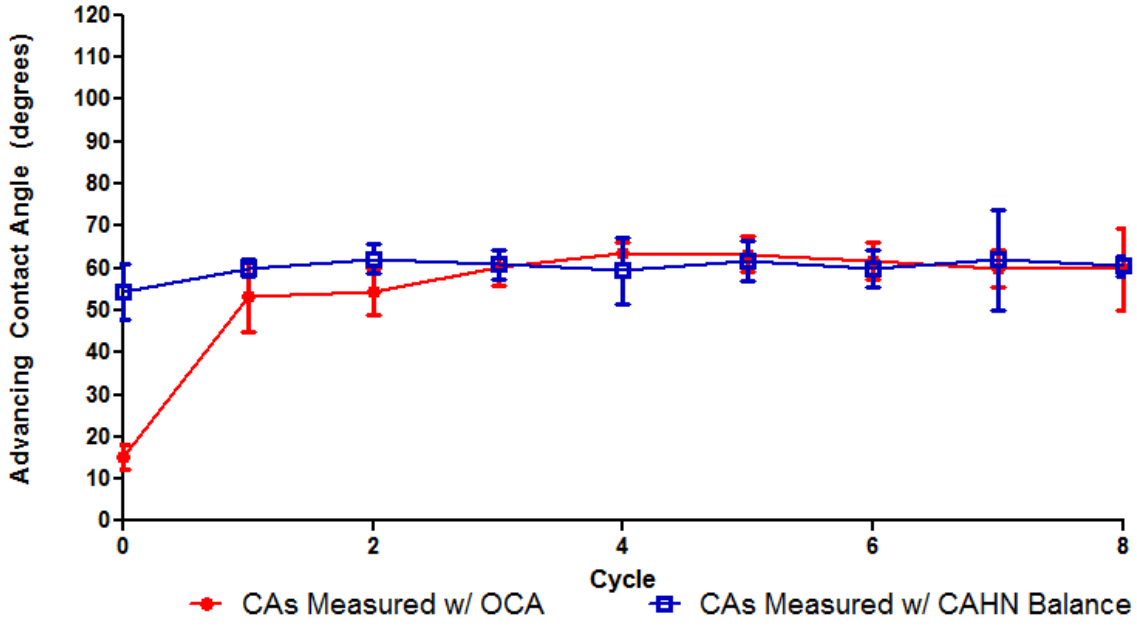
The advancing CAs for omafilcon A and narafilcon A were statistically higher than the remaining three lens materials ($p<0.02$), but these were not statistically different from each other ($p>0.05$). The two nelfilcon A lens materials were not significantly different from each other at any cycle examined ($p>0.10$). Etafilcon A advancing CAs were statistically lower than omafilcon A and narafilcon A ($p<0.001$) immediately upon removal from the packaging products and then

remained lower until the final 3 cycles, when their CA became no different from omafilcon A and narafilcon A ($p>0.60$). Etafilcon A exhibited a higher advancing CA than both nelfilcon A products immediately upon removal from the packaging and at the majority of the various soaking cycles ($p<0.05$), with the exception of cycles 1 and 3 ($p>0.05$).

Figure 4-2a-e are comparisons of the advancing CAs for each individual lens type measured by the sessile drop vs. the Wilhelmy balance method. Plots for nelfilcon A (Figure 4-2b) and m-nelfilcon (Figure 4-2c) show no difference in advancing CAs for the two techniques after cycle 2 ($p>0.05$). Prior to that, the CAs are lower with both lens materials with the sessile drop method ($p<0.05$). Analysis of plots for omafilcon A (Figure 4-2a) and narafilcon A (Figure 4-2e) show significant differences across all cycles ($p<0.05$) between the two methods, with lower CAs for the Wilhelmy method. Significant difference in CAs between the two methods for etafilcon A (Figure 4-2d) were seen after the 4th cycle ($p<0.05$), with advancing CAs measured using the sessile drop increasing until cycle 6 after which the CAs plateau.



a)


Advancing Contact Angle (degrees)
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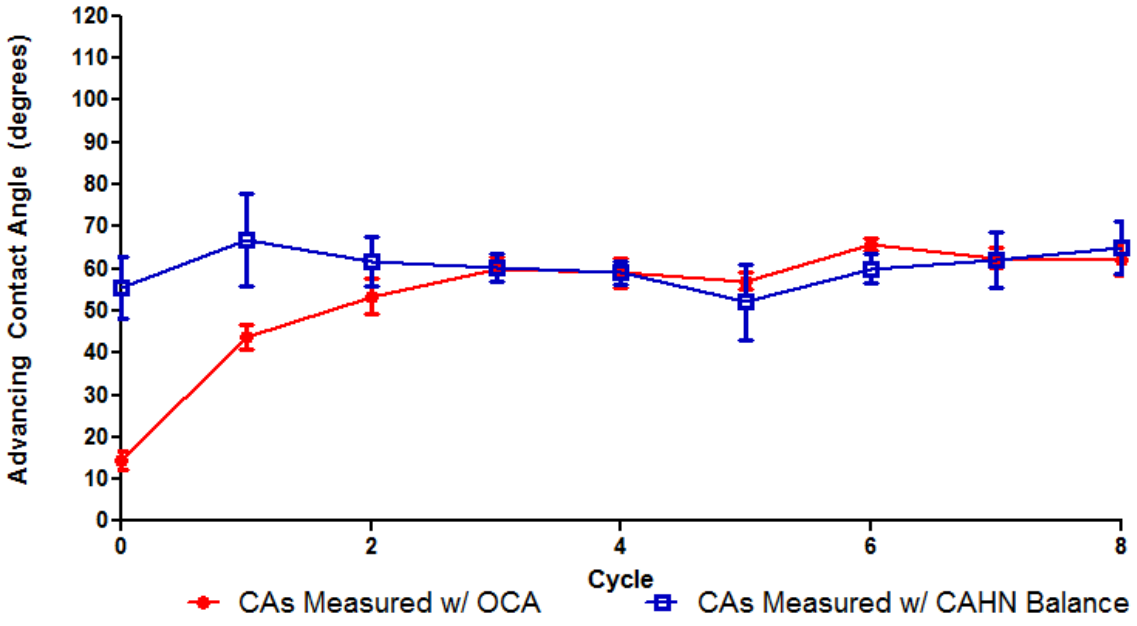
Cycle
CAs Measured w/ OCA
CAs Measured w/ CAHN Balance

b)


Advancing Contact Angle (degrees)
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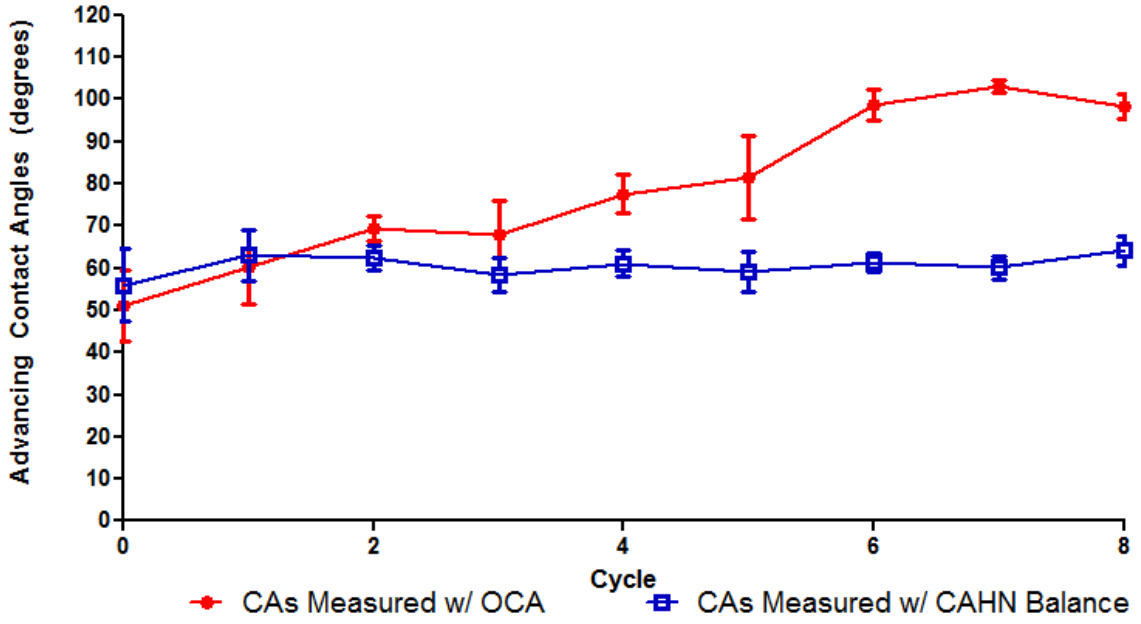
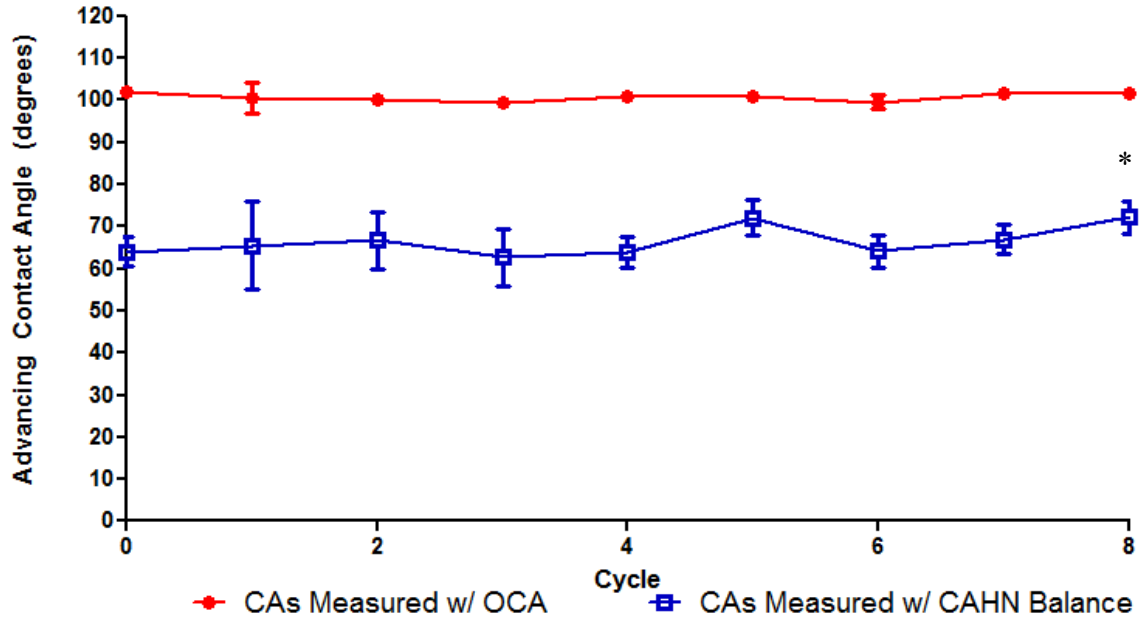
CAs Measured w/ OCA
CAs Measured w/ CAHN Balance

c)



d)



e)

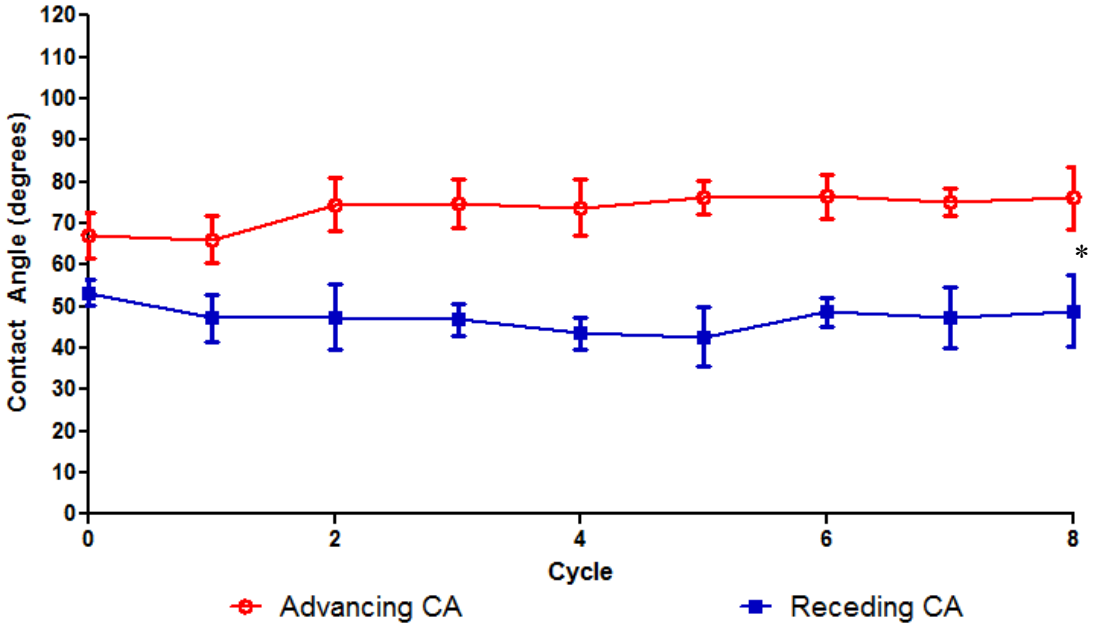
Figure 4-2: The advancing contact angle for each lens material upon removal from the packaging solution and after each progressive soaking period, as measured by sessile drop

and Wilhelmy balance methods for a) omafilcon A; b) nelfilcon A; c) modified-nelfilcon A; d) etafilcon A; and e) narafilcon A

Figure 4-3a-e describe the advancing and receding CAs determined using the Wilhelmy balance method. Figure 4-3a and Figure 4-3d graphically demonstrate using the Wilhelmy balance method that omafilcon A and etafilcon A exhibit fairly substantial degrees of hysteresis, as shown by their significant differences between the advancing and receding CAs across all cycles ($p<0.05$). Figure 4-3b, Figure 4-3c and Figure 4-3e demonstrate that hysteresis is minimal for both nelfilcon A products and the narafilcon A material, with very little difference between the advancing and receding CAs for these products ($p>0.05$).

When the Wilhelmy balance advancing CAs between products was directly compared (Figure 4-3a-e), it is seen that the CAs for omafilcon A remained statistically higher from all other lens types after the first two cycles ($p<0.05$). Etafilcon A, nelfilcon A and m-nelfilcon A advancing CAs were not statistically different from each other for any cycle ($p>0.05$).

Receding CAs for etafilcon A were statistically lower than all other lens types ($p<0.05$). Omafilcon A, nelfilcon A, m-nelfilcon A, and narafilcon A lenses were not significantly different from each other at the same cycles ($p>0.05$).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Cycle
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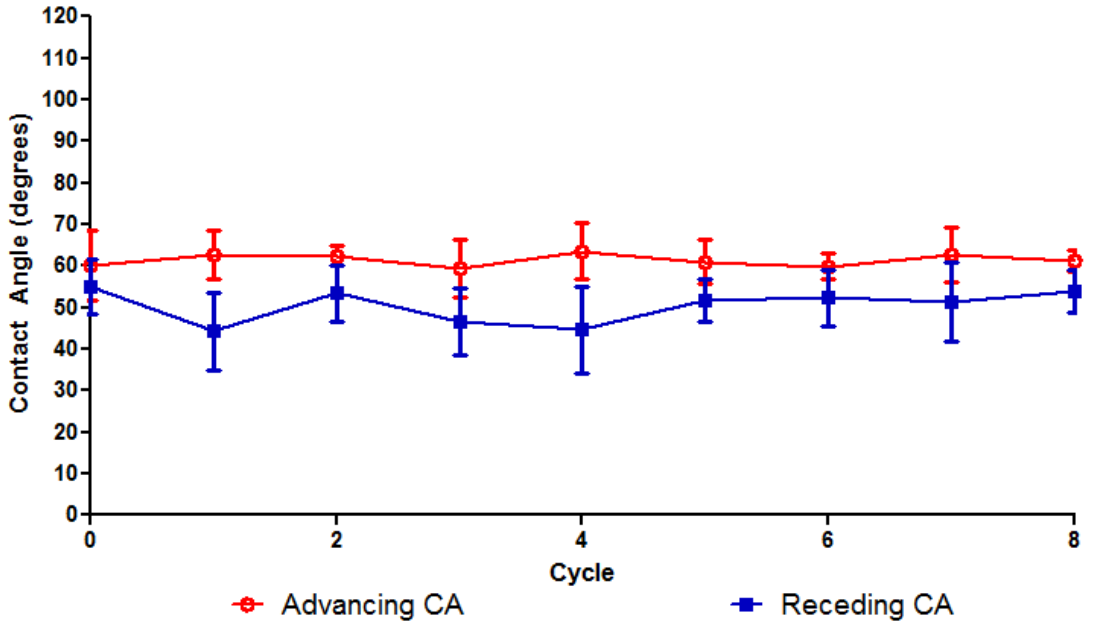
Advancing CA
Receding CA

a)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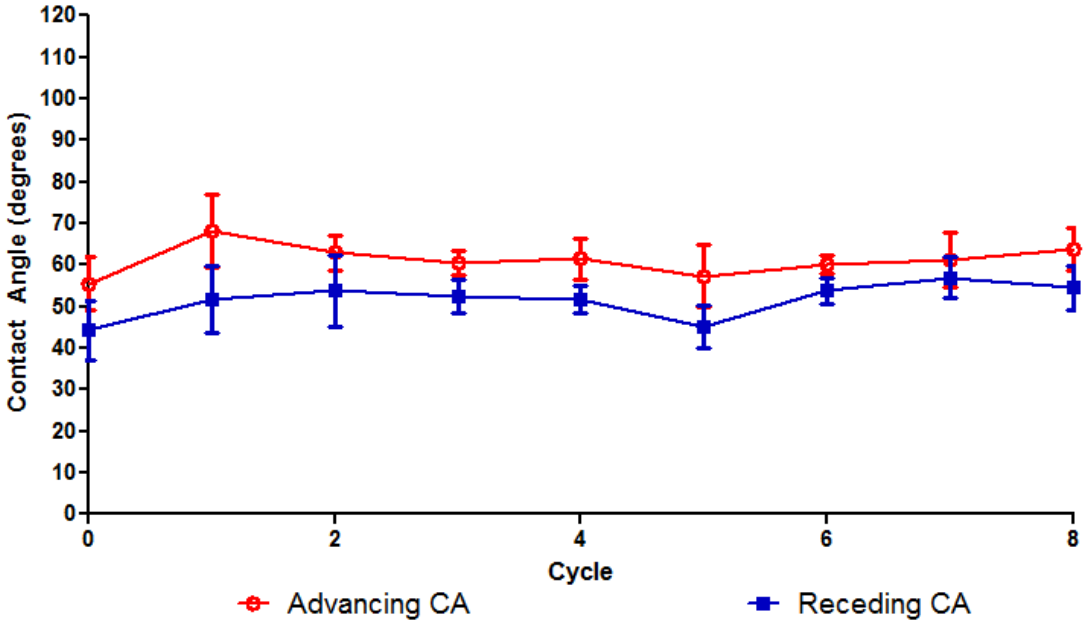
Cycle
Advancing CA
Receding CA

b)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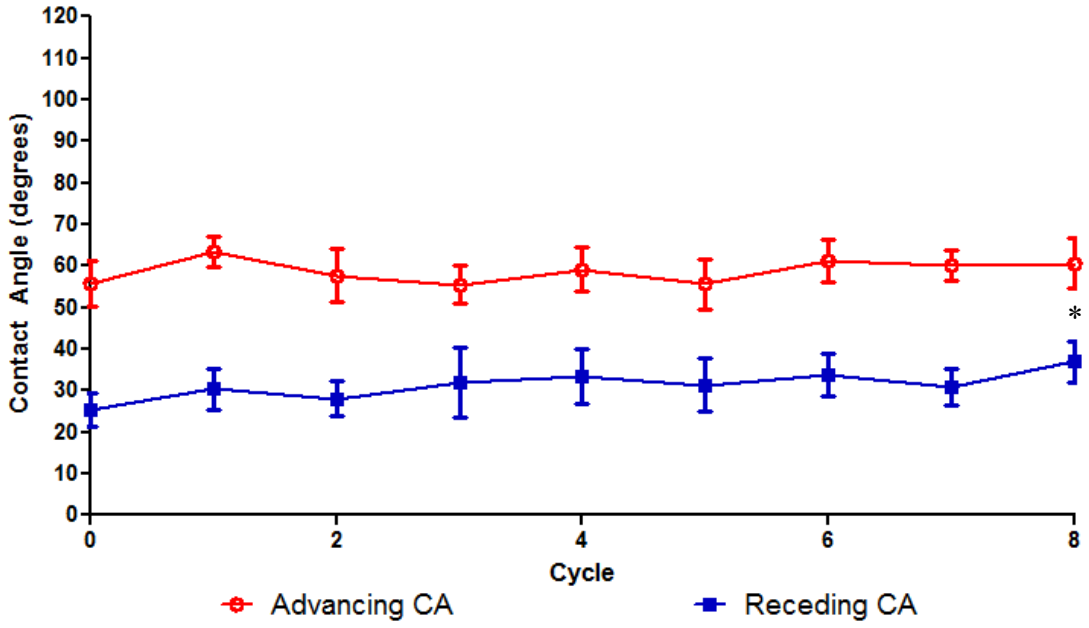
Cycle
Advancing CA
Receding CA

c)


Contact Angle (degrees)
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Advancing CA
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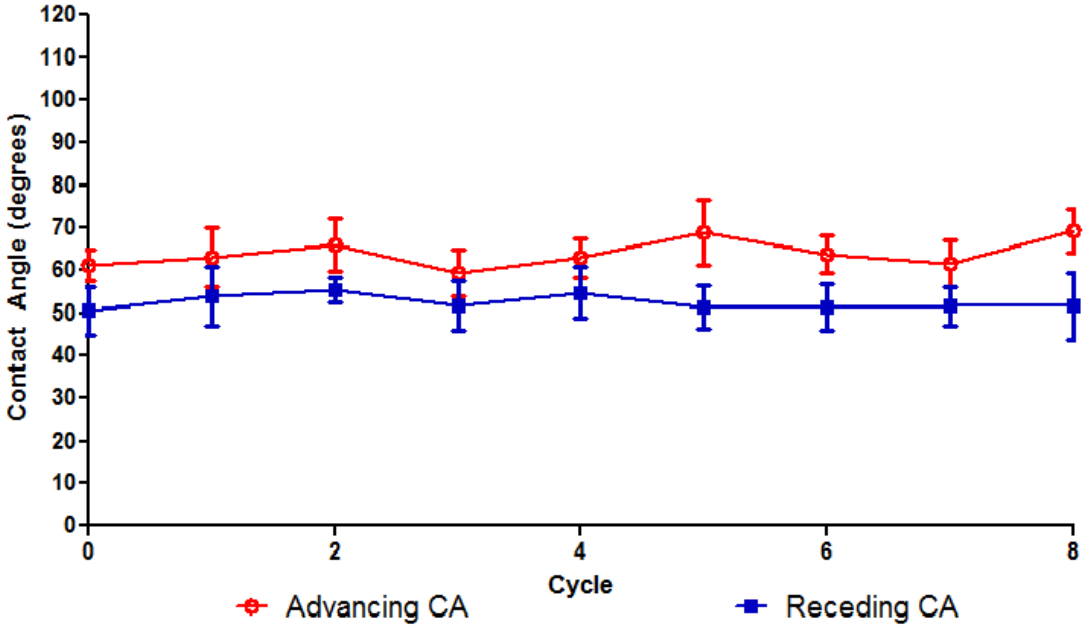
d)



e)

Figure 4-3: The advancing and receding contact angles for each lens material upon removal from the packaging solution and after each progressive soaking period, as measured by the Wilhelmy balance method for a) omafilcon A; b) nelfilcon A; c) modified-nelfilcon A; d) etafilcon A; and e) narafilcon A.

All blister pack solutions had pH values that remained within the quoted pH range of human tears (6.6-7.8), as shown in Table 4-2. There was a statistically significant difference between the pH values measured ($p=0.0003$), with omafilcon A exhibiting the highest pH and m-nelfilcon A the lowest. The pH values of the blister pack solutions for nelfilcon A, m-nelfilcon A and etafilcon A were not significantly different from each other ($p>0.05$).

Table 4-2: Average values for physical properties of blister pack solutions investigated

Lenses	pH	Osmolality (mmol/kg)	Surface Tension (dynes/cm)	Viscosity (cP)
omafilcon A	7.43±0.07	285.00±1.83	47.87±0.36	0.93±0.02
nelfilcon A	7.15±0.03	282.50±8.58	46.98±1.28	0.94±0.03
m-nelfilcon A	7.01±0.09	286.50±5.20	21.74±2.65	2.95±0.06
etafilcon A	7.14±0.06	433.25±1.26	39.57±1.22	0.87±0.02
narafilcon A	7.32±0.11	424.50±2.08	45.11±0.57	0.94±0.05
p-value	0.0003	<0.00001	<0.00001	<0.00001

The osmolality values (Table 4-2) exhibited a significant difference between products ($p<0.00001$) and these can be broadly separated into two groups. The osmolality of omafilcon A, nelfilcon A, and m-nelfilcon A blister pack solutions were all only slightly lower than that reported for human tears (305 mmol/kg)[136] and were no different to each other ($p>0.80$). In comparison, the osmolality of the etafilcon A and narafilcon A blister pack solutions were much higher ($p<0.005$), but they were not significantly different from each other ($p>0.10$).

Table 4-2 also reports the surface tension values of the packaging solutions, which also exhibited a significant difference between products ($p<0.00001$). The surface tension of the blister pack solution for m-nelfilcon A was significantly lower than all the other blister pack solutions ($p<0.001$). Surface tension for the etafilcon A blister pack solution was significantly lower ($p<0.05$) than the omafilcon A, nelfilcon A, and narafilcon A blister pack solutions, but significantly higher ($p<0.05$) than the blister pack solution of m-nelfilcon A. The surface tension of the blister pack solution for narafilcon A was significantly higher ($p<0.05$) than the surface tensions for the m-nelfilcon A and etafilcon A blister pack solutions, but was not significantly different ($p>0.05$) from the surface tensions of the omafilcon A and nelfilcon A blister pack solutions.

The viscosity of each blister pack solution is reported in Table 4-2. The viscosity of the m-nefilcon A blister solution (2.95cP) was significantly higher than the viscosity of all other blister solutions ($p<0.0002$). The viscosity of the blister solution for omafilcon A, nelfilcon A, etafilcon A, and narafilcon A were not statistically different from each other ($p>0.05$).

4.5 Discussion and Conclusion

DD lenses have a number of advantages compared to hydrogel lenses that are reused, including less deposit accumulation on the lens,[137, 138] improved comfort and increased patient compliance due to the lack of a maintenance routine.[137-141]

In certain countries around the globe, up to 50% of new patient fits are conducted with DD lenses.[142] However, despite the latest developments in lens materials, patients still cease to wear their lenses due to discomfort and dryness.[140, 143, 144] In an attempt to reduce this sensation of dryness many companies modify the lens material with internal wetting agents[145-147] or include surfactants into the blister packaging solution.[135, 146, 148] DD lenses do not require the use of multipurpose cleaning solutions thus, upon initial insertion of a DD contact lens onto the eye, the physical properties of the blister package solution and the wettability of the lens surface may jointly affect the comfort of the lens in-eye.

One approach is that taken by CIBA Vision, who incorporate "excess" non-polymerized PVA into the nelfilcon A lenses and even more "free" PVA into the m-nelfilcon A lenses, which is gradually released from the surface of the lens over the course of the day.[146, 149] In conventional nelfilcon A lenses, the PVA is polymerized into the lens material to *N*-formylmethyl acrylamide and termed as being "functionalised." The nelfilcon A lens material used in this study has functionalised PVA in the lens matrix as well as non-functionalised PVA

which floats free in the lens matrix, with additional non-functionalised PVA in the m-nelfilcon A lens material.[149] This non-functionalised PVA is slowly released from the lens and helps stabilize the tear film and should, theoretically, improve comfort.[149] The m-nelfilcon A material also incorporates another hydrophilic monomer (PEG) into the lens material and a wetting agent (HPMC) is additionally included in the packaging solution, in an attempt to enhance initial wettability and sustain surface wettability over the course of the day.[124, 145] Figure 4-1, Figure 4-2b and Figure 4-2c demonstrate that nelfilcon A and m-nelfilcon A had relatively low average advancing CAs over all cycles examined, averaging 53° (sessile drop) and 61° (Wilhelmy balance). The receding CA (Figure 4-3b and Figure 4-3c) of approximately 51° for both nelfilcon-based lenses, was also relatively low, and exhibited minimal hysteresis (Figure 4-3b and Figure 4-3c). Given our previous suggestion that lenses with low advancing CAs and minimal hysteresis should prove to exhibit excellent wettability, it would appear that the incorporation of leachable PVA does result in lenses with such properties. Figure 4-1 indicates that the two nelfilcon A products had very similar advancing CAs by the sessile drop technique and it would appear that the modifications made to the latest nelfilcon A product translate into relatively small differences between the two products. The osmolality of nelfilcon A (282.50mmol/kg) and m-nelfilcon A (286.50mmol/kg) blister pack solutions (Table 3-2) were lower than that reported for human tears,[136] but would not be expected to induce any discomfort on insertion. The surface tension of nelfilcon A (46.98dynnes/cm) was close to that reported for human tears.[136] The surface tension of the blister pack solution for m-nelfilcon A (21.74dynnes/cm) was significantly lower than that of human tears and the surface tension of the other blister pack solutions, including nelfilcon A ($p<0.05$). The likely reason for this is the incorporation of the HPMC and the presence of the excess leachable PVA into the packaging

solution, both of which are surface-active and will lower surface tension. The viscosity of the blister solution for the m-nelfilcon A material was higher than that of humans tears and the viscosities of the other blister pack solutions. This high viscosity is again likely due to the incorporation of HPMC into the blister solution.

Omafilcon A lenses had relatively high advancing CAs (Figure 4-1and Figure 4-2a) measured by the sessile drop (average 101°) and Wilhelmy balance techniques (average 73°). The average receding CA for omafilcon A was approximately 47° over all 8 cycles (Figure 4-3a), exhibiting a relatively high hysteresis. This relatively high degree of hysteresis may be caused by the chain rotation of hydroxyethyl methacrylate (HEMA) within the omafilcon A lens material. When HEMA is exposed to air (when the eye is open), the methyl groups rotate towards the hydrophobic interface by chain rotation.[33] This is a more favourable energetic state, thereby lowering the surface free energy of the lens surface. However, on exposure to polar liquids (ie. tears), the polymers will rotate so that the hydrophilic groups are pointing towards the polar phase. Thus, between blinks, the lens may have a tendency to exhibit reduced wettability relative to lenses which exhibit low degrees of hysteresis. As shown in Table 4-2, the osmolality (285mmol/kg) and the surface tension (47.87 dynes/cm) of the blister pack solution for omafilcon A was similar to the values reported for human tears. The viscosity (0.93cP) was close to the viscosity of water (1.00cP).

The etafilcon A material was the only one to exhibit progressively increasing advancing CAs over the 8 cycles when measured by sessile drop method (Figure 4-1 and Figure 4-2d), but had comparable advancing CAs to the nelfilcon A lenses when measured using the Wilhelmy balance (Figure 4-3d). Etafilcon A-based lenses had low receding angles (approximately 31°) compared to the other lenses (Figure 4-3d) but did exhibit a high degree of hysteresis. Etafilcon

A is also based on polyHEMA and the aforementioned chain rotation is the likely cause of the hysteresis determined. As shown in Table 4-2, the surface tension (39.57dynnes/cm) of the blister pack solution was slightly lower than that reported for human tears.[136] The viscosity of the blister solution (0.87cP) was lower than that of tears and lower than the viscosity of water (1.00cP) One significant difference from that measured in the majority of the packaging solutions was that the osmolality (433.25mmol/kg) was very high compared to the osmolality of tears. It is believed that this high osmolality is required to maintain the parameters of the etafilcon A lenses when stored in the blister pack, as placement of the lenses in a hypotonic solution similar to that of the other lenses (around 285 mmol/kg) results in a marked expansion of the lens and dramatic changes in lens diameter (approximately 20% increase in diameter) and thickness (data not shown).

The newest DD lens material (narafilcon A) is the only silicone hydrogel lens and, as such, exhibits substantially higher oxygen permeability (Table 4-1). However, previous studies have shown that silicone hydrogel materials typically exhibit lower levels of wettability and higher CAs than that seen with hydrogel materials.[31, 39, 150, 151] Advancing CAs were approximately 100° and 64° measured using the sessile drop and Wilhelmy balance methods respectively (Figure 4-1 and Figure 4-2e). The receding CA was approximately 52° degrees (Figure 4-3e), which interestingly was comparable to the receding angle for m-nelfilcon A (Figure 4-3c). The hysteresis (approximately 12°) of the narafilcon A lens was relatively low (Figure 4-3e). As would be predicted for a siloxane-based lens, the advancing CA was indeed quite high, but the hysteresis was relatively low. The surface tension (45.11 dynes/cm) was comparable to that of the surface tension of tears.[136] The viscosity of the blister solution (0.94cP) was lower than the viscosity of human tears (1.5cP) and similar to the viscosity of water

(1.00cP). However, the osmolality of the narafilcon A blister pack solution (424.50mmol/cm) was almost as high as that measured for etafilcon A, and much higher than that of the tear film. Interestingly, when narafilcon A lenses were placed into an hypotonic solution (data not shown) their parameters remained fairly similar to that measured in the packaging solution (increase in diameter of 3%), so the reason for the high osmolality remains unknown. As shown in Figure 4-2e, the narafilcon A material showed the greatest difference in advancing CA between the two methods, suggesting that it is most affected by methodological technique in its assessment of CA.

In addition to narafilcon A, the two polyHEMA-based lens materials omafilcon A and etafilcon A also show marked differences in the measurement of their advancing CAs (Figure 4-2a and 4-2d). As described above, polyHEMA-based materials tend to reorient themselves depending on the surrounding environment. Narafilcon A contains polyvinylpyrolidone (PVP), a high molecular weight wetting agent incorporated into the lens material.[126] PVP acts as a lubricant and a humectant, binding moisture and presenting a wettable lens to the ocular surface.[78, 152] Thus, when the lens is in an aqueous environment, the PVP retains moisture at the surface of the lens, making it more wettable. Preparation of the lenses for CA measurements by sessile drop requires blot drying the lens, as opposed to just shaking off the excess blister pack solution during preparation of the lens for the Wilhelmy balance method. Blot drying the lens would cause the polyHEMA to orient in the lens so that the hydrophobic polymers are facing the lens surface, making the lens less wettable, which could account for the high CAs measured using the sessile drop technique. Preparation of the lens for the Wilhelmy balance is time consuming and the lens can become dehydrated if it is blot dried before attachment to the electrobalance. Hence, the lens is prepared by just shaking off the excess blister pack solution. A

small film of blister pack solution on the lens would cause the hydrophilic moieties of HEMA to be exposed to the outside surface of the lens, accounting for the lower CAs. Thus, it is important when reporting the in vitro CAs that the method by which the CAs were measured is accurately reported and the methods suitably described.

The pH of all blister pack solutions was close to neutral (Table 4-2), and although there was a statistical difference, these differences were not considered clinically significant.[136]

Overall, the nelfilcon A and m-nelfilcon A lens exhibited low advancing and receding CAs, with minimal hysteresis, and the m-nelfilcon A blister pack solution had the lowest surface tension, which could be attributed to the HPMC in the blister pack and non-functionalised PVA released from the lens. The potential link between CA assessment and in eye comfort must be addressed. In 2007, Winterton et al.[123] investigated the effect of the elution of PVA on comfort of the nelfilcon A material. This was carried out as a clinical trial with patients wearing nelfilcon A lenses with non-functionalised PVA and nelfilcon A lenses in which the PVA was extracted before wear. Severe or moderate lens awareness, increased stinging and reduced wear time was reported by patients wearing lenses with the extracted PVA.[123] These results were attributed to the reduced amount of PVA in the extracted lenses. An additional experiment was also conducted looking at the rate of elution of PVA under in vitro and in vivo conditions. Results indicated an enhanced rate of elution of PVA from lenses worn in vivo compared to lenses tested in vitro. The hypothesis suggested by Winterton et al.[123] to this phenomenon was that in-eye, blinking provided the additional "energy" needed to "squeeze" excess fluid and PVA from the lens, resulting in the higher elution rate.[123]

A study by Peterson et al.[149] compared in-eye comfort of conventional nelfilcon A vs. nelfilcon A with the non-functionalised PVA. Comparisons between the two lenses were made

with a comfort rating and measuring the non-invasive tear break-up time (NIBUT). Comfort rating for nelfilcon A with non-functionalised PVA was consistently higher than that of the conventional nelfilcon A lens over 16 hours of wear time, and the NIBUT was longer for nelfilcon A compared to the conventional nelfilcon A. Both results could be attributed to the non-functionalised PVA incorporated in the nelfilcon A lens. A year later, a clinical trial was conducted comparing the second generation nelfilcon A lens and the newest nelfilcon A lens material (m-nelfilcon A).[153] The m-nelfilcon A lens material rated significantly higher than nelfilcon A in overall preference, overall comfort, all day comfort, lenses feeling fresh and clean throughout the day, lenses staying moist throughout the day, and clear vision.[153] These results were suggested by the researchers to be due to the added wetting agents into the m-nelfilcon A lens material (PEG) and into the blister pack solution (HPMC).

In 2008, Giles and Fahmy[145] compared the performance of m-nelfilcon A with a newer etafilcon A lens material which incorporates a wetting agent based on PVP. Performance was based on less dryness, overall comfort, all day comfort, and ease of handling.[145] The m-nelfilcon A lens rated significantly higher in all measured categories.[145] They suggested that these finding were due to the incorporated wetting agent (PEG) into the lens material and the lubricating agent, HPMC in the blister packaging solution.

This experiment effectively measured the physical properties of the blister pack solutions for the DD lenses used in this study and demonstrated the substantivity of the blister solutions through the 5 minute soak followed by blot drying method. As mentioned previously this method was conducted to mimic blinking, however drying the lens after a 5 minutes soak is not a true representation of the evaporation of the tear film off the surface of the contact lens after a blink that lasts less than a second. Therefore, a new technique which more effectively mimics blinking

needs to be developed. In Chapter 6, an experiment is described which investigates the effect of tear components on the wettability of contact lenses after lenses have been placed in a “model blink cell”.

5. Chapter 5: Validation of a Model Blink Cell

5.1 Introduction

In vitro experiments investigating the performance of a biomaterial are conducted to test the potential behaviour of the body to the biomaterials in vivo. As an example, blood contacting devices are generally exposed to plasma in vitro before human or animal testing commences.

A number of experiments have looked at the performance of different contact lens materials when exposed to tear components.[41, 61, 76, 79, 154] However, none of these studies have investigated the effect of tear components on the lens material when placed in an environment that mimics blinking. Typically, lens materials are merely "soaked" in a single protein solution for a set period of time. This is clearly very different to the true in vivo situation, in which lenses are bathed in a highly complex solution (the tear film) and intermittent drying of the lens surface takes place during the inter-blink period. In the previous chapter, a method was used in which lenses were cycled through intervals of being soaked in saline for 5 minutes, after which the CA was measured. This method was conducted to mimic the hydration and drying of the lens by the tear film during blinking, however, this technique was not a realistic representation of blinking, as drying of the lens occurs much faster than at 5 minute intervals.

In an attempt to mimic the complex in eye situation, a "model blink cell" was developed, which functions to mimic in eye blinking in an in vitro setting. It is composed of a pump/valve system, a "bath" which contains six pistons with convex surfaces, two sensors in the bath, a series of tubes for delivery of solutions, a container holding fresh solution, and a container which holds waste solution (Figure 5-1).

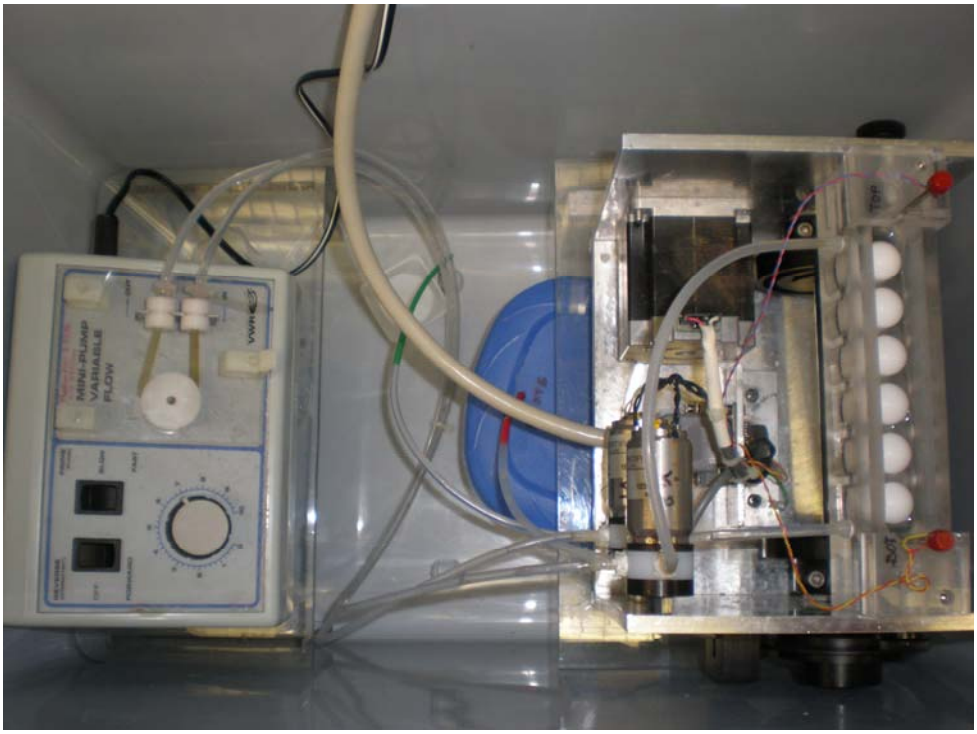


Figure 5-1: Picture of the model blink cell showing the pistons, valves, tubing series, pump, and sensors.

Six contact lenses can be placed posterior side down on the top of the pistons at one time (Figure 5-1). A solution, such as saline or a complex mimic of the tears, is brought up from the container holding the fresh solution (this container is missing in Figure 5-1) and cycled through the model blink cell until a purge time is reached. The solution is pumped through the tubing, into the bath containing the pistons and contact lenses, and then back into the tubing. As solution is cycled through the model blink cell, the pistons move up and down, consequently moving the contact lenses in and out of the solution, to mimic blinking. The amount of time the contact lenses spend in and out of the solution is controlled by the experimenter, by setting the time intervals on the control box resting on top of the model blink cell (Figure 5-2). Other settings that

are set by the experimenter are the purge and refill time, as well as the temperature inside the model blink cell.

Figure 5-2: Control box which controls temperature, purge and refill times, and amount of time lenses in and out of the fluid bath.

In the experiment described in Chapter 6, lenses were placed on the pistons in the model blink cell and then exposed to a saline solution, a lysozyme solution, and an artificial tear solution (see section 5.3.1 for their exact composition) for 5 minutes, 1 hour, 4 hour, and 8 hour time intervals. During these time intervals the pistons moved in and out of the solution so the lenses would be in the solution for 1 sec and out of the solution for 5 seconds to mimic blinking. However, before that experiment was undertaken, an experiment in which the lenses were soaked in the three solutions for the respective time intervals rather than being placed in the model blink cell was conducted to validate whether or not the model blink cell was a useful tool to investigate the effects of tear components on the wettability of contact lens materials.

5.2 Materials

Three DD lenses were examined in this study: omafilcon A (CooperVision, Pleasanton, California), modified (m-) nelfilcon A (CIBA Vision, Duluth, Georgia), and narafilcon A (Johnson & Johnson, Jacksonville, Florida). Please refer to Table 4-1 for the various properties of these materials.

The solutions used in the study were a saline solution (Unisol, Alcon, Fort Worth, Texas), lysozyme solution, and a complex tear solution. The saline solution was used as a control solution.

5.3 Methods

5.3.1 Preparation of Tear Solutions

The artificial lysozyme solution was prepared at a concentration of 1.9mg/ml by dissolving granular hen egg lysozyme (HEL) in sterilized phosphate buffered-saline (PBS) with a pH of 7.4. The HEL was purchased from Sigma (St. Louis, Missouri).

The complex tear solution was composed of five lipids (triolein, cholesterol, oleic acid, oleic acid methyl ester, and cholesteryl oleate), three proteins (β- lactoglobulin, albumin, and lysozyme) and mucin. All complex tear components were purchased from Sigma (St. Louis, Missouri).

A lipid stock solution was created by dissolving appropriate quantities of each lipid in 2ml of a hexane/ether solution. The concentration of the triolein, cholesterol, oleic acid, oleic acid methyl ester, and cholesteryl oleate were added at concentrations of 0.016mg/ml, 0.0018mg/ml, 0.0018mg/ml, 0.012mg/ml, and 0.024mg/ml respectively. Lipid stock solution was added to sterilized PBS that was heated to 37ºC. The hexanes and ether were allowed to

evaporate in a cell culture hood by repeatedly heating the solution to 37ºC until all the hexanes and ether evaporated.

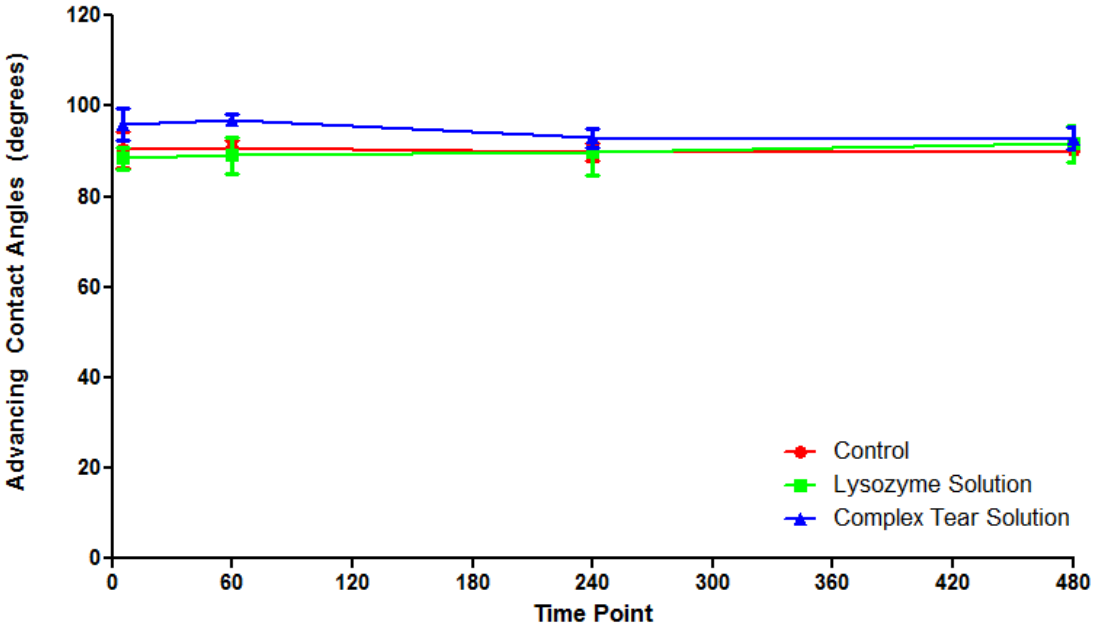
Bovine submaxillary mucin was placed on weighing paper and exposed to UV light for 20 minutes to sterilize. After sterilization the mucin was added to the lipid solution at a concentration of 0.15mg/ml.

Granular human albumin, HEL, and β-lactoglobulin were dissolved in the mucin/lipid solution. B-lactoglobulin was used as a substitute for lipocalin which is naturally found in the human tear film. Albumin was added at a concentration of 0.20mg/ml, HEL was added at a concentration of 1.9mg/ml, and β-lactoglobulin was added at a concentration of 1.6mg/ml. Lactoferrin was not added to the complex tear solution due to cost. The complex tear solution was stored at 4ºC when not in use.

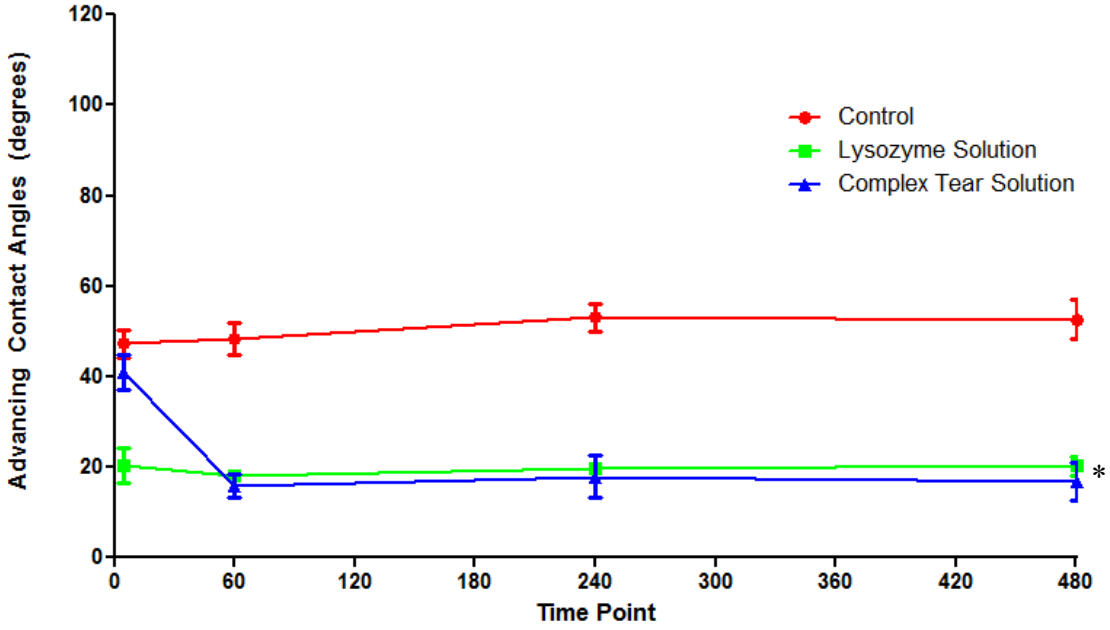
Lenses (n=4) were soaked in 5ml of the control, lysozyme, and complex tear solution for 5 minutes, 1 hour, 4 hours, and 8 hours in an incubator set at 37ºC. After lenses were soaked in each solution for the set time point the advancing CAs were measured using the sessile drop technique (see section 2.1.2.1.) and the Wilhelmy balance method (see section 2.2.2.2). The receding CAs were measured using the Wilhelmy balance method (see section 2.2.2.2).

5.4 Results

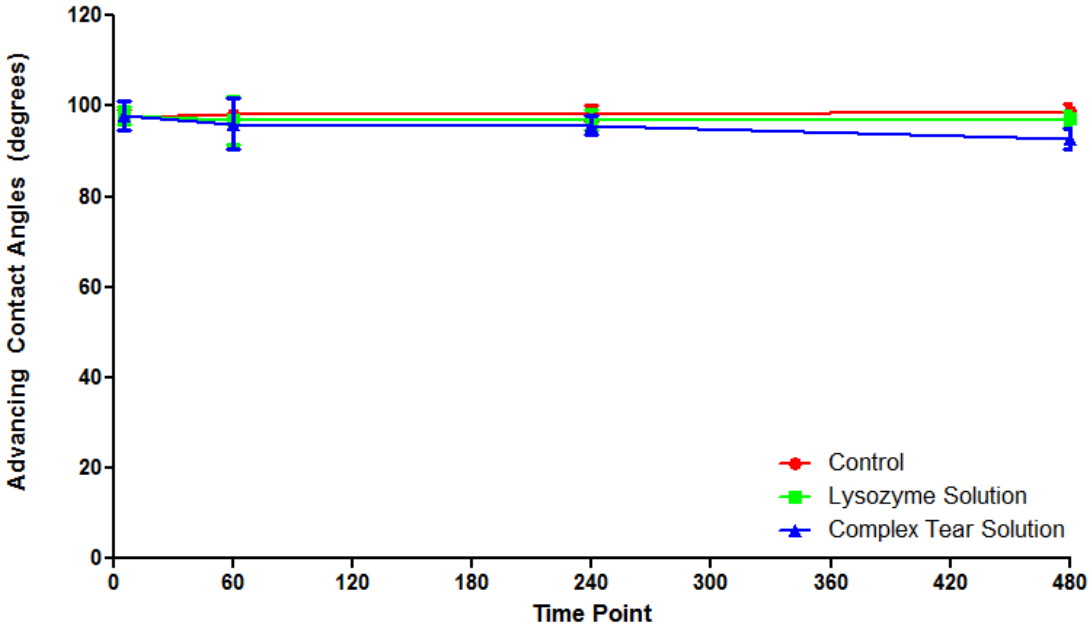
The advancing CAs measured by the sessile drop technique for omafilcon A, nelfilcon A, and narafilcon A after lenses were soaked in 5ml of the control solution, lysozyme solution and complex tear solution for 5 minutes, 1 hour, 4 hours, and 8 hours are shown in Figure 5-3a-c.


Advancing Contact Angles (degrees)
120
100
80
60
40
20
0
0
60
120
180
240
300
360
420
480
Time Point
Control
Lysozyme Solution
Complex Tear Solution

a)


Advancing Contact Angles (degrees)
120
100
80
60
40
20
0
0
60
120
180
240
300
360
420
480
Time Point
Control
Lysozyme Solution
Complex Tear Solution
*

b)



c)

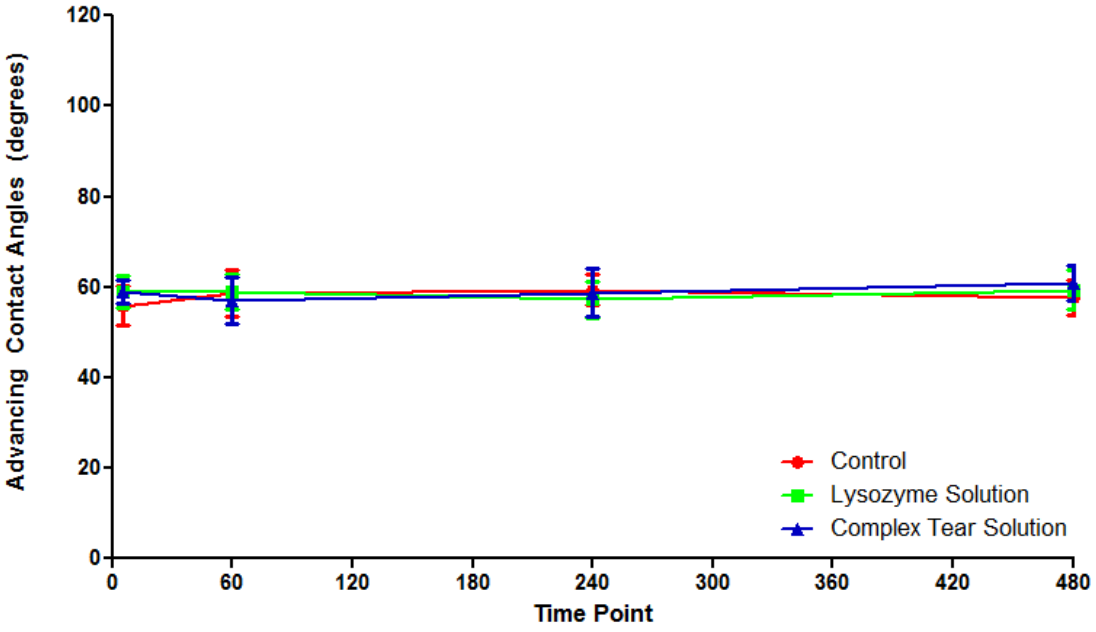
Figure 5-3: Advancing CAs measured by the sessile drop technique for a) omafilcon A b) nelfilcon A and c) narafilcon A, after being soaked in 5ml of control solution, lysozyme solution, and complex tear solution for 5 minutes, 1 hour, 4hours, and 8 hours.

There was no statistical difference between advancing CAs for omafilcon A after being soaked in any of the three solutions at any time point ($p>0.05$). The advancing CAs for nelfilcon A after being soaked in the control solution were statistically higher ($p<0.001$) than all other CAs, except for the CAs measured after nelfilcon A was soaked in the complex tear solution for 5 minutes ($p>0.05$). The CAs after nelfilcon A was soaked in the lysozyme solution for 5 minutes were statistically lower than the CAs measured after being soaked in the control solution and complex tear solution for 5 minutes ($p<0.001$). The CAs measured after nelfilcon A was soaked in the lysozyme and complex tear solutions for 1 hour, 4 hours, and 8 hours were not statistically different from each other ($p>0.90$). There was no statistical difference in advancing

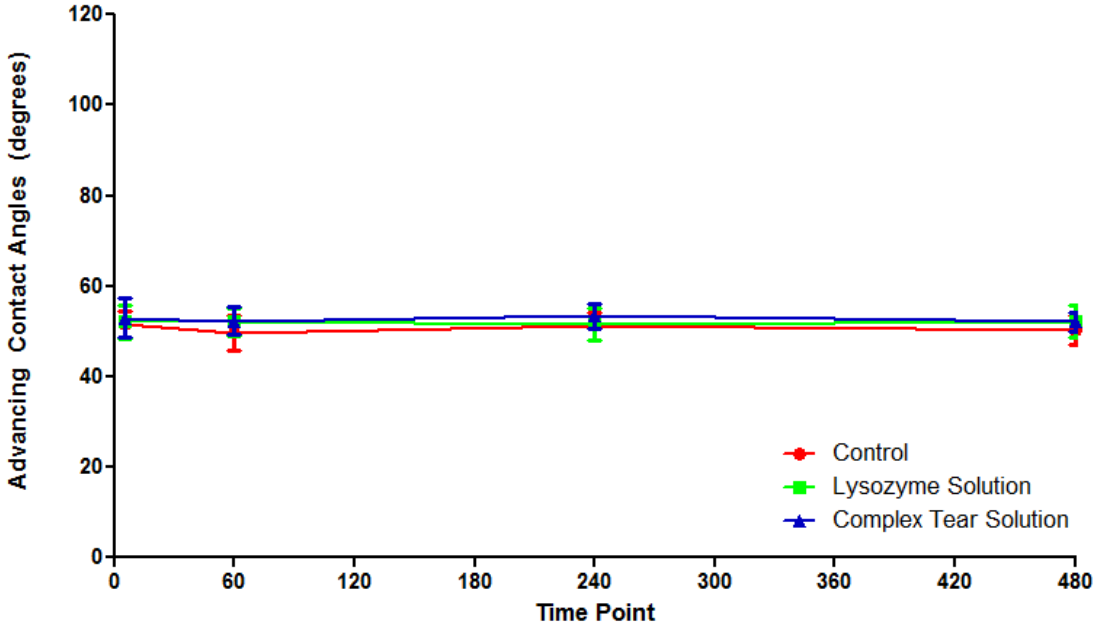
CAs measured after narafilcon A was soaked in any of the three solutions for any time point ($p>0.05$).

Comparing advancing CAs between lenses there was no statistical difference between the advancing CAs of omafilcon A and narafilcon A after either lens was soaked in the control solution, lysozyme solution, or complex tear solution at any time point ($p>0.05$). All advancing CAs for nelfilcon A were statistically lower than the CAs for nelfilcon A and omafilcon A ($p<0.05$) with the CAs after nelfilcon A was soaked in the lysozyme and complex tear solution for 1 hour, 4 hours and 8 hours, being the lowest CAs measured ($p<0.001$).

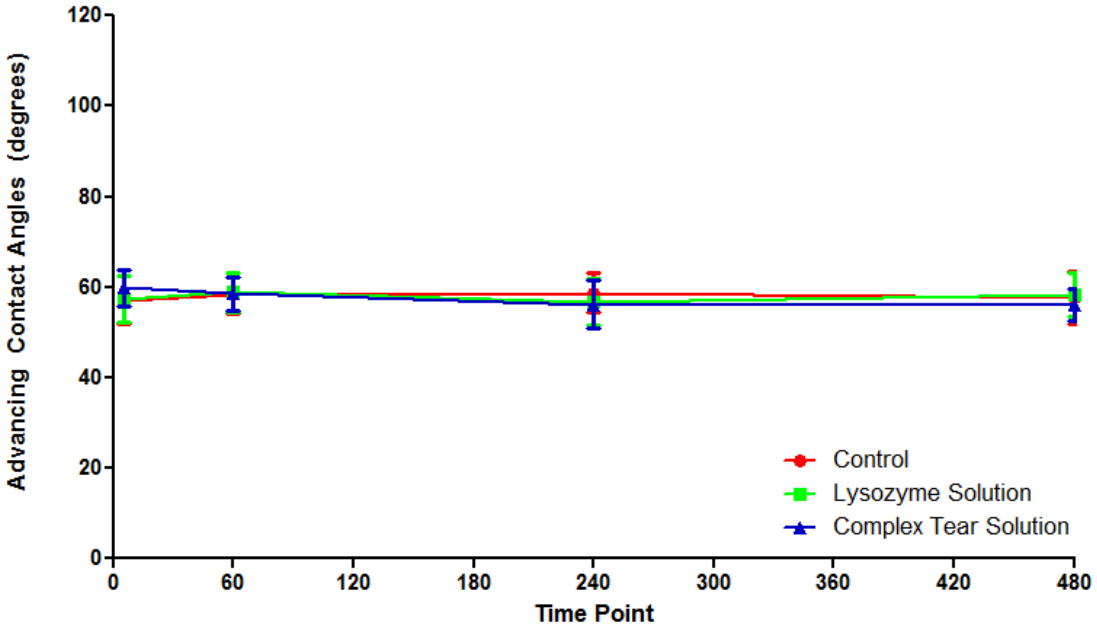
The advancing CAs measured by the Wilhelmy balance method for omafilcon A, nelfilcon A, and narafilcon A after lenses were soaked in 5ml of the control solution, lysozyme solution and complex tear solution for 5 minutes, 1 hour, 4 hours, and 8 hours are shown in Figure 5-4a-c.


Advancing Contact Angles (degrees)
120
100
80
60
40
20
0
0
60
120
180
240
300
360
420
480
Time Point
Control
Lysozyme Solution
Complex Tear Solution

a)


Advancing Contact Angles (degrees)
120
100
80
60
40
20
0
0
60
120
180
240
300
360
420
480
Time Point
Control
Lysozyme Solution
Complex Tear Solution

b)



c)

Figure 5-4: Advancing CAs measured by the Wilhelmy balance method for a) omafilcon A b) nelfilcon A and c) narafilcon A, after being soaked in 5ml of control solution, lysozyme solution, and complex tear solution for 5 minutes, 1 hour, 4hours, and 8 hours

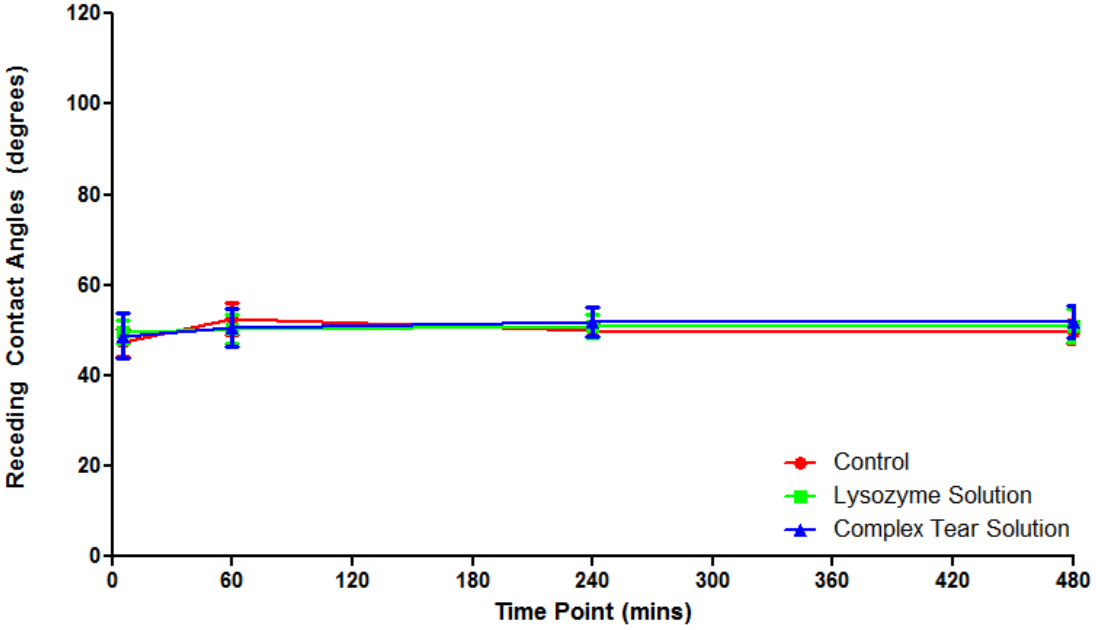
The advancing CAs measured by the Wilhelmy balance method for omafilcon A (Figure 5-4a) after being soaked in each solution for any of the time points, were not statistically different ($p>0.10$). This trend was similar for nelfilcon A (Figure 5-4b) and narafilcon A (Figure 5-4c).

Comparing CAs between lenses, the CAs for nelfilcon A after being soaked in the control solution were all statistically lower ($p<0.05$) than the CAs for omafilcon A and narafilcon A after being soaked in the control solution. The CAs for omafilcon A and narafilcon A after being soaked in the control solution were not statistically different from each other at any time point ($p>0.90$).

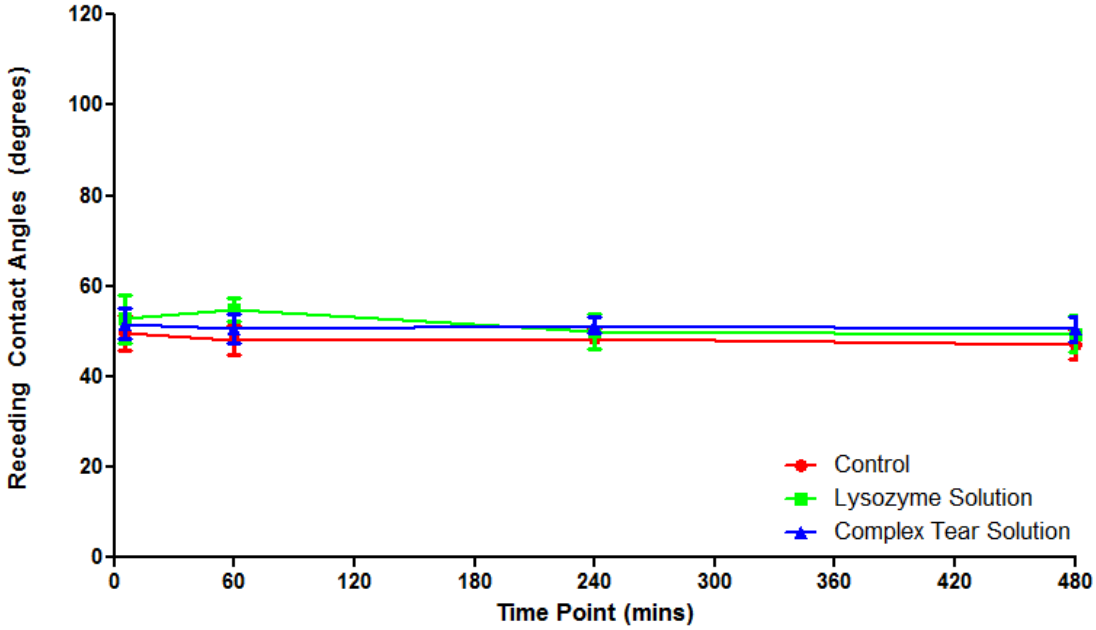
The advancing CAs for omafilcon A and narafilcon A after being soaked in the lysozyme solution were not statistically different from each other at any time point ($p>0.90$). The CAs for nelfilcon A were statistically lower than the CAs for omafilcon A and narafilcon A after being soaked in lysozyme solution for 1 hour, 4 hours, and 8 hours ($p<0.05$).

After all three lens materials were soaked in the complex tear solution, again there was no statistical different in CAs for the omafilcon A and narafilcon A lens materials at anytime point ($p>0.10$). The CAs for nelfilcon A were significantly lower than the CAs for omafilcon A after being soaked in the complex tear solution for 5 minutes and 1 hour ($p<0.01$), and were significantly lower than all CAs for narafilcon A after being soaked in the complex tear solution for any time point ($p<0.05$).

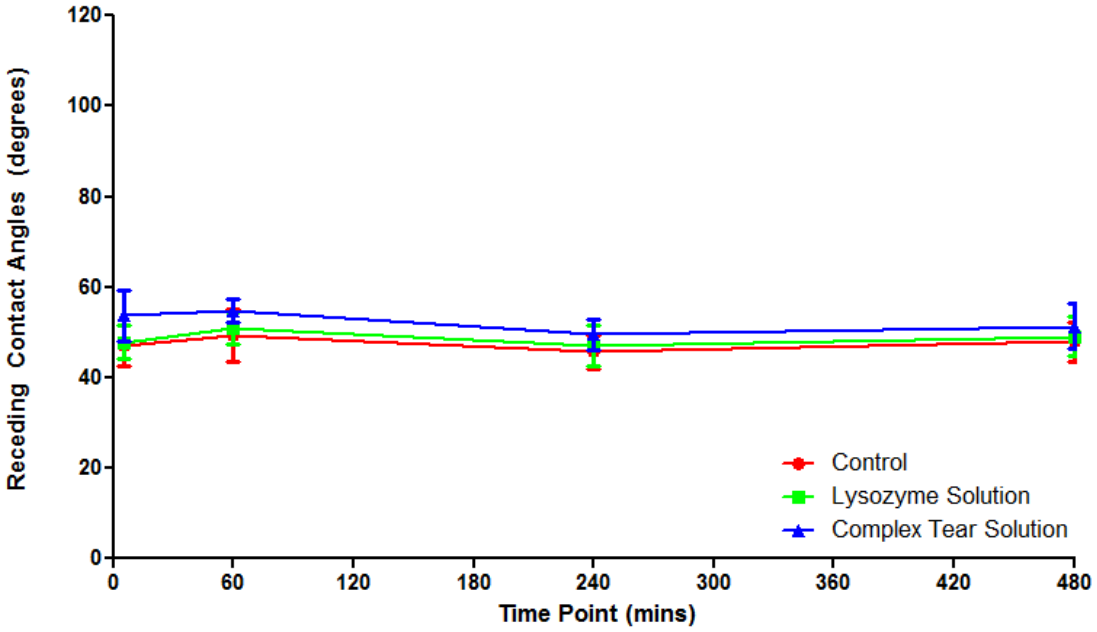
The receding CAs measure for omafilcon A, nelfilcon A, and narafilcon A after each lens materials was soaked in the three solutions for 5 minutes, 1 hour, 4 hours, and 8 hours, are shown in Figure 5-5a-c respectively.


Receding Contact Angles (degrees)
120
100
80
60
40
20
0
0
60
120
180
240
300
360
420
480
Time Point (mins)
Control
Lysozyme Solution
Complex Tear Solution

a)


Receding Contact Angles (degrees)
120
100
80
60
40
20
0
0
60
120
180
240
300
360
420
480
Time Point (mins)
Control
Lysozyme Solution
Complex Tear Solution

b)



c)

Figure 5-5: Receding CAs measured by the Wilhelmy balance method for a) omafilcon A b) nelfilcon A and c) narafilcon A, after being soaked in 5ml of control solution, lysozyme solution, and complex tear solution for 5 minutes, 1 hour, 4hours, and 8 hours.

There was no significant difference in receding CAs for any of the lens materials after soaking in the control solution, lysozyme solution, and complex tear solution for 5 minutes, 1 hour, 4 hours, and 8 hours ($p>0.05$). There was also no significant difference comparing the receding CAs between lenses at any time point after being soaked in any of the solutions ($p>0.05$).

5.5 Discussion and Conclusion

The very small change in advancing CAs measured by the sessile drop technique and Wilhelmy balance method for omafilcon A and narafilcon A after lenses were soaked in the three solutions for 5 minutes, 1 hour, 4 hours, and 8 hours suggest that there is little to no deposition of tear components on the surface of these lens materials. Advancing CAs measured by the sessile

drop technique for the omafilcon A lens material were slightly higher after the lens material was soaked in the complex tear solution, which may have been due to components from the complex tear solution depositing on the lens surface. However, these CAs were not statistically higher, which leads us to believe that whatever was deposited on the lens surface had little impact on the surface wettability of omafilcon A.

There was a significant increase in wettability measured by the sessile drop technique for the nelfilcon A lens material after the lens was soaked in the lysozyme solution and complex tear solution. This was probably due to deposition of lysozyme from both solutions onto the lens surface. An increase in wettability after nelfilcon A was soaked in the complex tear solution was only detectable by the sessile drop technique after the lens was soaked in the solution for 1 hour. The little increase in wettability after 5 minutes in the complex tear solution may be due to competitive binding between the components in the solution.

There were no detectable differences in wettability using the Wilhelmy balance method for any of the lens materials after they were soaked in the lysozyme and complex tear solutions. This may have been due to the lenses not being exposed to the tear components for a long enough period for a large enough amount of deposition to occur on the lens materials to further cause an effect of the surface wettability detectable by the Wilhelmy balance method. As will be discussed later, the majority of deposition studies dope lenses for periods of 48 hours to 2 weeks. These longer time periods would allow more deposition to occur on the lenses, which could lead to a greater impact on the surface wettability of the lens material.

Although this study showed some changes to the wettability of the lens materials due to deposition of tear components, it is generally believed that deposit formation is more influenced by tear thinning and drying of the lens surface between blinks, thus a similar study was

conducted to investigate if there are any differences in wetting behaviours from this study compared to a study using the model blink cell.

6. Chapter 6: Effect of Tear Components on the CA of DD Lenses

6.1 Introduction

The purpose of this experiment was to investigate the effect of tear components on the surface wettability of contact lenses after placing lenses in a "model blink cell". The model blink cell was used to see if there was an impact on the deposition of tear components from a technique in which the lenses were hydrated and then exposed to the open air, much like blinking in-eye as compared to just soaking the lenses in complex tear solutions (see Chapter 5: Validation of a Model Blink Cell).

6.2 Materials

Three DD lenses were examined in this study: omafilcon A (CooperVision, Pleasanton, California), modified (m-) nelfilcon A (CIBA Vision, Duluth, Georgia), and narafilcon A (Johnson & Johnson, Jacksonville, Florida). Please refer to Table 4-1 for the various properties of these materials.

The solutions used in the study were a saline solution (Unisol, Alcon, Fort Worth, Texas), lysozyme solution, and a complex tear solution. The saline solution was used as a control solution.

6.3 Methods

6.3.1 Preparation of Tear Solutions

The tear solutions used in this study were prepared exactly as described in Chapter 5 (please refer to section 5.3.1).

6.3.2 Model Blink Cell

Lenses were removed directly from the blister pack and placed posterior side down on the pistons and fastened into place with the anterior surface of the lens exposed to the solution in the model blink cell. One type of lens material was placed in the model blink cell at one time to prevent contamination of blister pack components from other lenses, which could potentially alter the wettability results.

The model blink cell was set to expose the lenses to the control solution, lysozyme solution, and complex tear solution every 5 seconds, which is approximately the average blink rate in vivo.[155, 156] Lenses were exposed to the solution for 1 second which is longer than the actual spreading of the tear film over the lens surface in vivo, however, this was the shortest exposure time available using the model blink cell. Wettability of each lens material was measured after lenses were placed in the model blink cell for 5 minutes, 1 hour, 4 hours, and 8 hours.

The model blink cell used in this study was designed by another graduate student (Holly Lorentz) and built by two Research Technicians within the School of Optometry (Robin Jones and Andrew Nowinski).

6.3.3 Sessile Drop Technique

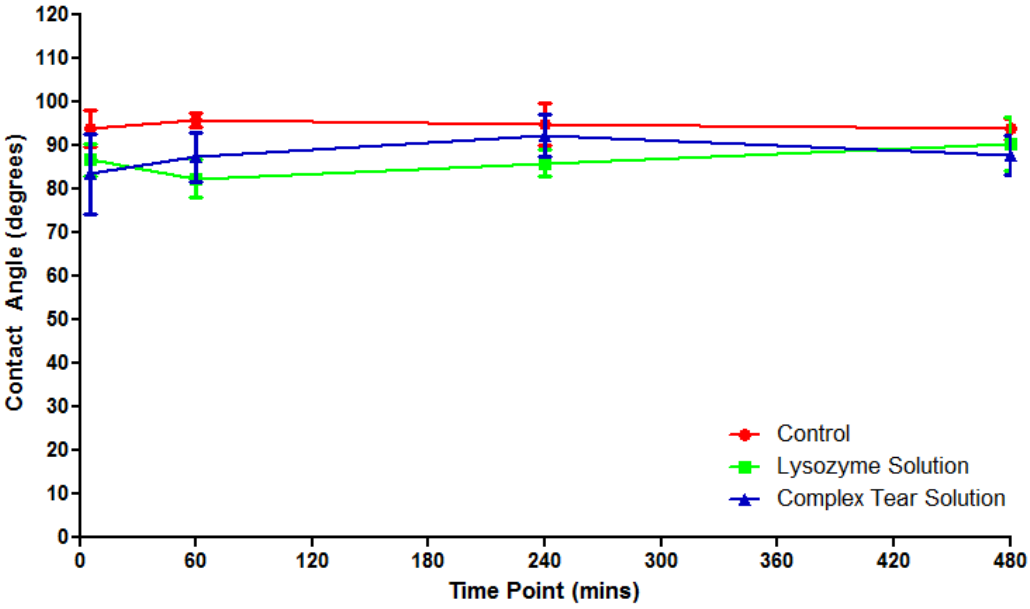
The advancing CAs of each lens material was measured using the sessile drop technique as described previously (please refer to section 2.1.2.1).

6.3.4 Wilhelmy Balance Method

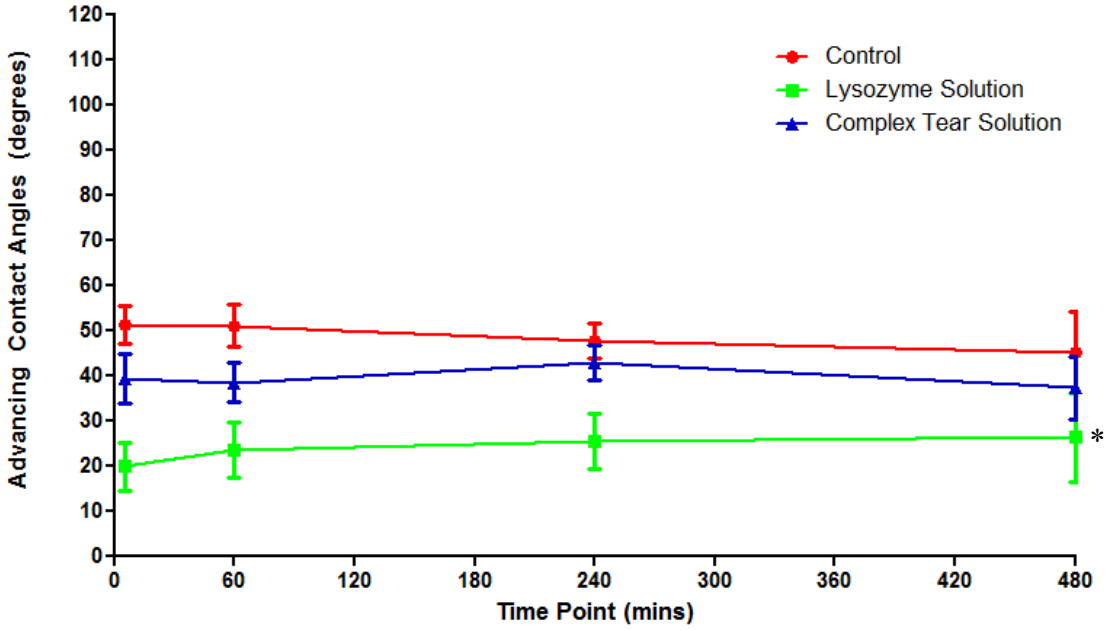
The advancing and receding CAs of each lens material were measured using the Wilhelmy balance method previously described (please refer to section 2.2.2.2).

6.4 Results

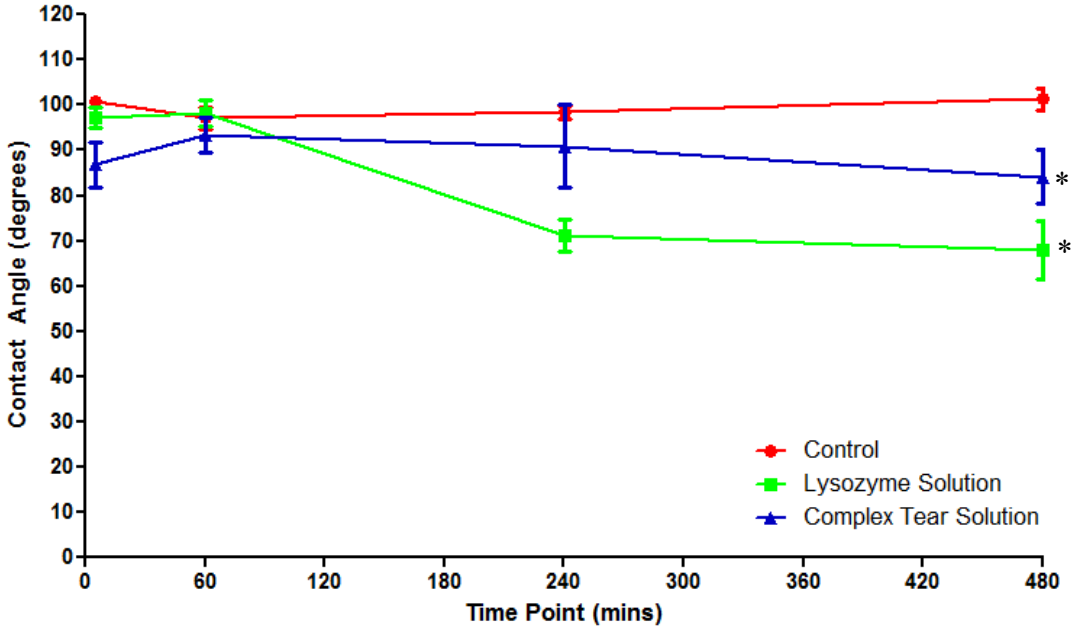
The advancing CAs of each lens material after the lenses were exposed to the control solution, lysozyme solution, and complex tear solution in the model blink cell for 5 minutes, 1 hour, 4 hours, and 8 hours are shown in Figure 6-1. The advancing CAs were measured using the sessile drop technique.



a)



b)



c)

Figure 6-1: Advancing CAs measured by the sessile drop technique for a) omafilcon A b) m-nelfilcon A and c) narafilcon A, after being exposed to the control solution, lysozyme

solution, and complex tear solution for 5 minutes, 1 hour, 4hours, and 8 hours in the model blink cell.

The advancing CAs of omafilcon A after the lenses were exposed to the lysozyme solution for 1 hour were statistically lower than the CAs after the lenses were exposed to the control solution for 1 hour ($p<0.01$), but not statistically different to the CAs after the lenses were exposed to the complex tear solution for 1 hour ($p>0.05$). The remainder of the CAs for omafilcon A were not statistically different from each other after exposure to all three solutions for 5 minutes, 4 hours, and 8 hours ($p>0.05$).

The advancing CAs for m-nelfilcon A after the lenses were exposed to the lysozyme solution for 5 minutes, 1 hour, 4 hours, and 8 hours, were all statistically lower compared to the CAs after the lenses were exposed to the control solution and complex tear solution for 5 minutes, 1 hour, 4 hours and 8 hours ($p<0.001$). The advancing CAs after m-nelfilcon A was exposed to the control solution and complex tear solution were not statistically different at the 5 minute, 4 hour, and 8 hour time points ($p>0.05$), but were statistically different at the 1 hour time point ($p<0.05$).

The advancing CAs for narafilcon A after being exposed to the complex tear solution for 5 minutes were statistically lower than the CAs after being exposed to the control solution and lysozyme solution for 5 minutes ($p<0.01$).There was no statistical difference in CAs after narafilcon A was exposed to any of the solutions for 1 hour ($p>0.90$). After 4 hours of exposure to the lysozyme solution the CAs were statistically lower than the CAs after narafilcon A was exposed to the control and complex tear solution for 4 hours ($p<0.001$). The CAs after narafilcon A was exposed to the control and complex tear solution for 4 hours were not statistically different from each other ($p>0.10$). The CAs after narafilcon A was exposed to each solution for

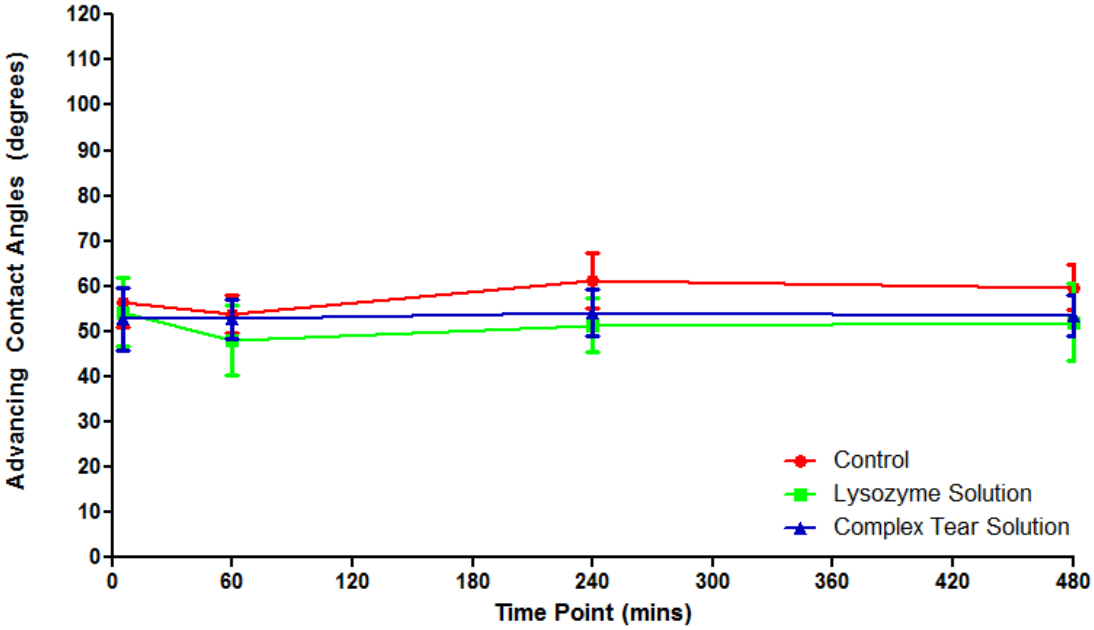
8 hours were all statistically different from each other with CAs after exposure to the control solution being statistically the highest ($p<0.001$) and CAs after exposure to the lysozyme solution for 8 hours being statistically the lowest ($p<0.001$).

The advancing CAs after m-nelfilcon A was exposed to the control solution were statistically lower at all time points compared to the CAs of narafilcon A and omafilcon A after exposure to the control solution ($p<0.001$). The CAs of narafilcon A and omafilcon A after exposure to the control solution were not statistically different from each other ($p>0.05$).

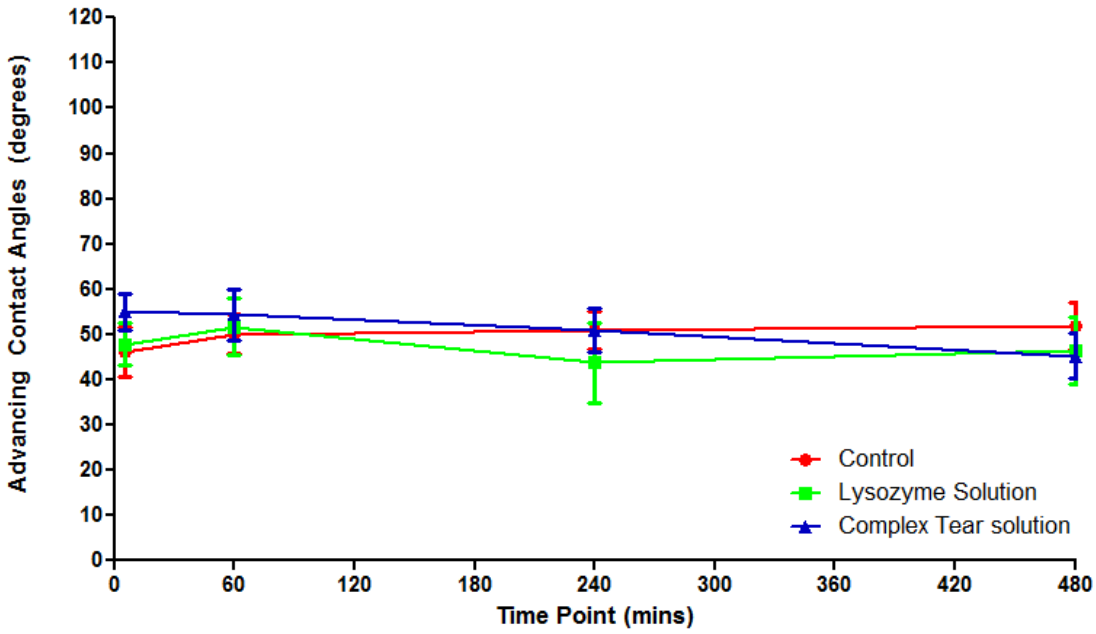
After lenses were exposed to the lysozyme solution, CAs for m-nelfilcon A were statistically lower at all time points compared to the CAs for omafilcon A and narafilcon A ($p<0.001$). CAs for narafilcon A and omafilcon A were also statistically different from each other at each time point after being exposed to the lysozyme solution ($p<0.05$). CAs for narafilcon A after being exposed to the lysozyme solution for 5 minutes and 1 hour were statistically higher than CAs after omafilcon A was exposed to the lysozyme solution for 5 minutes and 1 hour ($p<0.05$). CAs for narafilcon A after being exposed to the lysozyme solution for 4 hours and 8 hours were statistically lower than CAs after omafilcon A was exposed to the lysozyme solution for 4 hours and 8 hours ($p<0.001$).

The CAs for m-nelfilcon A after exposure to the complex tear solution were statistically lower at all time points compared to CAs for omafilcon A and narafilcon A after exposure to the complex tear solution ($p<0.001$). The CAs for omafilcon A and narafilcon A after exposure to the complex tear solution were not statistically different from each other at any time point ($p>0.30$).

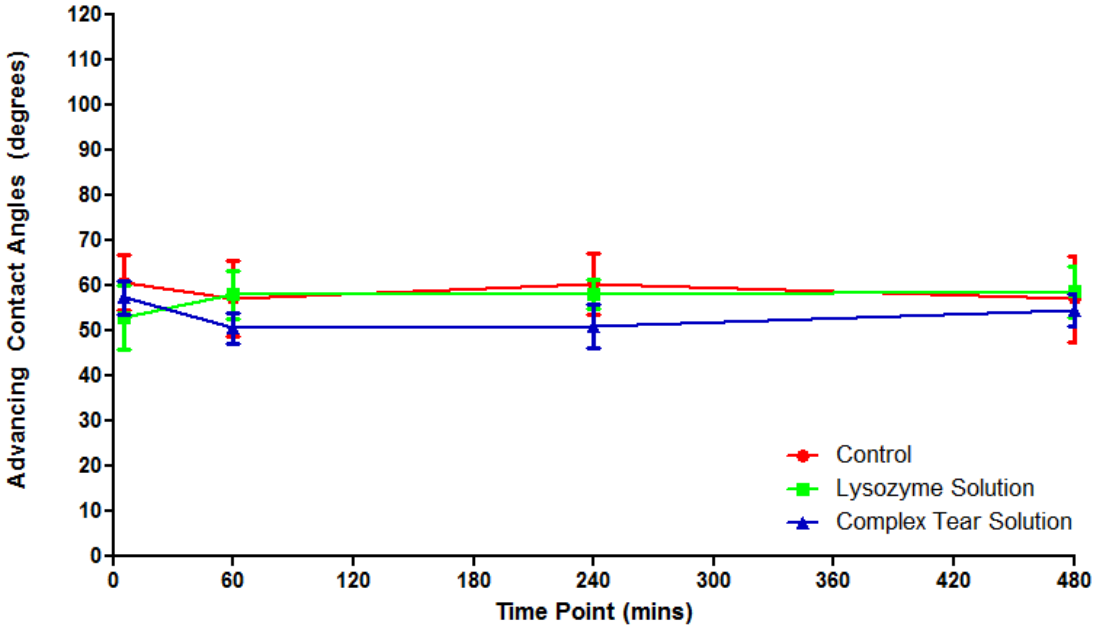
Figure 6-2 shows the advancing CAs measured by the Wilhelmy balance method after lenses were exposed to the control, lysozyme, and complex tear solutions for 5 minutes, 1 hour, 4 hours, and 8 hours.



a)



b)



c)

Figure 6-2: Advancing CAs measured by the Wilhelmy balance method for a) omafilcon A b) m-nelfilcon A and c) narafilcon A, after being exposed to the control solution, lysozyme solution, and complex tear solution for 5 minutes, 1 hour, 4hours, and 8 hours in the model blink cell.

The advancing CAs for omafilcon A after exposure to the control, lysozyme, and complex tear solution for 5 minutes and 1 hour were not statistically different from each other ($p>0.30$). The CAs after omafilcon A was exposed to the control solution for 4 hours and 8 hours were statistically higher than the CAs after omafilcon A was exposed to the lysozyme and complex tear solutions for 4 hours and 8 hours ($p<0.01$). There was no statistical difference between CAs at any time point when omafilcon A was exposed to the lysozyme and complex tear solutions.

The CAs for m-nelfilcon A after exposure to the lysozyme solution for 4 hours were statistically lower than the CAs after m-nelfilcon A was exposed to the control and complex tear

solution for 4 hours ($p<0.05$). There was no statistical difference between CAs at any other time point after m-nelfilcon A was exposed to the control, lysozyme, and complex tear solutions ($p>0.05$).

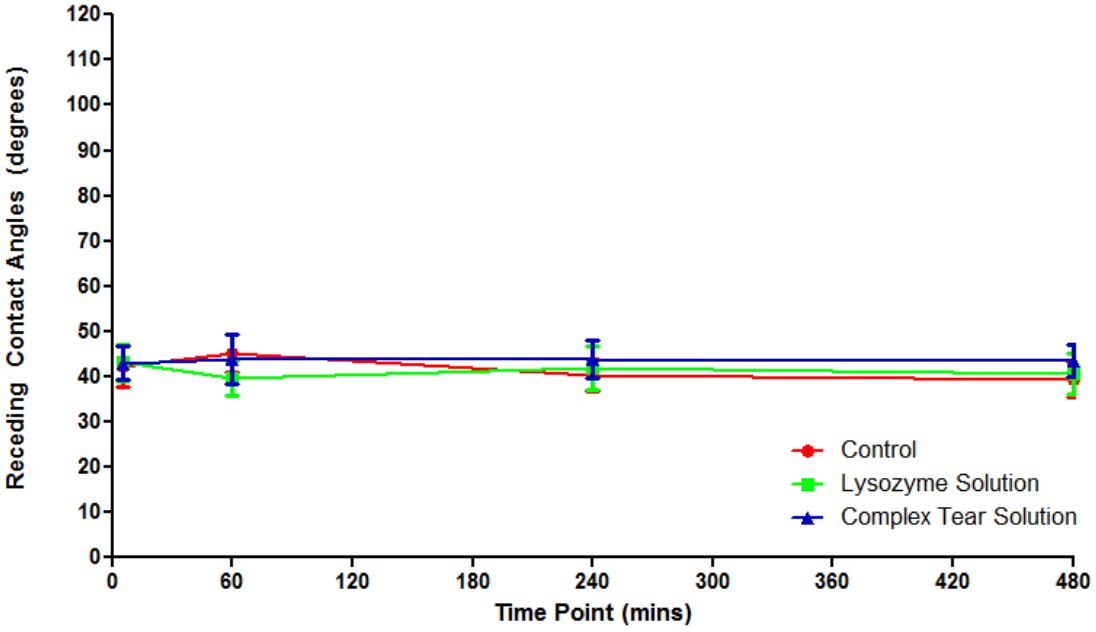
The CAs of narafilcon A after the lenses were exposed to the control, lysozyme and complex tear solution were not statistically different from each other at any time point ($p>0.05$).

Comparing CAs between lens materials after exposure to the control solution, the CAs for m-nelfilcon A were statistically lower than the CAs for omafilcon A and narafilcon A at the 5 minute and 4 hour time points ($p<0.05$). The CAs for omafilcon A and narafilcon A were not statistically different from each other after being exposed to the control solution for any time point ($p>0.05$).

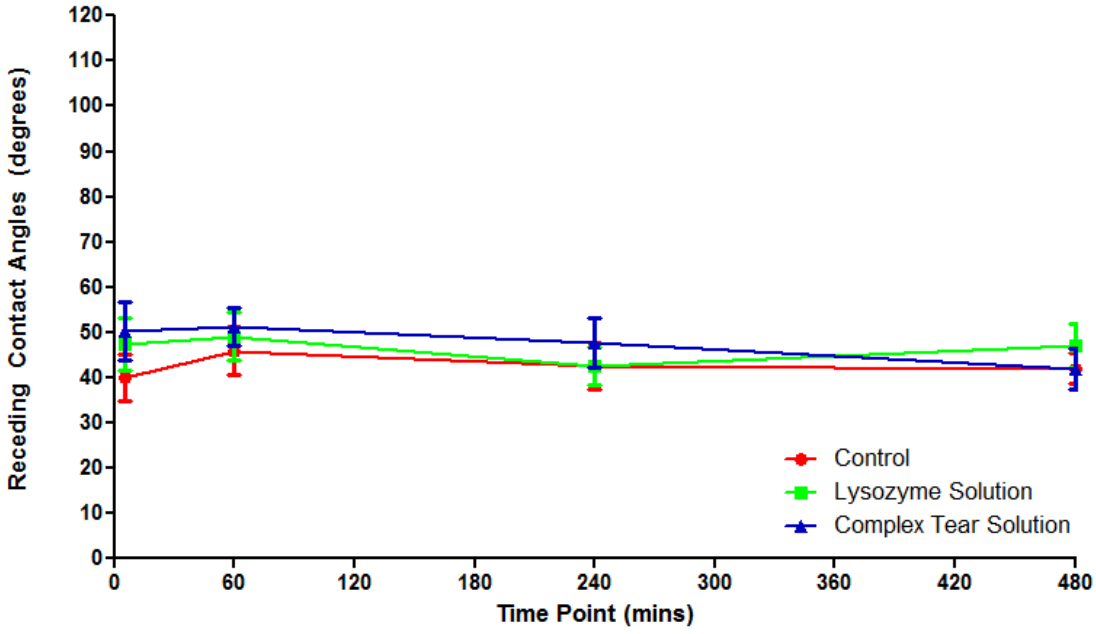
After lenses were exposed to the lysozyme solution, the CAs for m-nelfilcon A were statistically lower than the CAs for narafilcon A at the 4 hour and 8 hour time points ($p<0.001$). There was no statistical difference between CAs at any time point after omafilcon A and narafilcon A were exposed to lysozyme solution ($p>0.05$). There was also no statistical difference between CAs at any time point after m-nelfilcon A and omafilcon A were exposed to the lysozyme solution.

There was no statistical difference in CAs at any time point after omafilcon A, narafilcon A, and m-nelfilcon A were exposed to the complex tear solution for 5 minutes, 1 hour, and 4 hours ($p>0.05$). The CAs after m-nelfilcon A was exposed to the complex tear solution for 8 hours were statistically lower ($p<0.001$) than the CAs after narafilcon A and omafilcon A were exposed to the complex tear solution for 8 hours. CAs after narafilcon A, and omafilcon A were exposed to the complex tear solution for 8 hours, were not statistically different form each other ($p>0.05$).

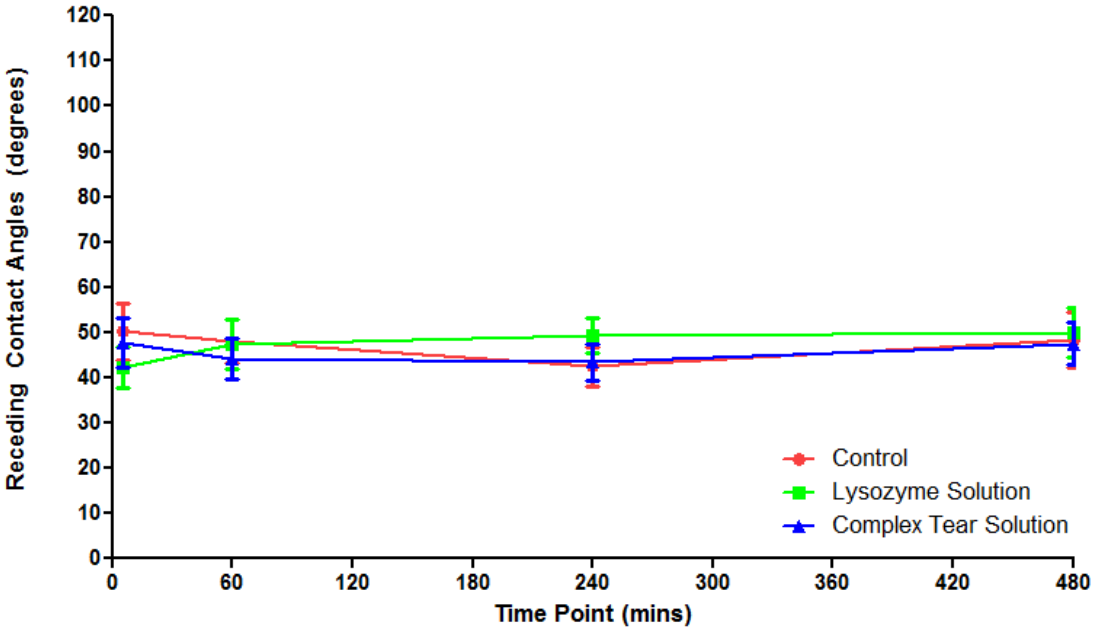
The receding CAs measured by the Wilhelmy balance method after the lenses were exposed to the control, lysozyme and complex tear solution for 5 minutes, 1hour, 4 hours, and 8 hours are shown in Figure 6-3.



a)



b)



c)

Figure 6-3: Receding CAs measured by the Wilhelmy balance method for a) omafilcon A b) m-nelfilcon A and c) narafilcon A, after being exposed to the control solution, lysozyme solution, and complex tear solution for 5 minutes, 1 hour, 4hours, and 8 hours in the model blink cell.

There was no statistical difference in receding CAs at any time point after omafilcon A was exposed to the control, lysozyme, and complex tear solutions ($p>0.30$).

The receding CAs after m-nelfilcon A were exposed to the control solution for 5 minutes were statistically lower than the receding CAs after m-nelfilcon A was exposed to the lysozyme and complex tear solutions for 5 minutes ($p<0.01$). There was no statistical difference between CAs after m-nelfilcon A was exposed to the control, lysozyme, and complex tear solutions for 1 hour, 4 hours, and 8 hours ($p>0.05$).

There was no statistical difference between receding CAs after narafilcon A was exposed to control, lysozyme, and complex tear solutions for 5 minutes, 1 hour, 4 hours, and 8 hours ($p>0.10$).

Comparing receding CAs between lenses, the CAs for narafilcon A after exposure to the control solution for 5 minutes were statistically higher than the CAs for omafilcon A and m-nelfilcon A after exposure to the control solution for 5 minutes ($p<0.001$). After lenses were exposed to the lysozyme solution, receding CAs for omafilcon A were significantly lower than the CAs for narafilcon A and m-nelfilcon A at all time points. Receding CAs for narafilcon and nelfilcon were not statistically different from each other ($p>0.05$). There was no statistical difference between the receding CAs at any time point when narafilcon A, m-nelfilcon A, and omafilcon A were exposed to the complex tear solution ($p>0.05$).

Comparing results from this study to the results from the validation study, there were some statistical differences between CAs measured in the two experiments.

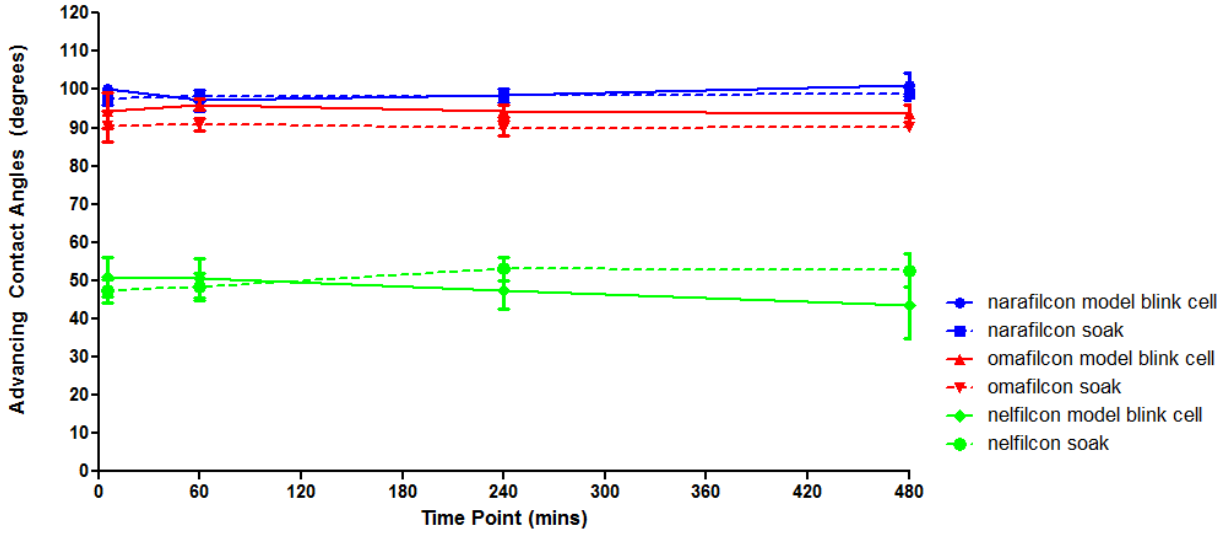


Figure 6-4: Comparing the advancing CAs measured by the sessile drop technique after omafilcon A, narafilcon A, and m-nelfilcon A were soaked in the control solution or exposed to the control solution in the model blink cell for 5 minutes, 1 hour, 4 hours, and 8 hours. (Please note, in the legend, "narafilcon model blink cell" indicates the CAs after narafilcon was placed in the model blink cell, and "narafilcon soak" indicates the CAs after narafilcon was soaked in solution for the respective time. This is the same for the other lens materials.)

Table 6-1: The advancing contact angles measured by the sessile drop technique after each lens materials is soaked in the control solution or exposed to the control solution in the model blink cell

	Contact Angles at each Time Point (degrees)			
Lens - Method	**5mins**	**1hr**	**4hr**	**8hr**
Narafilcon A-model blink cell	100.3	97.1	98.6	101.2
Narafilcon A-soak	97.6	98.2	98.4	98.9
p-value	0.3951	0.9773	1.0000	0.5588
Omafilcon A-model blink cell	94.5	96.1	94.4	93.8
Omafilcon A-soak	90.4	91.0	89.9	90.1
p-value	0.6311	0.3949	0.5129	0.7375
m-Nelfilcon A-model blink cell	50.8	50.6	47.5	43.5
m-Nefilcon A-soak	47.3	48.3	53.1	52.7
p-value	0.9739	0.9976	0.7970	0.3236

Figure 6-4 and **Error! Reference source not found.** compare the advancing CAs measured by the sessile drop technique after each lens material was soaked in the control solution for 5 minutes, 1 hour, 4 hours, and 8 hours (refer to section 5.4) and the CAs after the lenses were placed in the model blink cell and exposed to the control solution for 5 minutes, 1 hour, 4 hours, and 8 hours (Figure 6-1). Comparing the CAs between the two methods for any of the lens materials, showed no statistical difference at any time point ($p>0.30$).

The advancing CAs after lenses were soaked in lysozyme solution or exposed to lysozyme solution in the model blink cell are compared graphically in Figure 6-5.

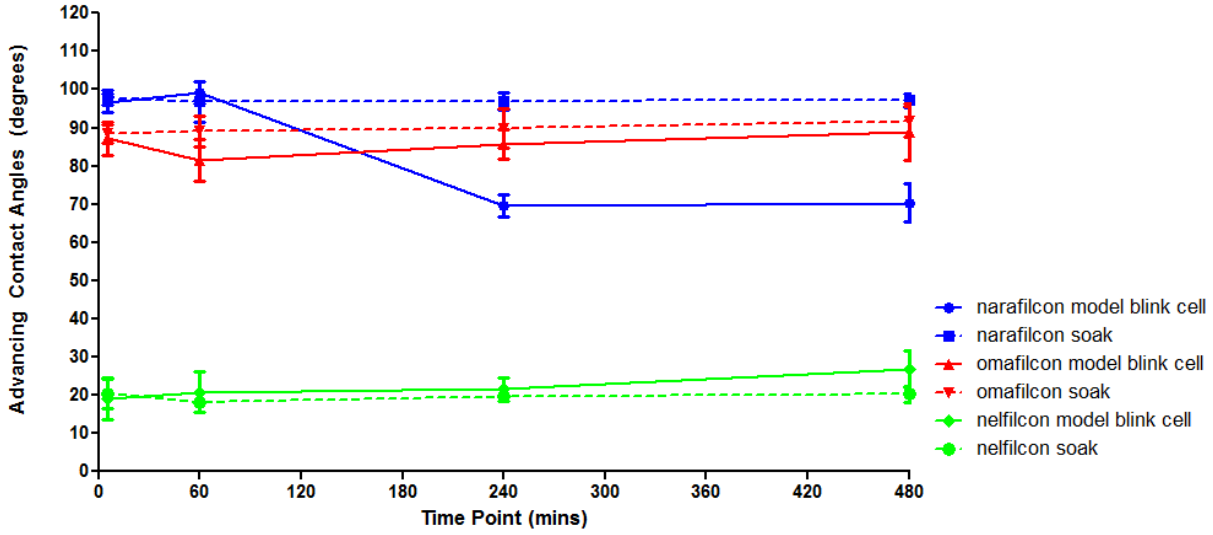


Figure 6-5: Comparing the advancing CAs measured by the sessile drop technique after omafilcon A, narafilcon A, and m-nelfilcon A were soaked in the lysozyme solution or exposed to the lysozyme solution in the model blink cell for 5 minutes, 1 hour, 4 hours, and 8 hours.

Table 6-2:The advancing contact angles measured by the sessile drop technique after each lens material was soaked in the lysozyme solution or exposed to the lysozyme solution in the model blink cell

	Contact Angles at each Time Point (degrees)			
Lens - Method	**5mins**	**1hr**	**4hr**	**8hr**
Narafilcon A-model blink cell	96.5	99.2	69.5	70.3
Narafilcon A-soak	97.8	96.8	97.0	97.2
p-value	0.9979	0.9434	0.0002	0.0002
Omafilcon A-model blink cell	87.2	81.4	85.7	89.0
Omafilcon A-soak	88.5	89.1	90.0	91.7
p-value	0.9987	0.1048	0.6306	0.9239
m-Nelfilcon A-model blink cell	18.9	20.8	21.6	26.9
m-Nefilcon A-soak	20.5	18.1	19.6	20.2
p-value	0.9987	0.9682	0.9939	0.3479

The CAs measured by either technique were not statistically different at any time point for omafilcon A and m-nelfilcon A. CAs after narafilcon A was exposed to the lysozyme solution for 4 hours and 8 hours were statistically lower than when narafilcon A was soaked in lysozyme solution for 4 and 8 hours ($p=0.0002$).

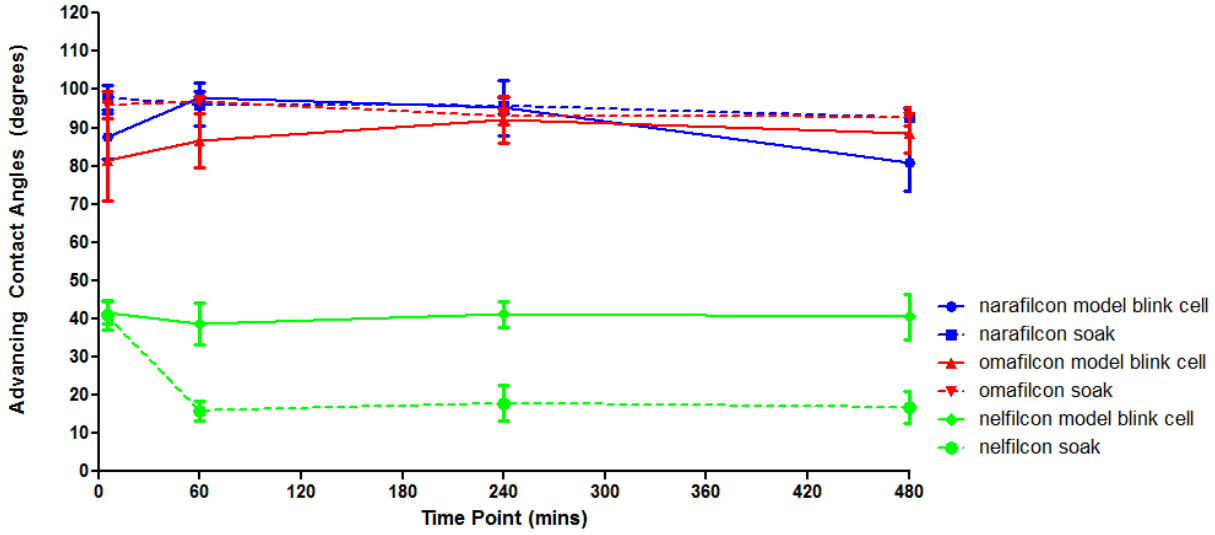


Figure 6-6: Comparing the advancing CAs measured by the sessile drop technique after omafilcon A, narafilcon A, and m-nelfilcon A were soaked in the complex tear solution or exposed to the complex tear solution solution in the model blink cell for 5 minutes, 1 hour, 4 hours, and 8 hours.

Table 6-3: The advancing contact angles measured by the sessile drop technique after each lens material was soaked in the control solution or exposed to the control solution in the model blink cell

	Contact Angles at each Time Point (degrees)			
Lens - Method	**5mins**	**1hr**	**4hr**	**8hr**
Narafilcon A-model blink cell	87.8	92.9	95.2	81.0
Narafilcon A-soak	97.9	96.1	95.8	92.6
p-value	0.3936	0.9936	1.0000	0.2642
Omafilcon A-model blink cell	81.6	86.7	92.2	88.7
Omafilcon A-soak	95.9	96.8	93.0	92.6
p-value	0.0348	0.1766	1.0000	0.9143
m-Nelfilcon A-model blink cell	41.6	38.8	41.2	40.6
m-Nefilcon A-soak	40.8	15.9	17.8	16.8
p-value	0.9991	0.0002	0.0002	0.0002

Figure 6-6 and Table 6-3 compares the advancing CAs measured by the sessile drop technique after lenses were soaked or exposed to the complex tear solution. There was no statistical difference in CAs at any time point between the two methods for narafilcon A ($p>0.20$). The CAs for omafilcon A after lenses were exposed to the complex tear solution for 5 minutes in the model blink cell, were statistically lower than the CAs measured after omafilcon A was soaked in the complex tear solution for 5 minutes ($p=0.0348$). The CAs for m-nelfilcon A after being soaked in the complex tear solution for 1 hour, 4 hours, and 8 hours were statistically lower than the CAs after m-nelfilcon A was exposed to the complex tear solution in the model blink cell for 1 hour, 4 hours, and 8 hours ($p=0.0002$). There was no statistical difference in CAs

when m-nelfilcon A was soaked in the complex tear solution compared to being exposed to the complex tear solution for 5 minutes (p>1.00).

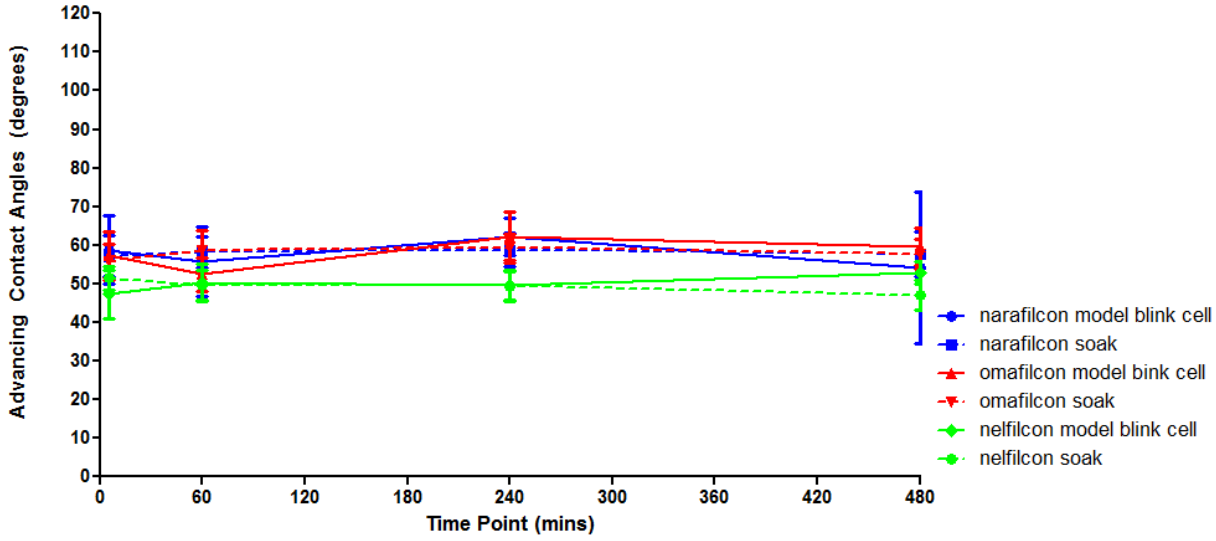


Figure 6-7: Comparing the advancing CAs measured by the Wilhelmy balance method after omafilcon A, narafilcon A, and m-nelfilcon A were soaked in the control solution or exposed to the control solution in the model blink cell for 5 minutes, 1 hour, 4 hours, and 8 hours.

Table 6-4: The advancing contact angles measured by the Wilhelmy balance method after each lens materials was soaked in the control solution or exposed to the control solution in the model blink cell

	Contact Angles at each Time Point (degrees)			
Lens - Method	**5mins**	**1hr**	**4hr**	**8hr**
Narafilcon A-model blink cell	58.8	55.9	62.2	54.1
Narafilcon A-soak	57.2	58.2	58.8	57.7
p-value	0.9994	0.9934	0.9403	0.9266
Omafilcon A-model blink cell	57.2	52.4	62.0	59.6
Omafilcon A-soak	55.8	58.7	59.4	57.7
p-value	0.9964	0.0500	0.8558	0.9690
m-Nelfilcon A-model blink cell	47.4	50.3	49.5	52.7
m-Nefilcon A-soak	51.4	49.6	49.4	48.7
p-value	0.3036	0.9999	1.0000	0.3083

Figure 6-7 and Table 6-4 compare the advancing CAs measured by the Wilhelmy balance method after each lens type is either soaked in the control solution or exposed to the control solution in the model blink cell. There was no statistical difference in CAs between methods for any of the lens types at any time point ($p>0.30$).

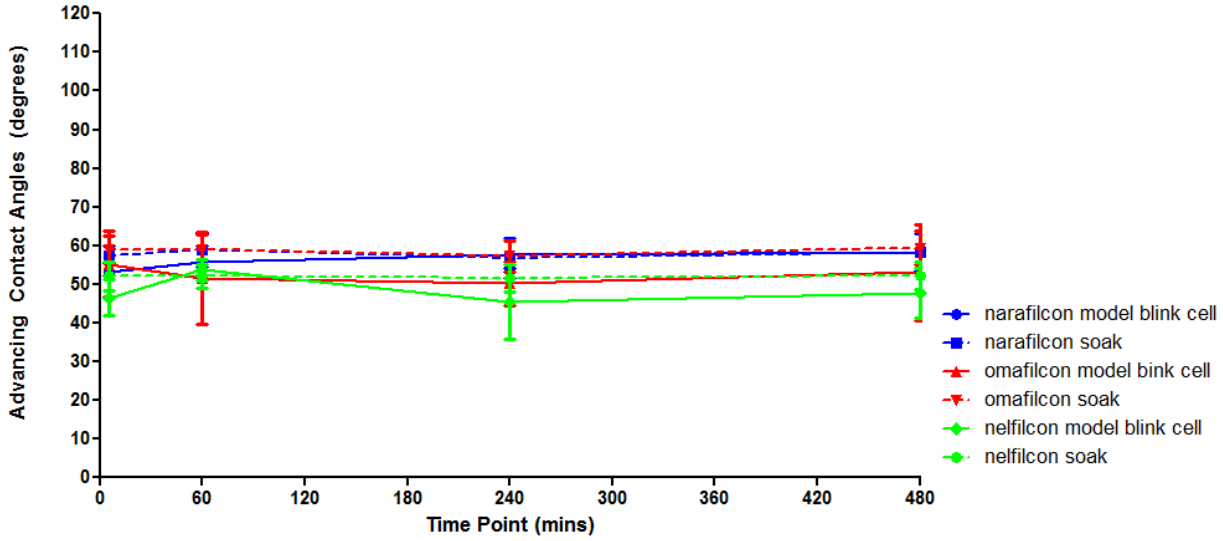


Figure 6-8: Comparing the advancing CAs measured by the Wilhelmy balance method after omafilcon A, narafilcon A, and m-nelfilcon A were soaked in the lysozyme solution or exposed to the lysozyme solution in the model blink cell for 5 minutes, 1 hour, 4 hours, and 8 hours.

Table 6-5: The advancing contact angles measured by the Wilhelmy balance method after each lens material was soaked in the lysozyme or exposed to the lysozyme solution in the model blink cell

	Contact Angles at each Time Point (degrees)			
Lens - Method	**5mins**	**1hr**	**4hr**	**8hr**
Narafilcon A-model blink cell	53.1	55.7	57.6	58.3
Narafilcon A-soak	57.3	58.9	56.7	58.2
p-value	0.5437	0.8398	0.9999	1.0000
Omafilcon A-model blink cell	55.1	51.6	50.1	53.1
Omafilcon A-soak	58.9	59.0	57.2	59.4
p-value	0.7624	0.0809	0.0995	0.1885
m-Nelfilcon A-model blink cell	46.5	53.6	45.4	47.8
m-Nefilcon A-soak	52.1	52.1	51.6	52.2
p-value	0.1249	0.9930	0.0643	0.3560

Figure 6-8 and Table 6-5 compare the advancing CAs when each lens type is either soaked in the lysozyme solution or exposed to the lysozyme solution in the model blink cell. There was no statistical difference in CAs between methods for any of the lens types at any time point ($p>0.06$).

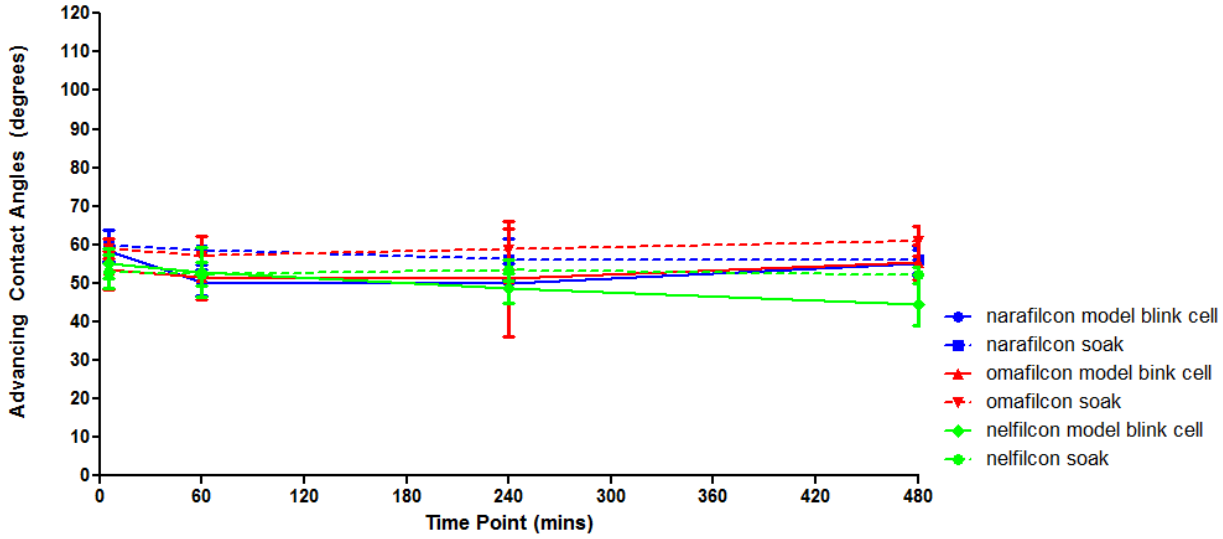


Figure 6-9: Comparing the advancing CAs measured by the Wilhelmy balance method after omafilcon A, narafilcon A, and m-nelfilcon A were soaked in the complex tear solution or exposed to the complex solution in the model blink cell for 5 minutes, 1 hour, 4 hours, and 8 hours.

Table 6-6: The advancing contact angles measured by the Wilhelmy balance method after each lens material was soaked in the complex tear solution or exposed to the complex tear solution in the model blink cell

	Contact Angles at each Time Point (degrees)			
Lens - Method	**5mins**	**1hr**	**4hr**	**8hr**
Narafilcon A-model blink cell	58.4	50.1	50.0	55.2
Narafilcon A-soak	59.8	58.5	56.2	56.1
p-value	0.9753	0.0002	0.0041	0.9986
Omafilcon A-model blink cell	53.4	51.6	51.1	55.4
Omafilcon A-soak	59.0	57.1	58.8	60.9
p-value	0.4177	0.4370	0.0982	0.4605
m-Nelfilcon A-model blink cell	55.1	52.8	48.7	44.5
m-Nefilcon A-soak	52.9	52.3	53.4	52.1
p-value	0.8223	0.9999	0.0597	0.0005

Figure 6-9 and Table 6-6 compare the advancing CAs between methods when each lens type is either soaked in the complex tear solution or exposed to the complex tear solution in the model blink cell. There was no statistical difference in CAs between methods for the omafilcon A lens material at any time point ($p>0.10$). Advancing CAs after narafilcon A was soaked in the complex tear solution for 1 hour and 4 hours were statistically higher than the CAs after narafilcon A was exposed to the complex tear solution for 1 hour and 4 hours ($p<0.005$). The CAs after m-nelfilcon A was soaked in the complex tear solution for 8 hours were statistically higher than the CAs after the lens materials was exposed to the complex tear solution in the model blink cell for 8 hours ($p=0.0005$).

Comparing receding CAs between methods, there was no statistical difference in receding CAs for narafilcon A and m-nelfilcon A at any time point after lenses were soaked or exposed the control solution ($p>0.05$). The receding CAs for omafilcon A were statistically higher at all time points after being soaked in the control solution ($p<0.05$). There was no statistical difference in receding CAs for narafilcon A and m-nelfilcon A at any time point after lenses were soaked or exposed to the lysozyme solution ($p>0.05$). The receding CAs for omafilcon A were statistically higher at all time points after being soaked in the lysozyme solution ($p<0.01$). Again, there was no statistical difference between CAs for narafilcon A and m-nelfilcon A after the lenses were soaked or exposed to the complex tear solution for 5 minutes, 1 hour, 4 hours, and 8 hours ($p>0.05$). The receding CAs after omafilcon A was soaked for 5 minutes, 1 hour, 4 hours, and 8 hours, were statistically higher than the CAs after omafilcon A was exposed to the complex tear solution in the model blink cell for the same time points ($p<0.05$).

6.5 Discussion and Conclusion

Contact lens wear typically has a negative effect on the stability and integrity of the tear film, by affecting the remoistening of the cornea by the tear film, and altering pre and post lens tear exchange.[5] Consequently these alterations to the tear film impact the performance characteristics of the contact lenses, resulting in reduced comfort, reduced visual acuity, and inflammation, all of which may be linked to deposition of tear components on the surface of the lens material.[68, 157, 158]

As mentioned previously, the majority of in vitro studies looking at the deposition of tear components on the lens surface, dope the lenses in different tear solutions. These studies have

shown differences in deposition over time, however none of them have taken into account the drying and rehydrating effect of the tear film on the surface of the lens material that occurs in vivo. In this study, the lenses were placed in a system (model blink cell) in which the lens materials were exposed to different tear solutions and then quickly exposed to the air, much like what happens in-eye.

The CAs after omafilcon A was exposed to the control solution were slightly higher compared to the CAs after omafilcon A was exposed to the other two tear solutions (Figure 6-1a). The wettability increased slightly, exhibited by lower CAs, after omafilcon A was exposed to the lysozyme and complex tear solutions with CAs being the lowest with exposure to the lysozyme solution. Similar results for omafilcon A were found when measuring the advancing CAs using the Wilhelmy balance method (Figure 6-2a). These CAs were statistically lower than the CAs measured using the sessile drop technique which as discussed previously, was probably due to the differences in lens preparation between techniques before CA analysis. However, despite the differences in CAs by the two methods, there again was little impact of tear components on the wettability of the lens material at all time points. Similarly, with CA analysis by the sessile drop technique, CAs measured by the Wilhelmy balance method after omafilcon A was exposed to the control solution were higher compared to the CAs after omafilcon A was exposed to the lysozyme and complex tear solutions. Again, CAs were the lowest after omafilcon A was exposed to the lysozyme solution.

The lower CAs measured by both methods after exposure to the lysozyme solution could be attributed to a small amount of lysozyme depositing onto the lens surface. Omafilcon A is a non-ionic lens material (FDA group II) thus the little attraction of lysozyme to the lens surface would not be due to a charge attraction. Lysozyme has outer hydrophilic moieties which would

be attracted to the hydrophilic HEMA polymers, which would be exposed at the lens surface when the lens was submerged into the solution, allowing the lysozyme to bind. This relatively small amount of lysozyme deposited on the lens surface is supported by results from other experiments looking at the deposition of proteins onto the surface of omafilcon A and other FDA group II lenses.[73, 76] Looking at the receding CAs measured by the Wilhelmy balance method there was no statistical difference between CAs after omafilcon A was exposed to any of the three solutions (Figure 6-3a).

The advancing CAs measured by the sessile drop technique for m-nelfilcon A were relatively lower than the advancing CAs for the other two lens materials (Figure 6-1b), which was similar to the results found in Chapter 4. The CAs after m-nelfilcon A was exposed to the lysozyme solution were significantly lower than the CAs after m-nelfilcon A was exposed to the control and complex tear solution, suggesting that lysozyme deposition onto the lens surface was maximal with the lysozyme only solution. The CAs after m-nelfilcon A was exposed to the complex tear solution were slightly lower than the CAs after m-nelfilcon A was exposed to the control solution, and slightly higher than CAs after exposure to the lysozyme solution. This result may have been due to competitive binding of the components in the complex tear solution to the lens surface, which would have limited the deposition of lysozyme on the material. Similarly to omafilcon A, the advancing CAs measured by the Wilhelmy balance method after m-nelfilcon A was exposed to all the solutions were not statistically differently from each other. The CAs after m-nelfilcon A were exposed to the lysozyme solution were slightly lower than the CAs after m-nelfilcon A was exposed to the other two solutions, once again probably due to deposition of lysozyme on the surface of the lens material. There was little effect of tear components on the receding angles of m-nelfilcon A. As mentioned in Chapter 4, m-nelfilcon A

is composed of PVA. A study by Wang et al[159] investigated the interaction of PVA and lysozyme and found that PVA and lysozyme form a complex which did not alter the activity of lysozyme. This complex could also form with lysozyme and the PVA in the lens material, accounting for the significant increase in wettability of m-nelfilcon A after exposure to the lysozyme solution.

The advancing CAs for narafilcon A measured by the sessile drop technique after the lenses were exposed to the control solution were higher than the CAs after narafilcon A was exposed to the lysozyme and complex tear solutions (Figure 6-1c). The CAs after narafilcon A was exposed to the lysozyme solution for 4 hours and 8 hours were significantly lower than the other CAs for narafilcon A. Again, this increase in wettability was probably due to lysozyme depositing onto the lens surface. The CAs after narafilcon A was exposed to the complex tear solution for 4 hours and 8 hours were lower than the CAs after exposure to the control solution but higher than the CAs after exposure to the lysozyme solution. The slight decrease in CAs is due to the deposition of proteins on the lens surface but not as low as the CAs after exposure to the lysozyme solution, due to the competitive binding of components in the complex tear solution. There was no statistical difference in advancing CAs (Figure 6-2c) or receding CAs (Figure 6-3c) measured by the Wilhelmy balance method after narafilcon A was exposed to the three solutions.

Other in vitro deposition studies have reported significantly lower amounts of protein deposition on silicone hydrogel lens materials compared to deposition onto conventional hydrogel materials.[61, 76, 160] Evaluating the results of CA analysis by the sessile drop technique (Figure 6-1), there appeared to more lysozyme deposition on the narafilcon A lens material compared to the omafilcon A lens material, which is interesting considering that results from deposition studies indicate that omafilcon A accumulates more protein on the lens surface

compared to deposition on materials similar to narafilcon A, galyfilcon A and senofilcon A.[76, 160] A study investigating the location of protein accumulation in the lens material indicated that tear components tend to accumulate in the bulk of omafilcon A rather than accumulate at the surface. This could account for the little change in surface wettability of omafilcon A.[160] This study also demonstrated that the little amount of protein that deposited on galyfilcon A and senofilcon A accumulated at the surface of the lens materials.[160] If deposition is similar for narafilcon A, then accumulation of proteins at the lens surface could account for the observed change in wettability. The change in wettability of narafilcon A could also be explained by the drying-hydrating effect of the model blink cell. Lysozyme has been reported to be primarily denatured onto the surface of silicone hydrogel lens materials.[76, 79, 154, 161] During the drying of the lens surface in the model blink cell, lysozyme may denature and fix onto the surface of the narafilcon A lens, which would form a substrate for protein build-up, subsequently causing the observed change of surface wettability. However, when proteins denature, typically the interior hydrophobic moieties become exposed, which theoretically should lead to a decrease in wettability or higher CAs. Thus, the denaturing of proteins on the surface of narafilcon A leading to improved wettability appears unlikely.

Interesting to note, there was very little difference in advancing CAs measured by the Wilhelmy balance method for any of the lens types after exposure to the tear solutions. All the lens materials were still hydrated before CA analysis which may be a reason for the little differences in CA analysis between lens materials. As seen in Chapter 4, there appears to be little impact of the surface characteristics of lenses on the force required to move the lens material in and out of probe fluid, and consequently little impact on the surface CA. This trend appears to be consistent when looking at the impact of tear components on the wettability of the lens materials

evaluated by the Wilhelmy balance method. The amount of time the lenses were exposed to the tear components may have been too short to allow enough accumulation of tear components onto the lens surfaces to be detectable by the Wilhelmy balance method. In the study by Subbaraman et al,[76] after 12 hours of doping lenses in a lysozyme solution, there was approximately 11µg of lysozyme deposited onto or into omafilcon A lens material and less than 2µg of lysozyme deposited onto galyfilcon A and senofilcon A. Dramatic increases in deposited lysozyme on the lens materials was not seen until doping for approximately 7 days. If lenses were exposed to the tear solutions in the model blink cell for longer periods of time, accumulation of tear components on the lens surface may be detectable by a change in wettability measured by the Wilhelmy balance method.

As mentioned in Chapter 4, the low advancing CAs, low hysteresis, and added wetting agents to the blister solution of the m-nelfilcon A lens material theoretically should enhance the comfort of the lens material compared to narafilcon A and omafilcon A lens materials. However, m-nelfilcon A appears to have more deposited protein on the surface compared to narafilcon A and omafilcon A. Studies have reported that deposition can lead to decreased visual acuity, decreased comfort, and inflammation in-eye,[68, 162, 163] however all of these studies observed these adverse reactions after at least 1 month of lens wear. A study by Donshik and Porazinski[71] looked at the incidence of giant papillary conjunctivitis (GPC) due to contact lens wear. Participants wore lenses on a daily to 4 week replacement schedule. Results showed that the incidence of GPC was 36% in patients who wore their lenses for 4 weeks and <4.5% in patients who wore their lenses for less than 4 weeks.[71] Based on the results of these studies, it would be unlikely that the deposition of tear components onto the surface of the m-nelfilcon A would cause adverse reactions in-eye, as it is worn as a daily disposable lens.

Comparing results of this chapter to those in Chapter 5, there were some differences in CAs between the soaking and model blink cell methods. There were no differences in CAs measured by the sessile drop technique between the two experimental methods using the control solution for omafilcon A, narafilcon A, and m-nelfilcon A. The CAs for omafilcon A and m-nelfilcon A after exposure or soaking in the lysozyme solution were not statistically different. The CAs for narafilcon A after exposure to the lysozyme solution in the model blink cell for 4 hours and 8 hours were lower than the CAs when narafilcon was soaked in lysozyme solution for 4 hours and 8 hours. As mentioned previously, the lower CAs after being placed in the model blink cell may be due to the hydrating-drying effect in the model blink cell causing a layer of lysozyme to deposit on the lens surface. The CAs after narafilcon A was soaked in the lysozyme solution exhibited little to no deposition on the surface of the lens material which is further supported by other in vitro studies looking at deposition on silicone hydrogel lenses.[61, 76, 79]

There was little impact of tear components on the surface wettability measured by the sessile drop technique, of narafilcon A and omafilcon A after the two lens materials were either soaked in the complex tear solution or exposed to the complex tear solution in the model blink cell. The CAs assessed by the sessile drop technique after m-nelfilcon A lenses were soaked in the complex tear solution was significantly lower at 1hour, 4 hour, and 8 hour time points, than the CAs after m-nelfilcon A was exposed to the complex tear solution in the model blink cell. Soaking of the lens in the solution allows a longer exposure time of the lens material to the tear components leading to enhanced deposition leading to the increased wettability.

There was no difference in receding CAs for m-nelfilcon A and narafilcon A between the two experimental methods. Receding CAs for omafilcon A were significantly higher when the lenses were soaked in any of the three solutions over all time points. This indicates that

hysteresis is decreased after omafilcon A was soaked in each solution. When omafilcon A was soaked in the two tear solutions, more protein may have accumulated in the lens material compared to the amount that may have accumulated after the lens was placed in the model blink cell. The more proteins accumulated after soaking, could have possibly prevented the rapid polymer rotation at the lens surface, thus reducing the amount of hysteresis. Similarly when omafilcon A was soaked in the control solution, disinfecting agents may have deposited on the lens surface, again prevented rapid polymer rotation and reducing the hysteresis of omafilcon A. However, there is no experimental evidence supporting these explanations and thus more research would need to be conducted exploring the reasoning behind the difference in hysteresis for omafilcon A after being soaked in solution compared to exposure to the solution in the model blink cell.

Overall, tear components may alter the surface wettability of contact lenses depending on the lens material, time of exposure to the tear solutions, and method used to evaluate the lens wettability. In this study, deposition of tear components, particularly lysozyme, was higher on the m-nelfilcon A material, as exhibited by the enhanced wettability after exposure to the lysozyme and complex tear solutions. The differences from this experiment using the model blink cell and the experiment in Chapter 5 indicate that a drying-hydrating mechanism can alter the deposition of tear components on the lens surface. Thus, the model blink cell may be a useful tool in future in vitro deposition studies.

7. Summary and Future Work

7.1 Overall Summary and Conclusions

This thesis has provided some answers to questions regarding factors that can influence wettability analysis of contact lenses in vitro. It also validated another method that could be used to investigate the effect of deposition of tear components on the surface wettability of contact lenses. Chapter 2 first demonstrated that blot-drying contact lenses on different drying materials for a range of time points can cause variation in advancing CAs measured by the sessile drop technique. Results demonstrated that lenses that were blot dried for approximately 20 seconds on lens paper showed the least variation in CAs measured by the sessile drop technique. This drying method was used for the remainder of the CA analyses by the sessile drop technique in this thesis. Chapter 2 also demonstrated that different methods of measuring contact lens wettability in vitro can produce different CAs. There were statistical differences in the CAs measured using the sessile drop technique and the Wilhelmy balance method. These results emphasize that the method used to measure the wettability of contact lenses in vitro should always be stated. The second part of Chapter 2 also demonstrated that the advancing CAs of contact lenses can be influenced by components in the blister pack solution, more specifically, components in the blister solution can enhance the wettability of contact lenses.

Chapter 3 looked at the physical properties of blister pack solution of silicone hydrogel lenses. The blister solutions that had added surfactants and wetting agents exhibited lower ST's and higher viscosities compared the other blister solutions. These differences should theoretically

improve the wettability of the contact lenses by improving the spreading and retention time of the tear film on the surface of the lens.

Chapter 4 looked at the wettability and physical properties of DD contact lenses.The effect of the blister solution on DD lenses is likely more important than it is for SH materials, as it is the primary component that will affect the comfort and stability of the tear film on initial insertion. Much like in Chapter 3, the blister solution with the added surfactants and wetting agents had a lower ST and higher viscosity, which should improve the initial wettability and subsequently initial comfort of the lens in-eye. The wettability of the DD lenses varied between lens materials. The lens material with the physically altered blister pack solution had the highest wettability among the lens materials tested.

Chapters 5 and 6 looked at the deposition of tear components and the impact of deposition on the wettability of three of the DD lens materials investigated in Chapter 4. The lens materials were all soaked in a control, lysozyme, and complex tear solutions and the wettability of the lens materials measured after 4 different time points. The m-nelfilcon A lens materials was the only material that apparently deposited proteins on the lens surface, as shown by the lower CAs after the lens material was soaked in the lysozyme and complex tear solutions. A similar experiment was conducted in Chapter 6, except lenses were not soaked in the tear solutions but rather exposed to the tear solutions in a model blink cell to see if there was any difference in deposition of tear components from the drying-hydrating environment in the model blink cell. There were differences in deposition of the proteins on the lens surfaces after being placed in the model blink cell compared to being soaked in the tear solutions. This result demonstrated that drying of tear solutions on the lens surface does impact the deposition of tear components on the lens surface.

General conclusions from this thesis can be summarized in the following points:

- Preparation of the lens material prior to CA analysis particularly by the sessile drop technique can lead to variation in results, thus the same procedure should be undertaken before CA measurement.
- Different methods of measuring the wettability of contact lenses in vitro produce different results, thus the method used for CA analysis should always be stated.
- Blister solutions can alter the wettability of contact lenses.
- Adding surfactants and wetting agents to the blister solutions could alter the physical properties of the blister solutions, which can also alter the comfort of the lens in-eye.
- Deposition of tear components on the lens surface can alter the wettability of contact lenses.
- The model blink cell may be a useful tool to use for deposition studies, as there are differences in protein deposition on the lens surface in a hydrating-drying environment compared to just soaking the lenses in the solutions.

7.2 Future Work

As determined in this thesis, the model blink cell can be used for future deposition experiments. However, some improvements to the model blink cell needs to be conducted. The model blink cell is not a sterile system in that it opens to the outside air and contaminates the solution inside the model blink cell. Thus solutions that were sterile prior to being placed in the model blink cell were contaminated during use in the model blink cell. As well, the humidity inside the model blink cell varied which could potentially affect the resulting CAs and deposition of the tear components. However the study in this thesis which used the model blink cell,

humidity inside the model blink cell varied between 11% and 71% and had no impact on the resulting CAs. However, before use of the model blink cell for further use, the model blink cell should be remade to be a sterile system with controlled humidity in the system.

It would be interesting to measure the wettability of lenses after they have been exposed to tear components in the model cell for longer periods of time than 8 hours. The etafilcon A lens material has been shown to accumulate large amounts of deposited protein on the lens surface in small amounts of time and it would be interesting to see if similar amounts of protein deposit on the lens surface after being placed in the model blink cell for varying time points

The experiment in Chapter 6 only investigated the effect deposition had on the wettability of the lens materials. It would be interesting to actually quantify the amount of protein that accumulated on the lens material after lenses were placed in the model blink cell and even determine the activity of the protein accumulated on the lens surface. Another interesting experiment would be to place different lens materials in the model blink cell for varying time points, and then investigate where in the lens material the tear components accumulated (ie. at the lens surface of in the bulk of the lens material). This could be done by using confocal microscopy which has been used previously for investigation of deposition of tear components in lens materials.[160, 164]

It would be interesting to compare the results of the experiments in Chapters 5 & 6 to results from an ex vivo study in which participants wore the m-nelfilcon A, omafilcon A, and narafilcon A and the wettability assessed after 5 minutes, 1 hour, 4 hours, and 8 hours of wear time. This could be taken even further and an in vivo experiment could be conducted in which again, participants wear m-nelfilcon A, omafilcon A, and narafilcon A lenses and wettability assessed in vivo. A method would need to be developed in which CAs would be assessed on the

lens surface in-eye. Currently there are techniques that have been developed to assess CAs of contact lenses in vivo, however these techniques are manual or use solutions with a higher viscosity to measure the CA (techniques not yet published). Using a solution with a different viscosity than that already used for in vitro studies negates the capacity of being able to compare the CA results in vivo with those already measured in vitro. Thus, a method measuring CAs in vivo in which is automated and uses HPLC water to drop on the lens surface would need to be developed.

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