Examination of Contact Lenses and Dry Eye Using Evaporimetry

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

Introduction

Evaporimetry is a non-invasive technique used to assess the stability of the tear film. The test measures the rate of tear evaporation, and has been used to investigate dry eye, contact lenses, and the efficacy of different treatments for dry eye and contact lens (CL) discomfort. There is currently only one modified dermatological instrument available for practitioners, and experts have stated a need to develop evaporimeters suitable for use in clinical practice. The purpose of this thesis was two-fold, namely to (i) evaluate the commercially available evaporimeter, and (ii) describe the design, development, and testing of a novel evaporimeter.

Overall Aims

- To assess the calibration of the only commercially available evaporimeter (Eye-VapoMeter), and to investigate its ability to detect *in vitro* differences between soft CLs;
- To describe the development, *in vitro*, and *in vivo* testing of a novel binocular evaporimeter.

Methods and Materials

- *In vitro* differences between 7 silicone hydrogel and 9 hydrogel CLs were measured with the Eye-VapoMeter. The change in evaporation rate per minute was calculated from the slope of the evaporation rate over time. Four sequential 10-minute time periods were investigated from 0 to 40 minutes;
- Calibration of the Eye-VapoMeter was investigated by simulating evaporation from different ocular surface areas and by modifying the air volume inside the evaporimeter goggle using two types of model eyes. The absolute evaporation rate was determined from the slope of water loss over time. The unadjusted evaporation rate from the instrument was measured with different areas and volumes inside the evaporimeter. A linear regression was used to determine the correction factor for each goggle volume based on the unadjusted evaporation rate and absolute evaporation rate;
- A novel binocular evaporimeter was developed to measure the tear evaporation rate (TER) from the ocular surface. *In vitro* testing of the new evaporimeter was performed using four

elliptical model eyes with different surface areas (1 to 2.5 cm^2 in 0.5 cm^2 steps) and air volumes within the evaporimeter. Measurements were recorded for each side of the goggle;

- *In vivo* pilot testing was performed by conducting a series of experiments on volunteers to determine the best way of performing evaporimetry with the new instrument. Measurements were taken with the eyes open and closed (n=5), with the effect of a liposomal spray (CALMO® Eye Spray), and with a single application of an artificial lubricant (Refresh Tears®) (n=5). Fixation was tested by comparing evaporation rates with the eyes open, and blinking normally in downgaze, primary gaze, and upgaze (n=1). Optimal blink rate was investigated using blink rates of three or five seconds in volunteers with self-reported dry eye (n=3);
- The effect of a lipid nano-emulsion was assessed. Thirty-six non-CL wearers were enrolled and screened. Twenty-one participants were suitable and classified as dry eye or non-dry eye using the Ocular Surface Disease Index (OSDI) and non-invasive break-up time (dry eye: OSDI ≥13 and break-up time ≤5 seconds in the worst eye). At the test visit, two baseline TERs were taken, 20 minutes apart. A single dose of Systane® Complete was instilled, and TER assessed at 10, 30, and 60 minutes post-instillation.
- The effect of CL wear was assessed. Twenty CL wearers were screened and classified using the Contact Lens Dry Eye Questionnaire (CLDEQ-8) as asymptomatic (CLDEQ-8<12) or symptomatic (CLDEQ-8≥12). Two baseline TERs were recorded after a 15-minute interval. Participants were randomized to wear delefilcon A in one eye and nesofilcon A in the other eye. TER was assessed after 15 minutes and 6 hours of CL wear.

Results

- *In vitro* measurements with the Eye-VapoMeter found a significant difference in evaporation rates reported for each 10-minute period for each CL. Evaporation rate varied with CL material, water content, and presence of an internal wetting agent;
- Calibration measurements showed that water loss occurred at a linear rate. Correction factors were calculated for the Eye-VapoMeter. All graphs of the correction factor and evaporimeter volume were fit with a second order polynomial non-linear regression;
- *In vitro* measurements with the novel evaporimeter measured a significantly lower evaporation rate with the smallest model eye compared to the larger ones, and a significantly lower evaporation rate for the 10 cm^3 volume compared to the 13 and 18.63 cm^3 volumes;

- Pilot testing demonstrated that the relative humidity (RH) significantly changed in each side of the goggle when the novel evaporimeter was placed over the open and closed eye. No significant differences in RH were detected between the goggles. The TER was significantly lower immediately after application of the liposomal spray compared to the second baseline measurement and 15 minutes after the spray was applied. Use of an artificial lubricant found significantly higher TER in both eyes after instillation compared to the first baseline measurement and 15 minutes post-instillation. Comparison of different positions of gaze revealed less change in RH over time in downgaze. Comparison of blink rate found that 2 out of 3 participants preferred a five second blink rate;
- Twenty people (10 non-dry eye, 10 dry eye) completed the lipid nano-emulsion study. Changes in TER were observed during the study. Nano-emulsion instillation produced an initial increase in TER after 10 minutes, and a reduction in TER after 30 minutes;
- Twenty people (10 asymptomatic, 10 symptomatic) completed the CL study. The TER was significantly higher after 6 hours of CL wear. No significant difference in TER was detected between the two groups, or between CL type (delefilcon A and nesofilcon A) after 6 hours of wear.

Conclusions

1. Using a new *in vitro* technique, the Eye-VapoMeter was able to detect differences in evaporation rate from a range of CLs differentiated by material, water content, and presence of wetting agent;
2. Calibration of the Eye-VapoMeter found the relationship between correction factor and volume was best fit with a second order non-linear regression;
3. A novel closed-chamber binocular evaporimeter was designed, developed, and tested;
4. *In vitro* testing of the novel evaporimeter detected lower evaporation rates with a smaller surface area and volume;
5. *In vivo* testing demonstrated that the novel evaporimeter was able to:
    a. measure higher TERs in dry eye participants compared to those without dry eye;
    b. measure significant decreases in TER following the instillation of a lipid eye drop;
    c. measure significantly higher TERs associated with CL wear.

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Dedication

To my parents and Danny. I cannot thank you enough for all that you have done for me.

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List of Abbreviations

°C	degrees Celsius
°F	degrees Fahrenheit
%	percent
ADDE	aqueous deficient dry eye
AER	absolute evaporation rate
ANOVA	analysis of variance
CLDEQ-8	Contact Lens Dry Eye questionnaire
CLEAR	Contact Lens Evidence-based Academic Reports
CL	contact lens
cm	centimeter
cm^2	square centimeter
cm^3	cubic centimeter
CORE	Centre for Ocular Research & Education
D	Diopter
DED	dry eye disease
DEWS	Dry Eye WorkShop
Dk/t	oxygen transmissibility
DS	diopter sphere
EDE	evaporative dry eye
et al.	et alia (“and others”)
EWC	equilibrium water content
FDA	Food & Drug Administration
g	gram

h	hour
Hy	hydrogel
IQR	interquartile range
ISO	International Organization for Standardization
K5M	Keratograph 5M
LLT	lipid layer thickness
logMAR	logarithm of the minimum angle of resolution
m^2	square meter
mg	milligram
MGD	meibomian gland dysfunction
MGYLS	meibomian gland yielding liquid secretion
MGE	Meibomian Gland Evaluator
min	minute
MLEO	Murphy Lab for Experimental Optometry
ml	milliliter
mm^2	square millimeter
n	sample size
NIKBUT	non-invasive Keratograph break-up time
NITBUT	non-invasive tear break-up time
nm	nanometer
OD	right eye
OS	left eye
OSDI	Ocular Surface Disease Index
OU	both eyes

p-value	probability value
PMMA	polymethyl methacrylate
PNG	portable network graphics
r	Pearson correlation coefficient
r_s	Spearman rank correlation coefficient
R^2	coefficient of determination
RGP	rigid gas permeable
RH	relative humidity
s	second
SD	standard deviation
SiHy	silicone hydrogel
TBUT	tear break-up time
TER	tear evaporation rate
TFOS	Tear Film & Ocular Surface Society
TMH	tear meniscus height
UER	unadjusted evaporation rate
µl	microliter
µm	micrometer (micron)
vs.	versus
W	Watt

Chapter 1
Literature Review

1.1 Introduction

Evaporimetry is a non-invasive method used to assess the stability of the tear film. The measurement monitors the change in relative humidity (RH) in front of the eye [1-4], to determine the rate of aqueous tear loss from the tear film [5-7] and the stability of the lipid layer [8-10]. The assessment of tear evaporation has been used to diagnose [11] and evaluate the efficacy of different treatments for dry eye disease (DED) [12-15]. Evaporimetry has also been used to examine the effect of different types of contact lenses on the tear film [16-18] and to investigate the effectiveness of dry eye treatments in contact lens (CL) wearers [19, 20]. Despite the technique previously being confined to use within research, one instrument was validated for ocular use in 2014 [21] and is now commercially available for practitioners to purchase.

This chapter will discuss the importance of the tear film and its role in preventing evaporation, describe different types of instrumentation that have been used to perform evaporimetry, and review factors that influence the rate of tear evaporation.

1.2 Tear Film

1.2.1 Structure

Wolff described the tear film as a three-layered structure composed of lipid, aqueous, and mucus layers [22]. The outermost lipid layer contains oils derived from the meibomian glands. Meibomian glands are modified sebaceous glands located in the upper and lower eyelid, posterior to the eyelashes. Meibum is released from the meibomian glands with each blink and blinking helps spread the lipid-rich secretion across the surface of the eye [10]. Lipids in the meibomian glands are primarily composed of sterol esters and wax esters, including cholesterol, fatty acids, and fatty alcohols [23-25]. The lipid layer contains a thick, outer non-polar phase on top of a single-layered polar phase [26]. The aqueous layer in the middle of the tear film contains watery secretions from the lacrimal glands, and the innermost mucus layer is comprised of secretions from the conjunctival goblet cells [22].

The three-layer concept of the tear film proposed by Wolff has since been replaced by the theory of a thin lipid layer that covers an aqueous-mucin gel phase, which is attached to transmembrane bound mucins in the glycocalyx (Figure 1-1) [26, 27]. The aqueous-mucin phase comprises the majority of the thickness of the tear film [27] and contains proteins, electrolytes, oxygen, glucose, and gel-forming mucins [27, 28]. The concentration of mucins increases towards the corneal epithelium [29] and the glycocalyx is tightly attached to the microvilli of the conjunctival and corneal epithelial cells [27, 28].

The average thickness of the tear film ranges from 2.7 to 46 µm [27, 30-38]. The mean thickness of the thin, outer lipid layer ranges from 13 to 170 nm [27, 36, 39-42], with a normal lipid layer thickness reported to be 32 to 46 nm [43] or 61.8 to 75.5 nm [41]. The thickness of the aqueous layer varies from 2.2 to 7 µm [28, 44] and the aqueous-mucin layer is 3.8 to 4.2 µm thick [42]. The mucin layer is 1 µm thick over the conjunctiva [45], although others have suggested that mucus accounts for the majority of the thickness of the tear film [31].

Variability in the reported mean thickness of the tear film can be due to the use of different instrumentation [46], the invasiveness of the test, and the duration between the last blink and the time of measurement [33]. Information on different measurement techniques to measure the thickness of the tear film can be found in a review by Bai and Nichols [46].

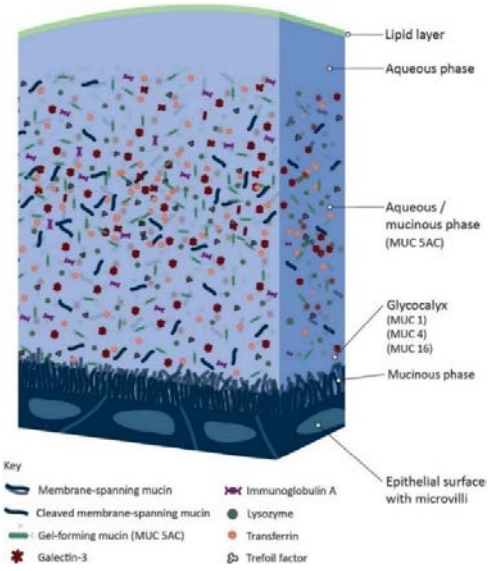

Figure 1-1: Cross-section of the structure of the tear film. Image from: Downie, LE et al. CLEAR - Anatomy and Physiology of the Anterior Eye. Cont Lens Anterior Eye 2021;44(2):132-156.

1.2.2 Function

The main functions of the tear film are to [47]:

- Provide a smooth optical surface between the cornea and air;
- Lubricate the eye to ensure the eyelids glide smoothly over the ocular surface during a blink;
- Mechanically remove dust and debris with each blink;
- Defend the eye against pathogens with the aid of proteins, antibodies, and phagocytes;
- Nourish the corneal epithelium with oxygen, glucose, and vitamins;
- Remove corneal waste products, including carbon dioxide and lactate.

Each layer of the tear film also has a specific role in supporting the tear film. The primary role of the lipid layer is to slow the evaporation of the inner layers of the tear film [28, 48-50], with the rate of evaporation occurring 4 [48, 49] to 17 times [50] faster when the lipid layer is absent. The primary function of the non-polar phase of the lipid layer is to control the rate at which water vapor, carbon dioxide, oxygen, and ions are lost, but also serves as a reservoir for non-polar lipids, such as wax and cholesterol esters [26]. The polar phase is crucial to creating a stable lipid layer by orienting the hydrophilic heads of molecules towards the aqueous phase [51]. The lipid layer is also an effective barrier for removing dust particles from the eye [28].

The aqueous component of the tear film lubricates the ocular surface, removes debris, nourishes the ocular surface epithelial cells, and contains antibacterial proteins, like lysozyme, to protect the eye against infection [22, 28]. The main function of mucin is to stabilize the tear film [52]. However, gel-forming mucins also lubricate the eye to decrease the amount of friction during a blink, smooth the optical surface, help remove debris and pathogens from the tear film, and aid in spreading the aqueous and lipid layers [27, 36, 52]. The underlying glycocalyx provides structural stability to the entire tear film [28].

Further information on the structure and function of the tear film can be found in the TFOS DEWS II Pathophysiology report [40] and the CLEAR – Anatomy and physiology of the anterior eye report [27].

1.3 Evaporimetry

1.3.1 Underlying Principles

Evaporation is a process that occurs when molecules leave the surface of a liquid and diffuse into the surrounding air [53, 54]. The rate of evaporation is dependent on the RH of the surrounding air. RH is the ratio of the actual vapor pressure to the saturation vapor pressure (Equation 1-1), or the ratio of vapor mass to the mass of saturated vapor at a particular temperature [54, 55]. The saturation vapor pressure is solely dependent on the temperature, and increases as the temperature increases [54]. In a closed system at 100% RH, the air is fully saturated and the rate of evaporation equals the rate of molecules condensing back into the liquid. However, in an open system evaporation will continue since the vapor molecules are not confined to an enclosed space and the majority will be lost to the ambient air.

$$relative\ humidity\ (\%) = \frac{actual\ vapor\ pressure}{saturation\ vapor\ pressure}\ x\ 100\%$$

Equation 1-1: Relative humidity formula as a percentage

1.3.1.1 Factors that Affect the Rate of Evaporation

1.3.1.1.1 Temperature

RH is strongly affected by the temperature [54]. The kinetic energy of a liquid molecule increases when heated, which raises the chances of the molecule being able to escape from the surface of the liquid into the surrounding air [56], and therefore increases the rate of evaporation.

1.3.1.1.2 RH

There is an inverse relationship between the rate of evaporation and RH, with slower rates of evaporation when the RH is high [53, 57]. Evaporation occurs at a slower rate because the air is more saturated under high levels of RH.

1.3.1.1.3 Surface Area

The rate of evaporation increases as the size of the surface area increases [53] because there is a greater amount of liquid exposed to air.

1.3.1.1.4 Air Flow

The rate of evaporation increases as the rate of air flow increases [53] because the air current carries liquid molecules away from the surface, reducing the RH of the air above the surface and promoting the evaporation of additional molecules [58].

1.3.2 Types of Evaporimeters

1.3.2.1 Types of Chambers

Evaporimeters have been commonly used in dermatology to measure the rate of trans-epidermal water loss from the skin and are classified as open-chamber, semi-open, or closed-chamber devices (Figure 1-2A) [59]. Open-chamber systems have an opening which constantly exposes the temperature and RH sensor to the ambient environment during the measurement [5]. In contrast, a closed-chamber system protects the sensor from the external environment once it has been placed over the surface to be examined (Figure 1-2B). Semi-open instruments contain an open grid that permits water to evaporate from the chamber, while simultaneously shielding the sensor from the ambient air flow [59].

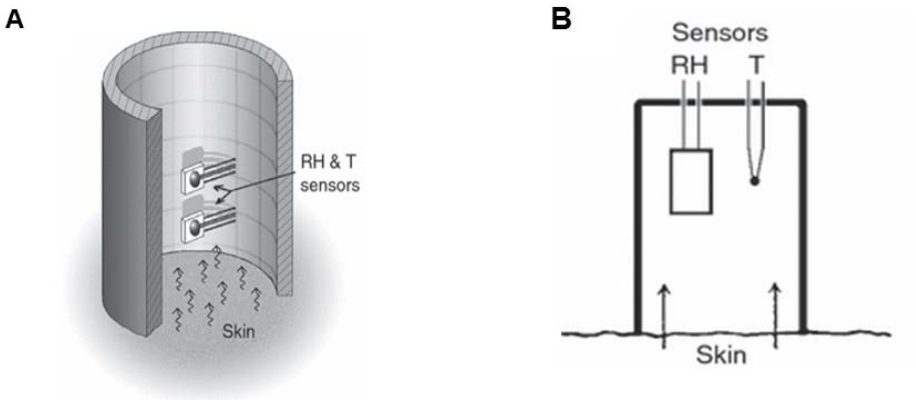

Figure 1-2: Schematic diagram of (A) an open-chamber and (B) a closed-chamber evaporimeter. In the open-chamber, the cross-section shows the instrument applied to the skin while the opposite end of the instrument remains exposed to the ambient environment. This open-chamber device uses two relative humidity (RH) and temperature (T) sensors to determine the rate of diffusion. In the closed-chamber, the open end of the instrument is placed against the skin and forms an enclosed system. A set of RH and T sensors are used to monitor the change in conditions inside the chamber. Images from Imhof, R.E. et al. Closed-chamber

transepidermal water loss measurement: microclimate, calibration and performance. Int J Cosmet Sci. 2009; 31(2):91-118.

A summary of the leading advantages and disadvantages of open-chamber and closed-chamber evaporimeters appears in Table 1-1. Both types are user-friendly [60, 61] and are available as portable devices [62, 63]. Although the compact, self-contained nature of one evaporimeter led to unsupported claims that they can be used in different positions [63, 64], this was later disproven [60].

The main advantage of an open-chamber device is that it allows for continuous measurement of the rate of water loss from the skin [62, 63]. However, open-chamber instruments need time to stabilize before a measurement [65] and measurements are only reproducible once the sensors have warmed up to the temperature of the skin [66]. Open-chamber devices are very sensitive to small fluctuations in the ambient environment (such as air currents from air conditioning, doors opening, breathing, or talking), which requires them to be tested in an area that is unaffected by air flow. They also tend to be expensive, and can be more difficult to transport [62, 63]. One group also reported that an open-chamber evaporimeter produced lower rates of evaporation when high amounts of water loss were tested in comparison to a closed-chamber device [64]. However, the researchers were unable to explain why this discrepancy occurred.

The advantages of closed-chamber evaporimeters are that they are not sensitive to external air flow [62, 63], require less time to take a measurement, are compact and portable, and are a less expensive option [62]. However, since the device is enclosed, continuous measurements cannot be taken because water vapor builds up in inside the system [62, 67]. The RH initially rises slowly, then increases at a linear rate [67]. This results in the instrument needing a break between each measurement to allow the RH inside the instrument to return to ambient conditions [62, 67]. One type of closed-chamber evaporimeter (VapoMeter, Delfin Technologies Ltd., Kuopio, Finland) automatically resets after a two-minute interval, although it has been suggested that this may not be an adequate amount of time for the RH to return to baseline levels if the rate of evaporation is high [60]. The closed chamber also interferes with the normal rate of water loss from the skin [59], although this can be minimized by keeping the measurement short [64].

Table 1-1: Advantages and disadvantages of open-chamber and closed-chamber evaporimeters used in dermatology

	Open-chamber	Closed-chamber
Advantages	Continuous measurements [62]	Faster measurement time [62] Not sensitive to external air flow from environment or the body [62, 63]
Disadvantages	Requires time to stabilize before measurement [65] Sensitive to environmental conditions [62] Underestimates high evaporation rates [64]	Requires time to return to ambient conditions before next measurement [62, 67] Could saturate under high rates of water loss [60] Blocks normal water loss [59, 64] Single measurement [60]

Semi-open chamber evaporimeters are beneficial as they overcome problems encountered with both open- and closed-chamber instruments. The covered opening at the top of the instrument permits evaporated water to escape from the system to prevent saturation [59]. Since the system is partially open, the instrument does not interfere with the normal rate of skin evaporation [59] and allows for continuous measurements to be recorded [68]. The addition of a grid also prevents air currents in the ambient environment from affecting the sensors. However, the system is not fully portable [59] and measurements can have large standard errors of the mean [69].

1.3.2.2 Types of Ventilation

Evaporimeters are also classified on whether ventilation is supplied to the chamber. In dermatology, ventilated instruments add a dry gas to a chamber at predetermined humidity level under a controlled speed [70]. The RH is simultaneously measured as it enters and exits the chamber (Figure 1-3) [70]. In contrast, unventilated instruments do not incorporate a method of ventilation. Unventilated evaporimeters either measure the change in water vapor inside a chamber using a hygrometer, or by gravimetrically determining the increase in mass of a hygroscopic salt over time [70].

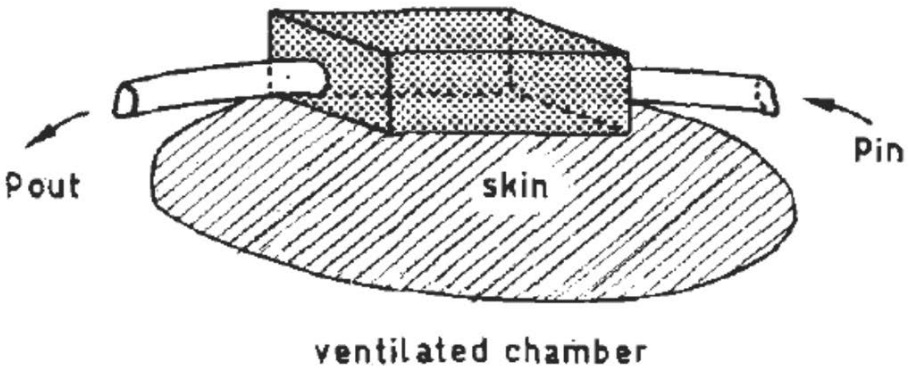

Figure 1-3: Schematic diagram of a ventilated chamber. The vapor pressure of a gas (P_{in}) is measured as it enters the chamber on the right side, at a known velocity, and passes over the skin. The vapor pressure is recorded as it exits (P_{out}) on the left side. Image from Nilsson, GE. Measurement of water exchange through skin. Med Bio Eng Comput 1977; 15(3):209-218.

A summary of the key advantages and disadvantages of ventilated and unventilated evaporimeters is shown in Table 1-2. The main advantage of a ventilated chamber is that it allows measurements to be made under controlled conditions that are not influenced by the ambient RH [61]. However, the exchange of water in a ventilated chamber is dependent on the velocity of air flow, with higher flow rates causing greater water loss [71]. The addition of a dry gas to the chamber can also lead to dehydration of the skin [61]. Ventilated chambers have been recommended for measurements of higher rates of water loss because the output sensors do not change by a large amount when the evaporation rate is low [70].

Advantages of unventilated chambers are that they are easy to use [61], have a short measurement time [63], and require less equipment, which makes them less expensive. However, the use of a hygroscopic salt will cause moisture to be absorbed from both the skin and the internal environment of the chamber [70]. The inability to control the initial RH within the evaporimeter also means that the rate of water loss is initially affected by the ambient environmental conditions [61], and the subsequent change in humidity within a closed-chamber is affected by the RH and temperature inside the chamber [61].

Table 1-2: Advantages and disadvantages of ventilated and unventilated evaporimeters used in dermatology

	Ventilated	Unventilated
Advantages	Controlled conditions between measurements [61]	Ease of use [61]
		Requires less equipment
Disadvantages	Water loss depends on the velocity of air in the chamber [70]	Water loss is affected by the ambient conditions [61] and internal chamber conditions [70]
	Can cause skin dehydration [61]	Hygroscopic salts absorb moisture from the skin and chamber [61]

1.3.3 Evaporimeters for Ocular Use

A few evaporimeters that were originally created to measure trans-epidermal water loss have also been used to measure the tear evaporation rate (TER). Semi-open or closed-chamber evaporimeters that use a hydroscopic salt do not seem to be methods that researchers have adopted to investigate the ocular evaporation rate.

Two open-chamber evaporimeters that have been used to measure the TER are the Evaporimeter (ServoMed AB, Stockholm, Sweden) [72, 73] and Tewameter® TM 300 (Courage + Khazaka electronic GmbH, Köln, Germany) [74]. Both instruments have two sets of temperature and RH sensors inside the chamber that are placed a known distance apart [66, 73]. The rate of diffusion is calculated using Fick's law of diffusion (Equation 1-2) [66, 70, 75], which states that in the absence of forced convection, the evaporation rate is proportional to the vapor pressure gradient between two sensors located in the layer of air adjacent to the surface being tested [70, 75]. The vapor pressure gradient is calculated as the difference in vapor pressure between the two sensors [66] and is based on the RH [70].

$$\frac{(1\ dm)}{(A\ dt)} = -D' * \frac{p}{x}$$

$\frac{(1\ dm)}{(A\ dt)}$	Evaporation rate (g/m^2/h) [A = area]
D'	Diffusion coefficient of water vapor in air
$\frac{p}{x}$	Vapor pressure gradient per meter in the air layer adjacent to evaporating surface

Equation 1-2: Fick's law of diffusion

1.3.3.1 Modified Dermatological Evaporimeters

In 1980, Hamano et al. [72] published a report in Japanese that used a ServoMed EP-1 Evaporimeter to measure the TER of rabbits and humans under different conditions. This appears to be the first time an evaporimeter was used in humans, since previous measurements of evaporation rate were only conducted with rabbits [48, 76, 77]. Although the measurement probe on the dermatological instrument contained a capsule designed to be placed on the skin, the researchers created new capsules with varying diameters to place the probe directly onto the cornea [73].

In 1990, Trees and Tomlinson [75] reported on the non-invasive use the ServoMed EP-1 Evaporimeter to measure the TER. The measurement probe was located 1 to 2 cm away from the eye and a swimming goggle was used to prevent the probe from contacting the eye (Figure 1-4). The participant was asked to lightly hold the goggle around the eye to ensure a good seal while their head was supported on a headrest and chinrest [49]. Over the years, different versions of the ServoMed Evaporimeter have been used to investigate the evaporation rate, including the EP-1 [14, 15, 49, 75, 78-80], EP-2 [81, 82], and EP-3 [17, 83, 84]. The vast majority of studies using the ServoMed Evaporimeter have been conducted by Tomlinson and colleagues [14, 15, 17, 49, 75, 78-81, 83-92].

Trees and Tomlinson [75] standardized their measurements by using the evaporation rate of a 31ºC water bath. A ratio of the average evaporation rate measured over a 2-week period and actual evaporation rate of the water bath during each measurement was used to correct the TER. This method was later updated to correct all values to an arbitrary temperature of 25ºC and 40% RH because the water bath was vulnerable to changes in the air current and resulted in fluctuating values [49]. Condensation could build up in the goggle if measurements lasted a few minutes, therefore, the evaporation rate was calculated using a best-fit line of the raw data at time zero.

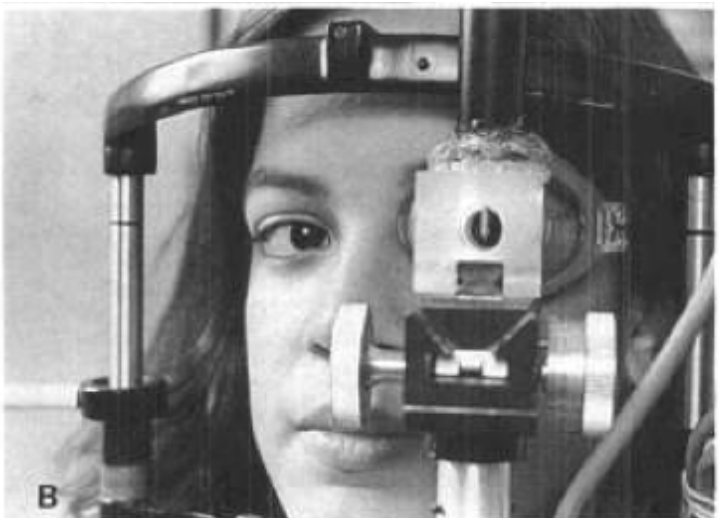

Figure 1-4: ServoMed EP-1 Evaporimeter showing a sensor in front of the left eye and the opening exposed to the ambient environment. Image from Trees, G.R., Tomlinson, A., Effect of Artificial tear solutions and saline on tear film evaporation. Optom Vis Sci 1990;67(13):886-890.

In 2014, Rohit et al. [21] validated the use of the self-contained, portable VapoMeter (Delfin Technologies Ltd., Kuopio, Finland) to measure the TER. The closed-chamber, unventilated instrument was modified for ocular use by attaching a swimming goggle to the end of the measurement probe. Personal communication with Delfin Technologies Ltd. reported that the total air volume within the goggle and the measurement cylinder is 20.1 cm^3. Since the total enclosed volume within the device is larger than normal, the researchers calculated an absolute TER using correction factors based on the evaporation rate measured from model eyes. The commercially available version of the instrument is currently marketed as the Eye-VapoMeter (Delfin Technologies Ltd., Kuopio, Finland) and has subsequently been used by other researchers to measure the TER [93-98]. However, disadvantages of unventilated closed-chamber evaporimeters include possible poor mixing of the air inside the goggle, possible temperature variations inside the goggle that can affect the RH, and blinking may cause small air currents inside the instrument [99].

In 2016, Jeon et al. [74] modified the Tewameter® TM 300 for use on the eye by adding a custom-made adapter cap that was shaped like a swimming goggle. Although they described the instrument as an open-chamber, ventilated device, this description may be incorrect. The instrument product brochure verifies that it is an open-chamber device, but does not mention ventilation [100]. Since the underlying theory relies on Fick's law and the absence of forced convection [70, 75], it is unlikely that ventilation

would be incorporated into an open-chamber device because this would alter the natural flow of water vapor.

1.3.3.2 Research-developed Evaporimeters

The remaining closed-chamber evaporimeters have all been developed for use in research, with the majority of them incorporating ventilation [1, 3, 99, 101, 102]. A summary of the different types of evaporimeters appears in Table 1-3.

Tomlinson and Cedarstaff [102] developed a binocular resistance hygrometer in the early 1980's. The instrument supplied 70% RH air at a constant flow rate to each goggle and measured the change in humidity as the air went over the eyes. Measurements were recorded for up to an hour in 30-second intervals. An additional 30-minute calibration session was required to determine the rate of water loss over time per eye, requiring the eyes to be open or closed in 10-minute intervals.

Rolando and Refojo [1] created a binocular, closed-chamber, ventilated evaporimeter using a pair of modified swimming goggles in the early 1980's. Dry air, set to an arbitrary value of 29.5% RH, was initially added to the goggles while the eyes were closed and air was mixed using a pump. Participants were then asked to open their eyes for a minute, before closing their eyes again. Once the eyes were closed, the air was mixed and removed from the goggle, where the new level of RH was detected by a sensor [1, 103].

In 1990, Yamada and Tsubota [104] published a report in Japanese showing a monocular, closed-chamber, unventilated evaporimeter that housed a sensor inside a cylinder. A couple of years later, Tsubota and Yamada [2] detailed the development of a binocular evaporimeter (Figure 1-5). The evaporimeter contained a temperature and RH sensor inside a cylinder, which was attached to each side of a pair of goggles.

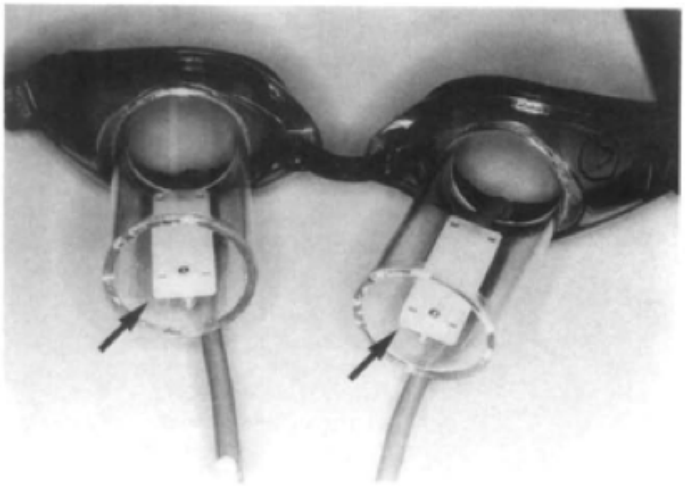

Figure 1-5: Tsubota-Yamada evaporimeterwith an arrow pointing to the sensor attached to each cylinder. The cylinders were attached to a goggle that was placed over the eyes. Image from Tsubota, K., Yamada, M. Tear evaporation from the ocular surface. Invest Ophthalmol Vis Sci 1992;33(10):2942-2950.

In 1993, Mathers et al. [3] reported on the development of a closed-cylinder, ventilated evaporimeter by placing a sensor inside a plastic cylinder that was 5 cm long and 3 cm in diameter. Dry air was added to the chamber until the RH reached 5%, then the air was shut off for 2 minutes to allow the RH to increase. The evaporation rate was arbitrarily calculated at 30% and 40% RH. The cylinder was later updated to improve the fit around the eye using an orbital impression [105]. The authors reported that the smaller volume of the chamber improved leakage and decreased the TER by 60% compared to their previous findings. Similar systems were subsequently used by McCulley and colleagues [6, 7, 12, 106-109] and Guillon and Maissa [110, 111].

In the early 2000's, Endo et al. [112] developed a ventilated, closed-chamber device using a quartz crystal sensor ("microbalance") [101] attached to a goggle. Measurements were recorded at 40% RH with a flow rate of 250 ml/min [112], although this was later decreased to 150 ml/min [101, 113]. Preliminary testing of the device reported a mean ± standard deviation (SD) TER of $83.0 \pm 1.1 \times 10^{-7}$ $g/cm^2/s$ [112]. However, no information was supplied regarding the test population, therefore it is unknown whether any of the participants had DED. The microbalance was sensitive enough to detect changes within the instrument caused by blinking [101].

The most recently designed evaporimeter is the Berkeley flow evaporimeter [99]. The monocular flow evaporimeter contains two cylinders attached to a swimming goggle. Air of a known RH and flow rate enters the inner cylinder whereupon it reaches the eye. Air leaves the system via the outer cylinder where two temperature and RH sensors measure the outflow. Both the flow rate and RH can be varied to simulate different environmental conditions.

Table 1-3: Classification of evaporimeters

Open-chamber		Closed-chamber	
Unventilated	**Ventilated**	**Unventilated**	**Ventilated**
ServoMed Evaporimeter	Tewameter[a]	Yamada-Tsubota Eye-VapoMeter	Resistance hygrometer Rolando-Refojo Mathers Quartz crystal "microbalance" Berkeley flow

[a]: classified as reported by Jeon et al. [74].

1.3.4 Differences in Instrumentation and Methodology

In addition to different types of evaporimeter being used over the years, the components comprising instruments and the methodology used to test participants varies between studies. An overview of differences appears below.

1.3.4.1 Sampling Rate

Technological advances have made it possible for sensors to record measurements at a more frequent rate. The sampling rate of different evaporimeters ranges from every 0.2 seconds [64], 0.25 seconds [101], 1 second [74], 2 seconds [15], to 10 seconds [2].

1.3.4.2 Duration of Measurement

Excluding any time required to ventilate an instrument, the measurement length required to assess the open or closed eye varies from <10 seconds [21], 1 minute [1], 100 seconds [101], 110 seconds [2], 2 minutes [3, 114], up to 5 minutes [99]. McCulley et al. [6] used a Mathers-based evaporimeter and reported that it took 14.5 seconds for the RH to change from 25% to 35% and 22.5 seconds to change from 35% to 45% RH.

1.3.4.3 Blink Rate

The majority of research groups have permitted participants to blink normally [2, 3, 74, 99, 101, 115] during the measurement, with the Tewameter methodology confirmed via personal communication. However, other researchers decided to control the blink rate and had participants blink every three seconds [7, 109] or five seconds [101], while other groups asked participants not to blink for up to one minute during the measurement [1, 21, 94, 95].

Despite the Eye-VapoMeter being the only commercially available instrument, the methodology varies between researchers. Some studies required the eyes to be held open [19, 21, 95, 116] during the <10 seconds test [21], while others have allowed participants to blink as needed [93, 94]. This highlights the need for standardization of the test to improve the ability to compare results between researchers.

1.3.4.4 Open and Closed Eye Measurements

The skin that surrounds the eye covers 77% of the area inside an evaporimeter [3] and represents 5% to 18% of the total evaporation rate [3, 16]. The mean ± SD evaporation rate of the skin has been reported as $3.73 \pm 2.4 \times 10^{-7}$ g/cm^2/s in a mixed group of dry eye and non-dry eye participants [3], $11.9 \pm 1.8 \times 10^{-7}$ g/cm^2/s in non-dry eye, and $16.1 \pm 5.9 \times 10^{-7}$ g/cm^2/s in floppy eyelid syndrome [117]. Rolando and Refojo [1] reported a 3.38 ± 0.40% increase in RH after an evaporimeter was placed over the closed eyes for a minute.

In order to determine the evaporation rate derived from the ocular surface, most researchers have calculated the difference between the evaporation rate with the eyes open compared to when the eyes are closed [2, 3, 6, 13, 19, 21, 75, 93, 94, 96, 98, 99, 101, 106, 114, 116]. However, others have only had participants close their eyes to calibrate the instrument [102] or as a baseline prior to ventilating the instrument [1]. Bilkhu et al. [95] did not include closed eye measurements because each participant served as their own control to examine the change in repeated measurements over time.

1.3.4.5 Petroleum Jelly

In order to minimize evaporation of the skin, researchers have applied petroleum jelly to the skin [3, 19, 21, 118]. Petroleum jelly reduces the skin evaporation rate by 70% [75] to 87.6% [1], with the combination of petroleum jelly and closed eye measurements found to decrease the evaporation rate by 64.1% [21].

Petroleum jelly has also been placed around the goggle in order to minimize the possibility of air leaking from the goggle [16].

1.3.4.6 Volume of Evaporimeter

The majority of evaporimeter chamber/goggle volumes previously investigated were larger, with swimming goggle volumes of 16 cm^3 (personal communication with Delfin Technologies Ltd) and 20 cm^3 [101], a chamber volume of 25 cm^3 [3], a goggle and cylinder volume of 44 cm^3 [2], or a total volume of 80 cm^3 encompassing swimming goggles and additional tubing to provide ventilation [1].

1.3.5 Application of Evaporimetry to the Field of Dry Eye

1.3.5.1 Dry Eye Disease (DED)

1.3.5.2 Definition

The TFOS DEWS II Definition and Classification report defined DED as:

> "…a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neuro-sensory abnormalities play etiological roles [119]."

1.3.5.3 Classification

DED is classified into three categories based on the etiology (Figure 1-6). The three sub-types of DED are: (i) aqueous deficient dry eye (ADDE), (ii) evaporative dry eye (EDE), and (iii) mixed dry eye [119]. ADDE is caused by insufficient lacrimal secretion and may be caused by Sjögren's syndrome [40, 120]. EDE occurs when there is normal tear volume and lacrimal function, but rapid evaporation occurs from the ocular surface [120]. EDE is the most prevalent type of DED [121-124] and is most commonly caused by meibomian gland dysfunction (MGD) [119, 125, 126]. Mixed DED occurs when both ADDE and EDE are present and becomes more frequent as DED worsens [119].

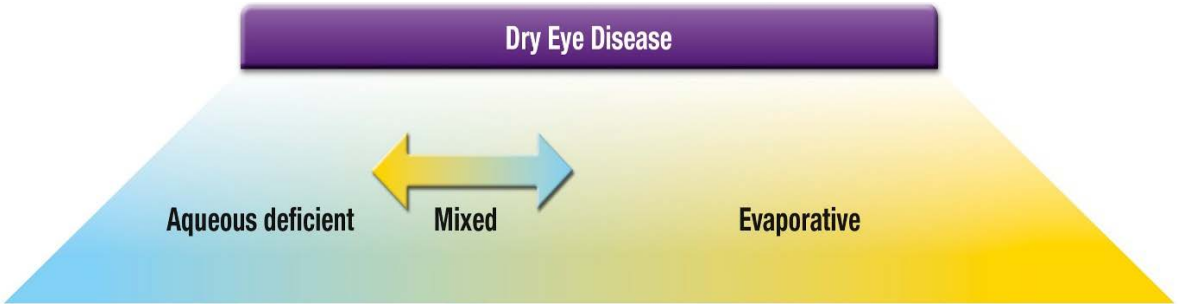

Figure 1-6: Classification of the three subtypes of dry eye disease. Evaporative dry eye encompasses a larger portion of the image because it is more prevalent than aqueous deficient dry eye. Image from Craig JP et al. TFOS DEWS II Definition and Classification Report. Ocul Surf 2017; 15(3):276-283.

1.3.5.4 Prevalence

The TFOS DEWS II Epidemiology report [121] analyzed 24 international studies and reported that the prevalence of DED based on symptoms, with or without the presence of signs, varied between 4.9% in females in Singapore [127] up to 52.9% in females in China [128]. The prevalence of DED based on signs alone ranged from 1.5 to 98.5% depending on how dry eye is defined [129].

Comparisons between studies is difficult due to the different criteria used to define DED, with some researchers using either symptoms or signs, or both symptoms and signs. However, more recent work has reported 6.8% of adults ≥18 years old in the United States have been diagnosed with DED [130], while symptoms of dry eye were reported in an estimated 21.3% of Canadians [131], 9.1% of Dutch [132], 12.8% of Brazilians [133], 32.1% of Saudi Arabians [134], and 62.6% of Emiratis [135]. Recent systematic reviews and meta-analyses have reported 42.0% of Africans (symptoms only or both signs and symptoms) [136] and 13.55% (signs and symptoms) to 31.40% (symptoms) of Chinese [137] have DED.

Further information on DED and MGD can be found in the TFOS DEWS II collection of reports [10, 40, 119, 121, 138-143] and in The International Workshop on Meibomian Gland Dysfunction reports [25, 126, 144-150].

1.3.5.5 Evaporimetry Cut-off Values for DED

A summary of suggested cut-off values to diagnose DED, ADDE, and EDE appears in Table 1-4 When evaporimetry is used as a standalone test to diagnose DED, Khanal et al. [4] reported that a TER

>33 g/m^2/h (>9.2 x 10^{-7} g/cm^2/s) has a sensitivity of 51% and a specificity of 96%. Sensitivity improved if both evaporimetry and fluorophotometry were assessed, and was maximized when the aforementioned tests were combined with tear osmolarity to achieve a sensitivity of 100% and a specificity of 66%.

A meta-analysis published by Tomlinson et al. [11] the following year found a sensitivity of 51.1% and a specificity of 89.9% when the cut-off value between non-dry eye and DED was increased to 22.0 x 10^{-7} g/cm^2/s. The cut-off value has been incorrectly reported in The International Workshop on Meibomian Gland Dysfunction: Report of the Diagnosis Subcommittee [150] as dry eye <22 x 10^{-7} g/cm^2/s, rather than >22 x 10^{-7} g/cm^2/s [11]. Wong et al. [5] suggested that the meta-analysis cut-off values may be too large because of discrepancies between some of the reported TERs in comparison to the values listed in the original papers.

The cut-off values for differentiating between ADDE and EDE range from 11.1 [88] to 27.5 x 10^{-7} g/cm^2/s [11], with higher rates of evaporation occurring in EDE [11, 88]. The sensitivities and specificities of the cut-off values vary between of 45.5 [11] to 77% [88] and 55 [88] to 79.8% [11], respectively.

Table 1-4: DED cut-off values

	Cut-off value (x 10^{-7} g/cm^2/s)	**Sensitivity (%)**	**Specificity (%)**
Non-DED versus DED [4]	9.2	51	96
Non-DED versus DED [11]	22.0	51.1	89.9
Non-DED versus ADDE [11]	20.6	55.8	85.9
Non-DED versus EDE [11]	22.3	61.2	90.6
ADDE versus EDE [11]	27.5	45.5	79.8
ADDE versus EDE [88]	11.1	77	55

DED: dry eye disease; ADDE: aqueous deficient dry eye, EDE: evaporative dry eye

1.3.6 Units of Measurement

In addition to researchers using various instruments and methodologies to measure evaporation, TER has been reported with different units of measurement. Evaporation rates are most commonly measured in x 10^{-7} g/cm^2/s [1, 3, 8, 18, 82, 105, 106, 110, 111, 113, 118, 151-160] or g/m^2/h [14, 17, 19, 21, 49, 72-74, 78-80, 84, 86, 88, 92-94, 97, 98, 112, 114, 161] if a modified dermatological device was used.

Other units that have been used to describe TER include x 10^{-6} g/cm^2/s [99], mg/h/mm^2 [76], mg/cm^2/h [77, 162], mg/min/cm^2 [16, 102, 163, 164], x 10^{-7} g/s per eye [2, 151], µl/min [3, 8, 158], and µl/cm^2/min

[6, 7, 12, 13, 107-109]. A summary of how to convert between different units into x 10^{-7} g/cm^2/s is shown in Table 1-5.

Spectral and infrared thermography have also recorded TER in units of µm/min [165-168], W/m^2 [169, 170] and W/min [171]. Since discussion of spectral and infrared thermography are beyond the scope of this literature review, the reported TERs from these studies have not been included in this thesis. Further information on infrared thermography can be found in a recent review by Shah and Galor [172].

Table 1-5: Conversion of tear evaporation rate to units of x 10^{-7} g/cm^2/s

Original unit	To convert to units of x 10^{-7} g/cm^2/s
g/m^2/h	Divide by 3.6 [5, 11, 173]
mg/cm^2/h	Divide by 0.36
mg/cm^2/min	Divide by 0.006
mg/mm^2/h	Divide by 0.0036
µl/min	Multiply by 100[a]
µl/cm^2/min	Divide by 0.006

[a]: when the area of the ocular surface area is 1.67 cm^2

1.3.7 Problems with Previously Published Tear Evaporation Rates (TERs)

Mistakes have been reported when calculating the TER and other inconsistencies were noted during the process of conducting this literature review. These changes are discussed in further detail below.

1.3.7.1 Specific Papers

Tomlinson et al. [11] reported in a meta-analysis that a miscalculation was made in one of his previous studies. This error led to a 100x overestimation in the TER. Although only one paper was cited as being incorrect [102], this thesis assumes that any work conducted by Tomlinson and Cedarstaff that was published using the same resistance hygrometer [16, 163] in units of mg/min/cm^2 was miscalculated. The values have been adjusted accordingly prior to using the conversion in Table 1-5 to create revised summary tables for DED and non-DED (Table 1-7) and CL wear (Table 1-12).

Tomlinson et al. [91] investigated the effect of three different ocular lubricants on TER. In Tables 1 and 2 the rates of evaporation were reported in units of g/m^2/s, but the graphs were labelled as g/m^2/h. A typographical error was presumably made when creating the tables. The correct unit is most likely g/m^2/h based on other work by the same group [14, 17, 49, 75, 78, 79, 88, 92] and because values in g/m^2/s would be approximately 10000x larger than expected.

Khanal et al. [81] studied the effect of an artificial lubricant on people who had undergone cataract surgery. Participants were categorized into 3 groups (control – no drop, Refresh® Soothe and Protect, or saline). In the results section, the overall study population was found to have significantly higher TERs at 3 days, 2 weeks, and 1 month post-surgery, and corresponding p-values were listed for each time point. The text referred to Table 2, which reported the mean ± SD TER of each group at every time point. The table indicated that significant changes in TER from baseline were shown in bold. Although the results for 3 days and 2 weeks were in bold font, none of the results from 1 month were bold. Therefore, it is unknown which of the groups had a significant increase in TER at 1 month.

1.3.7.2 Consensus Reports

Verification of TERs reported in consensus review papers against their original source was also occasionally problematic, as some of the values listed in the review were not found in the original paper or have been reported incorrectly. For example, in Table 1 of the TFOS International Workshop on CL Discomfort: Report of the CL Interactions with the Tear Film Subcommittee [36], mean and SD TERs were reported for Tomlinson et al. and Tomlinson and Cedarstaff, but the papers only contain graphs of the TER over time without any corresponding tables or specific values listed [80, 164]. The mean evaporation rate for Hamano et al. does not appear in the cited paper [174]. Similarly, the SD listed for Craig and Tomlinson was not in the original paper [49]. In addition, the values reported for Tomlinson and Giesbrecht [85] were reported as a single value, although the original values were reported separately for males and females [79, 85]. The TER in the summary table of the report [36] was neither an average of the sexes, or an average based on the ratio of male and female participants. The average TER reported for Dogru et al. should be labelled as a median value [18]. The units for Tsubota and Yamada [2] should be x 10^{-7} g/s and has not been labelled accordingly in multiple reviews [10, 11, 36, 51, 175].

Table 3 of the TFOS DEWS II Tear Film report [10] said that the researchers who performed measurements with a ServoMed Evaporimeter from 1990 onwards used a closed-chamber instrument. However, the instrument should have been listed as an open-chamber device [70, 75]. The same table also stated that the mean evaporation rate for Hamano et al. was 26.9 x 10^{-7} g/cm^2/s (SD not reported) [10]. However, a conversion of mean ± SD values in the cited paper [73] from g/m^2/h revealed a TER of 24.2 ± 5.8 x 10^{-7} g/cm^2/s. The reported TER for Cedarstaff and Tomlinson also does not match the conversion of values for the naked eye from mg/min/cm^2 to x 10^{-7} g/cm^2/s [16], even if the values were

not considered to be 100x larger than they should be. Updated TERs have been included in Table 1-12. A single mean ± SD TER was reported for Trees and Tomlinson, although three separate mean baseline TERs were reported prior to instillation of an artificial lubricant [75]. Averaging the three baseline values together yielded a different result than what was reported. The individual baseline TERs are also shown in Table 1-8. The report also stated that the mean TER for Mathers was $14.7 \pm 6.7 \times 10^{-7}$ g/cm^2/s [10], but the original paper lists a value of $14.8 \pm 6 \times 10^{-7}$ g/cm^2/s [8]. In addition, a mean TER of 26×10^{-7} g/cm^2/s at 40% RH [10] was reported for Peng et al., but the original paper found a low air velocity TER of 22×10^{-7} g/cm^2/s [99]. This value has been updated in Table 1-7.

1.3.8 Factors that Can Affect Tear Evaporation

1.3.8.1 Patient-related Factors

1.3.8.1.1 Age

An equivocal relationship exists between age and TER, with some finding that TER increases with age [111, 159]. Guillon and Maissa [111] reported people without dry eye ≥45 years old have a 31% higher TER at 30% RH, and a 55% higher TER at 40% RH, compared to those <45 years old. Mathers et al. [159] found a weak positive correlation (Pearson's r = 0.38) between TER and people in their second decade of life up to their ninth decade of life. Age is a consistent risk factor for DED [121] and increased TERs may be observed because tear instability worsens with age [176].

However, other researchers have been unable to find a significant difference in the TER in people without DED when comparing those <40 to ≥40 years old [1], and <41 to ≥41 years old [86]. In addition, no significant correlation (R^2 = 0.002) was reported between the TER of non-dry eye participants aged between 7 to 92 years old [79]. Differing results may be due to differences in sample size, ages, and the ratio of male to females participants [79].

1.3.8.1.2 Sex

A mixed relationship exists between sex and TER with some reporting that females have a higher evaporation rate [79, 111]. Guillon and Maissa [111] reported females without DED have a 24% higher TER at 30% RH, and a 47% higher TER 40% RH, compared to males. Tomlinson et al. [79] measured a similar result, with a 49% increase in TER in females versus males. Menstrual cycle may also affect TER, with higher rates reported on day 19 compared to day 2 [84]. Sex is a consistent risk factor for

DED [121], and higher TERs may occur in women due to hormonal differences and external influences, such as wearing eye make-up [111].

Other researchers were unable to find a significant difference between the TER of females and males without dry eye [1, 86]. Inconsistent findings between different researchers may be due to different sample sizes, participant ages, and varying ratios of male to female participants [79].

1.3.8.1.3 Race

Although Asians are at 1.5 to 2.2x greater risk of developing DED [121], no significant difference in TER was found between Caucasians and East Asians that did not have dry eye [97].

1.3.8.1.4 DED

Most studies have reported higher TERs in people with DED than those without dry eye [3, 4, 8, 9, 74, 78, 88, 93, 101, 105, 109, 113, 118, 152, 153, 156, 158, 177], with increased TERs found in ADDE [3, 88, 109, 118], EDE [8, 9, 88, 101, 156], and mixed dry eye [8, 109]. Higher TERs have been reported in EDE than ADDE [11, 88], although not all have been able to find a difference between the two subtypes [74]. EDE can cause increased evaporation due to decreased or altered meibum secretion, which causes disruption to the lipid layer of the tear film [147, 178]. ADDE can also result in a higher TER due to poor spreading of tears across the ocular surface, which leads to increased tear instability and an abnormally thickened lipid layer [88].

Increased rates of evaporation have also been found in Sjögren's syndrome compared to ADDE that is not related to Sjögren's syndrome [113, 179], people with hyperthyroidism or hypothyroidism compared to those without thyroid disease [93], blepharitis [114], severe DED due to chronic graft-versus-host disease [177], atopic keratoconjunctivitis [156], floppy eyelid syndrome [117], and ectrodactyly-ectodermal dysplasia-clefting syndrome [153].

Although some researchers found a difference between the TER of dry eye and non-dry eye participants [6, 106, 107, 177], others measured higher TERs in people without DED [2, 72, 73]. A higher TER in people without dry eye was attributed to tear dynamics, which depends on tear production, tear evaporation, and tear drainage [2]. Greater amounts of tears are produced when DED is absent, which allows for higher amounts of evaporation. However, since there are small amounts of tears being produced in DED, the relative contribution of evaporation to the overall tear dynamics is greater than in non-dry eye. Varying results may be due the use of different instrumentation, different RHs, the level

of severity of dry eye [2, 74, 177], the use of invasive methods to measure evaporation [72, 73], and the use of different criteria to define DED, with some using only signs [8, 101, 113] or symptoms [2], while others required the presence of both signs and symptoms [3, 78, 105-107, 109, 118, 158].

1.3.8.1.4.1 Evaporation Rate in DED and Non-DED

A summary of the range of TERs reported in non-DED and DED is shown in Table 1-6. Some of the variability can be attributed to the RH of these values being tested in a range from 20 [99] to 70% [92].

Table 1-6: Range of TERs in non-DED and DED

	Range of Values (x 10^{-7} g/cm^2/s)	
	Minimum	**Maximum**
Non-DED	0.02 [78]	29 [99]
DED	0.3 [92]	59.1 [8]

Despite higher TERs of 39.3 x 10^{-7} g/cm^2/s and 73.1 x 10^{-7} g/cm^2/s being found in non-DED and DED, respectively [117], these values have not been included in the main portion of Table 1-6. This was done in order to maintain consistency with previous researchers that used the same instrument who reported the difference between the open eye and closed eye [101, 113], rather than used an equation that accounted for the area of the ocular surface and the area of evaporimeter [117].

A list of different studies reporting the TER of people with and without DED appears in Table 1-7. Where possible the study populations have been classified into the three subtypes of DED as per TFOS DEWS II [119]. To aid in the comparison of the evaporation rate between different studies, values have been converted to units of x 10^{-7} g/cm^2/s (if possible).

Table 1-7: Published TERs for non-DED and DED

Year	Investigator	Animal	Non-Dry Eye Evaporation Rate (x 10^{-7} g/cm^2/s)	RH (%)	Dry Eye Evaporation Rate (x 10^{-7} g/cm^2/s)	Classification	Description
1941	Von Bahr [76]	Rabbit	41.7	NR			
1961	Mishima [77]	Rabbit	7.8	NR			
1969	Iwata [48]	Rabbit	10.1	<50			
1980	Hamano [72]	Rabbit	11.4[72, 73] ± 4.4[73]	42[72]/60[73]			
1980	Hamano [72, 73]	Human	24.2[72, 73] ± 5.8[73]	42[72, 73]	13.6 ± 3.1	DED	Trachoma, Stevens-Johnson's syndrome, etc.
1983	Cedarstaff [163]	Human	1.12^ ± 0.12^	70			
1983	Rolando [1]	Human	4.07 ± 0.40	29.5	8.17 ± 2.65	Tear film abnormalities	
1983	Rolando [118]	Human	4.07 ± 0.40	NR	7.87 ± 2.80	ADDE	Sicca
1985	Rolando [152]	Human		NR	6.38 ± 0.53*	EDE	Meibomitis
1990	Yamada [104]	Human	8.3 ± 1.9	40	4.6 ± 2.9	ADDE	
1992	Tsubota [2]	Human	15.6 ± 3.8[a]	40	9.5 ± 5.6[a]	DED	
1993	Tomlinson [79]	Human	12.2 ± 6.8[b][79, 85]	NR			
			8.2 ± 5.9[c][79, 85]	NR			
1993	Mathers [3]	Human	14.7 ± 6.4	30	47.6 ± 20.1	ADDE	Sicca
			12.1 ± 5.5	40	33.0 ± 12.4	ADDE	Sicca
1993	Mathers [8]	Human	14.8 ± 6	30	49.9 ± 21	EDE (MGD)	Dropout
				30	59.1 ± 28	Mixed	Sicca and MGD
1995	Tsubota [151]	Human	7.8 ± 2.2	40			
1995	Shimazaki [9]	Human	13.09 ± 1.35[a]	40	10.41 ± 1.28[a]	EDE (MGD)	Obstructive
				40	18.39 ± 1.43[a]	EDE (MGD)	Dropout
				40	14.43 ± 1.87[a]	EDE (MGD)	Obstructive and dropout
1996	Mathers [158]	Human	13 ± 6	30	25 ± 35	DED	ADDE or hyperosmolarity

Year	Investigator	Animal	Non-Dry Eye Evaporation Rate ($\times 10^{-7}$ g/cm^2/s)	RH (%)	Dry Eye Evaporation Rate ($\times 10^{-7}$ g/cm^2/s)	Classification	Description
1996	Mathers [105]	Human	15.1 ± 8.62[105, 159]	NR	23.9 ± 17.47	ADDE	
				NR	22.81 ± 16.33	Mean of all MGD types	
				NR	27.67 ± 18.25	Mixed	Obstructive
				NR	12.32 ± 8.76	Mixed	Seborrheic
				NR	16.06 ± 8.92	Mixed	Rosacea
				NR	20.05 ± 11.32	Mixed	Seborrheic and obstructive
1997	Craig [49]	Human	0.39 ± NR	50	1.64 ± NR	EDE	No visible lipid layer or abnormal colored fringes
1998	Craig [86]	Human	1.07 ± 1.23	NR			
2000	Craig [78]	Human	0.02 ± 0.14	50	0.41 ± 0.19	DED	7 out of 8 had a lipid-deficient tear film
2003	Goto [101]	Human	4.1 ± 1.4	10-15	5.8 ± 2.7	EDE (MGD)	Obstructive
			5.7 ± 1.4[d]	10-15	7.4 ± 2.8[d]	EDE (MGD)	Obstructive
2003	McCulley [106]	Human	10.92 ± 4.28	NR	11.67 ± 6.13	ADDE	Sicca
				NR	10.55 ± 7.08	Mixed	Sicca + turbid/difficult-to-express secretions
2004	Matsumoto [153]	Human		NR	6.98 ± NR		Ectrodactyly-ectodermal dysplasia-clefting syndrome
2004	Tomlinson [79, 85]	Human	12.2 ± 6.8[c]	NR			
			8.2 ± 5.9[d]	NR			
2005	Liu [117]	Human	4.6 ± 3.0[e]	NR	7.4 ± 3.2[e]		Floppy eyelid syndrome
2006	McCulley [107]	Human	10.8 ± 3.7	20-25	11.2 ± 5.2	ADDE	Sicca
			6.2 ± 2.7	40-45	6.2 ± 3.7	ADDE	Sicca
				20-25	11.3 ± 8.8	Mixed	Sicca + turbid/difficult-to-express secretions
				40-45	5.3 ± 3.0	Mixed	Sicca + turbid/difficult-to-express secretions

Year	Investigator	Animal	Non-Dry Eye Evaporation Rate (x 10^{-7} g/cm^2/s)	RH (%)	Dry Eye Evaporation Rate (x 10^{-7} g/cm^2/s)	Classification	Description
2006	McCulley [6]	Human	9.7 ± 3.0^b	25-35	8.0 ± 2.8^b	DED	
			7.2 ± 2.7^b	35-45	6.2 ± 1.8^b	DED	
			7.8 ± 3.7^c	25-35	7.3 ± 2.2^c	DED	
			4.8 ± 1.5^c	35-45	5.7 ± 1.5^c	DED	
2007	Uchiyama [108]	Human	10.8 ± 3.7	20-25	11.0 ± 5.0	ADDE	Sicca
			6.2 ± 2.7	40-45	6.0 ± 3.7	ADDE	Sicca
				20-25	9.5 ± 5.0	Mixed	Sicca + turbid/difficult-to-express secretions
				40-45	5.2 ± 3.2	Mixed	Sicca + turbid/difficult-to-express secretions
				10-15	2.9 ± 1.8	ADDE	Non-Sjögren's syndrome
2007	Goto [113]	Human		10-15	5.9 ± 3.5	ADDE	Sjögren's syndrome
				10-15	2.9 ± 1.8	ADDE	Non-Sjögren's syndrome
2008	Matsumoto [154]	Human	2.5 ± 0.9	50-60	7.7 ± 0.2	EDE	Smokers
2008	Matsumoto [180]	Human	4.30 ± 3.82	30-50	6.37 ± 3.72	EDE (MGD)	
2008	Rummenie [160]	Human	1.84 ± 1.19, 2.13 ± 0.91	50-60			
2008	Guillon [110]	Human	15.1 ± 7.3	25-35			
			11.3 ± 6.8	35-45			
2008	Uchiyama [12]	Human		25-35	14.3 ± 6.2	ADDE	Sicca
				35-45	10.4 ± 5.2	ADDE	Sicca
				25-35	14.6 ± 6.4	ADDE	Sicca
				35-45	10.5 ± 5.5	ADDE	Sicca
2008	Khanal [4]	Human	5.8 ± 2.8[4, 88]	NR	10.5 ± 7.4	ADDE, EDE	Sjögren's syndrome, GVHD, MGD

Year	Investigator	Animal	Non-Dry Eye Evaporation Rate (x 10^{-7} g/cm^2/s)	RH (%)	Dry Eye Evaporation Rate (x 10^{-7} g/cm^2/s)	Classification	Description
2009	Khanal [88]		5.8 ± 2.8[4, 88]	NR	9.6 ± 5.6	ADDE	Sjögren's syndrome, GVHD, rheumatoid arthritis
				NR	12.8 ± 10.5	EDE	Posterior blepharitis, partial blinking, related to visual display terminal use, lipid abnormalities
2009	Wojtowicz [7]	Human	11.5 ± 4.0	25-35			
			8.2 ± 3.0	35-45			
2010	McCann [114]	Human	5.0 ± 3.0	NR	12.9 ± 6.4		Blepharitis
2010	Wang [177]	Human	2.2 ± 1.53	10-15	3.6 ± 1.66	Mixed	GVHD mild DED + obstructive MGD
				10-15	5.98 ± 3.61	ADDE, Mixed	GVHD severe DED, GVHD severe DED + obstructive MGD
2010	Wojtowicz [13]	Human		25-35	8.2 ± 3.8	ADDE	Sicca
				35-45	5.3 ± 2.7	ADDE	Sicca
				25-35	7.8 ± 3.2	ADDE	Sicca
				35-45	5.2 ± 2.3	ADDE	Sicca
2010	Guillon [111]	Human	13.7 ± NR	25-35			
			16.6 ± NR	35-45			
2010	Ward [181]	Human	2.2 (1.2 – 2.4)[f]	50-60			
2011	Arciniega [109]	Human	5.5 ± 2.0	25-35	9.3 ± 2.7	ADDE	Sicca
			3.8 ± 1.3	35-45	6.7 ± 1.3	ADDE	Sicca
				25-35	9.2 ± 4.3	Mixed	Sicca + turbid/difficult-to-express secretions
				35-45	6.2 ± 3.2	Mixed	Sicca + turbid/difficult-to-express secretions

Year	Investigator	Animal	Non-Dry Eye Evaporation Rate (x 10^{-7} g/cm^2/s)	RH (%)	Dry Eye Evaporation Rate (x 10^{-7} g/cm^2/s)	Classification	Description
2012	Khanal [89]	Human		NR	13.3 ± 5.1	ADDE	GVHD
				NR	7.0 ± 4.4	ADDE	Sjögren's syndrome
				NR	12.8 ± 10.5	EDE (MGD)	
2012	Ibrahim [156]	Human	3.3 ± 1.5	30-50	9.8 ± 5.0		Atopic-keratoconjunctivitis
				30-50	7.4 ± 2.7	EDE (MGD)	Obstructive
2013	Tomlinson [91]	Human	4.7 ± 2.4[g]	20	13.9 ± 6.7[f]	ADDE	
			4.4 ± 2.1[g]	20	14.0 ± 5.5[f]	ADDE	
			4.2 ± 1.8[g]	20	15.3 ± 4.4[f]	ADDE	
2013	Madden [92]	Human	21.6 ± 4.0	5	28.1 ± 3.1	ADDE	
			6.6 ± 1.2	40	16.6 ± 2.9	ADDE	
			0.3 ± 1.8	70	0.3 ± 2.4	ADDE	
2013	Abusharha [182]	Human	28.2 ± NR	5			
			12.8 ± NR	40			
2014	Peng [99]	Human	29 ± NR	20			
			22 ± NR	40			
2016	Alghamdi [116]	Human	21.9 ± 9.2	45.5 ± 9			
2016	Abusharha [115]	Human	5.6 ± NR	40 at 5ºC			
			17.4 ± NR	40 at 25ºC			
2016	Jeon [74]	Human	15.2 ± 3.9	41, 40.2	17.8 ± 3.0	ADDE	
				40.2	17.0 ± 4.1	EDE	
2019	Abusharaha [93]	Human	4.4(3.8)[h]	<40%	11.4 (11.5)[h]	Thyroid-gland patients	
					8.1 (16.5)[h]	Hyperthyroid	
					14.7 (7.8)[h]	Hypothyroid	
2019	Ahmed Alanazi [94]	Human	4.3(3.6)[h]	<35%	10.5 (16.5)[h]	EDE	Smokers

Evaporation rates are reported as the mean ± standard deviation, unless specified otherwise.

RH: relative humidity; DED: dry eye disease; ADDE: aqueous deficient dry eye; EDE: evaporative dry eye; Mixed: aqueous deficient dry eye and evaporative dry eye; NR: not reported; MGD: meibomian gland dysfunction; GVHD: graft-versus-host-disease; MG: meibomian gland.

^: values were adjusted to be 100x smaller as per Tomlinson et al. [11] under the assumption that all studies published around the same time were overestimated.
*: standard error of the mean.
[a]: units = x 10^{-7} g/sec.
[b]: female.
[c]: male.
[d]: calculated using Rolando and Refojo's exposed ocular surface area calculation.
[e]: Evaporation rate was also reported as normal = $39.3 \pm 13.6 \times 10^{-7}$ g/cm^2/s and flopped eyelid syndrome = $73.1 \pm 29.7 \times 10^{-7}$ g/cm^2/s.
[f]: median (lower 95% confidence limit – upper 95% confidence limit).
[g]: evaporation rate calculated based on the assumption that the original units were g/m^2/h (not g/m^2/s).
[h]: median (IQR).

1.3.8.1.5 Dry Eye Treatments

Due to the multifactorial nature of DED [119, 120], the TFOS DEWS II Management and Therapy report recommended a four-step approach to treat and manage DED [140]. Table 1-8 shows a summary of the measured TERs from before and after different dry eye treatments were applied to people with and without DED. The majority of these studies were conducted on non-CL wearers with dry eye.

Further information regarding the treatment of DED can be found in the TFOS DEWS II Management and Therapy report [140], The International Workshop on MGD: Report of the Subcommittee on the Management and Treatment of MGD [145], and in recent review articles [183-187].

1.3.8.1.5.1 Artificial Lubricants and Liposomal Sprays

Some of the recommended treatments in the first step of managing DED include widely available over-the-counter products, such as ocular lubricants, lid hygiene, and warm compresses [140]. Ocular lubricants containing lipids or liposomal sprays can be particularly helpful for improving a poor lipid layer in the presence of MGD or EDE [90, 188, 189] to reduce evaporation from the surface of the eye [91]. A 34 to 42% decrease in TER was reported when artificial lubricants with lipids were used when compared to a formulation that did not contain a lipid [91]. McCann et al. [90] also found that an oil-in-water emulsion was able to significantly decrease the TER to a greater degree than other ocular lubricants. Information regarding specific components of ocular lubricants or types of artificial tears can be found in TFOS DEWS II Management and Therapy report [140], and other papers or literature reviews [190-192].

Dry eye treatments should decrease the TER by 24% in ADDE and by 46% in EDE to be considered clinically effective [91]. Previous studies have examined the short-term and long-term effect of artificial lubricants and liposomal sprays on TER. For the purpose of this literature review, a short-term effect was considered to be the change in TER following a single application of an eye drop or spray. The long-term effect of a treatment occurred after the test product was dispensed and used consecutively over a period of time.

A significant increase in TER occurs immediately after instillation of an artificial lubricant or drop of local anesthetic [1, 2, 15, 75]. This may be due to destabilization of the lipid layer and tear film [75] or due to an increase in fluid volume within the eye [1]. Elevated rates of evaporation can persist for 5 minutes [2] up to 37.5 minutes [15, 75, 193], with TERs returning to baseline levels by 45 minutes after

instillation of an eye drop [193]. Toda et al. [193] interpreted the increase in TER that occurred for 30 minutes after the instillation of hydroxypropyl methylcellulose as proof of its ability to stay on the ocular surface for a longer period of time and improve moisture to a greater degree than other ocular lubricants which did not change the TER. However, this contradicts the belief that therapeutics should decrease the TER [91].

Most studies have been unable to measure a significant change in TER between 10 minutes [82, 193] to 60 minutes [12] after instillation of artificial lubricants [13, 98, 193], saline [12, 193], or liposomal sprays [82, 95, 98]. Wojtowicz et al. [13] suggested that a change in evaporation rate was not detected due to a small sample size (n=20). Wang et al. [98] suggested that their results could have been affected by the use of eyeliner which disrupted the tear film, but that this change may have been counteracted by the application of a lipid emulsion and liposomal spray. Another reason why a change in TER may not have been observed was due to a poor choice of time points. If an ocular lubricant has a short residence time, the duration of the time interval between measurements may have been too long to detect a change in evaporation.

One short-term study conducted by Uchiyama et al. [12] was able to demonstrate a reduction in TER 30 minutes after the instillation of an artificial lubricant containing hydroxypropyl guar. However, more long-term studies have shown a decrease in TER after using artificial lubricants for up to 90 days [14, 90-92, 188]. Madden et al. [92] found that the effectiveness of the ocular lubricant varied with the ambient RH. Significant decreases in TER were observed in both DED and non-DED participants at 5% RH, but this effect was only seen in DED participants when the RH increased to 40%. There may be advantages to the prolonged use of an ocular lubricant since McCann et al. [90] were able to detect significant change in TER after 90 days of use, but this effect was not present after the first 30 days.

Other researchers have been unable to find a significant change in TER after using artificial lubricants for up to 2 weeks [91, 92]. However, significant differences in TER were detectable depending on the presence or absence of DED [91], the formulation of the artificial lubricant [91], and when tested at lower levels of ambient RH [92].

Khanal et al. [81] reported a significant increase in TER at 3 days, 2 weeks, and 1 month following cataract extraction. All participants experienced an increase in TER after surgery, regardless of whether artificial lubricants were used. The findings of this study can be difficult to compare to others since the invasive nature of the surgery will have affected the eyes and because all participants were also

prescribed antibiotics and steroids preserved with benzalkonium chloride. Although preservatives such as benzalkonium chloride have a toxic effect on the corneal epithelium [194-196], Tomlinson and Trees [15] did not find a significant change in TER by adding benzalkonium chloride or chlorobutanol to artificial tears.

Some studies have been unable to detect a difference in TERs amongst different types of ocular lubricants [13], or between an artificial lubricant and liposomal spray [98]. However, others have been able to detect significantly different TERs between different types of artificial lubricants [14, 90, 91], or have shown differences between artificial tears and saline [12, 193].

Varying results between studies may be due to the volume of product that was instilled, the frequency of use, the use of different instruments to measure the TER, and the length of time between the last instillation of the product and the evaporimetry measurement.

1.3.8.1.5.2 Other Treatments for DED

If the recommended first step treatments for DED prove to be inadequate, additional measures should be implemented. Some of the second level treatments suggested by the TFOS DEWS II Management and Therapy report include punctal occlusion, use of moisture chamber spectacles or goggles, and intense pulsed light therapy to treat MGD [140]. As discussed below, most studies have been unable to find a significant change in TER after the use of these products.

1.3.8.1.5.3 Punctal Plugs

Although a systematic Cochrane review published in 2017 reported that the evidence to support the use of punctal plugs to treat dry eye was inconclusive [197, 198], others have found that punctal occlusion can be an effective treatment to improve signs and symptoms of DED [199, 200]. Tsubota and Yamada [2] reported that the insertion of collagen punctal plugs without anesthetic resulted in a significantly higher TER 30 minutes post-insertion. This could be due to the evaporimeter detecting an increased tear volume in the eye or because participants could have experienced reflex tearing [201].

1.3.8.1.5.4 Increased Moisture

Increasing the RH surrounding the eye is another method of improving the signs and symptoms of DED [202-206]. Modification of the environment surrounding the eye can be achieved by using

modified spectacles or goggles [82, 95, 202-206], or by placing a source of moisture nearby, such as a humidifier [207, 208].

Pearce et al. [83] reported that a goggle-based eyelid warming device was able to significantly decrease TER after a 10 minute session. However, others were unable to find a change in TER after a 10 minute [82] or 30 minute treatment [95] using a similar device. Bilkhu et al. [95] suggested that a change may not have been found due to limitations of the evaporimeter, or due to the ability of the lipid layer to not only prevent evaporation, but also reduce the coefficient of friction between the ocular surface and eyelids [95, 209].

Hirayama et al. [157] were unable to measure a difference in TER following 5 days of use of a moist air device that was placed adjacent to a computer monitor. However, participants that were not given the moist air device had a significant increase in TER over the same period, therefore the device may have had a protective effect at preventing TER from worsening.

1.3.8.1.5.5 Warm Compresses

Warm compresses applied to the closed eye can be used to soften meibum, which is released into the tear film to improve the lipid layer and prevent evaporation [25, 145, 187]. Application of a warm compress for 10 minutes did not significantly alter the TER of people with and without meibomian gland dropout [82].

1.3.8.1.5.6 Intense Pulsed Light

Intense pulsed light has been suggested as a treatment when warm compresses have failed [210]. The exact mechanism of action is unknown. Three sessions of intense pulsed light therapy administered over 45 days did not significantly change the TER of people with mild or moderate MGD [96]. TERs were noted to exhibit large amounts of variability between visits. A lack of significant change in evaporation over the course of the study was thought to be due the majority of participants having an intact lipid layer, which prevented the TER from increasing [49]. Further information regarding the latest evidence-based treatments for MGD can be found in a recent review by Lam et al. [187].

Table 1-8: Published TERs involving different treatments for DED

Year	Investigator	Cohort	Treatment	RH (%)	Baseline Evaporation Rate (x 10^{-7} g/cm^2/s)	ΔEvaporation Rate with Dry Eye Treatment	Evaporation Rate After Treatment (x 10^{-7} g/cm^2/s)
1983	Rolando [1]	Non-DED	Proparacaine (0.5%)	29.5	3.91 ± 0.23	↑	Post-single drop: 9.41 ± 1.08
1990	Trees [75]	Non-DED	Liquifilm Tears	NR	11.9	↑	1.5 min: 21.6 ± 2.2[a,b]
						↔	37.5 min: 14.8 ± 1.8[a]
			Tears Naturale II	NR	11.4	↑	1.5 min: 24.1 ± 2.9[a,b]
						↔	37.5 min: 11.4 ± 1.9[a]
			Saline	NR	12.8	↑	1.5 min: 24.0 ± 2.9[a,b]
						↔	37.5 min: 15.5 ± 1.7[a]
1992	Tsubota [2]	DED symptoms	Artificial tears	40	10.8 ± 4.4[c]	↑	1 min: 21.3 ± 11.2[b,c]
							10 min: 10.6 ± 5.0[c]
			Sodium hyaluronate (0.05%)	40	11.2 ± 5.3[c]	↔	1 min: 20.3 ± 9.2[b,c]
							10 min: 9.8 ± 5.9[c]
			Sodium hyaluronate (0.1%)	40	10.4 ± 4.4[c]	↑	1 min: 20.1 ± 3.9[b,c]
							10 min: 10.3 ± 3.4[c]
			Sodium hyaluronate (0.3%)	40	10.2 ± 4.3[c]	↔	1 min: 24.9 ± 3.7[b,c]
							10 min: 9.9 ± 4.5[c]
			Punctal plugs	40	10.2 ± 5.5[c]	↑	30 min: 18.2 ± 4.8[c]
1996	Toda [193]	ADDE	HPMC (0.5%)	40	8.9 ± 10.1[c]	↑	10 min: 16.9 ± 9.5[c]
						↔	45 min: 14.9 ± 8.4[b,c]
			Sodium hyaluronate (0.1%)	40	NR	↔	10 min: NR
						↔	45 min: NR[b]
			Saline	40	NR	↔	10 min: NR
						↔	45 min: NR[b]
2002	Goto [188]	Mixed	Castor oil mixture	40	30 ± 9[c]	↓	2 weeks: 22 ± 11[c]
2006	Pearce [83]	DED symptoms	EyeCalm meibomian goggles	100	11.1[d] ± NR	↓	10 min: 7.5[d] ± NR

Year	Investigator	Cohort	Treatment	RH (%)	Baseline Evaporation Rate (x 10^{-7} g/cm^2/s)	ΔEvaporation Rate with Dry Eye Treatment	Evaporation Rate After Treatment (x 10^{-7} g/cm^2/s)
2007	Khanal [14]	Mild to moderate DED	Castor oil emulsion (1.25%)	NR	NR	↓	30 days: Δ–2.0 ± 1.5[e]
			Hypromellose (0.32%)	NR	NR	↓	30 days: Δ–0.56 ± 1.3[e]
2008	Uchiyama [12]	ADDE	Systane	25-35	14.6 ± 6.4	↓	30 min: 12.6 ± 7.3
						↔	60 min: 13.6 ± 6.2
			Systane	35-45	10.5 ± 5.5	↓	30 min: 9.5 ± 5.5
						↔	60 min: 9.8 ± 4.3
			Saline	25-35	14.3 ± 6.2	↔	30 min: 13.7 ± 5.0
						↔	60 min: 15.4 ± 8.3
			Saline	35-45	10.4 ± 5.2	↔	30 min: 10.1 ± 4.1
						↔	60 min: 11.3 ± 6.4
2008	Khanal [81]	Non-DED phaco	Refresh Soothe and Protect	NR	5.3 ± 3.7	↑	3 days: 16.5 ± 9.3
						↔	3 months: 6.1 ± 3.1[b]
			Saline		5.9 ± 4.2	↑	3 days: 12.4 ± 9.8
						↔	3 months: 7.1 ± 5.3[b]
			No drop		6.8 ± 4.9	↑	3 days: 14.9 ± 7.5
						↔	3 months: 8.1 ± 5.2[b]
2010	Wojtowicz [13]	ADDE	Systane	25-35	8.2 ± 3.8	↔	30 min: 8.5 ± 4.2
			Systane	35-45	5.3 ± 2.7	↔	30 min: 5.3 ± 2.3
			Optive	25-35	7.8 ± 3.2	↔	30 min: 8.7 ± 4.0
			Optive	35-45	5.2 ± 2.3	↔	30 min: 5.7 ± 2.5
2012	McCann [90]	EDE	Lubristil 0.15%	NR	NR	↓	90 days: 2.6 ± 2.5[b,e]
			Dacriosol	NR	NR	↓	90 days: 2.3 ± 5.0[b,e]
			Emustil	NR	NR	↓	90 days: 6.8 ± 5.5[b,e]

Year	Investigator	Cohort	Treatment	RH (%)	Baseline Evaporation Rate (x 10^{-7} g/cm^2/s)	ΔEvaporation Rate with Dry Eye Treatment	Evaporation Rate After Treatment (x 10^{-7} g/cm^2/s)
2013	Tomlinson [91]	DED	Aqueous drop with carmellose sodium (0.5%)	20 at 22°C	13.9 ± 6.7[f]	↓	2 weeks: 10.8 ± 5.3[f]
			Aqueous drop with carmellose sodium (0.5%) and lipid	20 at 22°C	14.0 ± 5.5[f]	↓	2 weeks: 8.1 ± 3.8[f]
2013	Tomlinson [91]	DED	Drop without lubricant polymer, with glycerine (1%) and lipid	20 at 22°C	15.3 ± 6.2[f]	↓	2 weeks: 9.9 ± 4.4[f]
		Non-DED	Aqueous drop with carmellose sodium (0.5%)	20 at 22°C	4.7 ± 2.4[f]	↔	2 weeks: 5.0 ± 3.8[f]
			Aqueous drop with carmellose sodium (0.5%) and lipid	20 at 22°C	4.4 ± 2.1[f]	↓	2 weeks: 3.1 ± 1.3[f]
			Drop without lubricant polymer, with glycerine (1%) and lipid	20 at 22°C	4.2 ± 1.8[f]	↔	2 weeks: 4.3 ± 3.0[f]
2013	Madden [92]	Mild-to-moderate DED	Refresh Ultra	5	28.1 ± 3.1	↓	7 days: 21.2 ± 2.8
				40	16.6 ± 2.9	↓	7 days: 13.2 ± 1.9
				70	0.3 ± 2.4	↔	7 days: 0.6 ± 1.3
		Non-DED	Refresh Ultra	5	21.6 ± 4.0	↓	7 days: 17.1 ± 3.1
				40	6.6 ± 1.2	↔	7 days: 5.7 ± 1.1
				70	0.3 ± 1.8	↔	7 days: 0.09 ± 1.7
2013	Hirayama [157]	DED[g]	Moist electro-spray device with sodium hyaluronate and castor oil	30-50	7.7 ± 6.0	↔	5 days: 8.7 ± 5.7

Year	Investigator	Cohort	Treatment	RH (%)	Baseline Evaporation Rate (x 10^{-7} g/cm^2/s)	ΔEvaporation Rate with Dry Eye Treatment	Evaporation Rate After Treatment (x 10^{-7} g/cm^2/s)
2017	Wang [98]	Non-DED	Systane Balance	NR	33.1 ± 15.3	↔	15 min: 28.6 ± 13.3
			Tears Again		30.6 ± 15.0	↔	15 min: 30.6 ± 15.6
2018	Turnbull [82]	No dropout	Tears Again,		NR	↔	10 min: Δ0.73 ± 4.66
		Mild EDE	Blephasteam, or		NR	↔	10 min: Δ–0.64 ± 5.71
		Pronounced EDE	EyeBag		NR	↔	10 min: Δ0.29 ± 4.75
2021	Bilkhu [95]	Non-DED and mild DED[h]	Blephasteam and Actimist Eye Spray		11.6 ± 3.4	↔	30 min: 12.3 ± 5.4

Evaporation rates are reported as the mean ± standard deviation, unless otherwise specified. TER after treatment is presented as the length of time that had elapsed since the treatment was instilled or applied or the duration of the treatment.

RH: relative humidity; DED: dry eye disease; TER: tear evaporation rate; NR: not reported; ↑: significant increase in TER post-treatment; ↓: significant decrease in TER post-treatment; ↔: non-significant change in TER post-treatment; HPMC: hydroxypropyl methylcellulose; phaco: undergoing phacoemulsification; ADDE: aqueous deficient dry eye; EDE: evaporative dry eye.

[a]: standard error.
[b]: interim time point(s) not included in the table.
[c]: units = x 10^{-7} g/sec.
[d]: median.
[e]: type of descriptive statistics not specified.
[f]: evaporation rate calculated based on the assumption that the original units were g/m^2/h (not g/m^2/s).
[g]: included eight contact lens wearers.
[h]: included two contact lens wearers.

1.3.8.1.6 Blink Rate

Tsubota and Nakamori [151] examined the effect of different blink rates on TER. Blink rate intervals of 1, 5, 10, 20, 30, and 60 times per minute were tested. Reflex tearing occurred in some participants at blink rates of 5 times per minute or less, and these results were excluded from their analysis. Preliminary analysis did not find a strong correlation between the blink rate and the TER. Additional analysis was conducted with participants divided into two groups: non-DED participants (TER >7.8 x 10^{-7} g/s per cm^2) and participants with dry ocular surfaces (TERs <7.8 x 10^{-7} g/s per cm^2). Non-DED participants were unaffected by varying the blink rate, but DED participants had a moderate positive correlation ($r = 0.54$) between the TER and blink rate, with higher TERs measured as the blink rate increased.

1.3.8.1.7 Concentrated Tasks

Blink rate decreases when performing tasks that require concentration [211-213], such as using a digital device. An increased inter-blink interval can cause the tear film to thin and can lead to increased evaporation from the ocular surface.

Hirayama et al. [157] reported increased TERs in visual display users after 5 days without the use of any intervention. However, Bilkhu et al. [95] found a significant decrease in TER after 30 minutes of playing a game on a tablet computer. Since TER was not measured while the game was played, and there was a delay after the task was completed, the authors suggested that participants could have compensated for the decreased the blink rate during this time.

Khanal et al. [87] created a novel stress test using a small target viewed in upward gaze for 15 minutes, but did not find a significant change in TER was after completing the stress test. This may be due to the short duration of the task, the testing of normal participants, or not immediately conducting measurements directly after finishing the tear stress test.

1.3.8.1.8 Fixation

Tsubota and Nakamori [151] reported that the TER was 3.4 times larger when participants looked up versus down, and 2.5 times larger when participants looked straight compared to looking down. This change was related to the increased amount of exposed ocular surface in upgaze and primary gaze compared to downgaze, which can lead to greater amounts of tear instability and drying of the ocular surface.

1.3.8.1.9 Ocular Surface Area

In vitro work has shown that TER increases as the size of the ocular surface increases [21] because as the eye size increases, the lipid layer thins [178], which leads to increased amounts of evaporation from the surface of the eye. Race can affect eye size [97]. Since photographing and manually analyzing the area of the ocular surface of each eye can be a time-consuming process, Rolando and Refojo [1] sought to determine a relationship between the ocular surface area and size of the palpebral aperture. A strong positive correlation (r = 0.99) was reported between the area of the exposed ocular surface and the palpebral aperture in a cohort of Caucasians and Asians. The relationship was mathematically described with a linear equation (Y = 0.28x - 0.44, where Y = area of the exposed ocular surface and x = palpebral aperture) and this formula has been used by other researchers to determine the size of the ocular surface [3, 12, 117, 151].

Goto et al. [101] examined Asian participants without DED and also found a strong positive correlation (r = 0.90) between the size of the ocular surface and the palpebral aperture (Y = 0.22x – 0.55, where Y = area of the exposed ocular surface and x = palpebral aperture).

However, Tomlinson et al. [80] did not find a correlation between TER and the ocular surface area. This may have been due to a small sample size.

Other researchers have used computerized digital methods to measure the size of the ocular surface [21, 75, 99, 101, 106, 107]. One group reported using an average ocular surface size for Asians and non-Asians based on previous work to calculate a corrected TER obtained from a modified dermatological device [161]. When it was attempted to confirm these values against its original source because the same value (0.000167 m^2) was reported for both the ocular surface area and the goggle area of non-Asians, a discrepancy was noted between the author mentioned in the text compared to the one cited in the references. Personal communication in 2018 with the first author revealed that the area of the goggle for non-Asians was incorrectly reported and should have been the same as the value reported for Asians (0.00094 m^2). A corrigendum does not seem to have been issued to correct this error.

1.3.8.1.10 Air Volume Within the Evaporimeter

In vitro work demonstrated an inverse relationship between TER and the air volume within the instrument, with an increase in TER as the volume decreases [21]. Additional work conducted by the same group used a predetermined value to represent the internal goggle volume for Asians and non-Asians [161]. These values were used to correct the rate of evaporation after adapting the dermatological device for use with the eye [21].

However, *in vivo* work by Mathers et al. [105] reported a 60% reduction in TER when a smaller volume chamber was used with their evaporimeter.

1.3.8.1.11 Contact Lenses (CLs)

There are an estimated 140 million CL wearers across the world [214, 215], with approximately 40.9 million American adults (16.7%) that wear CLs [216]. CLs are used to correct refractive error [217], to treat ocular surface disease [217-219], to treat corneal problems [217, 220-222], to slow the progression of myopia [223-225], and for cosmetic use.

CLs are available as soft, rigid corneal, hybrid (combination of a soft and rigid lens) [226, 227], or scleral lenses.

1.3.8.1.11.1 Materials

The first scleral lenses were manufactured in the 1880's and were made from glass [217, 228]. In the 1930's, scleral lenses began to be made from plastic, and rigid corneal lenses were also developed using the same material [228]. However, the polymethyl methacrylate (PMMA) plastic material that was used was impermeable to oxygen [229] and could cause hypoxia [230, 231]. Therefore, it is no longer commonly used following the advent of rigid gas permeable materials that allow more oxygen to reach the eye [228, 229, 232].

In the 1950's and 1960's highly oxygen permeable CLs were made from silicone elastomers that did not contain water [228, 233]. Although the lenses had good oxygen permeability, the surface of the CL was highly hydrophobic without the addition of a surface treatment [228]. Problems encountered with silicone elastomer lenses included poor wettability [233] and binding to the cornea [234].

In 1960, Wichterle and Lim [235] first described the use of hydrophilic gels to make soft hydrogel CLs. Soft CLs were initially made from hydroxyethyl methacrylate and were first made commercially available by Bausch + Lomb in 1972. In 1994, soft daily disposable CLs were first introduced, which eliminated the need of a daily cleaning regime [228].

Soft CLs are described by their equilibrium water content, which is calculated as a percentage using the formula in Equation 1-3 [228].

$$Equilibrium\ water\ content\ (\%) = \frac{weight\ of\ water\ in\ polymer}{total\ weight\ of\ hydrated\ polymer}\ x\ 100$$

Equation 1-3: Equilibrium water content formula

The first silicone hydrogel (SiHy) soft CLs were released in 1998 by Bausch + Lomb (balafilcon A) and CIBA Vision (lotrafilcon A) [228], which enabled soft CLs to have greater oxygen permeability [229]. However, since the addition of silicone to a material makes it more hydrophobic, manufacturers have had to improve the wettability by incorporating surface treatments, adding internal wetting agents or hydrophilic monomers that migrate to the surface of the CL, or by creating lenses with water gradient technology [236].

Different types of surface treatments include plasma oxidation to form glassy islands (balafilcon A) [237, 238], a 25 nm plasma coating (lotrafilcon A and B) [238-240], plasma treatment (asmofilcon A) [240], and water gradient technology (delefilcon A, verofilcon A) [240, 241].

Internal wetting agents are embedded into the CL, but wetting agents can also be embedded onto the surface of the lens or designed to be released over the course of the day to improve wettability [242, 243]. Types of wetting agents include polyvinyl alcohol, hyaluronic acid, polyvinyl pyrrolidone, polyethylene glycol, hydroxypropyl methylcellulose, and phosphorylcholine [236, 243-245].

Delefilcon A and verofilcon A are two types of CLs designed with surface technology to improve wettability. In contrast to other hydrogel and silicone hydrogel CLs that have a single water content throughout the lens, the surface technology creates a higher water content at the surface compared to the core. Delefilcon A is a water gradient CL with 33% water content at its core, enclosed within a 4 to 5 µm surface gel of ≥80% water content and a 1 to 2 µm transition zone [237, 239, 245, 246]. Similarly, verofilcon A has a 51% inner core which is surrounded by a 2 to 3 µm surface with a water content ≥80% [241].

1.3.8.1.11.2 Classification of Soft CLs

Hydrogel and SiHy CLs were originally classified into four groups by the United States Food & Drug Administration (FDA) based on the water content and how the lenses interacted with proteins in the tear film and CL care solutions [240, 247]. Ionic CLs have negatively charged surfaces that attract positively charged proteins [248] and are sensitive to care systems [247].

Due to the advent of SiHy CLs, soft CLs are now categorized into one of five groups by the FDA and the International Organization for Standardization (ISO) 18369-1:2017 [240, 249]. Classification is based on the water content and ionic charge of the CL [240, 250]. The ISO classification system is shown in Table 1-9 [249], with the SiHy sub-groups shown in Table 1-10 [250]. The categorization of the sub-groups was based on a scheme proposed by Hutter et al. [251].

Table 1-9: ISO classification system for soft contact lens materials

Group	Water Content	Ionicity	Weight % of Monomers that are Ionic at pH 6-8
I	Low (<50%)	Non-ionic	<0.5
II	High (>50%)	Non-ionic	<0.5
III	Low (<50%)	Ionic	>0.5
IV	High (>50%)	Ionic	>0.5
V	Silicone hydrogel materials		

Table 1-10: ISO sub-classification system for silicone hydrogel materials

Group	Water Content	Ionicity at pH 6-8	Surface Tested?	Other
V-A	Not specified	Ionic		
V-B	High (>50%)	Non-ionic		
V-C	Low (<50%)	Non-ionic		Hydrophilic monomer only
V-Cm	Low (<50%)	Non-ionic	Yes	
V-Cr	Low (<50%)	Non-ionic	No	Semi-interpenetrating network

Conventional spherical hydrogel CLs are currently available in a range of water contents from 38% (polymacon) to 78% (nesofilcon A), and are replaced on daily, bi-weekly, or monthly basis [252]. Higher amounts of water in a hydrogel CL allow more oxygen pass through the lens and reach the eye [253, 254], but is associated with greater amounts of dehydration [255-257], a lower modulus [240], and more discomfort [258, 259].

Spherical SiHy CLs are produced in a range of water contents from 24% (lotrafilcon A) to 56% (somofilcon A), and are also replaced on daily, bi-weekly, or monthly frequency [252].

SiHy CLs can also be described based on their generation. Generations vary based on their polymer chemistry, type of surface treatment, and the relationship between the material and various properties of the CL [260]. First generation SiHy lenses were derived from tris(trimethylsiloxy) silypropylmethacrylate (TRIS) monomers, had surface plasma treatments to improve the wettability,

and a high modulus [261]. Second generation SiHy lenses, like lotrafilcon B, senofilcon A and galyfilcon A, were composed of a modified Tanaka monomer [262] which did not require surface coatings because of the addition of internal wetting agents, and had a higher oxygen permeability than would be predicted from its water content [260]. Third generation SiHy CLs, such as comfilcon A and enfilcon A, do not have surface treatments or wetting agents, and have a lower modulus of elasticity.

1.3.8.1.11.3 Prescribing Trends

The International CL Prescribing Survey Consortium has collected data from around the world since 2000. In 2001, the International Contact Lens Prescribing report surveyed six countries [263]. Soft CLs were used in the majority of fits (86%), with high water content CLs used 25% of the time and SiHy CLs only accounting for 7% of fits. In Canada, mid-water content (40–60%), monthly replacement lenses were the most commonly prescribed lenses.

Over the years, SiHy CLs have increased in popularity, particularly between 2004 and 2010 in Canada (Figure 1-7) [264]. The most recent international report surveyed 24 countries in 2020 and soft CLs were still the most popular type of lens used, accounting for 87% of new CL fits [265]. SiHy CLs are prescribed more frequently (72%) than hydrogels, with planned replacement CLs slightly more popular (54%) than daily disposable lenses. Similar rates of prescribing patterns were observed in Canada, with soft CLs also used in 87% of new fits and SiHy lenses prescribed 78% of the time. However, daily disposables were prescribed slightly more often (54%) than planned replacement CLs.

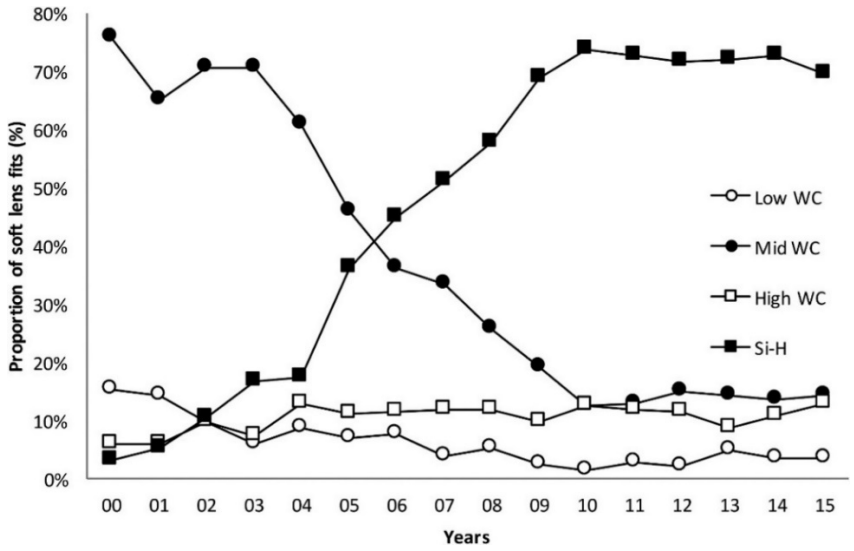

Figure 1-7: Canadian trends in soft contact lens prescribing from 2000 to 2015. Lenses are classified by water content (WC) or as a silicone hydrogel (Si-H). Image from Jones, D. et al. A sixteen year survey of Canadian contact lens prescribing. Cont Lens Anterior Eye 2016; 39(6):402-410.

1.3.8.1.11.4 Contact Lens Discomfort

Despite the innovation and creation of new types of the CLs, CL discomfort continues to be a problem. Therefore, one of the objectives of this thesis is to examine whether evaporimetry can detect differences in evaporation amongst CLs, as lenses that result in less evaporation should be more comfortable.

1.3.8.1.11.4.1 Definition

The 2013 TFOS International Workshop on CL Discomfort: Report of the Definition and Classification subcommittee [266] defined CL discomfort as:

> "…a condition characterized by episodic or persistent adverse ocular sensations related to lens wear, either with or without visual disturbance, resulting from reduced compatibility between the CL and the ocular environment, which can lead to decreased wearing time and discontinuation of CL wear."

1.3.8.1.11.4.2 Classification

CL discomfort can either caused by the lens or the environment. Lens-related factors include the material (water content, friction), design of the CL (base curve, shape of the edge), fit of the lens, frequency of lens wear, the replacement schedule, and how the care system interacts with the lens [36, 242]. Environmental factors that can impact a lens include non-modifiable factors (age, gender, disease, ethnicity), modifiable factors (medication, smoking, compliance), the environment of the eye (tear film stability, lipid layer), and the external environment (RH, temperature, air conditioning) [119, 214]. Further discussion of these topics can be found in The TFOS International Workshop on CL Discomfort reports [36, 214, 242] and CLEAR reports [243, 267].

1.3.8.1.11.4.3 Prevalence

McMonnies and Ho [268] were the first to report increased symptoms in CL wearers in comparison to non-wearers, and found that soft CL wearers were more symptomatic than rigid lens wearers.

Dryness is often reported as the most common symptom of CL discomfort [269-271], with 23 to 77% [269, 271, 272] of CL wearers suffering from dryness. Symptoms typically worsen over the course of the day [273-276].

A survey conducted by Doughty et al. [277] in 1994 found that 50.1% of Canadian CL wearers had symptoms of dry eye. This study also confirmed that CL wearers were more likely to have dry eye than non-wearers. A more recent study conducted in the United States, Canada, and the United Kingdom reported that soft lens wearers in North America had higher rates of CL-related dry eye (North America 39% versus United Kingdom 31%), and experienced more frequent discomfort to a greater degree than those in the United Kingdom [278].

1.3.8.1.11.4.4 Prevalence of Dropout

Between 15.9 and 40% of CL wearers stop wearing lenses [279-282], with 12 to 23% choosing to permanently discontinue wear [279, 280]. The most common reasons for CL discontinuation, or "dropout", are discomfort and dryness [214, 279-281, 283-286]. When people were asked why they stopped wearing CLs, 24.4 to 61% said that it was due to discomfort [279, 280, 282, 283, 286], 19.9% said dryness [279], and 64% said that it was because of both dryness and discomfort [281]. Dryness is the most frequently experienced type of discomfort [269, 283], followed by generalized discomfort [283]. Some of the other more commonly reported reasons for ceasing CL wear include red eyes, problems with their vision, difficulty handling the CLs, the cleaning regime, the financial expense, and because they had run out of lenses and not purchased an additional supply [279, 280, 283]. Further information on CL dropout can be found in a recent review by Pucker and Tichenor [284].

1.3.8.1.11.5 CLs and Tear Film

Insertion of a CL into the eye disrupts the tear film and causes the tear film to be divided into two parts (Figure 1-8). This division results in the creation of a pre-lens tear film (between the air and anterior surface of the CL) and a post-lens tear film (sandwiched between the posterior lens and the ocular surface), and leads to greater instability of the tear film [287]. The reported thickness of the pre-lens tear film on a soft CL ranges from 1.5 to 5.5 µm [30, 34, 37, 39, 288], whereas the post-lens tear film thickness ranges from <1 (after closing the eyes for 30 minutes) to 12 µm [30, 34, 35, 288, 289]. Rigid CLs have a thinner pre-lens tear film than soft CLs [39]. Variability in the estimated range of

thicknesses can be due to the use of different methodologies and instrumentation, the duration of CL wear when the measurements were obtained, and the type of CL worn.

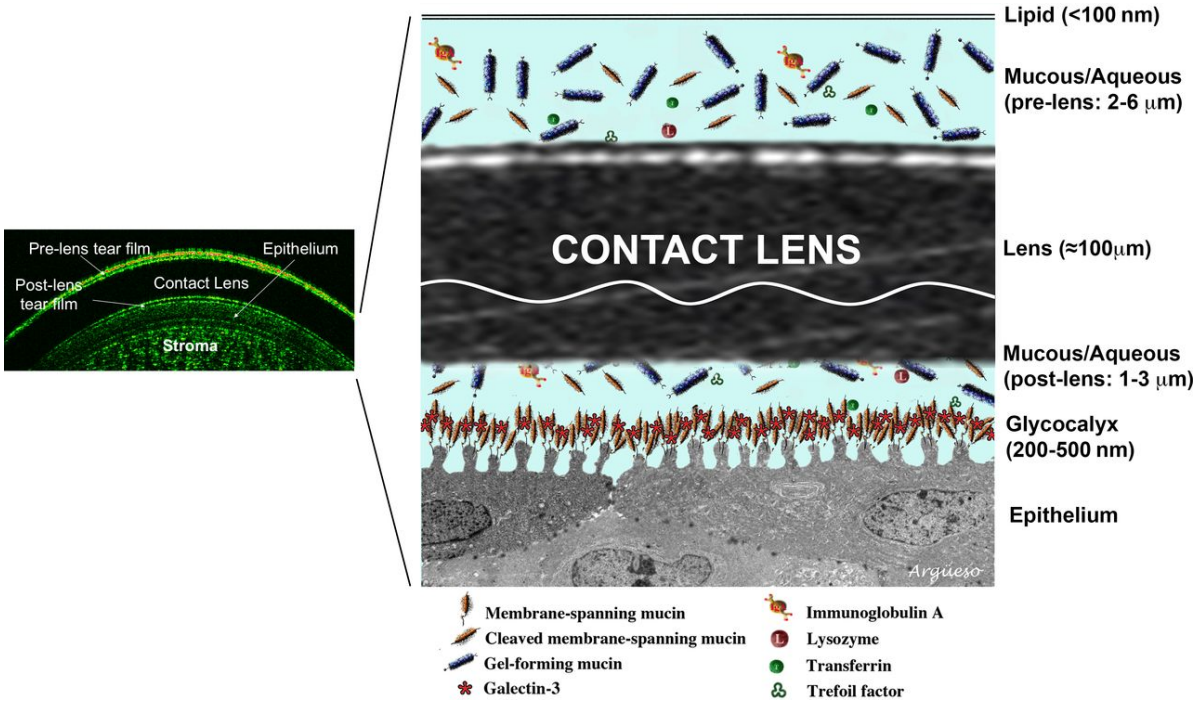

Figure 1-8: Insertion of a contact lens causes the tear film to be divided into the pre-lens and post-lens tear film, as visualized with optical coherence tomography (left) or depicted schematically (right). Image from Craig JP et al. The TFOS International Workshop on Contact Lens Discomfort: Report of the Contact Lens Interactions with the Tear Film Subcommittee. Invest Ophthalmol Vis Sci 2013;54(11):TFOS123-TFO156.

Following insertion of a CL, and after reflex tearing has stopped and excess fluid has drained from the eye, the pre- and post-lens tear film are thinner than a tear film that would be encountered in the absence of a CL [290]. Effects of the thinner tear film include decreased mucin at the CL surface, which results in poorer wettability [291], a more rapid break-up time [18, 292-295], increased friction between the CL and lid margin (which can be visualized as lid wiper epitheliopathy) [296-300], and increased evaporation [16, 17, 21, 73, 102]. Increased friction between the posterior surface of the CL and ocular surface [209, 301] can lead to signs of corneal and conjunctival desiccation [302-306], and lid parallel conjunctival folds [298, 301, 307]. A thinner tear film is also associated with symptoms of dryness or

discomfort [29, 298, 308, 309], which can lead to CL dropout [214]. A comprehensive review of complications associated with CL wear can be found in the CLEAR CL Complications report [287].

1.3.8.1.11.5.1 Percentage Change Formula

Table 2 of the TFOS International Workshop on CL Discomfort: Report of the CL Interactions With the Tear Film subcommittee [36] reported TER increased by 123 to 258% when CLs were worn. Although the original TERs were not provided in the report, calculation of the percentage increase with CL wear using the TERs found within the cited papers [17, 102, 110, 163] revealed that the percentages have been over-estimated by 100%.

The TFOS International Workshop on CL Discomfort: Report of the CL Interactions With the Tear Film subcommittee [36] calculated the percentage increase in TER during lens wear as a ratio of the TER when CLs were and were not worn (Equation 1-4):

$$\frac{TER\ with\ CL}{TER\ without\ CL}\ x\ 100\%$$

Equation 1-4: Percentage increase formula in tear evaporation rate with contact lenses expressed as a ratio.

However, the percentage change is best calculated with the following formula (Equation 1-5):

$$\frac{TER\ with\ CL - TER\ without\ CL}{TER\ without\ CL}\ x\ 100\%$$

Equation 1-5: Percentage change formula in tear evaporation rate with contact lens wear.

For example, Guillon and Maissa [110] reported a mean TER of 15.1 x 10^{-7} g/cm^2/s in non-CL wearers and 23.5 x 10^{-7} g/cm^2/s in CL wearers at 30% RH. Although the TFOS International Workshop on CL Discomfort: Report of the CL Interactions With the Tear Film subcommittee [36] calculated a 156% increase in evaporation rate, the actual increase was 56% because the TER of CL wearers was not more than double the rate of those that did not wear CLs.

The percentage change in TER in this thesis has been calculated using the formula in Equation 1-5.

1.3.8.1.11.6 Evaporation Rate of CL Wearers versus Non-CL Wearers

A summary of studies that measured the evaporation rate of CL wearers without a CL in their eyes is shown in Table 1-11. Guillon and Maissa [110] measured an 11 to 67% higher TER than non-CL

wearers, even when a CL was not worn. With a CL *in situ*, the TER was 41 to 48% higher than that of CL wearers who were not wearing a lens, and 56 to 67% higher than evaporation rate of people that do not wear CLs. Ward et al. [181] also reported that asymptomatic soft CL wearers with a CL *in situ* had a 77% higher TER than non-CL wearers.

However, Dogru et al. [18] only found a 2% non-significant change in TER after dispensing neophytes with senofilcon A for 2 weeks. TER was measured at least 10 hours after CLs were last worn, by which time the evaporation rate could have returned to baseline levels. Alghamdi et al. [116] were also unable to find a significant difference between the TER of non-CL wearers, previous wearers who had not worn CLs for at least 2 years, and habitual CL wearers that had worn CLs either a short period of time (2 ± 1 year), a moderate period (5 ± 1 year), or a long period (10 ± 2 years). CLs were not worn on the day of study visit. The lack of significant change in TER in these studies could be due to small sample sizes because Guillon and Maissa [110] concluded that an increased TER was detectable the day after CLs had been removed.

Table 1-11: Published TERs from studies involving CL wearers without a lens *in situ* and eyes without a CL

Year	Investigator	CL Type	Sample size	RH (%)	Evaporation Rate without CL (x 10^{-7} g/cm^2/s)	Evaporation Rate CL Wearers – No CL Worn (x 10^{-7} g/cm^2/s)	Evaporation Rate CL Wearers – CL *in situ* (x 10^{-7} g/cm^2/s)	ΔEvaporation Rate No CL versus CL Wearers (%)
2008	Guillon [110]	Soft daily CL[a]	Non-CL wearers = 139 CLW (no CL) = 129 CLW (CL *in situ*) = 111	30	15.1 ± 7.3	16.7 ± 7.5	23.5 ± 6.8	CL removed: ↑ 11 CL *in situ*: ↑ 56
				40	11.3 ± 6.8	12.8 ± 7.0	18.9 ± 6.2	CL removed: ↑ 13 CL *in situ*: ↑ 67
2011	Dogru [18]	Senofilcon A	17	30–50	4.1 (3.6 – 4.9)[b]	4.2 (3.7 – 5.0)[b]	NM	↔ 2%
2016	Alghamdi [116]	SiHy and hydrogels[c]	100 (20 per group)	45.5 ± 9	Non-CL wearers: 21.9 ± 9.2 Previous CLW: 25.0 ± 8.1	Short duration CLW: 31.9 ± 15.8 Moderate duration CLW: 30.6 ± 17.8 Long duration CLW: 23.9 ± 11.1	NM	↔ –4.4 to 46

Evaporation rates are reported as the mean ± standard deviation, unless otherwise specified.

CL: contact lens; RH: relative humidity; CLW: contact lens wearers; SiHy: silicone hydrogels; NM: not measured.

[a]: daily disposable, planned replacement, and unplanned replacement.
[b]: median (lower 95% confidence limit – upper 95% confidence limit).
[c]: daily disposable, planned replacement up to 1 month.

1.3.8.1.11.7 Comparison of CL Wearers Before and During CL Wear

The diffusion of water occurs in two directions during CL wear. Osmotic pressure or movement of the upper lid causes water to move from the pre-lens tear film, through the CL, into the post-lens tear film [310]. This helps maintain lens movement to prevent binding and facilitate the removal of debris [310, 311] to help prevent the development of CL complications, such as staining [302, 312-315], inflammation [316, 317], and infection [318-320]. Although the anterior-posterior movement of water through a CL is beneficial, the pervaporation (permeation and evaporation) [321] of water in the opposite direction is problematic because water is lost from post-lens tear film and evaporates from the front surface of the lens into the surrounding air [234]. This leads to CL dehydration [255, 256, 322] and increased evaporation [16, 17, 102, 110].

A summary of the TER when comparing the same participants with and without CLs appears in Table 1-12. The percentage change in TER varies between −25% with a rigid gas permeable lens [175] and 158% with a 38% water content CL [102]. Examination of TER by lens type shows that rigid CLs change the TER by −25 [175] to 116% [102], silicone elastomer CLs change the TER by ~ −10 [73] to 91% [102], and soft CLs change the TER by −12 [175] to 158% [102].

However, it should be noted that the 25% decrease in TER that occurred after a few minutes of wear with a new rigid corneal lens was only measured on one person [175]. This may not be a representative value because individual variations in evaporation can occur with CL wear [16, 17, 73]. In addition, Mathers concluded that there was no significant change in overall TER when the data from the rigid lens was combined with soft CLs [175], although there seems to be a trend for old CLs to cause an increase in TER. Hamano et al. [73] also showed a small decrease in TER after a PMMA CL was worn for 30 minutes; however, it is unknown if this change was statistically significant. These findings contradict the results of Tomlinson and Cedarstaff [102] that found two different PMMA CLs caused a 116% increase in TER when they were worn for an hour. Although all the participants in the study were habitual CL wearers, it is unknown if they normally wore hard or soft CLs. If the participants usually wore soft CLs, a higher TER could have been related to reflex tearing or discomfort with the rigid CL.

Mixed results were also observed with silicone elastomer lenses. Similar to the rigid lenses, it is not known whether the approximately 10% decrease in TER after 30 minutes of CL wear was significant [72, 73]. However, Hamano and al. [73] did note that there was a significant increase in TER for 3

minutes after the CL was inserted due to evaporation from the lens surface, but that the surface became completely dry after 5 minutes and TER decreased back towards baseline levels. These results are in contrast to the 91% increase in TER observed by Tomlinson and Cedarstaff [102]. The authors suggested that the difference in results may be due to the invasive nature of the open-chamber evaporimeter used by Hamano et al. [72, 73] that measured from a smaller area over the cornea and could have interfered with blinking.

The majority of results found that soft CLs cause an increase in TER [16, 17, 21, 73, 102, 110, 323] by 23 [17] to 158% [102]. Similar to the results above, Mathers did not state whether the −12% decrease in TER observed after the insertion of new soft CLs designed to be replaced every week was significant [175]. Likewise, it is unknown whether the 37% increase in TER observed with new CLs on a 6 week replacement scheme was statistically significant. However, since the sample size for the soft lenses was small (n = 4) for each type of lens, and this may have affected the results.

Although differences between TER have been detected amongst CLs [16, 73, 102], the effect of water content is unclear. Hamano et al. [73] included a graph of a participant whose TER was higher after insertion of a high water content hydrogel lens (62%) than with a low water content lens (30%). However, Tomlinson and Cedarstaff [102] reported that low water content hydrogel lenses (38%) have a higher TER than high water content lenses (78%). Although Cedarstaff and Tomlinson [16] were also able to detect differences between high and low water content lenses, the results showed variability between individuals. In contrast, Thai et al. [17] were unable to find significant differences in TER between 5 different types of soft CLs (38% to 62% water content).

Differences between studies may be due to the use of different instrumentation and types of CLs, the duration of wearing time, the age of the CLs, and the material.

Table 1-12: Published TERs comparing the effect of a CL *in situ* to no CL wear

Year	Investigator	CL Type	Sample size	RH (%)	Baseline Evaporation Rate - No CL (x 10^{-7} g/cm^2/s)	Evaporation Rate – CL *in situ* (x 10^{-7} g/cm^2/s)	ΔEvaporation Rate with CL Wear (%)
1981	Hamano [73]	HEMA	5[a]	38	NR	NR	↑ ~30
		PMMA	5[a]	38	NR	NR	↓? <–5[b]
		Silicone rubber	5[a]	38	NR	NR	↓? ~–10[b]
1982	Tomlinson [102]	Cibasoft 38%	5	70	1.09[a] ± 0.34[c]	2.83[c] ± 0.36[c]	↑ 158
		Paragon-18	5	70	1.09[c] ± 0.34[c]	2.37[c] ± 0.33[c]	↑ 116
		PMMA	5	70	1.09[c] ± 0.34[c]	2.36[c] ± 0.27[c]	↑ 116
		Silsoft	5	70	1.09[a] ± 0.34[c]	2.09[c] ± 0.37[c]	↑ 91
		Sauflon 70%	5	70	1.09[a] ± 0.34[c]	1.93[c,d] ± 0.28[c]/ 2.04[c,d] ± NR	↑ 76/ ↑ 87
1983	Cedarstaff [16]	Cibasoft 38%	5	70	1.24[e] ± 0.22[e]	1.93[e] ± 0.36[e]	↑ 55[f]
		Cibasoft 55%	5	70	1.30[e] ± 0.18[e]	1.79[e] ± 0.32[e]	↑ 38[g]
		Sauflon 70%	5	70	1.52[e] ± 0.31[e]	2.04[e] ± 0.31[e]	↑ 35[h]
2004	Mathers [175]	Daily wear for 6 weeks	4	NR	10.8[i] + 0.9[i]	New: 14.8[i] + 3.0[i]	↑? 37
						Old: 23.0[i] + 11.8	↑? 113
		Daily wear for 1 week	4	NR	25.5[i] + 20.0[i]	New: 22.4[i] + 12.6[i]	↔? –12
						Old: 33.4[i] + 12.2[i]	↑? 31
		RGP	1	NR	17.6[i]	New: 13.2[i]	↓? –25
						Old: 28.9[i]	↑? 64
		Single Use	1	NR	17.6[i]	23.7[i]	↑? 35
						Old: 37[i]	↑? 110
		Combined CLs	10	NR	18.0[i] + 13.4[i]	New: 18.5[i] + 8.7[i]	↔ 2.8
						Old: 28.9[i] + 11.3[i]	↑ 61
2004	Thai [17]	Balafilcon A	20	NR	10.85 ± 5.29	Δ4.09 ± 3.51	↑ 38
		Etafilcon A	20		10.85 ± 5.29	Δ4.38 ± 3.65	↑ 40
		Omafilcon A	20		10.85 ± 5.29	Δ2.50 ± 3.03	↑ 23
		Polymacon	20		10.85 ± 5.29	Δ2.96 ± 2.24	↑ 27
		Phemfilcon A	20		10.85 ± 5.29	Δ4.53 ± 3.11	↑ 42
2014	Rohit [21]	Nelfilcon A	15	56%	15.4[j]	25.2[j]	↑ 63

Year	Investigator	CL Type	Sample size	RH (%)	Baseline Evaporation Rate - No CL (x 10^{-7} g/cm^2/s)	Evaporation Rate – CL *in situ* (x 10^{-7} g/cm^2/s)	ΔEvaporation Rate with CL Wear (%)
2018	Siddireddy [323]	Etafilcon A, Comfilcon A, Balafilcon A, Lotrafilcon A, Lotrafilcon B	13 asymptomatic 17 symptomatic		21.1 (13.3)[k] 24.4 (12.5)[k]	26.7 (7.8)[k] 31.1 (15.0)[k]	↑? 26 ↑? 27

Evaporation rates are reported as the mean ± standard deviation, unless otherwise specified.

CL: contact lens; RH: relative humidity; HEMA: hydroxyethyl methacrylate; NR: not reported; PMMA: polymethyl methacrylate; n: sample size; ?: presumed level of significance based on the reported values; RGP: rigid gas permeable.

[a]: did not specify whether measurements were conducted on habitual contact lens wearers.
[b]: values estimated from a graph of percentage change.
[c]: values were adjusted to be 100x smaller as per Tomlinson et al. [11].
[d]: two different sets of data were reported.
[e]: values were adjusted under the assumption that the original values were 100x larger than they should be [11] because some of the same values for Sauflon 70% appear in Tomlinson and Cedarstaff [102].
[f]: individual analysis found one person had a significant decrease in evaporation rate.
[g]: individual analysis found one person had a non-significant change in evaporation rate.
[h]: individual analysis found two person had a non-significant change in evaporation rate.
[i]: type of descriptive statistic was not specified.
[j]: median.
[k]: median (IQR).

1.3.8.1.11.8 Effect of Environment

Dry ambient conditions can affect the TER of CL wearers. A summary of studies investigating the effect of the ambient environment on CLs is shown in Table 1-13.

Kojima et al. [155] fitted neophytes with etafilcon A and narafilcon A. After a week of wear, the researchers reported that etafilcon A wearers had an 82% significant increase in TER after 20 minutes in a windy, controlled adverse chamber set to a low RH. However, narafilcon A wearers had a non-significant increase in TER of 29%, which led the authors to suggest that SiHy lenses can help those who typically encounter adverse environments during their day-to-day activities.

Ward et al. [181] examined the effect of 5 minutes of exposure to passive smoking. No significant change in TER was found between baseline and 2 hours after the exposure to smoke in habitual lens wearers. They suggested that the CL may have acted as a barrier to protect the ocular surface from the smoke.

Table 1-13: Published TERs comparing the effect of an adverse environmental condition on CLs

Year	Investigator	CL (sample size)	RH (%)	Baseline Evaporation Rate – CL *in situ* (x 10^{-7} g/cm^2/s)	Evaporation Rate – After Adverse Condition (x 10^{-7} g/cm^2/s)	ΔEvaporation Rate After Adverse Condition (%)
2010	Ward [181]	Etafilcon A (4) Senofilcon A (5) Balafilcon A (3)	50–60	3.9 (2.90 − 6.65)[a]	4.3 (3.00 − 5.66)[a]	↔ 10
2011	Kojima [155]	Etafilcon A (16)	Ambient 30–40, CACE 18.5 at 18ºC	5.0 ± 2.8	9.1 ± 3.1	↑ 82
		Narafilcon A (15)		4.5 ± 3	5.9 ± 3.3	↔ 29

Evaporation rates are reported as the mean ± standard deviation, unless otherwise specified.

CL: contact lens; RH: relative humidity; CACE: controlled adverse chamber environment.

[a]: median (lower 95% confidence limit – upper 95% confidence limit).

1.3.8.1.11.9 Effect of Treatments for Dry Eye

The results from different studies that investigated the effect of a dry eye treatment on the TER of habitual CL wearers are mixed. A summary appears in Table 1-14. Rohit et al. [20] conducted a crossover study that dispensed a lipid spray, saline spray, lipid emulsion eye drop, and a saline drop for 2 weeks. No significant change in TER was detected with any of the treatments after 1 day or 14 days

of use. The authors suggested that a change in evaporation may not have been found with the lipid treatments because CLs were worn for 6 hours prior to the visit and that participants may have adapted to the use of the treatment. They also suggested that differences in formulation of the treatments, the amount of product that was dispensed, and the use of a spray compared to a drop may have been a factor.

In MGD, keratinized material can build up around the meibomian glands and obstruct the orifice [25]. Lid debridement helps remove keratinized material from the lid margin and can improve the expression of the meibomian glands [324, 325], decrease the symptoms of dry eye [324-326], and improve corneal and conjunctival staining [325]. Use of cleansers helps reduce the bacterial load and remove debris from the lid [140, 327]. Cleansers can improve dry eye symptoms [328, 329], decrease corneal staining [328], and decrease the amount of oily discharge around the eye [328].

Siddireddy et al. [19] conducted a crossover study to examine the effect of a single treatment of lid debridement compared to the use of a foam cleanser. TER was measured initially with CLs, then 20 minutes after removal of CLs. Although no significant change in TER occurred 7 to 10 days after the treatment when CLs were worn, a significant decrease was observed when CLs were not worn. Lid debridement was more effective at decreasing the TER than the foam cleanser in symptomatic and asymptomatic lens wearers.

Table 1-14: Published TERs comparing the effect of a dry eye treatment on CL wearers

Year	Investigator	CL	Sample size	RH (%)	Baseline Evaporation Rate (x 10^{-7} g/cm^2/s)	Evaporation Rate After Treatment (x 10^{-7} g/cm^2/s)	ΔEvaporation Rate After Treatment (%)
2016	Rohit [20]	Baseline: Habitual soft CL Post-treatment: Air Optix	24 asymptomatic	NR	35.6 ± 20.0	Lipid spray: 47.5 ± 22.5[a]	↔ 33
						Saline spray: 45.6 ± 26.4[a]	↔ 28
						Lipid drop: 40.8 ± 23.1[a]	↔ 15
						Saline drop: 44.7 ± 26.4[a]	↔ 26
			16 symptomatic		41.4 ± 21.4	Lipid spray: 33.1 ± 16.9[a]	↔ –20
						Saline spray: 36.4 ± 26.7[a]	↔ –12
						Lipid drop: 37.2 ± 20.0[a]	↔ –10
						Saline drop: 49.2 ± 34.7[a]	↔ 19
2019	Siddireddy [19]	Habitual soft daily wear CL	13 asymptomatic [19, 330]	NR	20 (11.1)[b,c]	Foam cleanser: Δ5.1[c,d]	↓ 26
					21.4 (13.6)[b,c]	Lid debridement: Δ8.6[c,d]	↓ 40
			17 symptomatic [19, 330]		23.3 (12.2)[b,c]	Foam cleanser: Δ6.0[c,d]	↓ 26
					24.7 (12.8)[b,c]	Lid debridement: Δ12.6[c,d]	↓ 51

Evaporation rates are reported as the mean ± standard deviation, unless otherwise specified.

CL: contact lens; RH: relative humidity; NR: not reported.

[a]: interim visit not included in the table.
[b]: median (IQR).
[c]: measured without contact lenses.
[d]: median difference.

1.3.8.2 Environmental Factors

1.3.8.2.1 Temperature

TER increases as the ambient temperature increases [115]. Abusharha et al. [115] found the TER at 25ºC was three times larger than the TER at 5ºC, and twice as high as the TER at 10ºC. This is due to the increased kinetic energy of water molecules as temperature increases, which causes the molecules to leave the ocular surface at a faster rate and result in increased evaporation [56].

1.3.8.2.2 Relative Humidity

TER increases as the RH decreases [6, 13, 92, 99, 107, 108, 110, 111, 182], with a 10% change in RH able to significantly alter the TER [6, 7, 13, 109]. Researchers tested non-DED and DED participants under dry environmental conditions, similar to those experienced in an airplane, and reported a 99.4 [107] and 99.7% [108] increase in TER when going from a 40-45% RH to a 20-25% RH. When examining more extreme changes in RH, Abusharha et al. [182] found higher TERs after being in a 5% RH for 20 minutes and 60 minutes compared to 40% RH. After being placed in a 5% RH environment for an hour, the TER was twice as high as that recorded at 40% RH. Madden et al. [92] measured higher TERs at 5% RH compared to 40% and 70% RH, and found TERs of approximately 0 $g/m^2/h$ at 70% RH. Higher TERs can occur at low RHs due to the thinning of the lipid layer, which causes an increase in evaporation [182].

1.3.8.2.3 Smoking

Smoking increases TER. Five minutes of exposure to passive cigarette smoke significantly increased TER in non-CL wearers without dry eye [160], with an increased TER present 2 hours after exposure to smoke [181]. However, a significant change in TER was not observed in CL wearers before and 2 hours after exposure to passive smoke [181].

Higher TERs have also been measured in chronic smokers compared to non-smokers [94, 154], which may be due to poor spreading of the lipid layer [154, 331] and shorter tear film break-up times [154, 332]. Further information on the effect of smoking on the tear film can be found in a recent review by Miglio et al. [333].

1.3.8.3 Other Factors

1.3.8.3.1 Diurnal Variation

Tomlinson et al. [164] measured a significantly lower TER upon waking, and this was believed to be due to the presence of a thicker lipid layer. The TER rapidly increased over the first 2 hours of the day, and then remained stable over the next 12 hours.

Wojtowicz et al. [7] reported mixed results when testing participants in the morning (between 8 to 9 AM) in comparison to 8 hours later. The first day of testing revealed a significantly lower TER in the morning. However, repeated measurements on a separate day did not show a significant difference between the two time points. The authors recommended assessing TER in the afternoon because the measurements were less variable.

Rohit et al. [21] tested TER in the morning (between 8:30 to 10:30 AM), at mid-day (12:30 to 2:30 PM), and in the afternoon (4:30 to 6:30 PM) on two different days. It was advised that to measure TER, the repeatability of the evaporimeter should be analyzed during different times of the day. TER was the most repeatable in the afternoon without CL wear, but became the least repeatable in the afternoon when CLs were worn.

1.4 Rationale and Objectives

DED is common reason that compels people to seek the advice of an eye care practitioner [121, 334], with 28.7% of people who attended an optometric practice in Canada reporting symptoms of dry eye [277]. DED is a significant economic burden [335-338], which can result in decreased work productivity [336, 337] and a poorer quality of life [336]. Suffering from moderate dry eye has been likened to suffering from moderate angina, while severe dry eye has been described as more debilitating than a disabling hip fracture [339].

Although the prevalence of DED increases with age [124, 130, 131, 340], EDE due to MGD accounted for 49.27% of dry eye cases in children and adolescents that attended an ophthalmology clinic from 2010 to 2018 [341]. The prevalence of EDE increased to 66% in 19 to 21-year-olds [341], and may be due to linked to the use of digital devices [342, 343]. As the amount of time spent on digital devices increases, so does the frequency of dry eye [344, 345].

Due to the multifactorial nature of DED, multiple tests are available to diagnose and treat dry eye. Ideally, a test should be quick, non-invasive, inexpensive, and easy to use. The TFOS DEWS II Tear Film Report [10] and a subsequent review paper from 2019 [346] both seem to have overlooked the availability of Eye-VapoMeter when stating that a commercial instrument to detect tear evaporation did not exist. One suggestion of the TFOS DEWS II Tear Film Report was that it would useful to develop evaporimeters that could be used in clinical practice under different temperatures and humidities [10].

The objectives of this thesis are two-fold. Firstly, to assess the calibration of the only commercially available evaporimeter and to investigate its ability to detect *in vitro* differences between CLs. Secondly, to describe the development, *in vitro*, and *in vivo* testing of a new evaporimeter. In contrast to previous closed-chamber systems that placed the sensor in a cylinder behind the goggle [2, 21, 99, 101] or connected the sensor via tubes to the swimming goggle [102, 103], the novel evaporimeter is believed to be the first to incorporate a sensor into the front lens plate of the goggle.

In addition to the application of evaporimetry to the dry eye field, it is also useful for investigating different types of CLs [16, 73, 102, 155]. If the evaporimeter is able to detect differences in the evaporation rate of CLs, this could identify lenses that are more comfortable, that can prolong the wearing time, and result in less discomfort [266]. The portability and short measurement time of the Eye-VapoMeter [21] means that it might be able to measure *in vitro* differences amongst CLs, which would be a more cost-effective method than conducting a clinical study. Chapter 2 examines the ability of the Eye-VapoMeter to measure different evaporation rates amongst various soft CL materials. Only soft CLs were examined because these were the most common type of lens used for new fits in 2020 [265].

Due to a difference in the volume of the swimming goggle attached to the commercially available evaporimeter in comparison to the one used during the validation of the instrument [21], Chapter 3 repeats the *in vitro* validation tests that were originally used to calibrate the instrument.

Since the Eye-VapoMeter does not show how RH changes over time when it placed over the eye, Chapter 4 describes the design and components of a novel binocular evaporimeter that might be able to be used in different temperatures and humidities. Chapter 5 investigates *in vitro* testing of the evaporimeter with different surface areas and air volumes, and compares measurements obtained with

each side of the instrument. Chapter 6 investigates the *in vivo* pilot testing of the new device and describes the optimization of the methodology used to measure evaporation.

Chapters 7 and 8 use the findings from Chapter 6 to conduct *in vivo* evaporimetry measurements on a varied group of participants. In order to determine whether the new instrument can be used in both dry eye and CL research, Chapter 7 investigates whether the instrument can differentiate between people with and without dry eye, and whether the instrument can detect changes after the instillation of an eye drop. Chapter 8 examines whether the novel evaporimeter is able to measure a change in evaporation with CL wear, tests whether it can differentiate between asymptomatic and symptomatic CL wearers, and examines whether two types of CLs have a different evaporation rate after 6 hours of wear.

Finally, Chapter 9 summarizes the findings of the thesis and provides suggestions for future work.

Chapter 2

In Vitro Measurement of Contact Lens Evaporation Rates Using the Eye-VapoMeter

2.1 Overview

PURPOSE: To investigate the ability of a commercially available evaporimeter to detect a difference in the *in vitro* evaporation rate of water from hydrogel and silicone hydrogel contact lenses (CLs) of different water contents.

METHODS: An *in vitro* study was conducted to measure the evaporation rate of water from 7 silicone hydrogel CLs (comfilcon A, delefilcon A, lotrafilcon B, narafilcon A, senofilcon A – 1 day and 2 week, somofilcon A), 9 hydrogel CLs (etafilcon A – 1 day and 2 week, hilafilcon B, nelfilcon A, nesofilcon A, oculfilcon D, omafilcon A, omafilcon B, polymacon), and 1 rigid corneal contact lens (itabisfluorofocon A). All CLs were –3.00 DS back vertex power. The evaporation rate from each soft lens was measured with the Eye-VapoMeter every 2-minutes until 3 consecutive measurements of 0 g/m^2h were obtained (n=5). The rigid contact lens was used as a control and evaporation rate was measured every 2-minutes for 10 minutes (n=5). The change in evaporation rate per minute was calculated from the slope of the evaporation rate over time. Four sequential 10-minute time periods were investigated from 0 to 40 minutes.

RESULTS: There was significant difference in the evaporation rates reported for each 10-minute period between all of the soft CLs (0 to 10 minutes: $p<0.0001$, 10 to 20 minutes: $p<0.0001$, 20 to 30 minutes: $p<0.0001$, 30 to 40 minutes: $p<0.0001$). Post-hoc testing found significant differences between types of CLs (0 to 10 minutes: all $p<0.046$, 10 to 20 minutes: all $p<0.048$, 20 to 30 minutes: all $p<0.050$, 30 to 40 minutes: all $p<0.045$). The CLs with the largest difference in evaporation rate were: delefilcon A and nesofilcon A (–1.55 vs. 0.17 (g/m^2h)/minute, $p=0.002$) from 0 to 10 minutes, comfilcon A and hilafilcon B from 10 to 20 minutes (–1.68 vs. –0.19 (g/m^2h)/minute, $p=0.001$), omafilcon B and narafilcon A (–2.14 vs. –0.34 (g/m^2h)/minute, $p<0.001$) from 20 to 30 minutes, and nesofilcon A and narafilcon A (–2.06 vs. –0.28 (g/m^2h)/minute, $p=0.006$) from 30 to 40 minutes.

CONCLUSIONS: The Eye-VapoMeter:

- Was able to measure evaporation rates of various soft CLs over time;

- Detected different rates of evaporation between various types of soft CLs;
- Did not detect any evaporation from a rigid corneal lens.

2.2 Introduction

Soft contact lenses undergo water loss throughout the day [47, 255, 322, 347-351] due to the increased temperature of the eye [47, 257, 350], evaporation from the front surface of the contact lens (CL) [47, 352], and pervaporation from the post-lens tear film [234]. Higher water content CLs are reported to dehydrate faster than low water content CLs [255, 257, 353, 354]. CLs with a higher water content contain greater amounts of water and therefore are able to lose more water [355], and also have a lens matrix that contains more space which allows water to more easily diffuse from the bulk of the lens material to the surface [321]. However, not all researchers have found that higher water content CLs have higher rates of dehydration [245, 349] and this may be due to differences in CL thickness [349], varying ratios of free to bound water within a material [356], and the inclusion of wetting agents [245].

Consequences of water loss from a CL include steepening of the base curve [350, 357], decreased total diameter [350, 355], reduced movement [358], a change in refractive index [245, 351, 359], and decreased oxygen transmission [350, 360]. Poor wetting of the front surface of a CL can lead to increased evaporation and greater amounts of friction between the CL and eyelid, which can result in ocular signs of lid-wiper epitheliopathy [298, 301, 361] and lid parallel conjunctival folds [161, 298, 301]. Thinning of the post-lens tear film can also lead to corneal staining [302, 313, 315, 362, 363], with the loss of water resulting in symptoms of dryness or discomfort [161, 298, 301, 348, 363].

The amount of water loss from a CL is traditionally measured using gravimetry or refractometry. Gravimetric methods use a microbalance to weigh a CL in order to determine how much water is lost [257, 353, 364, 365], while a refractometer can be used to estimate water content based on the refractive index of the CL [245, 255, 322, 348, 349]. An inverse relationship exists between refractive index and water content [245, 366, 367]. CLs with larger amounts of water have lower refractive indices, which are more similar to the refractive index of water (1.33) [245], whereas higher refractive indices indicate the presence of less water in a CL [245, 366, 367].

Evaporimetry may be an alternative method for measuring CL water loss, or dehydration, by measuring the rate of water loss from the CL. A higher evaporation rate indicates a faster rate of dehydration of

the CL. To our knowledge, evaporimetry has not previously been used to investigate the *in vitro* characteristics of CLs. This may be due to poor instrument portability, relatively long measurement times, and the prior lack of availability of a suitable device. However, one instrument with the potential to be used in this way is the Eye-VapoMeter (Delfin Technologies Ltd., Kuopio, Finland).

Published *in vivo* studies using the Eye-VapoMeter have reported on changes in the tear evaporation rate observed with and without CL wear [21, 323], or on the effect of a liposomal spray on the tear evaporation rate of CL wearers with their habitual CLs compared to 1 or 14 days of wearing Air Optix (Alcon, Fort Worth, Texas, USA). One non-peer-reviewed student project reported a significant increase in tear evaporation with Proclear CLs (CooperVision, San Ramon, California, USA), but not MyDay CLs (CooperVision, San Ramon, California, USA) [368].

Due to the lack of *in vitro* work investigating CL dehydration using evaporimetry, the purpose of this chapter was to examine the ability of the Eye-VapoMeter to measure different evaporation rates in a variety of soft CL materials.

The aims of this chapter are to use the Eye-VapoMeter to:

- Measure the evaporation rate of hydrogel and silicone hydrogel CLs while the lenses are dried in a controlled environment;
- Examine whether there are differences in the pattern of change in evaporation rate for each soft CL.

2.3 Methods and Materials

Soft CLs (7 silicone hydrogels, 9 hydrogels, Table 2-1) were selected for the study based on the material and water content. All lenses had –3.00 DS back vertex power (BVP) in an attempt to reduce variation in CL center thickness. Five CLs of each material were tested, and all five CLs originated from the same manufacturer's batch number. The order of testing each CL material was randomized.

CLs were removed from the storage blister pack using a pair of blunt end tweezers and each side was gently dabbed on lens paper (Fisherbrand, Fisher Scientific, Waltham, Massachusetts, USA) until liquid was no longer visibly absorbed by the lens paper. The back surface of the CL was then placed at the center of an aluminum model eye (Figure 2-1A). The curvature of the model eye was designed to match the shape of the swimming goggle attached to the base of the Eye-VapoMeter to prevent loss of air

when the evaporimeter was placed over the CL. The cylindrical holes of the model eye were covered with clear tape to form a uniform surface.

Lens preparation and testing were conducted inside a 61x46x38 cm environmental chamber (Model 5503-11 Package E, electro-tech systems inc., Glenside, Pennsylvania, USA) set to 40% relative humidity (RH) under automatic control.

With the CL in position on the model eye within the environmental chamber, the Eye-VapoMeter (Delfin Technologies Ltd., Kuopio, Finland) was placed over the CL. The instrument works by measuring the temperature and RH within the instrument goggle and cylinder. The duration of the measurement varies depending on how quickly the RH changes within the instrument. Lower rates of evaporation change at a slower rate and have a longer measurement time. The evaporation rate, ambient temperature, and ambient RH output values were recorded and logged using the manufacturer's proprietary software (DelfWin 4 version 3.1.14, Delfin Technologies Ltd., Kuopio, Finland) every 2-minutes. The time interval chosen between measurements was determined by the time interval required for the RH air within the Eye-VapoMeter goggle to ventilate, and for the temperature and RH within the goggle to return to ambient conditions following a measurement [369].

The measurement sequence for each CL was stopped when three consecutive measurements of 0 g/m^2h were obtained. The time of the first consecutive 0 g/m^2h measurement was considered to be the time when the water from the CL had stopped evaporating. The duration of evaporation (in minutes) was calculated as the time interval between the initial measurement and when the CL ceased to evaporate.

The rate of change of evaporation per minute was calculated as the slope of the Eye-VapoMeter evaporation rate over time. Four different time intervals were investigated: 0 to 10 minutes, 10 to 20 minutes, 20 to 30 minutes, and 30 to 40 minutes.

One rigid corneal CL (BVP: –3.00 DS) was used as a control and tested in 2-minute intervals for 10 minutes (n=5).

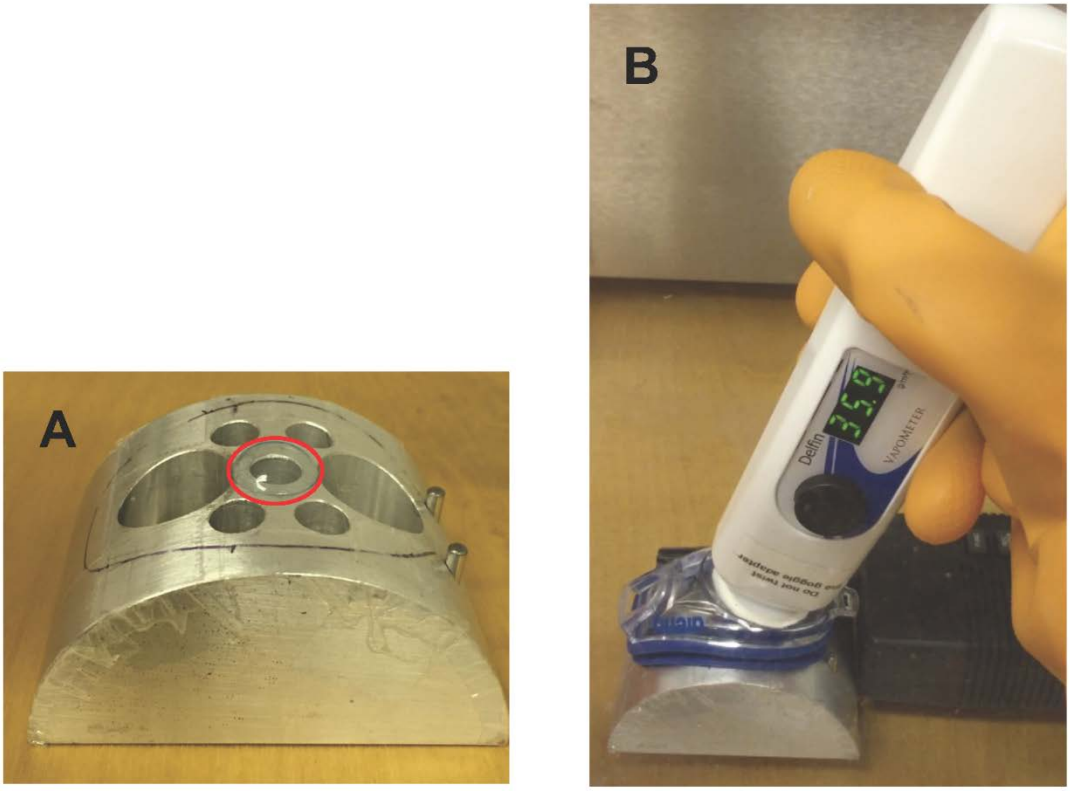

Figure 2-1: Placement of a soft contact lens with the back surface against the model eye (A), with a red outline highlighting the location of the soft contact lens. The black line outline shown in (A) helped align the Eye-VapoMeter over the model eye (B) to ensure the instrument was held in the same position during each measurement.

Table 2-1: Contact lens specifications

Material	Lens Name	Lens Type	Base Curve	Diameter	Equilibrium Water Content (%)	Center thickness (mm)
comfilcon A	Biofinity®	SiHy	8.6	14	48	0.080
delefilcon A	DAILIES TOTAL1®	SiHy	8.5	14.1	33	0.090
etafilcon A	1•DAY ACUVUE® MOIST®	Hy	8.5	14.2	Surface water content ≥80 58	0.084
etafilcon A	ACUVUE® 2	Hy	8.3	14	58	0.084
hilafilcon B	SofLens®	Hy	8.6	14.2	59	0.090
itabisfluorofocon A	NA	Rigid	7.8	9.6	NA	0.110
lotrafilcon B	AIR OPTIX® AQUA	SiHy	8.6	14.2	33	0.080
narafilcon A	1•DAY ACUVUE® TruEye® with HYDRACLEAR® 1	SiHy	8.5	14.2	46	0.085
nelfilcon A	DAILIES® AquaComfort PLUS®	Hy	8.7	14	69	0.100
nesofilcon A	Biotrue® ONEday	Hy	8.6	14.2	78	0.100
ocufilcon D	Biomedics® 55 Premier	Hy	8.6	14.2	55	0.070
omafilcon A	Proclear® 1 day	Hy	8.7	14.2	60	0.090
omafilcon B	Proclear®	Hy	8.6	14.2	62	0.065
polymacon	SofLens® 38	Hy	8.7	14	38.6	0.035
senofilcon A	ACUVUE OASYS® with HYDRACLEAR® PLUS	SiHy	8.4	14	38	0.070
senofilcon A	ACUVUE OASYS® with HydraLuxe™ 1-Day	SiHy	8.5	14.3	38	0.085
somofilcon A	clariti™ 1 day	SiHy	8.6	14.1	56	0.070

Hy = hydrogel; SiHy: silicone hydrogel; NA: not applicable

2.3.1 Statistical Analysis

Statistical analysis was conducted with GraphPad 8.3.0 for Windows (GraphPad Software, LLC, San Diego, California, USA). Data was tested for normality using the Shapiro-Wilk test with α=0.05. Comparisons between types of CLs were conducted with a Kruskal-Wallis test and a post-hoc Dunn's multiple comparisons test. Spearman's rank correlation (r_s) was used to determine the linear correlations between the evaporation rate per minute, water content, and duration of evaporation rate.

2.4 Results

2.4.1 Environmental Chamber Temperature and Relative Humidity

The mean ± standard deviation (SD) ambient temperature within the environmental chamber was 25.1 ± 0.5°C and RH was 40.3 ± 1.1%. The range of ambient temperature was 23.0 to 26.5°C and RH ranged between 37.2 to 45.0%.

2.4.2 Change in Evaporation Rate over Time

The change in median and interquartile range (IQR) evaporation rate of the rigid and soft CLs over time is shown in Figure 2-2. A summary of the median (IQR) initial evaporation rate of each soft CL is shown in Table 2-2, with rates of evaporation ranging from 35.3 (3.0) for nelfilcon A to 42.4 (3.6) g/m^{2}h for ocufilcon D. There was no significant difference in the initial evaporation rate of the different types of soft CLs (p=0.19). The soft CLs exhibited two types of evaporation curves: (i) a brief initial period of stabilization, before a rapid decrease to a moderate evaporation rate, followed by a long, gradual decrease to zero (Figure 2-3A); and (ii) a longer initial period of stabilization, before a rapid decrease to a low evaporation rate, followed by a gradual decrease to zero (Figure 2-3B). The evaporation rate of the rigid gas permeable corneal lens (itabisfluorofocon A) remained stable at 0 (0) g/m^{2}h over a 10-minute period.

It should be noted that the abrupt increase in evaporation rate that occurred at 50 minutes for nesofilcon A in Figure 2-3B was due to four out of five lenses reaching 0 g/m^{2}h between 42 to 50 minutes after measurements began. The graph of nesofilcon A at 52 minutes onwards therefore represents the evaporation rate of the single CL that took longer to fully dehydrate. Although two additional evaporation measurements were taken to ensure that each CL maintained a 0 g/m^{2}h evaporation rate for six minutes, and it can be assumed that each CL would have maintained this rate over time, the plot

only includes the first 0 g/m^{2}h value to provide consistency with the values used to calculate the duration of evaporation (Section 2.4.4).

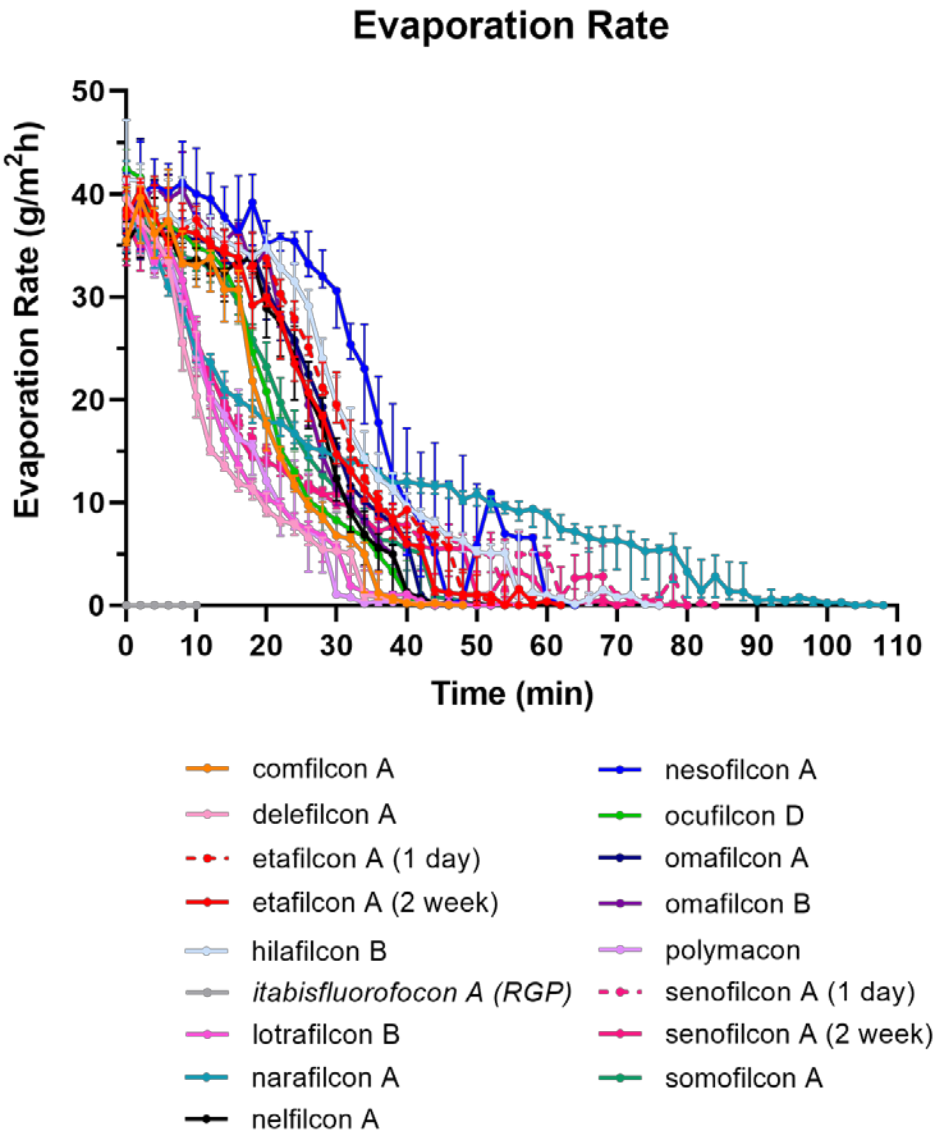

Figure 2-2: Median evaporation rate of each contact lens material over time. Error bars indicate interquartile range (IQR). Rigid gas permeable material (itabisfluorofocon A) is in italics.

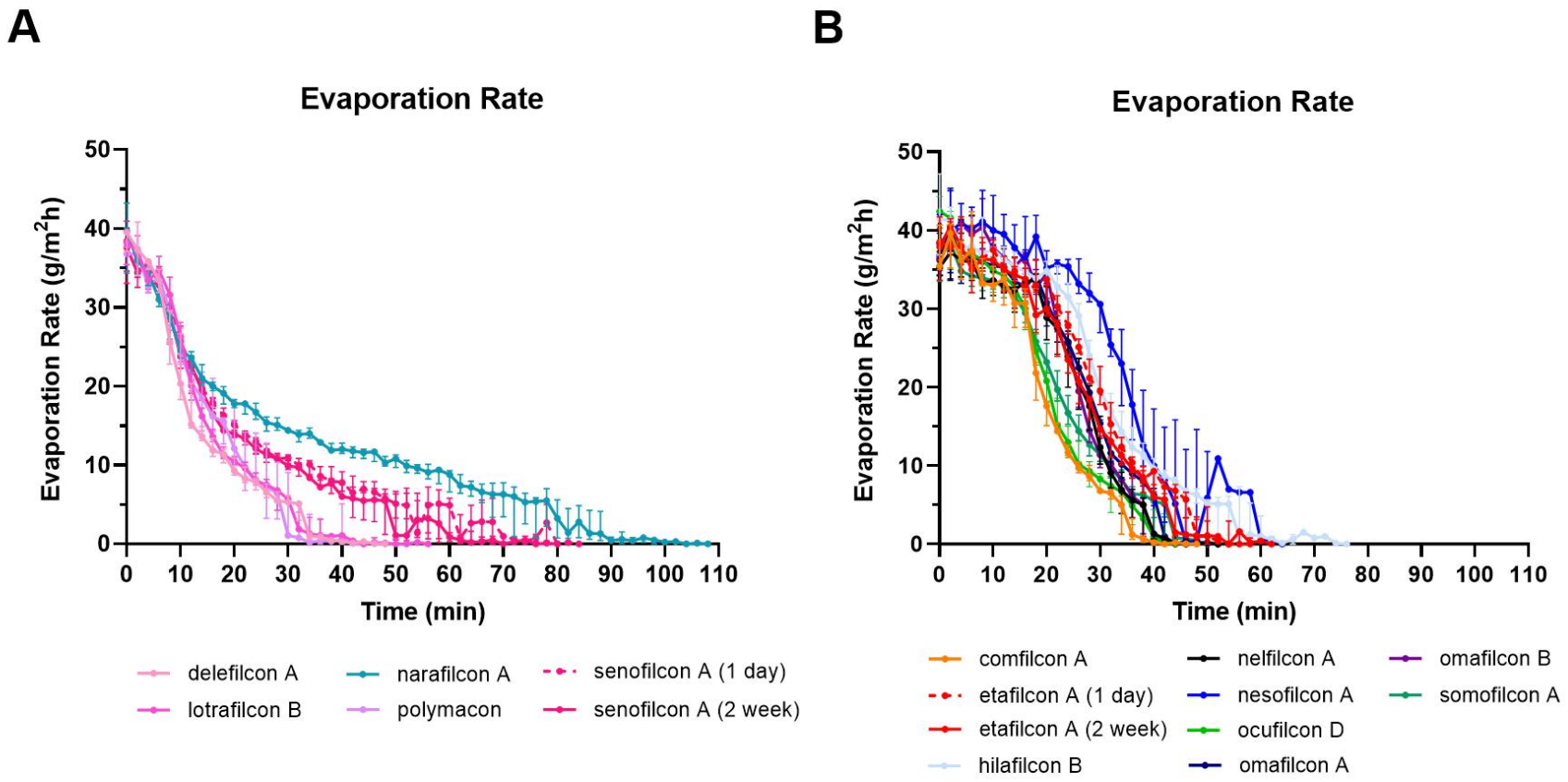

Figure 2-3: Median evaporation rate of each soft contact lens material over time. (A) showing a short initial stable period, before a rapid decrease to a moderate evaporation rate, followed by a long, gradual decrease to zero (0 g/m^{2}h). (B) showing a more prolonged period of stabilization, before a rapid decrease to a low evaporation rate, followed by a gradual decrease to zero (0 g/m^{2}h). Error bars indicate IQR.

Table 2-2: Median (IQR) initial evaporation rate of each soft lens material

Material	Evaporation Rate (g/m^2h) (n=5)
comfilcon A	35.3 (5.7)
delefilcon A	39.5 (2.6)
etafilcon A (1 day)	38.5 (6.7)
etafilcon A (2 week)	37.9 (5.5)
hilafilcon B	41.1 (10.3)
lotrafilcon B	38.2 (4.3)
narafilcon A	39.7 (8.9)
nelfilcon A	35.3 (3.0)
nesofilcon A	38.3 (7.1)
ocufilcon D	42.4 (3.6)
omafilcon A	36.2 (5.5)
omafilcon B	36.8 (8.1)
polymacon	36.8 (2.2)
senofilcon A (1 day)	38.4 (7.9)
senofilcon A (2 week)	37.6 (5.5)
somofilcon A	36.5 (3.7)
p-value	0.19

2.4.3 Change in Evaporation Rate per Minute over Time

The median (IQR) change in evaporation rate per minute for each soft CL material is shown in Table 2-3. There were significant differences in the rate at which each material dehydrated over time (0 to 10 minutes: $p<0.0001$, 10 to 20 minutes: $p<0.0001$, 20 to 30 minutes: $p<0.0001$, 30 to 40 minutes: $p<0.0001$). Post-hoc testing found significant differences between types of materials (0 to 10 minutes: all $p<0.046$, 10 to 20 minutes: all $p<0.048$, 20 to 30 minutes: all $p<0.050$, 30 to 40 minutes: all $p<0.045$, Appendix A), (Figure 2-4).

The two CL materials that exhibited the greatest difference in median evaporation rate per minute were: delefilcon A and nesofilcon A (–1.55 vs. 0.17 (g/m^2h)/min, $p=0.002$) from 0 to 10 minutes, comfilcon A and hilafilcon B from 10 to 20 minutes (–1.68 vs. –0.19 (g/m^2h)/min, $p=0.001$), omafilcon B and narafilcon A (–2.14 vs. –0.34 (g/m^2h)/min, $p<0.001$) from 20 to 30 minutes, and nesofilcon A and narafilcon A (–2.06 vs. –0.28 (g/m^2h)/min, $p=0.006$) from 30 to 40 minutes.

Table 2-3: Median (IQR) change in evaporation rate per minute for each soft lens material

Material	ΔEvaporation rate per minute (g/m^2h)/min			
	0 to 10 Minutes	10 to 20 Minutes	20 to 30 Minutes	30 to 40 Minutes
comfilcon A	–0.64 (0.82)	–1.68 (0.27)	–1.09 (0.29)	–0.66 (0.02)
delefilcon A	–1.55 (0.91)	–1.14 (0.64)	–0.43 (0.13)	–0.51 (0.10)
etafilcon A (1 day)	–0.17 (0.72)	–0.39 (0.46)	–1.21 (0.73)	–1.18 (0.30)
etafilcon A (2 week)	–0.19 (0.49)	–0.45 (0.28)	–1.67 (0.38)	–0.80 (0.21)
hilafilcon B	–0.28 (1.09)	–0.19 (0.31)	–1.30 (0.46)	–1.10 (0.15)
lotrafilcon B	–1.12 (0.61)	–1.55 (0.48)	–0.51 (0.12)	–0.47 (0.04)
narafilcon A	–1.34 (0.97)	–0.77 (0.24)	–0.34 (0.11)	–0.28 (0.13)
nelfilcon A	–0.24 (0.36)	–0.39 (0.61)	–1.64 (0.58)	–1.16 (0.58)
nesofilcon A	0.17 (0.46)	–0.64 (0.34)	–0.51 (0.45)	–2.06 (1.23)
ocufilcon D	–0.75 (0.29)	–1.48 (0.45)	–1.48 (0.45)	–0.72 (0.44)
omafilcon A	–0.07 (0.34)	–0.46 (0.38)	–1.67 (0.62)	–1.00 (0.51)
omafilcon B	0.02 (0.83)	–0.37 (0.29)	–2.14 (0.24)	–0.96 (0.33)
polymacon	–1.26 (0.61)	–1.13 (0.44)	–1.14 (0.63)	–0.11 (0.55)
senofilcon A (1 day)	–1.42 (0.68)	–1.04 (0.25)	–0.44 (0.08)	–0.26 (0.16)
senofilcon A (2 week)	–1.09 (0.80)	–1.08 (0.50)	–0.44 (0.18)	–0.38 (0.15)
somofilcon A	–0.29 (0.62)	–1.02 (0.34)	–1.17 (0.20)	–0.56 (0.49)

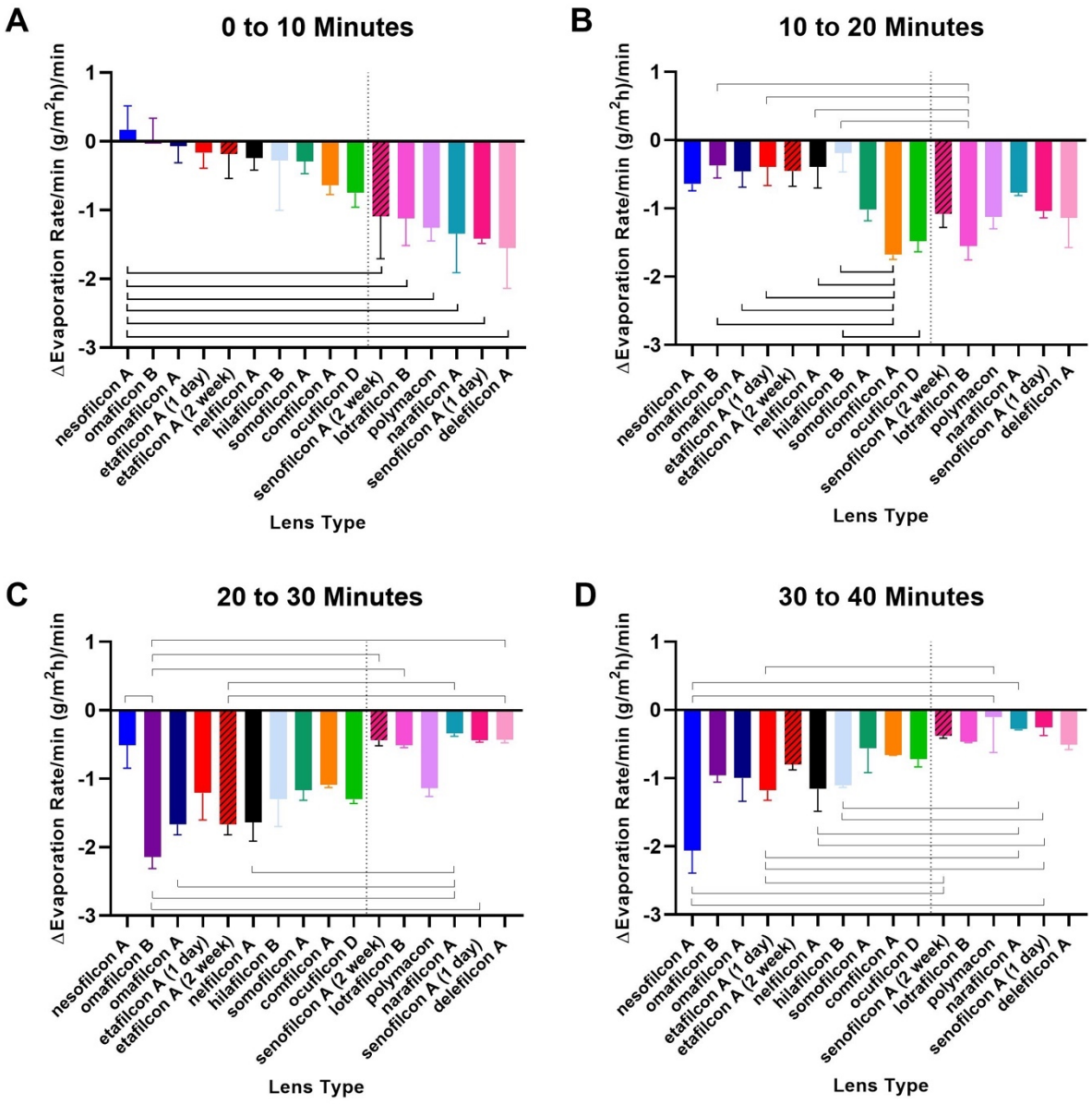

Figure 2-4: Median change in evaporation rate per minute for each soft lens material over 0 to 10 minutes (A), 10 to 20 minutes (B), 20 to 30 minutes (C), and 30 to 40 minutes (D). Error bars indicate IQR and significance lines indicate $p<0.05$ (0 to 10 minutes: all $p<0.046$, 10 to 20 minutes: all $p<0.048$, 20 to 30 minutes: all $p<0.050$, 30 to 40 minutes: all $p<0.045$). The dotted line delineates materials that had a longer initial period stable evaporation rate during the first 10 minutes (left) compared to those that had shorter initial period of evaporation (right).

2.4.3.1 Correlation Between Change in Evaporation Rate per Minute and Equilibrium Water Content

Significant correlations were found between the change in evaporation rate per minute and the equilibrium water content (EWC) of the CL material (Figure 2-5). Strong positive correlations were found during the first 20 minutes (0 to 10 minutes: r_s=0.91, p<0.0001, 10 to 20 minutes: r_s=0.75, p<0.001), followed by strong negative correlations over the subsequent 20 minutes (20 to 30 minutes: r_s=–0.70, p<0.003, 30 to 40 minutes: r_s=–0.85, p<0.001).

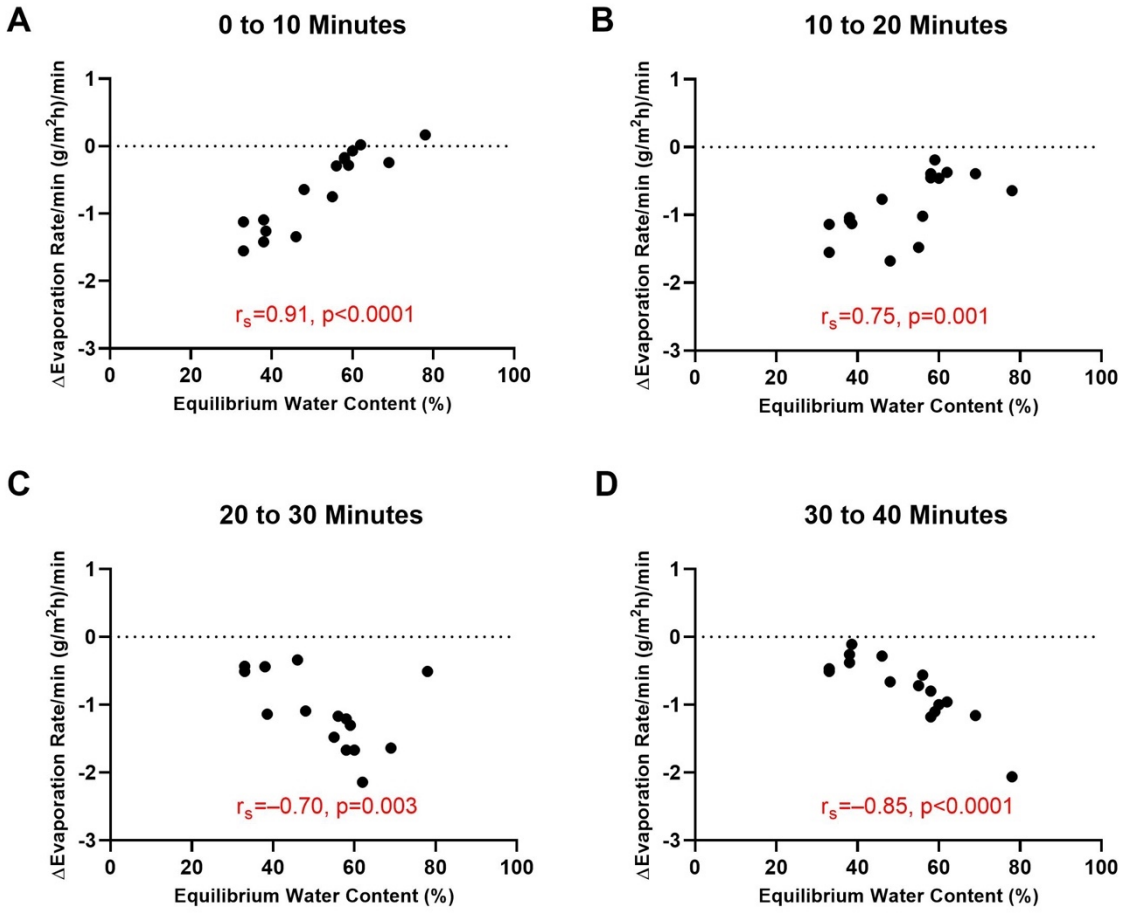

Figure 2-5: Correlations between the change in evaporation rate per minute and the material equilibrium water content (EWC) over: 0 to 10 minutes (A), 10 to 20 minutes (B), 20 to 30 minutes (C), and 30 to 40 minutes (D). All correlations were significant (0 to 20 minutes: all r_s>0.75, all p<0.002, 20 to 40 minutes: all r_s<–0.69, all p<0.004).

2.4.4 Duration of Evaporation Rate

Individual analysis of each CL material revealed that it took between 36 minutes (ocufilcon D and polymacon) and 108 minutes (narafilcon A) to reach a zero evaporation rate (0 g/m^2h). The median (IQR) amount of time it took for each material to cease evaporating is shown in Table 2-4. There was a significant difference in the length of time that each material took to reach 0 g/m^2h ($p<0.0001$). Post-hoc testing revealed significant differences between 10 combinations of CL material (all $p<0.039$, Appendix A, Figure 2-6). The two materials that exhibited the greatest difference in the duration of evaporation were polymacon and narafilcon A (40 vs. 100 min, $p<0.001$).

Table 2-4: Median (IQR) duration of evaporation for each soft contact lens material

Material	Duration of Evaporation (min)
comfilcon A	42 (6)
delefilcon A	44 (6)
etafilcon A (1 day)	56 (3)
etafilcon A (2 week)	62 (10)
hilafilcon B	64 (8)
lotrafilcon B	50 (10)
narafilcon A	100 (21)
nelfilcon A	46 (6)
nesofilcon A	48 (13)
ocufilcon D	42 (5)
omafilcon A	48 (7)
omafilcon B	44 (4)
polymacon	40 (6)
senofilcon A (1 day)	72 (16)
senofilcon A (2 week)	70 (21)
somofilcon A	50 (3)

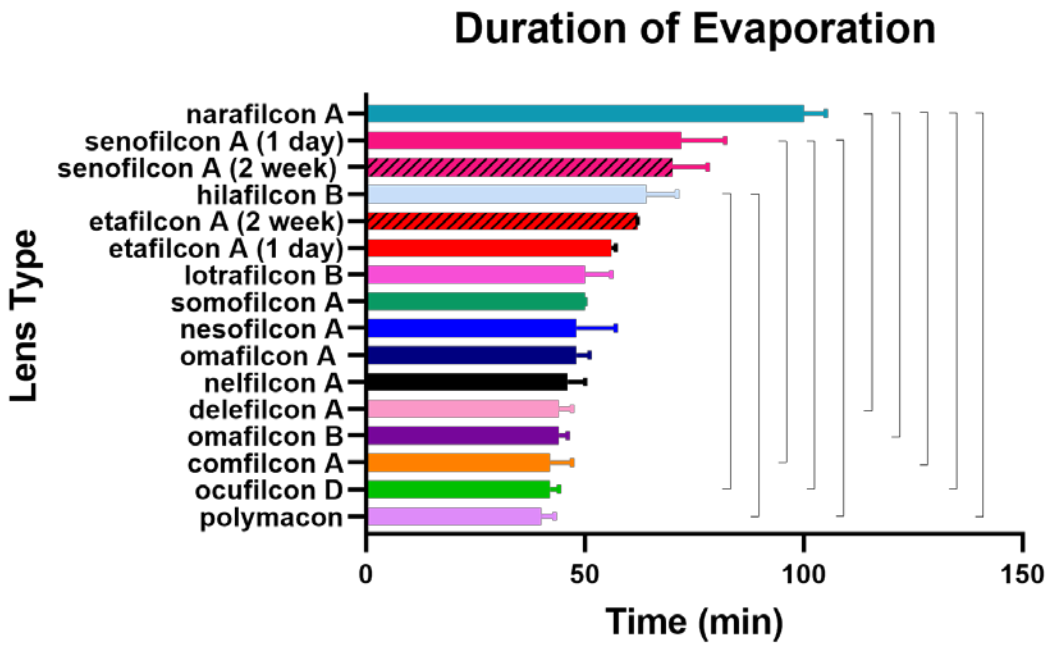

Figure 2-6: Median duration of measurable evaporation from each soft contact lens material. Error bars indicate IQR and significance lines indicate p<0.05 (all p<0.039).

2.4.4.1 Correlation Between Duration of Evaporation and Equilibrium Water Content

There was no significant correlation between the length of time over which a soft CL material could sustain some level of evaporation and the EWC (r_s=–0.12, p=0.65, Figure 2-7).

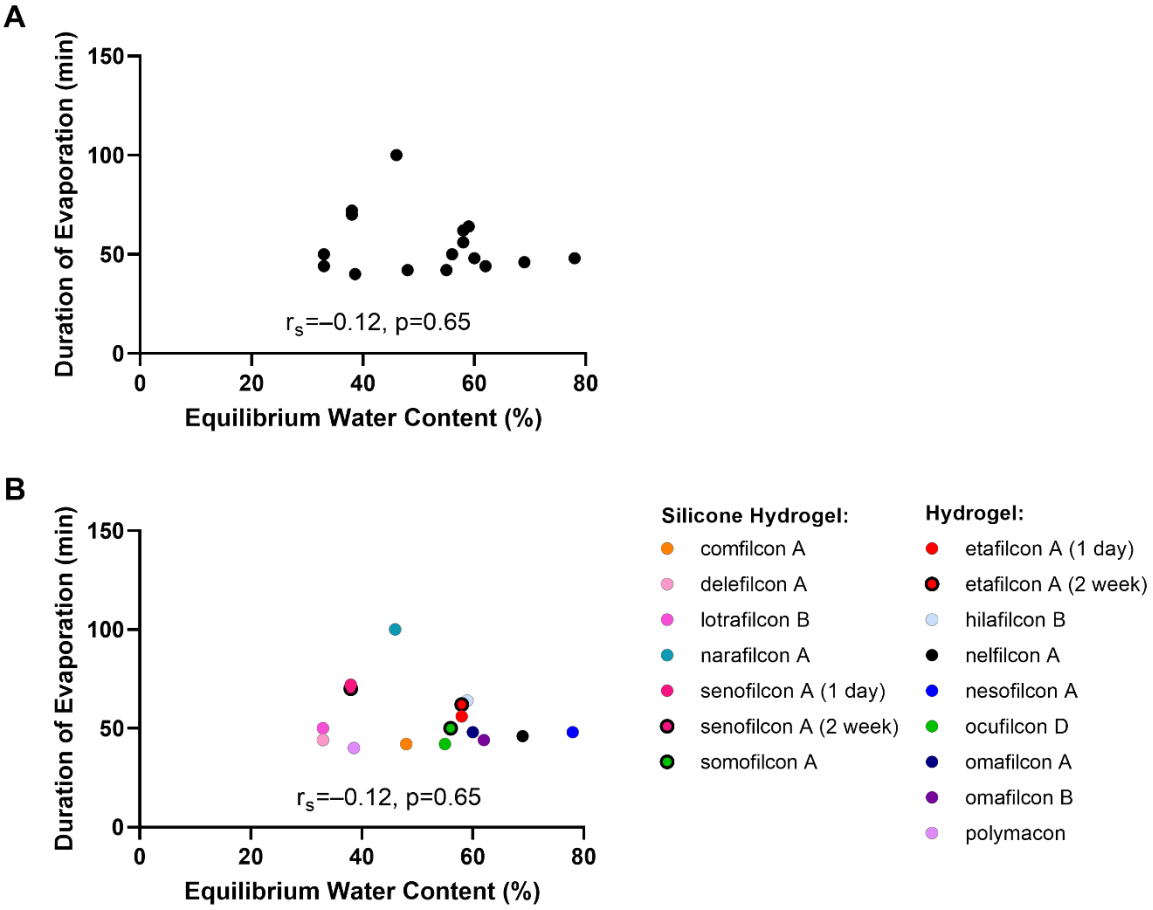

Figure 2-7: Correlation between the total time over which each contact lens material could sustain some level of evaporation and EWC (r_s=–0.12, p=0.65) (A), and shown for each lens material (B).

2.4.4.2 Correlation between Duration of Evaporation and Rate of Evaporation per Minute

No significant correlations were found between the total time over which a contact lens material could sustain some level of evaporation and the change in evaporation rate per minute (0 to 10 minutes: r_s=–0.16, p=0.55, 10 to 20 minutes: r_s=0.33, p=0.21, 20 to 30 minutes: r_s=0.41, p=0.12, 30 to 40 minutes: r_s=0.09, p=0.74, Figure 2-8).

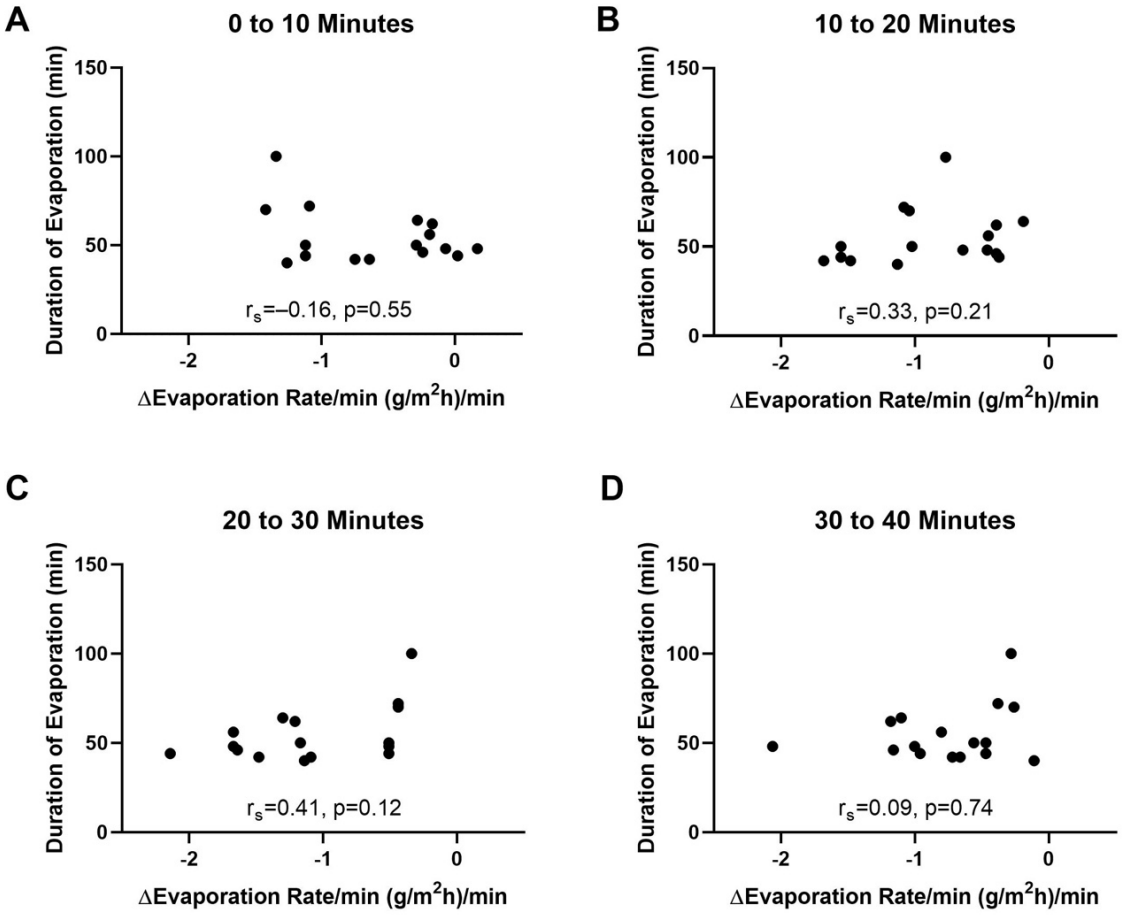

Figure 2-8: Correlations between total time the contact lens material could sustain some level of evaporation and the change in evaporation rate per minute (all r_s<0.42, all p>0.11).

2.5 Discussion

This study has demonstrated that the Eye-VapoMeter was able to measure the *in vitro* evaporation rate for hydrogel (Hy) and silicone hydrogel (SiHy) CL materials, and had the ability to detect significant differences in evaporation rate between materials. The instrument was also able to provide repeatable measurements of 0 g/m^{2}h (no evaporation) when tested on a rigid corneal CL material with a water content <1% [370, 371].

2.5.1 Evaporation Rate

All soft CL materials had a similar initial evaporation rate (median difference 7.1 g/m^{2}h) after being placed on the model eye. This suggests that each CL had a comparable amount of packaging solution removed after it was taken out of the blister pack and blotted. McConville and Pope [353] also reported

that the initial rate of evaporation rate of low water content CL materials (37.5%, 38.6%) were similar to high water content CL materials (55%, 58%), although the higher water content materials were noted to have a slightly higher rate of evaporation.

The plot of the evaporation rate over time for each CL material showed a non-linear profile that can be categorized by how the material acts during the initial period of stability and its subsequent change in evaporation rate. Six CL materials (delefilcon A, lotrafilcon B, narafilcon A, polymacon, senofilcon A −1 day and 2 week) had a short period of stability that lasted for less than 10 minutes, while the remaining 9 CL materials (comfilcon A, etafilcon A – 1 day and 2 week, hilafilcon B, nelfilcon A, nesofilcon A, oculfilcon D, omafilcon A, omafilcon B, somofilcon A) had a longer initial stable period of approximately 20 minutes. All CL materials then underwent a period where the level of evaporation linearly decreased, prior to slowing down until no relevant evaporation could be detected. The rate at which the evaporation rate changed per minute was also a defining characteristic of each CL material, with significant differences detected amongst materials (Section 2.5.2).

In vitro gravimetric work investigating CL dehydration has described similar patterns of water loss to those observed in this study. González-Méijjome et al. [364] investigated 12 soft Hy and SiHy CL materials (balafilcon A, etafilcon A, galyfilcon A, hioxifilcon A, hioxifilcon B, lidofilcon A, lotrafilcon A, lotrafilcon B, omafilcon A, polymacon, senofilcon A, vasurfilcon A). They reported three distinct phases in the dehydration curve: (i) an initial stable period, indicating a sustained dehydration rate; (ii) a rapid decrease in dehydration rate; and (iii) a period over which the dehydration rate went to zero. The only material that did not follow this pattern was lotrafilcon A, which did not have an initial period of stable dehydration. This was thought to be due to the very low EWC of the material (24%), the higher siloxane content of the material in comparison to other SiHy materials, and the presence of a thin hydrophilic membrane “coating” on the CL surface [364]. For each SiHy material, there would be a reduced water reservoir within the CL that limited the time over which evaporation could be sustained. Jones et al. [257] described a similar ogival pattern (shaped like the nose cone of a rocket) [372] of dehydration for 5 CL materials (balafilcon A, etafilcon A, lotrafilcon A, omafilcon A, polymacon) when the CLs were exposed to different ambient environment RHs and airflows. As shown in this study, the CL materials that had a longer initial period of stable evaporation also had higher EWCs ((comfilcon A (EWC: 48%), etafilcon A (EWC: 58%), hilafilcon B (EWC: 59%), nelfilcon A (EWC: 69%), nesofilcon A (EWC: 78%), ocufilcon D (EWC: 55%), omafilcon A (EWC: 60%), omafilcon B (EWC: 62%), somofilcon A (EWC: 56%)). McConville and Pope [353] also reported a graph of the absolute evaporative rate over time (g/min) that was very similar to the change in evaporation rate versus time observed in this study. Lower EWC materials showed slightly lower rates of evaporation

than higher EWC materials over the initial period of 5 to 10 minutes, then underwent a linear decrease in the rate of evaporation that was faster than high water content CL materials, before entering a slower phase where the rate of evaporation decreased to 0 g/min. The ability of high water content CL materials to sustain high levels of evaporation over a longer period of time was thought to be due to diffusion limited evaporation. Since the diffusion rate within the material is related to EWC [373], water will diffuse to the surface at a slower rate in low water content CL materials. As the water continues to evaporate, the surface water content will also decrease, along with the ability of water to diffuse to the surface. This reduces the amount of water at the surface of the CL that is available for evaporation. In contrast, high EWC CLs have higher reserves of water at the surface and also have faster rates of diffusion, which enables a longer initial period of high evaporation.

Although soft CL materials are described according to their EWC, the chemistry of the CL materials means that the water does not interact with the lens material in a uniform manner. Rather, the water held within a soft CL is classified as either bound or free [374, 375], depending on how well it is attached to the lens material. Bound water can either be loosely or tightly-bound to a CL. Tightly-bound water forms hydrogen bonds to polar groups [373, 374] in the lens material and cannot easily diffuse through the lens. In contrast, loosely-bound water is less tightly attached to the lens matrix via hydrogen bonds [376]. Since a high EWC material has a lower material-to-water ratio, there is less material for the water to bind to, and therefore more loosely-bound water as the water content increases [374, 376]. Free water is not bound to the lens matrix [374] and is therefore able to evaporate faster than bound water [375]. Higher EWC CLs have more free water [374, 376], more loosely-bound water [376], and greater amounts of free-to-bound water [374]. By comparison, and because the water content is lower, water in a low EWC CL is tightly-bound to the lens, therefore leaving less free water for evaporation [374]. These chemical features were revealed in the different patterns for evaporation rate shown in this study.

The results of this study support the idea proposed by González-Méijome et al. [364] that free water is lost during the first period of water loss. The shorter initial duration of evaporation observed with delefilcon A, lotrafilcon B, narafilcon A, polymacon, and senofilcon A could be attributed to their low water contents (33 to 46%). The decreased amounts of free water present in these low EWC materials means that the initial period of evaporation at the maximum rate cannot be sustained, compared to higher EWC CL materials (48 to 78%), where the availability of loosely-bound water on the surface of the lens and the higher water diffusion rate within the material enabled the maximum evaporation rate to be sustained for longer periods.

A higher diffusion rate enables free water within the deeper regions of the CL to be more easily drawn along the diffusion gradient to the superficial regions of the CL from which the water evaporates. This sustains the maximum evaporation rate, but also depletes the overall water content of the CL material more rapidly. The consequence of this effect was seen when the longer period of sustained maximum evaporation ended and the rate of evaporation rapidly decreased towards zero. In contrast, lower EWC CLs, or CLs with a chemistry that promotes stronger binding of water molecules, move more quickly from a period of maximum evaporation to lower rates of evaporation. The rate of evaporation now relates to the rate of diffusion within the material. The lower rate of evaporation, which changed at a slower rate over time, enabled evaporation to be sustained for longer overall period compared to higher EWC CLs.

2.5.2 Change in Evaporation Rate per Minute

Delefilcon A, lotrafilcon B, narafilcon A, polymacon, and senofilcon A (1 day and 2 week) (EWC: 33 to 46%) materials all demonstrated significantly faster changes in evaporation rate per minute compared to nesofilcon A (EWC: 78%) (–1.55 to –1.09 vs. 0.17 (g/m^2h)/min, all $p<0.0046$) over the first 10 minutes. Therefore, the low EWC materials had shorter periods of sustained maximum evaporation than the CL with the highest EWC. In agreement with *in vitro* dehydration studies [257, 364], this change is likely due to differences in water content rather than the type of material, since comparable rates of change were observed in materials that were fundamentally different in composition that had similar EWCs ((senofilcon A (SiHy, EWC: 38%) vs. polymacon (Hy, EWC: 38.6%), etafilcon A (Hy, EWC: 58%) vs. somofilcon A (SiHy, EWC: 56%) vs. hilafilcon B (Hy, EWC: 59%)). Further evidence as to the importance of EWC to the rate of evaporation was observed with the lack of a significant difference between the two types of branded contact lenses composed of the same material ((etafilcon A (1 day and 2 week); senofilcon A (1 day and 2 week)).

However, others have suggested that differences in the CL surface and the arrangement and spacing of polymers are more predictive of the performance of a SiHy material than the water content [354].

Looking again at the six low EWC CLs which all performed in a similar way, delefilcon A had the greatest amount of change in evaporation rate over the first 10 minutes. Delefilcon A is a 33% bulk water content water gradient CL with a 90 μm hydrophobic core [245, 377]. The CL material changes into an ≥80% water content hydrophilic gel, which is approximately 4-5 μm thick, at the surface [239]. Following the hypothesis outlined above, the high water content of the thin superficial gel was unable to maintain a high evaporation rate, nor was there free water available in the bulk of the CL to diffuse to the surface to support evaporation. Consequently, the initial change in evaporation rate per minute

was high, followed by a rate of evaporation that fell more quickly than the other low EWC materials. *In vivo* measurements using a refractometer support these findings and found that the water content of delefilcon A quickly changed from an >80% water content lens to a low water content material within 15 minutes of wear, whereas the water content of nesofilcon A remained stable over the same period [245].

Polymacon (EWC: 38.6%), narafilcon A (EWC: 46%), and senofilcon A (EWC: 38%) also rapidly changed over the first 10 minutes, but none of these materials had a surface treatment [354, 364] to improve wettability, nor did they contain any modifications to produce a high water content gel at the surface of the CL. However, both narafilcon A and senofilcon A have polyvinyl pyrrolidone firmly bound and incorporated within the CL material [354, 364, 378, 379] to promote binding of water, improve moisture retention [380], and to prevent hydrophobic silicone from migrating towards the CL surface [354]. The higher rates of change in evaporation per minute subsequently observed in polymacon from 10 to 30 minutes could be due to the lack of incorporation of an internal wetting agent [364]. The low amount of water in the CL and lack of a wetting agent in polymacon failed to prevent the diffusion of free water from the bulk of the CL to the surface, which resulted in increased water loss from the CL and faster change in the rate of evaporation per minute. In comparison, the presence of a wetting agent in narafilcon A and senofilcon A was able to create a reservoir that was able to sustain a slower rate of change in evaporation per minute during the intermediate time periods of the study. The reservoir allowed an increased rate of diffusion from the core to the surface of the CL to try to prevent dehydration. However, the presence of the wetting agent was not strong enough to fully overcome the attractive evaporative force of water to the superficial regions of the CL, and so a higher level of evaporation was maintained for a longer amount of time. Differences between the evaporation rate of the two senofilcon A CL materials could be due to crosslinking differences between the silicone and polyvinyl pyrrolidone [381].

Lotrafilcon B is a 33% EWC SiHy with 25 nm plasma surface treatment used to create a hydrophilic surface [239, 377]. It is not clear how the hydrophilic surface may influence the rate of evaporation, although the pattern was similar to that for delefilcon A. The hydrophilic nature of the surface may have drawn water from the interior of the CL towards the surface from where it will have evaporated. Since the surface is hydrophilic, it may have more available binding sites for water, but the conditions promoting evaporation were sufficient to easily overcome this water retention. The low EWC of the CL material means that the maximum level of evaporation could not be sustained for a prolonged amount of time, hence the rate of change in evaporation per minute fell rapidly.

In contrast to these low EWC CLs, nesofilcon A slightly increased its rate of evaporation per minute over the first 10 minutes. Nesofilcon A is a 78% EWC Hy CL that contains polyvinyl pyrrolidone as an internal wetting agent [382] and poloxamer 407 to maintain moisture at the surface of the lens. Using the hypothesis described above, the high EWC of the material provides a greater availability of free water and a high rate of diffusion to sustain the evaporation, which produces the longer initial period of maximum evaporation and slower rate of change per minute. The increased rate of evaporation during this period may be attributed to the effect of the poloxamer 407 helping to draw free water from the deeper regions of the CL towards the surface, which increased the rate of diffusion and therefore resulted in an increased the rate of evaporation. This also suggests that the diffusion rate may be a key factor in controlling the maximum evaporation rate for the material.

As time progressed, the other CLs also passed through phases where their rate of evaporation per minute changed. From 10 to 20 minutes, the majority of the six CLs with the lowest EWC began to slow their rate of evaporation per minute. During this time period, comfilcon A, the single low EWC CL that demonstrated a longer initial stable period of evaporation from 0 to 10 minutes, underwent a faster change in the rate of evaporation per minute compared to hilafilcon B (–1.68 vs. –0.19 $g/m^2h)/min$, p=0.001). Comfilcon A is a SiHy CL (EWC: 48%) made with Aquaform technology that helps maintain water within the lens [377] by bonding to the silicone chains. The Aquaform technology may have acted to promote the diffusion of free water from the bulk of the CL to the surface. The loss of water from the surface will have sustained the maximum evaporation rate for longer than expected amount of time for a low EWC material. However, after the initial stable period, comfilcon A could no longer maintain the high evaporation rate and subsequently quickly decreased in evaporation rate over the following 10 minutes. In contrast, hilafilcon B is a 59% EWC Hy that does not have any internal wetting agents, but has poloxamine added to the packaging solution to improve comfort [244] and to attract water to the CL surface [383]. The pattern of change in evaporation rate for this CL was therefore unaffected by the action of additives to the internal chemistry of the lens material. However, the ability of hilafilcon A to sustain a longer period of high evaporation compared to other CLs of a similar water content suggests that the poloxamine was able to assist in retaining water within the CL.

From 20 to 30 minutes, narafilcon A (EWC: 46%) had the slowest change in evaporation rate per minute compared to omafilcon B (–0.34 vs. –2.14 $g/m^2h)/min$, p<0.001). Omafilcon B is a biomimetic 62% EWC CL containing phosphorylcholine [363]. Phosphorylcholine acts as a surface and lens matrix binding agent for free water [384, 385] in a similar way to that previously described for narafilcon A and senofilcon A. The binding agent created a reservoir within the CL to promote diffusion rate within the material and also strongly bound water to the surface of the CL to create a layer of moisture that

enabled the material to sustain a high rate of evaporation for 20 minutes. After 20 minutes, omafilcon B was unable to maintain this high level of evaporation and underwent a decrease in evaporation rate. The effect of phosphorylcholine on maintaining the rate of evaporation during the intermediate time period of the study did not seem as pronounced as observed in narafilcon A or senofilcon A. The subsequent effect of phosphorylcholine on the change in evaporation rate per minute beyond 40 minutes was not analyzed because some of the CL materials had fully dehydrated by 40 minutes.

From 30 to 40 minutes, narafilcon A also had the smallest change in evaporation rate compared to nesofilcon A (–0.28 vs. –2.06 g/m^2h)/min, $p=0.006$). By this stage, narafilcon A had used up the majority of its available water and could not sustain a high evaporation rate. In contrast, nesofilcon A (EWC: 78%) had a large water reserve that enabled it to maintain surface evaporation for a longer duration, albeit it at a lower rate than during the initial period. However, between 30 to 40 minutes, the water reserve began to diminish, which resulted in a faster change in evaporation rate per minute.

Although it has been suggested that free water is the only type of water that is important to CLs [364] and the greatest difference was observed between 10 to 20 minutes [365], analysis of *in vitro* evaporation rate over prolonged periods could demonstrate how CLs would react in the eye when exposed to stressful conditions, such as wind or low RH.

2.5.2.1 Correlation Between Change in Evaporation Rate per Minute and Equilibrium Water Content

Strong positive correlations were found between the change in evaporation rate per minute and the EWC over the first 20 minutes of measurement (0 to 10 minutes: $r_s=0.91$, $p<0.0001$, 10 to 20 minutes: $r_s=0.75$, $p<0.001$), followed by strong negative correlations over next 20 minutes (20 to 30 minutes: $r_s=–0.70$, $p<0.003$, 30 to 40 minutes: $r_s=–0.85$, $p<0.001$). The shift in direction of the correlations after 20 minutes reflects the more prolonged stable rate of evaporation initially observed in the higher EWC CLs.

Although this technique has not been used before to measure *in vitro* dehydration, the closest comparisons would be to gravimetric measurements of water loss. González-Méijome et al. [364] were unable to find a significant correlation between the mean rate of dehydration measured over an initial period of stability and EWC, although the authors noted a trend towards higher dehydration rates as EWC increased. However, significant positive corrections were found between the valid dehydration and EWC over the first 10 minutes, and between the cumulative dehydration and water content over 11 to 15 minutes and 16 to 20 minutes. However, making comparisons been rates of dehydration can be complicated. Since there is no accepted standard calculation, researchers may choose to report the dehydration rate [364, 365], relative percentage of dehydration [257, 348], cumulative dehydration [364] or valid dehydration (amount of weight lost at a specific time point compared to the total amount of weight lost) [364, 365].

2.5.3 Duration of Evaporation Rate

All of the CLs stopped evaporating by 108 minutes, which is similar to McConville and Pope [353] who found that Hy CLs all dehydrated within 120 minutes. These findings help confirm Martín-Montañez et al.'s [365] assumption that all of their Hy and SiHy CLs had completely dried out by 120 minutes when the front surface of the lens was exposed to ambient conditions.

2.5.3.1 Correlation Between Duration of Evaporation and Equilibrium Water Content

Although a significant correlation was not found in this study between the time required for a CL to fully dehydrate and the EWC (r_s=–0.12, p=0.65), strong positive correlations have been reported between dehydration time and EWC [257, 364]. Aside from using a different methodology to determine the rate of loss, failure to find a significant correlation in this study may have been due to examining the entire duration of lens dehydration, compared to others that investigated shorter time intervals [257, 364]. It may also be due to the effect of binding agents within the CL material, and due to the effect of to surface treatments. However, a strongly negative correlation has also been reported between the absolute change in water content versus time [349].

2.5.3.2 Correlation Between Duration of Evaporation and Rate of Evaporation per Minute

Analysis of the change in evaporation rate per minute in increments of 10 minutes revealed no significant correlations with the total amount of time it took a CL to dehydrate (all r_s<0.42, all p>0.11). Interestingly, narafilcon A was one of the CLs that had a fast rate of change over the initial 10 minutes, but ultimately took the longest amount of time to dehydrate. The cause for this is unknown, but it may be related to the diffusion rate within the material and incorporation of a wetting agent.

2.5.4 Clinical Impact

The level of hydration of a CL is one important factor in predicting the success of a CL [364]. The results of the study suggest that CLs that quickly lost their surface water, such as delefilcon A, resulted in a faster change in evaporation rate per minute. This inability to maintain the high level of surface hydration may be due to reduced rates of diffusion from the bulk of the CL or decreased amounts of free-to-bound water. The loss of hydration could result in more discomfort due to the CL dehydrating at a faster rate and causing greater amounts of friction between the CL and eyelid. However, further work will need to be done to confirm this theory because *in vivo* work has reported higher levels of comfort and less dryness after 7 hours of wear with delefilcon A compared to nesofilcon A [359].

2.5.5 Limitations

In vitro testing can be poorly correlated with *in vivo* findings [349] and the *in vitro* conditions used in this study do not represent the normal environment of the eye. When a CL is worn, it will be surrounded by the pre- and post-lens tear film, and each blink will help replenish the tear film and wet the lens. Testing was also conducted on a model eye which was not heated to eye temperature [386], which could affect the rate at which the lens evaporated.

The rate of evaporation could also have been affected by the different way each CL dehydrated. Although the back surface of each CL was placed against the model eye, as the CL dehydrated the shape of the CL changed during the measurements. When some CLs dried, they lay flat against the model eye and were difficult to remove, while other CLs curled up and stood off from the model eye. This could have resulted in higher rates of evaporation if both the front and back surface of the CL contributed to the measurement, rather than just the front surface of the CL.

It is possible that the measurements could also have been affected by differences in lens thickness. However, it does not seem to have had a significant effect on the rate of evaporation since both comfilcon A (EWC: 48%, 0.08 mm thickness) and delefilcon A (EWC: 33%, 0.09 mm thickness) dehydrated significantly faster than narafilcon A (EWC: 46%, 0.085 mm thickness).

Although linear regressions have previously been used to analyze the rate of dehydration [349, 364], it may not be the best way to describe the change in evaporation that occurred. Other future methods of analyzing the data could include double exponential non-linear regression models [257] or second order polynomial functions [364].

In addition, there were some technical problems encountered with the DelfWin 4 version 3.1.14 software when measurements were occasionally not logged by the computer, even though the

evaporation rate, ambient temperature, and ambient RH were displayed on the instrument. To overcome this problem, all values displayed by the instrument were written down and used to replace any data missing in the computer program. However, there may be slight discrepancies between what the instrument would have recorded versus what the instrument displayed since the evaporation rate, temperature, and RH are recorded in 0.1 steps in the software. However, the instrument always displays the temperature to the nearest whole number. The rate of evaporation is not believed to have been affected by this problem because the instrument can only display a maximum of three numbers at a time. All of the values in this study were <100 g/m^2h, therefore, the displayed evaporation rate should reflect the one that would have been recorded in the system.

2.5.6 Future Work

Further work should be conducted to examine whether differences in evaporation rate can also be detected when a CL is worn in the eye. The results of this chapter showed that delefilcon A and nesofilcon A had the greatest difference in rate of evaporation over the first 10 minutes, which is most likely to be the time period most predictive of changes that would occur in the eye. The shorter time period is likely to best reflect *in vivo* changes because CLs do not fully dehydrate when they are worn [47, 256, 348, 359] and because the tear film helps replenish lost moisture with each blink [352]. The effect of faster dehydration of the CLs could be stimulated with the use of a goggle to produce a low RH and the use of a digital device [387] to a decrease the blink rate [388-390], or by subjecting them to changes in environmental conditions involving low RH [155, 182, 387], increased temperature [115], or increased air flow [391, 392]. In addition to performing evaporimetry, the water content of the CL could also be tested to determine how it changes during contact lens wear.

2.6 Conclusions

A new technique has been described to measure *in vitro* the rate of evaporation from soft CLs. Significant differences in the change in evaporation rate were found between different types of soft SiHy and Hy CLs.

The rate of change of evaporation was significantly correlated to the water content, with the 6 lowest water content CL materials unable to sustain evaporation at a high rate and therefore produced a faster reduction in evaporation rate over the first 10 minutes of the measurements compared to the highest water content lens material. The higher water content lenses were able to sustain a higher evaporation rate for a longer period, but all CLs eventually experienced a reduction in evaporation rate as the water reservoir within the lens was depleted. The differences in the rate of water loss are likely to be due to

the amount of free water and ability of water to diffuse through the lens material. Further work is needed to determine whether these changes can also be seen in the eye.

Chapter 3

Eye-VapoMeter Correction Factors

3.1 Overview

PURPOSE: To calculate correction factors for a commercially available evaporimeter, and to mathematically describe the relationship between the correction factor and the air volume within the instrument.

METHODS: An *in vitro* study was performed to investigate the effect of two parameters on the measured rate of evaporation from two different model eye systems: (i) simulated ocular surface area, and (ii) air volume inside the evaporimeter goggle. MODEL A: a single eye containing 5 small holes (0.52 cm^2 surface area per hole) and 2 large holes (2.70 cm^2 surface area per hole); MODEL B: a series of seven model eyes with each model eye containing a single elliptically shaped hole (similar to the shape of the palpebral aperture), with a surface area ranging from 1 to 2.5 cm^2 (in 0.25 cm^2 steps).

MODEL A STUDIES: WATER LOSS (610 μl): Using a combination of the holes in the single model eye, four total surface areas were tested (0.52, 1.03, 1.55, and 2.06 cm^2). For every hole combination, each hole was filled with a total of 610 μl of 34°C distilled water, and the model eye was placed on a 34°C heating plate. The rate of water loss (n=1) was measured under ambient room conditions by weighing the model eye every 15 minutes for 1.5 hours. The ABSOLUTE EVAPORATION RATE was determined from the slope of water loss over time. EVAPORATION RATE (610 μl): A test matrix was developed using 4 surface areas (0.52, 1.03, 1.55, and 2.06 cm^2) and 9 evaporimeter goggle volumes (4, 5, 6, 8, 10, 13, 14, 15, and 16 cm^3) inside an environmental chamber. The volume inside the evaporimeter was reduced by placing increasing amounts of modelling clay inside the goggle. Five consecutive measurements of evaporation rate (UNADJUSTED EVAPORATION RATE), each lasting 17 seconds or less, were recorded with 2-minute intervals between measurements. A linear regression was used to determine the CORRECTION FACTOR for each volume based on the unadjusted evaporation rate and absolute evaporation rate. A non-linear regression was used to describe the relationship between the correction factor and evaporimeter volume. WATER LOSS (750 μl): The same method as described in water loss (610 μl) was followed, except 750 μl of water added was to each hole. EVAPORATION RATE (750 μl): The same procedure as described in evaporation rate (610 μl) was followed, except each hole was filled with 750 μl of water. Four surface areas (0.52, 1.03, 1.55, and 2.06 cm^2) and 11 evaporimeter volumes (4, 5, 6, 8, 10, 11, 12, 13, 14, 15, and 16 cm^3) were investigated.

MODEL B STUDIES: WATER LOSS (ELLIPTICAL): Each model eye (1 to 2.5 cm^2) was filled with 800, 900, 1050, 1200, 1350, 1550, or 1750 μl of water, respectively. The same procedure described water loss (610 μl) was followed. MODEL EVAPORATION RATE (ELLIPTICAL): The same procedure as in evaporation rate (610 μl) was followed. Seven surface areas (1, 1.25, 1.5, 1.75, 2, 2.25, and 2.5 cm^2) and 17 evaporimeter volumes (4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 16.88, 17.75, 18.63, and 19.5 cm^3) were tested.

RESULTS: WATER LOSS (610 μl/750 μl/ELLIPTICAL): Water loss occurred at a linear rate in all three testing conditions (610 μl: all $R^2>0.99$, all $p<0.0001$, 750 μl: all $R^2>0.99$, all $p<0.0001$, elliptical: all $R^2>0.99$, all $p<0.0001$). EVAPORATION RATE (610 μl): Correction factors ranged between a minimum of 699.7/m^2 for a 4 cm^3 volume ($R^2=0.95$, $p=0.03$) and a maximum of 1625/m^2 for a 10 cm^3 volume ($R^2=0.97$, $p=0.01$). Not all linear regressions used to determine the correction factors were significant (volume 5, 8, 16 cm^3: all $R^2>0.86$, all $p=0.06$). The graph of the correction factor versus evaporimeter volume was fit with a second order polynomial non-linear regression ($Y = –17.6x^2 + 387.5x – 512.8$). EVAPORATION RATE (750 μl): Correction factors ranged between a minimum of 763.3/m^2 for a 5 cm^3 volume ($R^2=0.98$, $p=0.01$) and a maximum of 1408/m^2 for a 10 cm^3 volume ($R^2=0.99$, $p<0.01$). All linear regressions used to calculate the correction factors were significant (all $R^2>0.92$, all $p<0.04$). The graph of the correction factor and evaporimeter volume was fit with a second order polynomial non-linear regression ($Y = –8.1x^2 + 205.8x + 1.2$). MODEL EYE EVAPORATION RATE (ELLIPTICAL): Correction factors ranged between a minimum of 455.5/m^2 for a 4 cm^3 volume ($R^2=0.41$, $p=0.12$) and a maximum of 1686/m^2 for a 16.88 cm^3 volume ($R^2=0.86$, $p<0.01$). All of the linear regressions used to determine the correction factors were significant (5 to 19.5 cm^3: all $R^2>0.60$, all $p<0.03$), except for the 4 cm^3 volume. The graph of the correction factor and evaporimeter volume was fit with a second order polynomial non-linear regression ($Y = –3.6x^2 + 135.5x + 211.7$).

CONCLUSIONS: Correction factors were developed to convert the evaporation rate provided by the Eye-VapoMeter to an absolute tear evaporation rate. Correction factors were calculated for a range of air volumes from 4 up to 19.5 cm^3. Despite three different methods being tested, the smallest correction factors (455.5 to 763.3/m^2) occurred when the evaporimeter volume was reduced to either 4 or 5 cm^3. The original model eye had the largest correction factors (1408 and 1625/m^2) at 10 cm^3, while the elliptical model eye had a maximum correction factor of 1686/m^2 for a 16.88 cm^3 volume. The relationship between correction factor and volume was best fit with a with a second order non-linear regression in all three test conditions:

- 610 μl: $Y = –17.6x^2 + 387.5x – 512.8$;

- 750 µl: $Y = -8.1x^2 + 205.8x + 1.2$;
- Elliptical model eye: $Y = -3.6x^2 + 135.5x + 211.7$.

3.2 Introduction

The VapoMeter (Delfin Technologies Ltd., Kuopio, Finland) is a validated [63] instrument originally designed for dermatological use to measure the rate of water loss from the skin [64]. The device was subsequently validated for ocular use [21] with the addition of a swimming goggle to allow the instrument to conform to the shape of the face (Figure 3-1).

Figure 3-1: Original goggle and instrument used to validate the Eye-VapoMeter (A) compared to the commercially available instrument (B). Image A from Rohit A, et al. Validating a new device for measuring tear evaporation rates. Ophthalmic Physiol Opt. 2014;34:53-62.

Although previous researchers have accounted for individual differences in the size of the ocular surface when performing evaporimetry [1-3, 21, 49, 102], the contribution of the internal air volume within the device has largely been ignored and has been assumed to be constant [1-3, 101]. The effect of ocular surface area is straightforward – a larger surface area, with a constant rate of evaporation across the surface, will produce a larger apparent evaporation rate compared to a smaller surface area with the same constant rate of evaporation [53]. For internal air volume, the apparent evaporation rate

will vary in relation to the volume, for a constant surface area with a constant rate of evaporation. This relates to how the evaporation rate is determined. The internal sensor records the relative humidity (RH) in the air and as the water evaporates from the surface it increases the RH in the air volume. It can be hypothesized that if the volume is small, the RH in the air volume will increase more rapidly, producing an apparently faster rate of evaporation. In comparison, for a larger volume, the increasing RH from evaporation has more air of a lower RH in which the RH can diffuse, producing an apparently slower rate of evaporation.

The importance of accounting for anatomical variations in ocular surface area and volume within the instrument was demonstrated during validation of the Eye-VapoMeter by Rohit et al. [21], with higher evaporation rates measured as the volume decreased. Since the evaporation rate of the instrument is based on the change in RH that occurs inside a closed cylinder with a 2 cm^3 internal volume and a 1 cm^2 opening [64], the values were converted to a corrected tear evaporation rate (TER) to compensate for the larger internal volume of the instrument caused by the incorporation of the swimming goggle [21]. Readings were converted with a correction factor based on the rate of water loss from a model eye and the volume of air inside the instrument (Figure 3-2), and the area of the ocular surface.

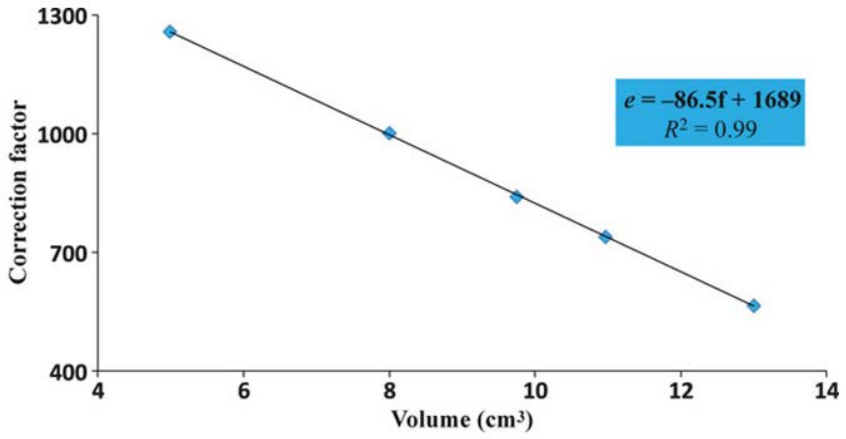

Figure 3-2: Linear relationship between correction factor versus evaporimeter volume as reported by Rohit A, et al. Validating a new device for measuring tear evaporation rates. Ophthalmic Physiol Opt. 2014;34:53-62.

However, the reported correction factors were poorly derived. The first step of their calibration process measured the rate of water loss from a model eye configured to test three different surface areas. The second step measured the actual rate of evaporation from each surface area with different evaporimeter goggle volumes. Crucially, the largest surface area tested during the water loss measurements was not

included in the second step of the calibration process because the results fell outside of the expected parameters. This left only two measured sets of data and so a third set of evaporation rates was interpolated from the values obtained from the two smaller surface areas [21]. To further extend the data range, the calibration curves were forced through the origin, which is not appropriate.

In addition, although visual observation of the original goggle used in the validation process appears to be similar to the commercially available one, the evaporimeter volumes tested with the model eye and the measured range of air volumes inside of the goggle of their participants [21] were between 5 to 11 cm^3 smaller than the size of the goggle on the marketed version of the Eye-VapoMeter. This may render the calibration curves invalid for the commercial instrument.

In view of how these issues may have affected the calibration curve for the device, the purpose of this study was to repeat the *in vitro* validation tests used to calibrate the instrument [21] to further investigate how a wide range of surface areas and evaporimeter volumes affect the correction factor.

The aims of this chapter are to:

- Calculate correction factors for different volumes within the evaporimeter;
- Describe the relationship between the correction factor and evaporimeter volume with a mathematical equation.

3.3 Circular Model Eye (610 μl/hole)

3.3.1 Materials and Methods

An aluminum model eye (Mr. Harmen Vander Heide, University of Waterloo, Waterloo, Ontario) functioned as a source of evaporation (Figure 2-1A). The model eye contained five small and two large cylindrical holes. The surface area of each small and large hole was 0.52 cm^2 and 2.70 cm^2, respectively. Four different cumulative surface areas (0.52, 1.03, 1.55, and 2.06 cm^2) were investigated.

3.3.1.1 Water Loss over Time

The model eye and a 480 ml glass storage bottle (Fisher Scientific, Waltham, Massachusetts, USA) filled with distilled water were heated overnight to 34°C in an Isotemp oven (Fisher Scientific, Waltham, Massachusetts, USA) to represent the temperature of the eye [257, 393, 394]. One to four small holes were each filled with 610 μl distilled water to create combined surface areas of 0.52, 1.03, 1.55, and 2.06 cm^2. Any holes that were not filled with water were covered with clear tape to ensure the volume of the hole did not contribute to total air volume inside the instrument. A thermocouple

(HH21A, Omega Engineering, Norwalk, Connecticut, USA) was used to monitor the internal temperature of the oven, and ambient temperature and RH were monitored with a digital thermo-hygrometer (RH411, Omega Engineering, Norwalk, Connecticut, USA).

Prior to any measurements, the model eye was tared on an analytical balance (Precision Balance B303-S, Mettler Toledo, Greifensee, Switzerland, Figure 3-3) that measures to 0.001 g. The model eye was then placed back in the oven for at least 10 minutes to compensate for any heat loss during this step, prior to starting water loss measurements.

The glass bottle filled with warm distilled water was removed from the oven and placed on a 34°C heating plate (Digital Heatblock, vwr, Radnor, Pennsylvania, USA). Water was added to the model eye, while it was still in the oven, using a pipette (Fisherbrand® 5000DG 100-1000 µl, Fischer Scientific, Waltham, Massachusetts, USA). Once it was prepared, the model eye was transferred from the oven to the balance and the mass of water was weighed in 15-minute intervals for 90 minutes. The bottle of water was placed back in the oven to keep warm after the first weighing measurement.

Between each measurement, the model eye was moved from the balance to the heating plate to maintain the desired 34°C temperature and exposed to ambient conditions to simulate evaporation from the eye under normal room conditions. This procedure was repeated once for each surface area in a random order, and the model eye was handled with nitrile gloves (HandPRO FreeStyle 1100, Hourglass International, Inc., Bernicia, California, USA) to reduce heat conduction between the model eye and the experimenter's hands to prevent changing the temperature of the model eye.

Figure 3-3: Experimental set-up for water loss measurements with an analytical balance, heating plate, model eye, and oven (left to right).

3.3.1.2 Measurement of Evaporation Rate

The effect of surface area and evaporimeter internal volume on the evaporation rate of the Eye-VapoMeter (Delfin Technologies Ltd., Kuopio, Finland) was investigated by testing four surface areas (0.52, 1.03, 1.55, and 2.06 cm^2) with nine evaporimeter volumes (4, 5, 6, 8, 10, 13, 14, 15, and 16 cm^3). The choice of evaporimeter goggle volumes used in this study was based on the work by Rohit et al. [21] conducted as part of the validation process when modifying the instrument for ocular use. They measured the in-goggle volume for their participants when the evaporimeter was applied to the closed eye, and found a range of volumes between 5 and 11 cm^3 [21]. The 5 cm^3 volume appears to be particularly small, but there is no reason to assume that anyone with significant proptosis would have been included in their study because participants with healthy eyes were recruited. Although it is not expected that goggle air volumes of 5 cm^3 would typically be encountered during testing, because the size of the goggle attached to the commercially available Eye-VapoMeter is 16 cm^3, small volumes were tested to replicate the test conditions used during the Rohit et al. [21] validation of the device.

The holes in the model eye that did not have water added to them were filled with custom-designed plastic inserts (Miss Han Qiao, CORE, Waterloo, Ontario, Figure 3-4A and B) to control the volume inside the evaporimeter. The internal air volume within the evaporimeter was decreased by adding modelling clay (Sculpey®^{III}, Polyform Products Co. Inc., Elk Grove Village, Illinois, USA) to the evaporimeter goggle (Figure 3-4C and D). To ensure a consistent amount of modelling clay was added, a 1 cm^3 cube of modelling clay was measured using Vernier calipers and weighed on a B303-S balance. Additional cubes of modelling clay were created with the same weight and added to the Eye-VapoMeter. The evaporimeter volume was tested from the largest to smallest volume to ensure the modelling clay was placed in the same position during the measurements. Initially, the modelling clay was added to the side of the goggle, and then spread out evenly to cover the surface of the evaporimeter as volume was reduced. The order of testing each area for a particular volume was randomized.

The model eye and distilled water were heated in an Isotemp oven, as described in the water loss experiment (Section 3.3.1.1). The same holes were tested for each area to ensure consistent measurements. After pipetting 610 µl water per hole into model eye with a 5000DG pipette, the model eye was carefully transferred from the oven to a 34°C Digital Heatblock heating plate inside an environmental chamber (Model 5503-11 Package E, electro-tech systems inc., Glenside, Pennsylvania, USA) set to 40% RH and five consecutive Eye-VapoMeter measurements were taken and logged with the manufacturer's DelfWin 4 version 3.1.14 software (Delfin Technologies Ltd., Kuopio, Finland). There was a two-minute interval between measurements to allow the sensor to re-stabilize at the

ambient RH as the instrument ventilated. Following each set of measurements, the remaining water was removed from the model eye with lens paper (Fisherbrand, Fisher Scientific, Waltham, Massachusetts, USA) and placed back in the oven for at least 15 minutes to re-heat the model eye and promote the evaporation of any remaining moisture in the hole before testing the next surface area. Ambient temperature and RH were monitored inside the environmental chamber with a RH 411 digital thermo-hygrometer. HandPRO gloves were worn during the measurements to reduce heat transfer by conduction.

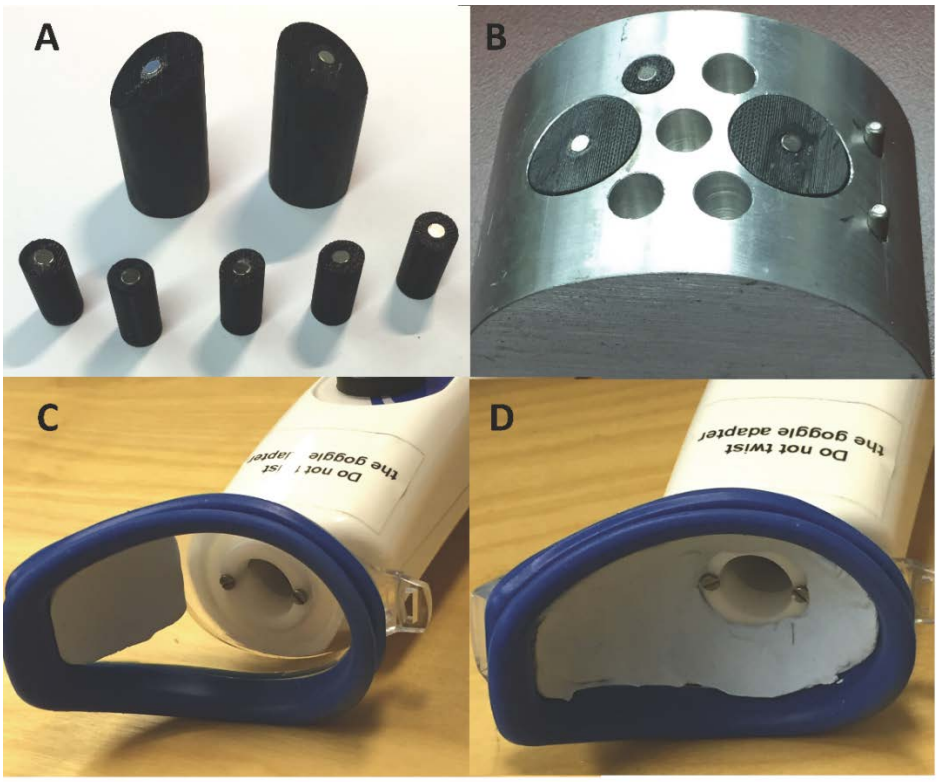

Figure 3-4: The area of the model eye was controlled by placing plastic inserts (A) into the holes of the model eye (B). The air volume of the evaporimeter was decreased by adding 1 cm^3 of modelling clay (C) or larger amounts of modelling clay (D) to decrease the air volume in the evaporimeter.

3.3.1.3 Statistical Analysis

Statistical analysis was conducted using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, LLC, San Diego, California, USA). Linear regressions were used to determine the ABSOLUTE EVAPORATION RATE (AER) of water. Linear regressions were also used to calculate correction factors for each volume based on the Eye-VapoMeter evaporation rate and the AER of water.

A second order polynomial non-linear regression was used to determine the relationship between the correction factor and volume within the evaporimeter. A p-value <0.05 was considered statistically significant.

3.3.2 Results

3.3.2.1 Water Loss over Time

The mean ± standard deviation (SD) ambient temperature was 25.57 ± 0.50°C and RH was 42.36 ± 0.91%. The range of ambient temperature was 25 to 26°C and RH ranged between 41 to 43%. The mean ± SD temperature of the oven was 34.08 ± 0.10°C (range: 34.0 to 34.2°C).

The change in weight of water over time for each hole combination appears in Figure 3-5. A summary of the rate of water loss is shown in Table 3-1. Water loss occurred at a linear rate over time (all R^2>0.99, all p<0.0001), and the slope was used to determine the AER of water for each surface area.

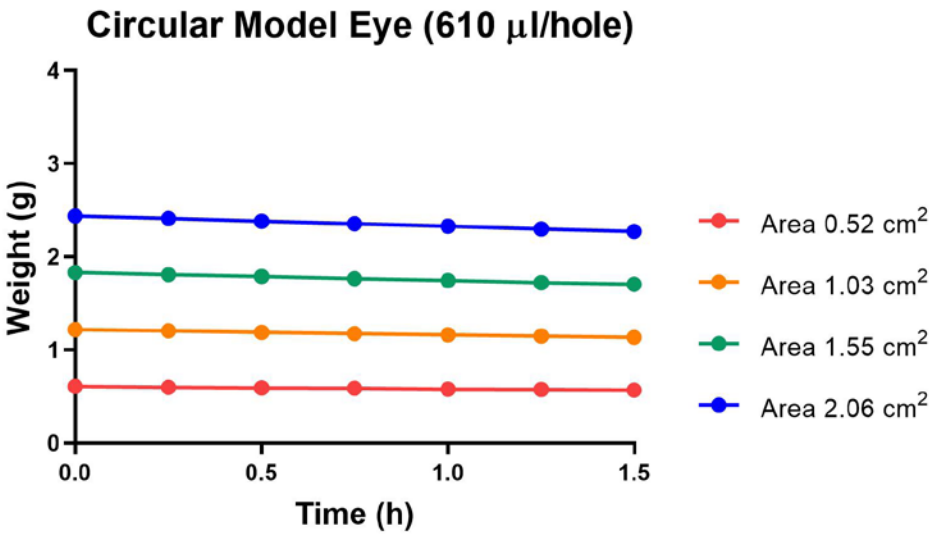

Figure 3-5: Weight of water over time filled with 610 µl of water per hole.

Table 3-1: Rate of water loss (610 µl of water per hole)

Area (cm^2)	Linear regression	AER (g/h)	R^2	p-value
0.52	Y = –0.03x + 0.61	0.03	0.99	**<0.0001**
1.03	Y = –0.06x + 1.21	0.06	0.99	**<0.0001**
1.55	Y = –0.09x + 1.83	0.09	0.99	**<0.0001**
2.06	Y = –0.11x + 2.44	0.11	0.99	**<0.0001**

AER: Absolute evaporation rate. **Bold** indicates statistical significance.

3.3.2.2 Eye-VapoMeter Evaporation Rate and Correction Factors

The mean ± SD ambient temperature was 25.76 ± 0.44°C and RH was 42.14 ± 0.96%. The range of ambient temperature was 25 to 26°C and RH ranged between 41 to 43%.

A summary of the mean ± SD evaporation rate measured for different volumes within the evaporimeter and different surface areas on the model eye is shown in Table 3-2. A graph of the UNADJUSTED EVAPORATION RATE (UER) measured with the Eye-VapoMeter compared to different AERs of water loss for various evaporimeter volumes appears in Figure 3-6.

Table 3-2: Mean ± standard deviation (SD) evaporation rate for different volumes and areas (610 µl of water per hole)

	Eye-VapoMeter Evaporation Rate (g/m^2/h)			
Volume (cm^3)	AER 0.03 *(0.52 cm^2)*	AER 0.06 *(1.03 cm^2)*	AER 0.09 *(1.55 cm^2)*	AER 0.11 *(2.06 cm^2)*
4	40.9 ± 2.7	50.3 ± 4.9	84.9 ± 7.1	95.9 ± 3.0
5	50.4 ± 4.2	58.3 ± 3.8	87.4 ± 3.1	135.4 ± 8.1
6	47.0 ± 2.1	59.4 ± 3.8	125.2 ± 6.9	143.1 ± 4.5
8	50.0 ± 2.0	55.3 ± 4.1	144.8 ± 11.1	160.4 ± 2.6
10	44.8 ± 1.5	70.4 ± 9.6	133.1 ± 4.9	179.0 ± 12.4
13	43.7 ± 1.1	63.2 ± 6.8	123.8 ± 7.0	168.9 ± 4.8
14	41.6 ± 2.9	68.1± 4.2	123.6 ± 3.2	157.0 ± 4.5
15	36.6 ± 5.5	46.4 ± 3.4	95.2 ± 8.1	137.9 ± 10.4
16	37.1 ± 2.6	50.5± 1.8	127.0 ± 4.9	134.8 ± 5.2

AER: Absolute evaporation rate.

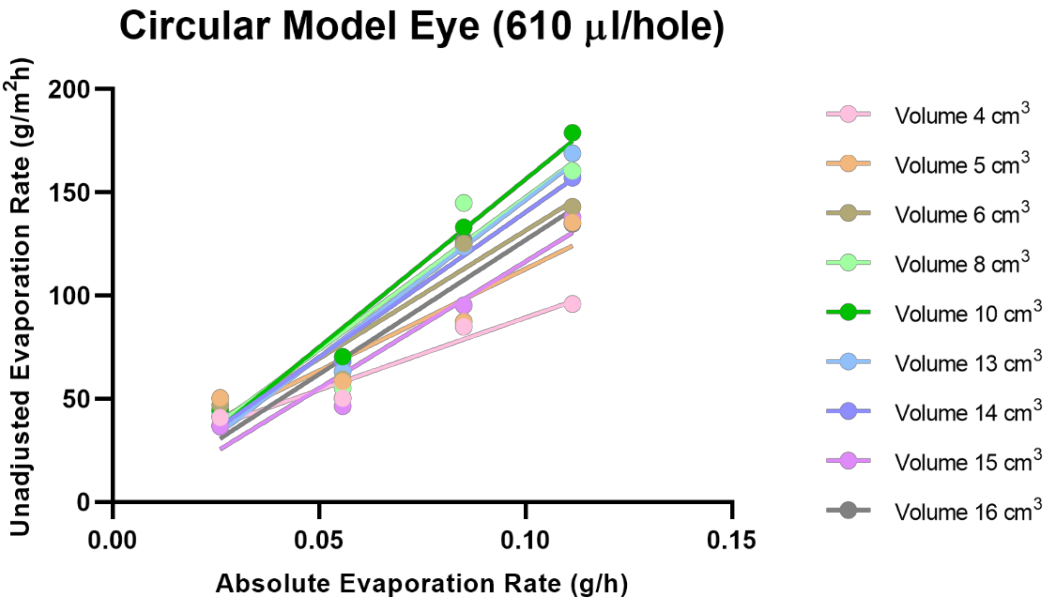

Figure 3-6: Unadjusted evaporation rate versus absolute evaporation rate filled with 610 µl of water per hole. Regression lines are shown for each evaporimeter volume (volumes 4, 6, 10, 13, 14, and 15 cm^3: all R^2>0.92, all p<0.05; volumes 5, 8, and 16 cm^3: all R^2>0.86, all p=0.06).

Linear regressions were calculated for each volume to derive a correction factor (volumes 4, 6, 10, 13, 14, 15 cm^3: all R^2>0.92, all p<0.05; volumes 5, 8, 16 cm^3: all R^2>0.86, all p=0.06, Table 3-3).

Table 3-3: Correction factor for each volume (610 µl of water per hole)

Volume (cm^3)	Linear regression	Correction Factor ($1/m^2$)	R^2	p-value
4	Y = 699.7x + 19.33	699.7	0.95	**0.03**
5	Y = 985.5x + 14.37	985.5	0.89	0.06
6	Y = 1241x + 7.41	1241	0.93	**0.04**
8	Y = 1476x + 0.04	1476	0.87	0.06
10	Y = 1625x - 6.19	1625	0.97	**0.01**
13	Y = 1524x - 6.01	1524	0.96	**0.02**
14	Y = 1406x - 0.20	1406	0.98	**0.01**
15	Y = 1229x - 6.45	1229	0.94	**0.03**
16	Y = 1299x - 3.01	1299	0.89	0.06

Bold indicates statistical significance.

A graph of the correction factor versus the evaporimeter volume is shown in Figure 3-7, with the data best fit with a second order polynomial non-linear regression ($Y = –17.6x^2 + 387.5x – 512.8$, where x = the volume inside the evaporimeter and Y = correction factor for a specific volume).

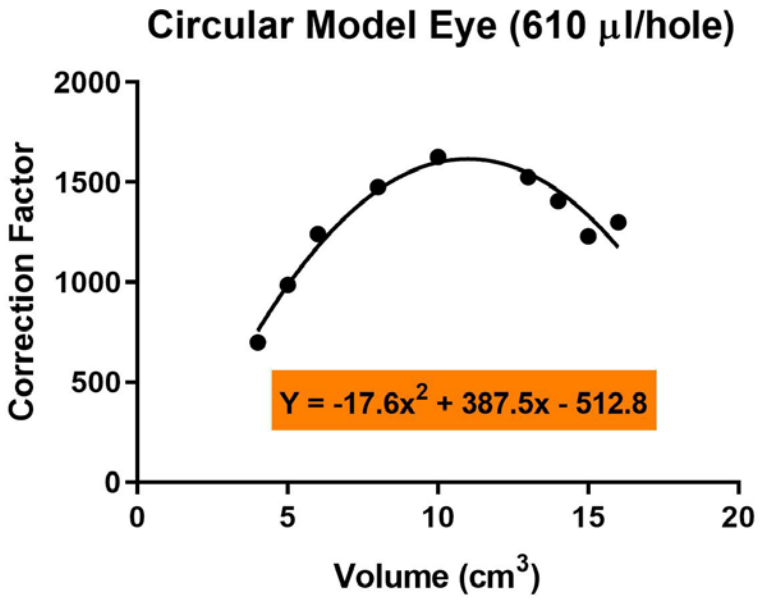

Figure 3-7: Correction factor versus evaporimeter volume filled with 610 µl of water per hole.

3.3.3 Discussion

Water loss occurred from the model eye over time, due to evaporation, with greater amounts lost as the surface area increased. Rohit et al. [21] also observed a negative linear loss of water in a stainless steel model eye when surface areas of 0.28, 1.12, and 1.96 cm^2 were investigated. However, their reported range of AERs (0.06 to 0.29 g/h for surface areas of 0.28 to 1.96 cm^2) was higher than the range found in this study (0.03 to 0.11 g/h for surface areas of 0.56 to 2.06 cm^2, Figure 3-8), even though the ambient RH was similar between the two studies. The higher ambient temperatures of 25 to 26°C in this study were higher than the 19 to 21°C reported by Rohit et al. [21]. This would have been expected to promote evaporation [53], resulting in greater amounts water loss than the other study. Instead, the opposite effect was observed.

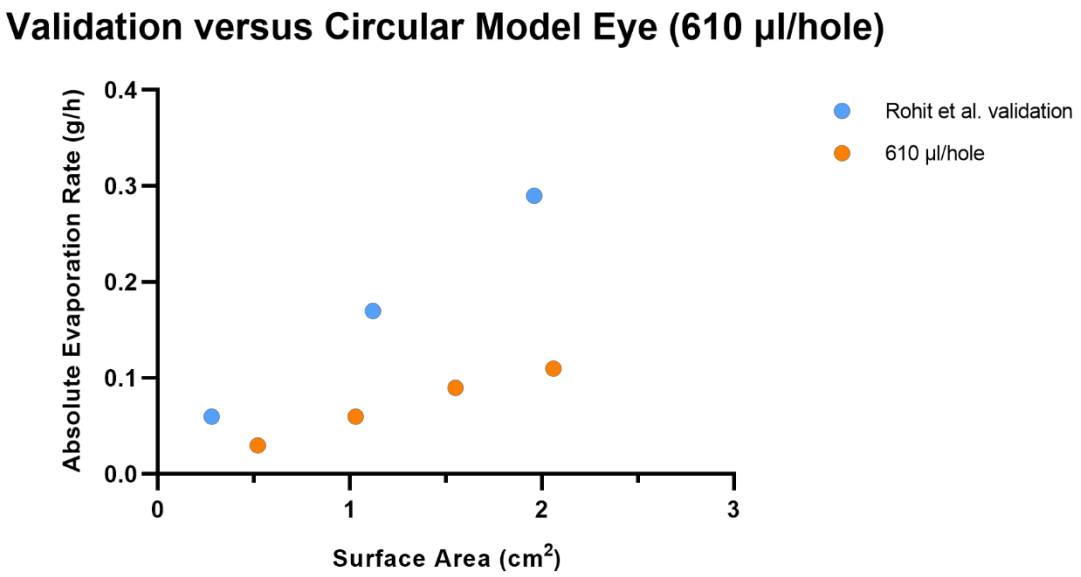

Figure 3-8: Absolute evaporation rate versus exposed surface area for the model eye with circular holes, filled with 610 µl of water per hole, compared to values reported by Rohit A, et al. Validating a new device for measuring tear evaporation rates. Ophthalmic Physiol Opt. 2014;34:53-62.

It is likely that these differences in evaporation rates occurred due to variations in the design and position of the holes between the two model eyes. The model eye used by Rohit et al. [21] seemed to have seven small, shallow holes positioned close together towards the center of the model eye (Figure 3-9), whereas the model eye used in this experiment had five small, deep holes distributed across the majority of the length (Figure 3-2). The more central location of the water source in the Rohit et al. [21] model eye placed them closer to the evaporimeter sensor, which may have promoted a more rapid

movement of the water molecules to the sensor. The difference may also be an unexpected effect due to the larger number of smaller holes used in the Rohit et al. [21] model eye. Each hole filled with water had a surrounding water meniscus around the edge of the hole that effectively increased the surface area of the hole. By having more holes, the effective surface area of the Rohit et al. [21] model eye may be larger than predicted, therefore producing a more rapid evaporation rate and a greater loss of water.

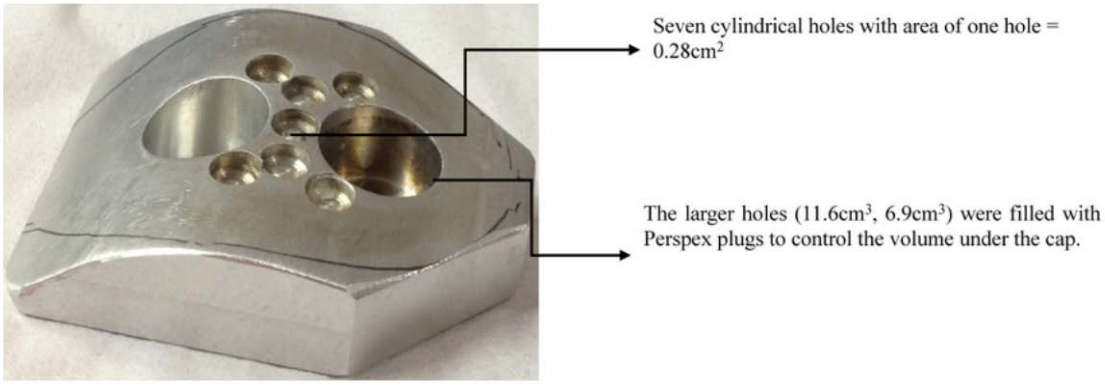

Figure 3-9: Stainless steel eye model eye used to originally validate the Eye-VapoMeter. Image from Rohit A, et al. Validating a new device for measuring tear evaporation rates. Ophthalmic Physiol Opt. 2014;34:53-62.

When testing the instrument on the model eye, Rohit et al. [21] observed an increase in the evaporation rate of the Eye-VapoMeter as the evaporative surface area increased. A similar strong positive relationship between surface area and evaporation rate was found in this study.

For the effect of goggle volume, Rohit et al. [21] found a strong negative relationship between the evaporation rate correction factors and volumes, with the equation e = –86.5f + 1689 (f = the volume inside the evaporimeter and e = correction factor for a specific volume). The highest evaporation rates were reported with the 5 cm^3 volume, and then evaporation rates linearly decreased as the evaporimeter volume increased to 13 cm^3. This supports the theory that increasing the goggle volume will produce a lower apparent rate of evaporation, since there is now a greater volume within the goggle for diffusion of the evaporated water molecules, leading to a reduced RH activating the sensor.

In contrast, for this study, the highest evaporation rate was obtained with the largest surface area (2.06 cm^2) and goggle volume of 10 cm^3, with lowest evaporation rate measured with the smallest surface area (0.52 cm^2) and a goggle volume of 15 cm^3. This reveals a subtler effect of goggle volume, with a positive linear relationship present for smaller volumes, and a negative relationship for larger goggle volumes. Based on these evaporation rates, this study found that a second-order polynomial non-linear

relationship could be fitted between the correction factors and volume inside the instrument ($Y = -17.6x^2 + 387.5x - 512.8$), which peaked at the 11 cm^3 volume.

The findings from the two experiments are thus in opposition to each other. However, the strong linear relationship found by Rohit et al. [21] was calculated only after excluding the third surface area of 1.96 cm^2 over which they measured. They did so because the AER of water (0.29 g/h) was higher than the anticipated TER [395]. This suggests some inconsistency in the experimental set-up. Since the remaining data covered measurements for only two surface areas, the authors interpolated the AER and instrument evaporimeter readings for an intermediate surface area of 0.56 cm^2, based on values obtained from the 0.28 and 1.12 cm^2 surface areas. In comparison, four surface areas were investigated in this study. Another reason why there may be a difference in results is because the linear regression of the UER versus the AER in the Rohit et al. [21] study was forced through the origin. This may be inappropriate since there may be further unexpected influences on evaporation rate for very small surface areas that are non-linear. The methodology in the validation paper [21] also did not specifically mention that a surface area of 0 cm^2 was tested, nor was a SD listed, which could be due to measurements either not being taken or repeated measurements not being taken.

Having set aside the results from Rohit et al. [21], and considering the results of this study alone, the relationship between goggle volume and evaporation rate is the opposite of the hypothesis proposed that smaller volumes should produce a faster evaporation rate. This hypothesis is based on the assumption that the measured RH at the instrument sensor is affected by the presence of the ambient RH in the goggle at the start of data collection. Once the goggle is placed over the model eye, water evaporating from the surface of the model eye enters the air just above the surface. It must then diffuse through the air towards the sensor for detection. For a small volume, the diffusion would be less than for a large volume, and so the RH at the sensor will increase more quickly, producing a more rapid rate of evaporation. This would support the findings of Rohit et al. [21].

However, it is likely that the diffusion effect may be aided by air movement within the goggle prompted by local shifts in RH. These effects improve the ability of the water vapor to be dispersed within the air within the goggle. It is also likely that the air movement is affected by the goggle volume. For very small volumes, the diffusion rate will be slower since the movement of the air within the goggle is restricted by the small volume due to the addition of modelling clay. As the air volume within the goggle increases, the circulation of water vapor within the goggle improves, which allows the RH at the sensor to increase at a faster rate, thereby resulting in a higher measured evaporation rate. However, there appears to be an optimal goggle volume through which the water vapor can diffuse within the

goggle, where the rate of evaporation from the surface matches with the diffusion rate within the goggle. As the goggle volume increases further, the diffusion rate becomes greater than the rate with which the evaporation from the surface can push water vapor into the goggle. This is the effect we see modeled in Figure 3-7.

The strong volume effect found in this study suggested that further investigation would be useful. The slower rate of water loss and lower rates of evaporation could be due to not completely filling each hole of the model eye with water. This will have produced an additional air volume of 0.14 to 0.56 cm^3 within the experimental set-up that may have contributed to how the RH diffuses in the available air. Therefore, the study was repeated by instilling a larger volume of water in each hole.

3.4 Original Model Eye (750 µl/hole)

3.4.1 Materials and Methods

See Section 3.3.1. The same procedure was followed to test four surface areas (0.52, 1.03, 1.55, and 2.06 cm^2) with 750 µl of distilled water per hole.

3.4.1.1 Water Loss over Time

See Section 3.3.1.1.

3.4.1.2 Measurement of Evaporation Rate

See Section 3.3.1.2. Eleven volumes within the evaporimeter were tested (4, 5, 6, 8, 10, 11, 12, 13, 14, 15, and 16 cm^3), with 4 surface areas (0.52, 1.03, 1.55, and 2.06 cm^2). Five consecutive Eye-VapoMeter measurements were taken and logged with the manufacturer's DelfWin 4 version 3.1.14 software. Based on the results from Section 3.3.2.2, the highest evaporation rate was measured with a 2.06 cm^2 surface area and 10 cm^3 volume. Therefore, additional volumes inside the evaporimeter were tested to further investigate the change in evaporation rate that occurred around the 10 cm^3 volume.

3.4.1.3 Statistical Analysis

See Section 3.3.1.3.

3.4.2 Results

3.4.2.1 Water Loss over Time

The mean ± SD ambient temperature was 25.21 ± 0.83°C and RH was 40.61 ± 1.87%. The range of ambient temperature was 24 to 26°C and RH ranged between 37 to 44%. The mean ± SD temperature of the oven was 33.96 ± 0.05°C (range: 34.0 to 34.1°C).

The change in the weight of over time with each hole filled with 750 µl of distilled water appears in Figure 3-10. A summary of the rate of water loss is shown in Table 3-4. Water loss occurred at a linear rate over time (all R^2>0.99, all p<0.0001).

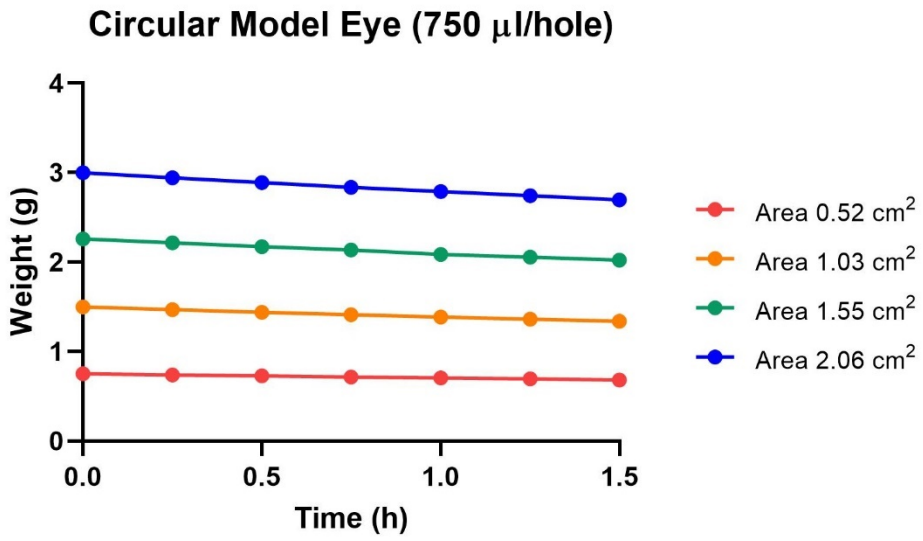

Figure 3-10: Weight of water over time filled with 750 µl of water per hole.

Table 3-4: Rate of water loss (750 µl of water per hole)

Area (cm^2)	Linear regression	AER (g/h)	R^2	p-value
0.52	Y = –0.05x + 0.75	0.05	0.994	**<0.0001**
1.03	Y = –0.11x + 1.50	0.11	0.997	**<0.0001**
1.55	Y = –0.16x + 2.26	0.16	0.997	**<0.0001**
2.06	Y = –0.20x + 2.99	0.20	0.999	**<0.0001**

AER: Absolute evaporation rate. **Bold** indicates statistical significance.

3.4.2.2 Eye-VapoMeter Evaporation Rate and Correction Factors

The mean ± SD ambient temperature was 25.43 ± 0.81°C and RH was 39.76 ± 1.30%. The range of ambient temperature was 24 to 26°C and RH ranged between 37 to 41%.

A summary of the evaporation rate measured for different volumes within the evaporimeter and various surface areas on the model eye is shown in Table 3-5. A graph of the UER from the Eye-VapoMeter versus different AERs of water loss for different volumes within the evaporimeter appears in Figure 3-11.

Table 3-5: Mean ± SD evaporation rate of different volumes and areas (750 µl of water per hole)

	Eye-VapoMeter Evaporation Rate ($g/m^2/h$)			
Volume (cm^3)	AER 0.05 *(0.52 cm^2)*	AER 0.11 *(1.03 cm^2)*	AER 0.16 *(1.55 cm^2)*	AER 0.20 *(2.06 cm^2)*
4	97.8 ± 14.5	124.1± 6.9	188.0 ± 7.4	213.9 ± 13.7
5	105.0 ± 14.1	133.0 ± 7.1	182.8 ± 6.8	217.1 ± 7.4
6	103.0 ± 10.2	119.3 ± 13.3	183.6 ± 22.4	218.0 ± 10.4
8	91.0 ± 11.3	140.4 ± 10.5	212.3 ± 11.0	251.6 ± 10.2
10	104.9 ± 5.0	181.2 ± 17.2	257.9 ± 12.5	314.6 ± 10.4
11	92.2 ± 10.7	132.4 ± 19.3	227.7 ± 11.5	287.8 ± 14.9
12	103.6 ± 8.7	165.4 ± 5.6	240.2 ± 9.8	289.0 ± 23.8
13	76.6 ± 13.2	153.0 ± 23.2	214.7 ± 11.8	273.0 ± 13.8
14	93.7 ± 7.6	137.3 ± 31.3	224.0 ± 3.5	283.3 ± 23.1
15	47.3 ± 1.2	110.0 ± 19.4	182.1 ± 9.2	217.7 ± 8.7
16	60.7 ± 5.9	105.2 ± 16.4	187.1 ± 11.7	252.6 ± 27.2

AER: absolute evaporation rate

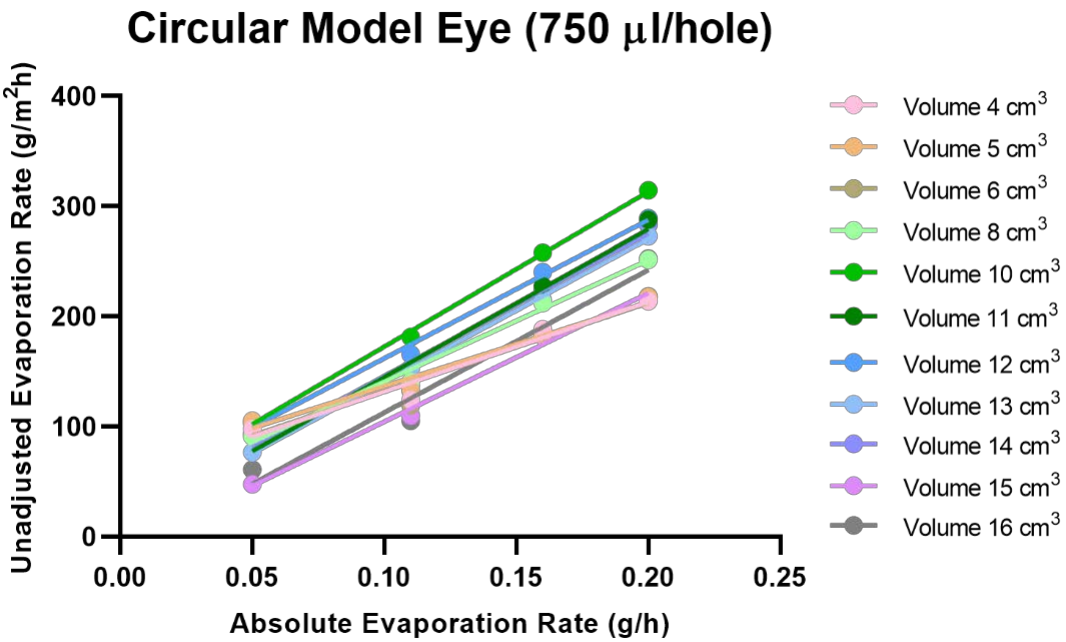

Figure 3-11: Unadjusted evaporation rate versus absolute evaporation rate filled with 750 µl of water per hole. Regression lines are shown for each evaporimeter volume (all R^2>0.92, all p<0.04).

Linear regressions were calculated for each volume to derive a correction factor (all R^2>0.92, all p<0.04, Table 3-6).

Table 3-6: Correction factor for each volume (750 µl of water per hole)

Volume (cm^3)	Linear regression	Correction Factor ($1/m^2$)	R^2	p-value
4	Y = 817.9x + 49.64	817.9	0.96	**0.02**
5	Y = 763.3x + 60.25	763.3	0.98	**0.01**
6	Y = 805.2x + 51.30	805.2	0.93	**0.03**
8	Y = 1103x + 30.46	1103	0.99	**<0.01**
10	Y = 1408x + 31.58	1408	0.99	**<0.01**
11	Y = 1345x + 10.17	1345	0.96	**0.02**
12	Y = 1258x + 36.04	1258	0.99	**<0.01**
13	Y = 1298x + 10.55	1298	0.99	**<0.01**
14	Y = 1294x + 16.38	1294	0.97	**0.02**
15	Y = 1168x - 12.55	1168	0.99	**<0.01**
16	Y = 1296x - 17.09	1296	0.97	**0.02**

Bold indicates statistical significance.

A graph of the correction factor versus the evaporimeter volume is shown in Figure 3-12, with the data best fit with a second order polynomial non-linear regression ($Y = –8.1x^2 + 205.8x + 1.2$).

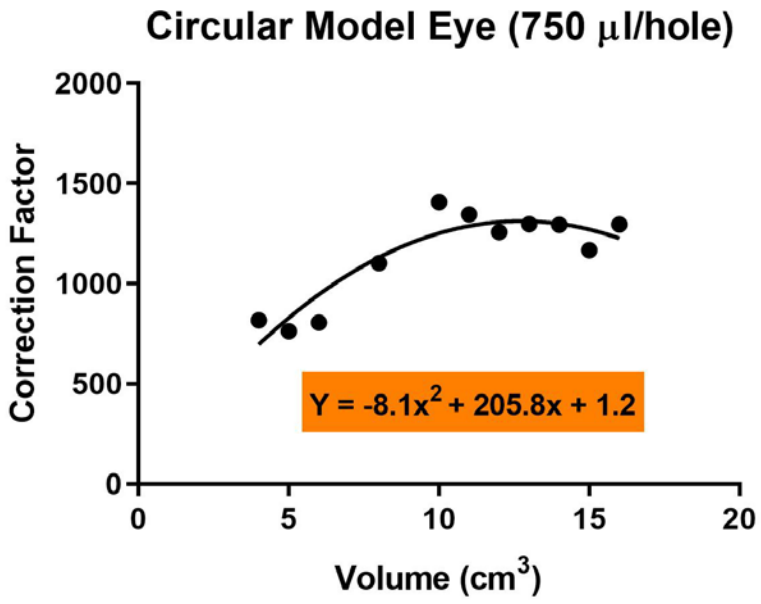

Figure 3-12: Correction factor versus evaporimeter volume filled with 750 µl of water per hole.

3.4.3 Discussion

As reported in Section 3.3.2.2, the UER increased as the surface area increased. Once again, the highest rate of evaporation occurred with the largest surface area (2.06 cm^2) and 10 cm^3 evaporimeter volume, and the lowest evaporation rate occurred with the smallest surface area (0.52 cm^2) and 15 cm^3 evaporimeter volume.

A similar relationship between evaporation rate and goggle volume was found as in the first experiment: a second order polynomial non-linear regression was found to best fit the relationship between the correction factor and evaporimeter volume ($Y = -17.6x^2 + 387.5x - 512.8$), which peaked at the 13 cm^3 volume. Although the peak correction factor occurred at 11 cm^3 when the model eye was filled with 610 µl of water, the peak correction factor shifted to 13 cm^3 with the model eye filled with 750 µl of water. The rate of change was slower for the smaller goggle volumes when each hole was filled with 750 µl of water, with the larger goggle volumes producing less of an apparent reduction in evaporation rate than before. This can be attributed to the reduction of air volume within the holes of the model eye, which improved the diffusion of water molecules from the surface of the model eye into the goggle. This further supports the theory that air volume within the goggle has a crucial effect on the measured evaporation rate.

Interestingly, the rate of water loss was faster with the model eye filled with 750 µl distilled water compared to 610 µl. It is not immediately apparent why this effect should have occurred, other than by having fully filled the holes in the model eye, the water surface was more exposed to ambient air circulation that promoted evaporation. However, the AERs (0.05 to 0.20 g/h for surface areas of 0.56 to 2.06 cm^2) in this study were still lower than the values reported by Rohit et al. [21] (0.06 to 0.29 g/h for surface areas of 0.28 to 1.96 cm^2, Figure 3-13).

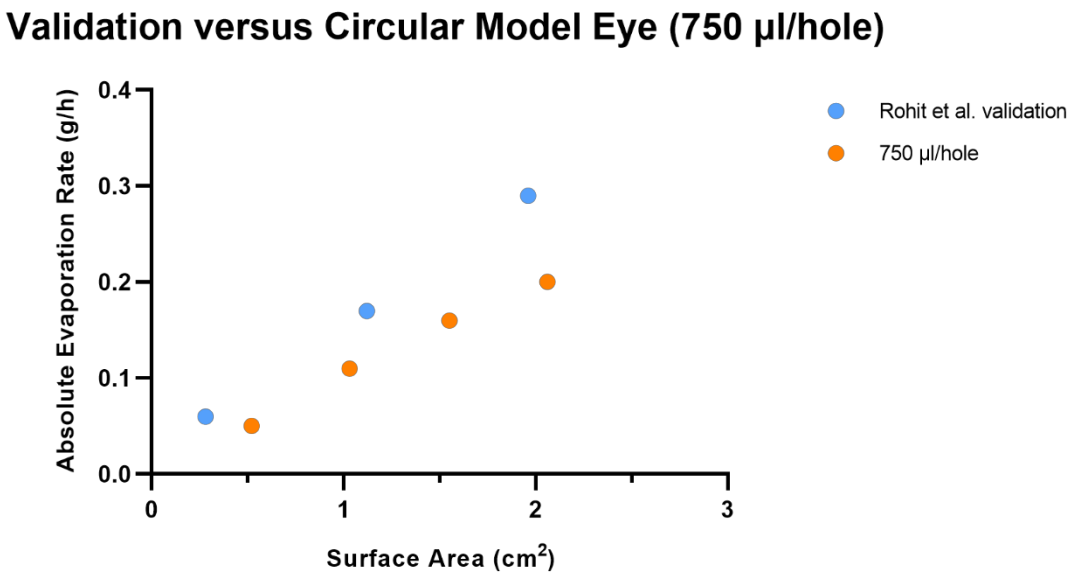

Figure 3-13: Absolute evaporation rate versus surface area for a circular model eye filled with 750 µl of water per hole in this study compared to values reported by Rohit A, et al. Validating a new device for measuring tear evaporation rates. Ophthalmic Physiol Opt. 2014;34:53-62.

Having identified that the air volume within the goggle was a key factor in the measurement of evaporation rate, and having also considered that the arrangement of the holes in the model eye may have an influence on the evaporation rate, a series of new model eyes was developed that more closely mimicked the true palpebral aperture shape of the eye. The results from these experiments are reported in the next sections of the chapter.

3.5 Elliptical Model Eyes

3.5.1 Materials and Methods

In order to better simulate evaporation from the surface of the eye, seven new aluminum model eyes were created with a single central elliptical hole (Mr. Daniel Knappert, Greig City Academy, London, UK, Figure 3-14). The width of each ellipse was 29 mm and based on an average female palpebral fissure size found in multiple ethnicities [396-398]. The size of a female eye was chosen because most studies have concluded that the prevalence of dry eye disease is higher in females [121, 399]. The size of the surface area of the model eyes was calculated to range from 1 to 2.5 cm^2 (in 0.25 cm^2 increments) and were chosen to represent a range of ocular surface areas encountered in younger [97] and older adults [400].

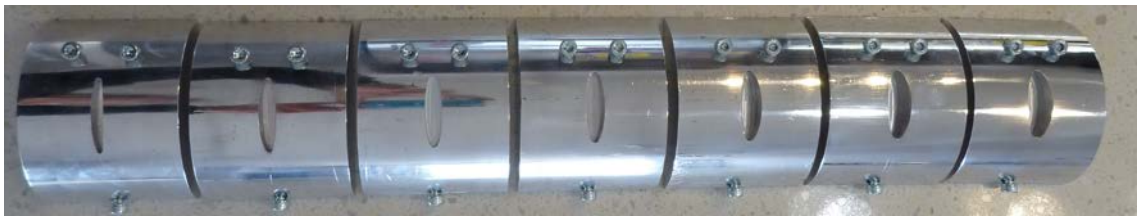

Figure 3-14: Elliptical model eyes with surface areas of 1 cm^2 to 2.5 cm^2 (left to right).

3.5.1.1 Water Loss over Time

See Section 3.3.1.1. Since the elliptical hole in each model eye was a different size, varying volumes of water were added to each model eye, but with the same intent to fully fill each hole, as shown in Table 3-7. An Eppendorf pipette (Reference 100-1000 µl, Eppendorf, Hamburg, Germany) was used to pipette the two smallest model eyes and 1000 µl into the other model eyes. The 5000DG pipette (Fisherbrand® 5000DG 100-1000 µl, Fischer Scientific, Waltham, Massachusetts, USA) was used to add any remaining volume into the model eye. Two pipettes were used when the volume was >1000 µl because it was faster to use two pipettes, rather than change the pipette tip between each instillation of water into the model eye. This allowed the evaporation rate to be measured within a shorter time interval for the larger volumes, which made the measurement time closer to the time required to fill the two smallest model eyes.

Table 3-7: Amount of water pipetted into each elliptical model eye

Area of model eye (cm^3)	Volume of Water (µl)
1	800
1.25	900
1.5	1050
1.75	1200
2	1350
2.25	1550
2.5	1750

3.5.1.2 Measurement of Evaporation Rate

See Section 3.3.1.2. A range of evaporimeter volumes were tested from 4 to 16 cm^3 (in 1 cm^3 intervals), and for volumes of 16.88, 17.75, 18.63, and 19.5 cm^3. The volume range from 4 to 15 cm^3 was modified using modelling clay, as described for the two previous studies, while the four volumes greater than 16 cm^3 were produced by positioning 1 to 8 thin, aluminum inserts (Mr. Daniel Knappert, Greig City Academy, London, UK, Figure 3-15) between the evaporimeter goggle and the model eye. Each insert contained a cut-out section of an internal volume 0.44 cm^3 and the inserts could be stacked together to create different volumes.

Each volume was tested with seven surface areas from 1 to 2.5 cm^2 in 0.25 cm^2 steps, with the 16 cm^3 volume tested first. The remaining volumes were tested from largest to smallest. The order of testing each area for a particular volume was randomized. When the evaporimeter volume was greater than 16 cm^3, only a single measurement was taken for each area per day to allow the inserts to heat up between measurements. All seven surface areas were tested on the same day if the evaporimeter volume less than 16 cm^3. No more than one evaporimeter volume was tested per day. Five consecutive Eye-VapoMeter measurements were taken for each surface area and volume combination, and logged with the manufacturer's DelfWin 4 version 3.1.14 software.

Figure 3-15: Aluminum inserts placed on the model eye to increase the air volume inside the evaporimeter.

3.5.1.3 Statistical Analysis

See Section 3.3.1.3.

3.5.2 Results

3.5.2.1 Ambient Temperature and Relative Humidity

The mean ± SD ambient temperature was 25.62 ± 0.48°C and RH was 41.12 ± 0.97%. The range of ambient temperature was 25 to 26°C and RH ranged between 40 to 43%.

3.5.2.2 Water Loss over Time

The change in the weight of over time with each elliptical model eye filled with 800 to 1750 µl of distilled water appears in Figure 3-16. A summary of the rate of water loss shown in Table 3-8. Water loss decreased at a linear rate over time (all $R^2>0.99$, all $p<0.0001$).

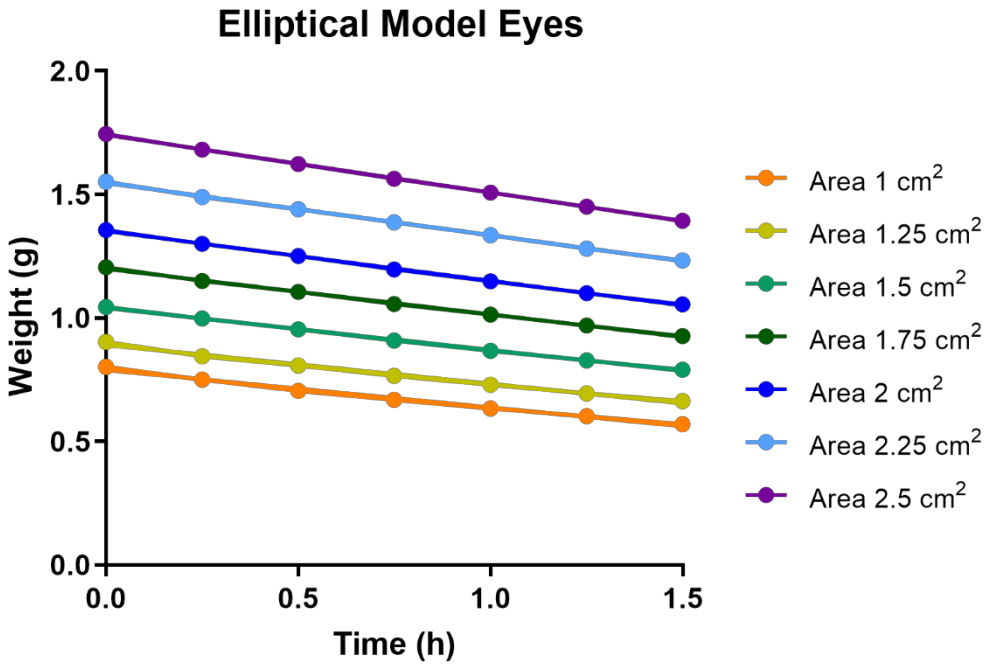

Figure 3-16: Weight of water over time in the elliptical model eyes.

Table 3-8: Rate of water loss (elliptical model eyes)

Area (cm^2)	Linear regression	AER (g/h)	R^2	p-value
1	Y = –0.15x + 0.79	0.15	0.991	**<0.0001**
1.25	Y = –0.16x + 0.89	0.16	0.992	**<0.0001**
1.5	Y = –0.17x + 1.04	0.17	0.998	**<0.0001**
1.75	Y = –0.18x + 1.20	0.19	0.999	**<0.0001**
2	Y = –0.20x + 1.35	0.20	0.999	**<0.0001**
2.25	Y = –0.21x + 1.55	0.21	0.999	**<0.0001**
2.5	Y = –0.23x + 1.74	0.23	0.999	**<0.0001**

AER: Absolute evaporation rate. **Bold** indicates statistical significance.

3.5.2.3 Eye-VapoMeter Evaporation Rate and Correction Factors

The mean ± SD ambient temperature was 25.62 ± 0.48°C and RH was 41.10 ± 0.94%. The range of ambient temperature was 25 to 26°C and RH ranged between 40 to 43%. The mean ± SD temperature of the oven was 33.96 ± 0.05°C (range: 33.9 to 34°C).

A summary of the evaporation rate measured for different volumes within the evaporimeter and varying surface areas on the model eye is shown in Table 3-9. A graph of the UERs from the Eye-VapoMeter versus different AERs of water loss for different volumes within the evaporimeter appears in Figure 3-17.

Table 3-9: Mean ± SD evaporation rate of different volumes and areas (elliptical model eyes)

	Eye-VapoMeter Evaporation Rate ($g/m^2/h$)						
Volume (cm^3)	AER 0.15 *(1 cm^2)*	AER 0.16 *(1.25 cm^2)*	AER 0.17 *(1.5 cm^2)*	AER 0.19 *(1.75 cm^2)*	AER 0.20 *(2 cm^2)*	AER 0.21 *(2.25 cm^2)*	AER 0.23 *(2.5 cm^2)*
4	186.8 ± 9.1	195.5 ± 11.5	176.6 ± 10.9	205.3 ± 13.2	195.0 ± 7.5	184.5 ± 16.3	241.0 ± 7.1
5	224.0 ± 10.1	233.8 ± 15.0	226.6 ± 22.9	246.9 ± 12.9	224.4 ± 6.9	289.7 ± 6.9	307.9 ± 16.7
6	240.0 ± 13.1	244.0 ± 24.2	247.5 ± 9.5	234.8 ± 7.5	247.2 ± 11.0	310.1 ± 13.3	297.2 ± 31.2
7	236.4 ± 6.1	263.0 ± 20.7	248.2 ± 12.0	270.3 ± 8.3	284.9 ± 14.0	309.5 ± 8.5	328.7 ± 14.2
8	239.9 ± 9.9	253.3 ± 21.3	262.1 ± 19.6	263.9 ± 18.9	297.0 ± 7.9	289.1 ± 10.0	356.3 ± 13.6
9	259.6 ± 5.2	262.6 ± 14.2	261.0 ± 12.5	281.7 ± 16.5	282.3 ± 12.4	334.8 ± 29.7	352.6 ± 20.6
10	241.8 ± 22.0	246.8 ± 9.7	285.8 ± 18.0	311.5 ± 8.6	305.6 ± 9.0	342.6 ± 18.6	366.2 ± 20.1
11	266.9 ± 8.0	250.4 ± 27.8	282.9 ± 17.2	303.4 ± 20.0	312.8 ± 19.7	337.1 ± 29.7	356.3 ± 11.8
12	248.6 ± 33.4	240.9 ± 29.1	257.8 ± 27.3	283.9 ± 15.0	256.7 ± 32.9	321.2 ± 17.8	335.3 ± 29.7
13	262.5 ± 11.2	266.9 ± 12.7	286.0 ± 12.4	298.9 ± 18.0	310.1 ± 17.0	351.3 ± 22.2	378.7 ± 19.8
14	236.2 ± 18.9	254.4 ± 22.1	286.0 ± 12.9	271.6 ± 22.8	278.7 ± 19.7	326.5 ± 33.8	362.0 ± 27.7
15	230.7 ± 26.6	241.8 ± 26.7	247.0 ± 42.2	252.9 ± 17.8	275.0 ± 24.6	296.2 ± 19.8	337.6 ± 20.0
16	264.5 ± 19.7	305.6 ± 30.0	284.8 ± 29.3	307.6 ± 16.8	298.6 ± 18.0	315.5 ± 15.8	394.0 ± 30.7
16.88	254.3 ± 31.4	207.3 ± 18.1	255.6 ± 17.5	287.5 ± 9.1	333.6 ± 12.7	343.8 ± 17.0	350.1 ± 22.9
17.75	233.1 ± 12.8	227.1 ± 13.2	262.3 ± 12.6	266.2 ± 24.9	304.6 ± 24.6	343.1 ± 14.9	349.1 ± 17.2
18.63	206.7 ± 6.7	250.5 ± 18.6	202.3 ± 18.0	240.2 ± 17.6	296.9 ± 25.1	303.1 ± 32.3	330.3 ± 24.8
19.5	215.2 ± 4.8	208.4 ± 21.6	218.1 ± 12.8	265.0 ± 18.4	263.0 ± 12.1	294.9 ± 23.1	334.2 ± 34.1

AER: Absolute evaporation rate.

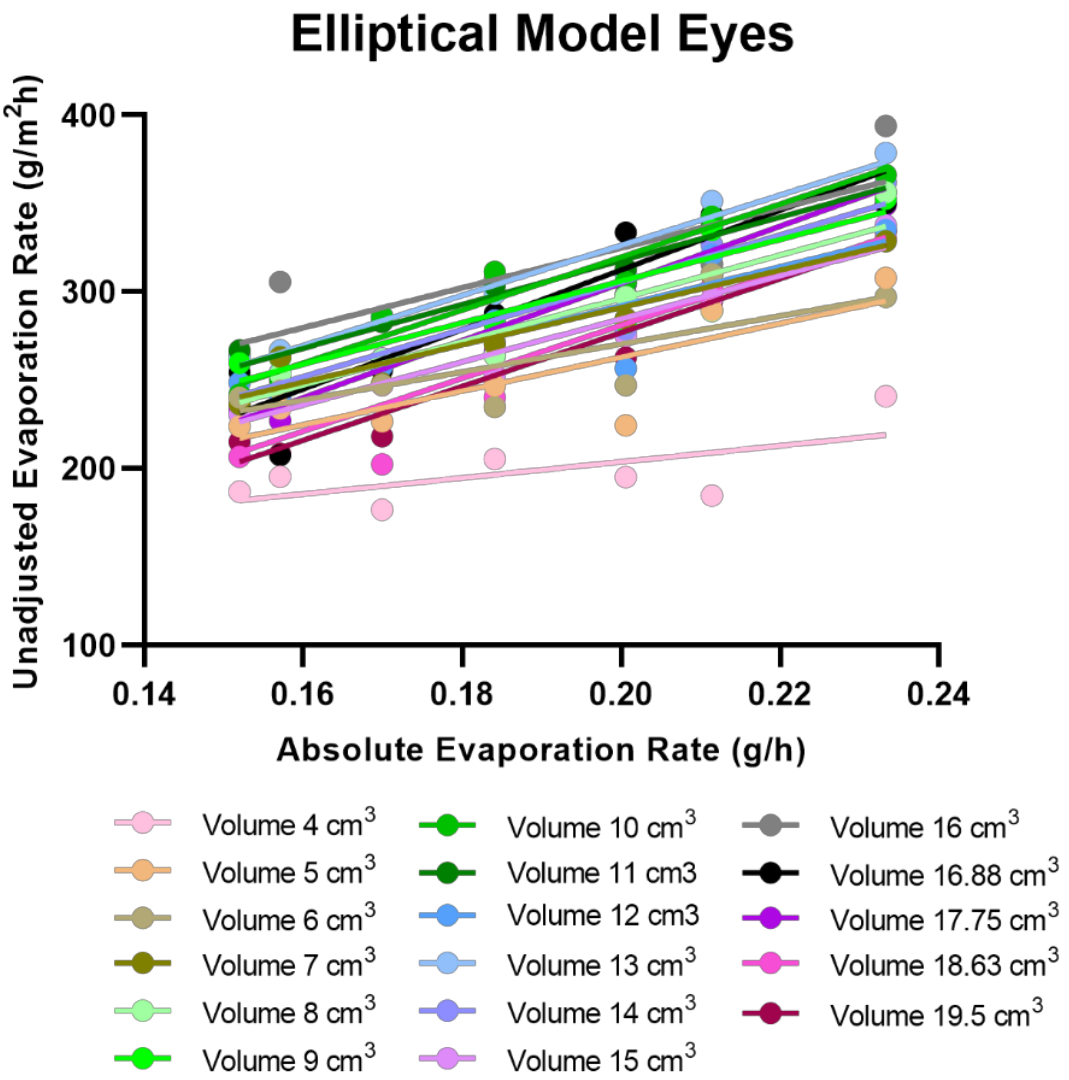

Figure 3-17: Unadjusted evaporation rate versus absolute evaporation rate for the elliptical model eyes. Regression lines are shown for each evaporimeter volume (volume 4 cm^3: R^2=0.41, p=0.12; volumes 5 to 19.5 cm^3: all R^2>0.60, all p<0.05).

Linear regressions were calculated for each volume to derive a correction factor (volume 4 cm^3: R^2=0.41, p=0.12; volumes 5 to 19.5 cm^3: all R^2>0.60, all p<0.05, Table 3-10).

Table 3-10: Correction factor for each volume (elliptical model eyes)

Volume (cm^3)	Linear regression	Correction Factor ($1/m^2$)	R^2	p-value
4	Y = 455.5x + 112.7	455.5	0.41	0.12
5	Y = 956.1x + 71.76	956.1	0.69	**0.02**
6	Y = 792.8x + 111.9	792.8	0.61	**0.04**
7	Y = 1056x + 79.94	1056	0.92	**<0.01**
8	Y = 1225x + 51.36	1225	0.88	**<0.01**
9	Y = 1177x + 70.73	1177	0.86	**<0.01**
10	Y = 1500x + 19.72	1500	0.94	**<0.01**
11	Y = 1238x + 70.06	1238	0.96	**0.02**
12	Y = 1100x + 72.19	1100	0.78	**<0.01**
13	Y = 1419x + 42.60	1419	0.96	**<0.01**
14	Y = 1336x + 38.20	1336	0.86	**<0.01**
15	Y = 1216x + 41.55	1216	0.94	**<0.01**
16	Y = 1128x + 99.16	1128	0.68	**0.02**
16.88	Y = 1686x - 24.75	1686	0.86	**<0.01**
17.75	Y = 1617x - 18.67	1617	0.95	**<0.01**
18.63	Y = 1498x - 18.57	1498	0.81	**<0.01**
19.5	Y = 1523x - 27.72	1523	0.95	**<0.01**

Bold indicates statistical significance.

A graph of the correction factor versus the evaporimeter volume is shown in Figure 3-18, with the data best fit with a second order polynomial non-linear regression ($Y = –3.6x^2 + 135.5x + 211.7$).

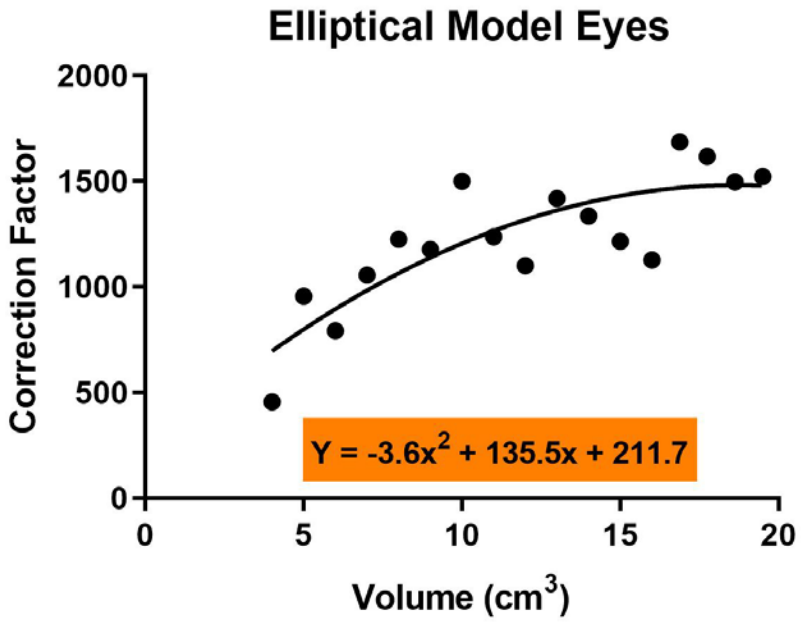

Figure 3-18: Correction factor versus evaporimeter volume for the elliptical model eyes.

3.5.3 Discussion

As observed with the original model eye, water loss decreased at a linear rate for the elliptical model eyes. However, the AERs (0.15 to 0.23 g/h for surface areas of 1 to 2.5 cm^2) continued to be lower than the values that Rohit et al. [21] reported (0.06 to 0.29 g/h for surface areas of 0.28 to 1.96 cm^2, Figure 3-19).

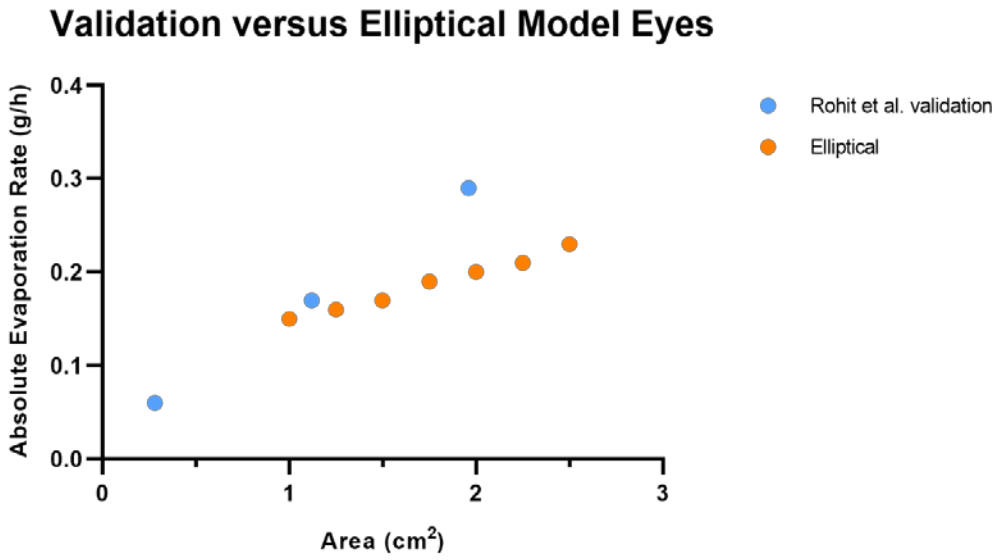

Figure 3-19: Absolute evaporation rate versus surface area for elliptical model eyes compared to values reported by Rohit A, et al. Validating a new device for measuring tear evaporation rates. Ophthalmic Physiol Opt. 2014;34:53-62.

Similar to the results found in Sections 3.3.2.2 and 3.4.2.2, the rate of evaporation rate was higher when the surface area was larger. The highest rate of evaporation was recorded with the largest surface area (2.5 cm^2) and a 16 cm^3 evaporimeter volume. The lowest evaporation rate was measured with the smallest surface area (1 cm^2) and the smallest evaporimeter volume (4 cm^3). Larger correction factors were obtained when the evaporimeter volume was greater than 16 cm^3. It is not known whether this trend would have also been observed with the original model eye because these volumes were not tested. As found in the original model eye, the graph of the correction factors versus the evaporimeter volume was best described with a second order polynomial non-linear regression ($Y = –3.6x^2 + 135.5x + 211.7$), which peaked at a volume of 19 cm^3.

Although testing was conducted with using two different model eyes, none of them resulted in a truly linear relationship between the correction factors and evaporimeter volume, but the elliptical model eyes were able to shift the curve further to the right and remove the large volume effect seen with the

original model eye. The overall effect produced a more linear relationship between the goggle volume and evaporation rate, albeit it with some variation. Interestingly, although larger volumes were tested with this model eye, the previously observed decrease in measured evaporation rate was not noted. This suggests that the single elliptical surface area model is more appropriate for testing and calibrating ocular surface rates of evaporation.

3.6 Testing the Correction Factors

Having developed the correction curve based on the elliptical model eye, it is possible to test the resulting correction factors. Rohit et al. [21] used the negative linear equation they found to calculate a correction factor based on the volume of each participant to determine an individual's corrected TER. Correction factors were based on the evaporation rate of water and the amount of air volume within the evaporimeter, based on the range of air volumes that they measured from 5 to 13 cm^3 with the model eye. A comparison of the correction factors reported for the instrument used by Rohit et al. [21] and the three studies in this chapter is shown in Table 3-11.

Table 3-11: Summary of correction factors: Rohit et al. [21] and aluminum model eyes

		Correction Factor ($1/m^2$)		
Volume (cm^3)	**Rohit [21]**	**Circular Model Eye (610 μl/hole)**	**Circular Model Eye (750 μl/hole)**	**Elliptical Model Eye**
6	1170.4	1178.6	944.4	895.1
7	1085	1337.3	1044.9	983.8
9	908.9	1549.1	1197.3	1139.6
10	820.1	1602.2	1249.2	1206.7
11	737.5	1620.1	1284.9	1266.6

It should be noted that the original paper [21] included an incorrect formula to calculate the evaporation rate from the skin. Since that formula was wrong, the sample calculations listed in the paper may also be incorrect. If the formula in the 2019 corrigendum is used, the skin evaporation rate would be 6.25 g/m^2h, rather than the reported value of 16 g/m^2h. However, if the skin evaporation rate was calculated incorrectly, then the resulting TER would also be wrong because this was calculated from the difference between the evaporation rate of the open eye and the skin evaporation rate.

Nevertheless, assuming the TER was 16.5 g/m^2h with an evaporimeter volume of 6 cm^3 and ocular surface area of 0.00024 m^2, then the TER would be 58.74 g/m^2h based on Rohit et al.'s [21] participant correction factor. For comparison, if the correction factor calculated from the non-linear regression from the elliptical model eyes was applied, the TER would be 76.81 g/m^2h.

Using the same TER and ocular surface area as listed above, if the evaporimeter volume was increased to 11 cm^3, Rohit et al. [21] would have reported a TER of 93.22 g/m^2h, whereas the correction factor from the elliptical model eye would have found much a lower rate of 54.28 g/m^2h.

3.7 Possible Sources of Error

The measurements of water loss were not conducted in an environmental chamber because the balance could not be moved. To compensate for the lack of ambient temperature and RH control, testing was planned for days that would most closely match the conditions of the environmental chamber. However, evaporation measurements were performed in an environmental chamber since the evaporimeter is more sensitive to fluctuations in ambient RH.

Each hole in the model eye was not refilled with water between consecutive evaporation measurements. Some water loss will have occurred during and between each measurement, which may have caused a gradual change in the measured evaporation rate, since the volume will also be changing as a result. However, this will be a consistent effect for each series of consecutive measurements. A better methodology may have been to record a single measurement of each model eye and to refill the hole before each measurement. However, this would then have introduced a different variable of not knowing exactly how much water was added to the model eye, which could produce variable rates of evaporation. In addition, this was not done due to the length of time it would have taken to complete the experiment.

Some of the evaporation rates obtained with the elliptical model eyes were >300 g/m^2h. The accuracy of these high values has yet to be established because the reported range of the dermatological device is 0 to 300 g/m^2h [369]. Since these experiments were only conducted with water, a 4x [48, 49] increase in evaporation rate is expected from an absent lipid layer compared to measurements obtained from an intact tear film.

It is unknown how much heat was able to be transferred from the heating plate to the top of each model eye, although it is hoped that the aluminum was able to transfer the heat well and keep the water warm. Future work could use an infrared thermometer to monitor to the temperature at the top of the model eye since lower temperatures would result in less water loss. However, by having consistent overall dimensions for each elliptical model eye, the effect of heat transfer should be similar for each surface area.

A significant amount of modelling clay needed to be added to the goggle to produce the small test volumes. It is possible that the modelling clay could have impeded the flow of water vapor from the

surface of the model eye to the sensor located in a chamber above the goggle. This is of particular concern when testing small volumes. However, it is not thought that the modelling clay absorbed any of the evaporated water because the material is hydrophobic, as demonstrated by a droplet of water maintaining its shape when placed on a piece of modelling clay.

There is also the possibility that some water vapor could have leaked from the air gaps between the stacked aluminum plates used to increase the volume underneath the evaporimeter. Although each plate was aligned as well as possible to reduce any air gaps, the system may not have been a completely closed system when volumes greater than 16 cm^3 where tested. This may have resulted in lower evaporation rates.

3.8 Future Work

Improvements to the evaporimeter volume testing could be made by using 3D printed plastic inserts to reduce the volume of the evaporimeter. This would be a better method of mimicking the size of the eye that would protrude into the goggle versus placing modelling clay on the lens of the evaporimeter. Modelling clay was not placed on the model eye to reduce the volume because it would be difficult to conform to the irregular shape of the goggle and could also prevent forming a tight seal around the edge of the goggle if the evaporimeter was not placed in the correct position. Plastic inserts could also be 3D printed to raise the height of the evaporimeter, which would eliminate any potential air gaps that would have occurred with the metal inserts.

Further testing will need to be done to determine the normal range of evaporimeter volumes found when people use the commercially available goggle.

3.9 Conclusions

The two aims for this chapter were to calculate correction factors for different volumes within the evaporimeter, and to describe the relationship between the correction factors and the evaporimeter volume with a mathematical equation. Through the series of studies reported, an improved elliptical eye model has been used to produce a mathematical correction factor that can take into account the surface area and air volume within the goggle.

For the three different models tested in this study, the smallest correction factors (455.5 to 763.3/m^2) were found when the evaporimeter volume was reduced to 4 or 5 cm^3. The original model eye had the largest correction factor at 10 cm^3, while the elliptical model eye had a maximum correction factor at a volume of 16.88 cm^3. Although calculating the corrected evaporation rate does not seem to have been widely adopted amongst researchers, the resulting non-linear equations seem to better represent the

characteristics of the marketed version of the Eye-VapoMeter compared to the ones obtained from the instrument during its validation.

The results of this chapter indicate that making comparisons between values obtained with the first version of the instrument that was validated for use with the eye and the marketed instrument is difficult, because the original correction factors do not seem to be optimized for the larger air volumes found when using the commercially available goggle. The results also show that using the linear regression equation to correct for the volume of the goggle will considerably overestimate or underestimate the corrected TERs. Making comparisons between the values obtained from different Eye-VapoMeters is further complicated by the fact that some researchers report the corrected TER [19, 116, 161, 323], whereas others appear to report the UER obtained directly from the instrument [93, 94, 96-98, 116].

Delfin Technologies Inc. were asked for permission to access the raw data in the instrument to deduce why the results of this study were different to the validation study [21], but this request was denied. This led to further work on developing a new in-house evaporimeter that would provide full control of the settings and access to the data that it records.

Chapter 4
Novel Evaporimeter Design

4.1 Overview

PURPOSE: To describe the design and components of a novel binocular evaporimeter.

MATERIALS: A new instrument was developed to measure the rate of evaporation from the ocular surface. The evaporimeter comprises a pair of swimming goggles, two temperature and relative humidity (RH) sensors, and a sensor microcontroller. A computer program logs and plots the output of each sensor and allows the data to be saved.

CONCLUSIONS: A new unventilated, closed-chamber, binocular evaporimeter was designed that enabled access to the temperature and RH data recorded during a measurement. *In vitro* and *in vivo* testing is needed to investigate the instrument's ability to detect changes in RH over time.

4.2 Introduction

Although the measurement time of the VapoMeter is short [21, 63, 64], one limitation of the instrument (as noted in Chapter 3) is that the output of the system does not allow the user to visualize how the relative humidity (RH) changes over time [60]. The instrument simply presents a final calculated evaporation rate for the measurement time period that does not show how the RH within the goggle chamber varies over that time. In addition, although the total duration of the measurement is displayed by the instrument, the specific time period over which the evaporation rate is calculated is not displayed. The time period is a critical variable that can influence the reported evaporation rate. Following unsuccessful attempts to replicate the correction factors reported when the VapoMeter was modified for ocular use [21], and because a subsequent request to Delfin Technologies to permit access to the raw data was declined, a new evaporimeter was developed.

A decision was made to develop a binocular instrument so that the tear evaporation rate (TER) from both eyes of a participant can be recorded simultaneously. The novel binocular instrument will allow the change in temperature and RH within each goggle to be monitored throughout the measurement period, while also permitting control over the length of the measurement and frequency of the sampling rate.

The aims of this chapter are to:

- Describe the components and design of the binocular evaporimeter;
- Describe the computer equipment associated with the device.

4.3 Materials

4.3.1 Description of the Novel Evaporimeter

The new evaporimeter was assembled by Science Technical Services at the University of Waterloo (Figure 4-1). The instrument consists of a pair of swimming goggles (Zoom X-Fit, Arena Distribution SA, Lugano, Switzerland) with polycarbonate lenses and a silicone seal [401], two Sensirion SHT31-DIS temperature and RH sensors (Sensirion AG, Zurich, Switzerland), and an Arduino Uno R3 microcontroller (Arduino, Scarmagno, Italy). The temperature and RH sensor was fully calibrated by the manufacturer [402]. An electrode system with a polymer will absorb or release water based on the RH within the environment and this change in electrical charge allows the RH to be measured [403].

One sensor was incorporated onto the front surface of each goggle by drilling three small holes. The middle 3 mm diameter hole allows the sensor to monitor the temperature and RH inside the goggle, while the remaining two outer holes are used to attach the sensor to the exterior front surface of the goggle. Air is prevented from leaking around the holes by covering the printed circuit board in sealant.

The sensor is accurate to ±2% RH and ±0.3°C, and the sampling rate can be programmed to record as frequently as 10 times per second or as slow as once every 2 seconds [402]. The horizontal distance separating the two sensors is approximately 64 mm and the combined thickness of the goggle front lens and blue silicone seal, adjacent to the sensor, is 1.5 cm.

Figure 4-1: View of the evaporimeter with a sensor attached to the front surface of each goggle, as seen from the front (top) and back (bottom).

4.3.2 Computer Equipment

The Arduino microcontroller was connected via a USB-cable to a Lifebook Series S710 laptop (Fujitsu, Tokyo, Japan). Custom-built software (Mr. Ehsan Zare-Bidaki, “Goggle”, MLEO, Waterloo, Canada, Figure 4-2) was created to enable the user to visualize the temperature and RH output from each sensor, and to view/record the values. Pressing the control software “Start” button triggers the system to report the temperature and RH output from each sensor and to generate a graph of the temperature and RH. Output boxes at the top of the software management screen display the current temperature and RH for each sensor, while a timer shows the time elapsed since the "Start” button was pressed. A log on the right-hand side of the screen contains the count corresponding to the frequency at which the values are sampled alongside the temperature and RH for each sensor (mounted on the right goggle and left goggle) of the evaporimeter. The red values in the log represent the recordings taken when the evaporimeter was held over the eyes. A log of the temperature and RH is also produced as a graph.

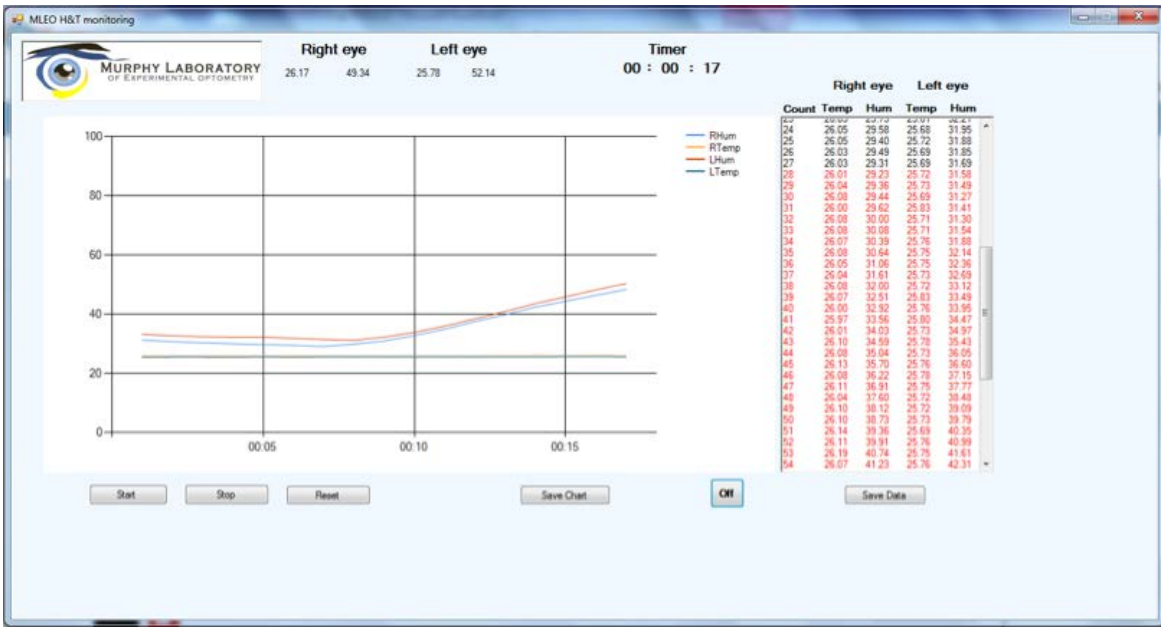

Figure 4-2: Computer program management screenused to monitor the temperature and relative humidity changes in each sensor of the evaporimeter.

The function of each button in the program is summarized below:

- **Start**: Signals the software to begin logging and graphing the temperature and RH of each sensor;
- **Stop**: Signals the software to cease logging and graphing the temperature and RH of each sensor;
- **Reset**: Erases the log and graph to allow a new measurement to be performed;
- **On/Off**: The “on” button changes the values in the log to red, in order to signify the time corresponding to when the evaporimeter is placed over the eyes. The “off” button changes the values to black, to denote when the evaporimeter has been removed from the face;
- **Save Data**: Allows the user to save the log as a word document;
- **Save Chart**: Allows the chart to be saved as a PNG file.

4.4 Discussion

The most recent evaporimeters described in the literature only measure the rate of evaporation from one eye at a time [21, 74, 99], and the most recent binocular evaporimeter seems to have first been described in 1992 [2]. Due to technological advances that have occurred since the 1990's, the new binocular evaporimeter was created to be light-weight and portable. The novel evaporimeter is designed to be comfortable to wear due to a silicone seal around the goggle opening that rests against the skin surrounding the orbit. The seal prevents leakage of air from within the goggle, while also protecting the closed environment within the goggle from external airflow. There is also the option of using the strap on the swimming goggle to allow measurements to be recorded without the need for the patient or practitioner to hold the instrument. However, the strap was eventually removed after additional testing because it could become trapped underneath the side of the goggle and prevent the evaporimeter forming a complete seal around each eye. It was intended that the binocular design would reduce a practitioner's chair time because it would allow both eyes to be measured simultaneously, rather than each eye in turn. The instrument also has the potential to be used in different positions, such as with a patient reclining or in a supine position due to its compact, lightweight design which is not attached to any fixed equipment.

Unfortunately, there are also some potential disadvantages to the choice of an unventilated, binocular closed-chamber device. The lack of ventilation in the instrument may make it difficult to compare measurements recorded at different ambient RHs, with lower RHs resulting in higher TERs [3, 6, 107, 108] and higher RHs suppressing evaporation [3]. A further issue is that moisture may build up in the device because the air in the goggle begins to saturate during the measurement period [60]. This can be minimized by limiting the time that the device is held over the eye and by ensuring the RH within the goggle has returned to the ambient baseline RH prior to starting the next measurement. In addition, the placement of a sensor in front of each eye may make it difficult to control fixation due to size of the circuit board obscuring a fixation target. This could affect the measurement since the palpebral aperture size varies when looking in different directions of gaze, and the rate of evaporation changes as the size of the palpebral aperture changes [151].

Future *in vitro* and *in vivo* work will need to be conducted with the device to investigate the ability of the sensors to detect a change in RH over time. During testing the ambient temperature and RH will need to be controlled as much as possible to prevent changes in TER caused by RH [182] and temperature [115].

4.5 Conclusions

A new instrument has been designed to measure the rate of evaporation. The next step is to conduct *in vitro* testing to investigate whether each sensor can detect a change in RH after exposure to a source of evaporation. If *in vitro* testing is successful, examination of the *in vivo* rate of tear evaporation should be conducted.

Chapter 5

In Vitro Testing of a Novel Evaporimeter

5.1 Overview

PURPOSE: To investigate the ability of a new binocular evaporimeter to measure a change in relative humidity (RH) when exposed to heated distilled water; to investigate how changing the surface area and air volumes within the evaporimeter affects the rate of evaporation; and to compare the change in RH and rate of evaporation between the right and left goggles of the instrument.

METHODS: An *in vitro* study was conducted to investigate the effect of area and air volume within the evaporimeter on evaporation rate and to compare the right goggle to the left goggle. AREA: The effect of area was examined by placing the right side of the evaporimeter over four separate aluminum model eyes with surface areas of 1, 1.5, 2, or 2.5 cm^2. Each model eye was filled with 34°C distilled water and placed in an environmental chamber (40% RH). The first four seconds of RH data obtained after the evaporimeter was placed over the model eye was excluded from the analysis. The evaporation rate was calculated as the change in RH for different time periods lasting 2 to 16 seconds in 2-second intervals. VOLUME: Air volume was decreased from 16 cm^3 to 10 or 13 cm^3 by adding modelling clay to the back surface of the lens or increased to 18.63 cm^3 by placing aluminum inserts between the goggle and model eye. Measurements of the temperature and RH were recorded in 1-second increments (n=7), with the goggle on the model eye, until the RH reached 85%. After removing the goggle, the recording continued until the difference in RH between the right and left goggle had recovered to <1%. Slope was calculated as the change in RH over a series of time periods from 2 to 16 seconds in 2-second intervals. The first four seconds of reported data after the evaporimeter was placed over the model eye were excluded from the analysis. RIGHT VERSUS LEFT GOGGLE: The same model eyes (n=5) were used to compare the evaporation rate measured by each goggle by separately testing the right and left goggle with an evaporimeter volume of 16 cm^3. The change in temperature and RH were recorded at a rate of once per second for 30 seconds. Slope was calculated as the change in RH over time in 5-second increments. Testing revealed a significant difference between the right and left goggle, which could be due to inadvertent removal of the anti-fog coating from the right goggle. The anti-fog coating was removed from the left goggle and the measurements repeated for the left goggle only. SIMULTANEOUS MEASUREMENTS: Simultaneous change in RH was investigated in 5-second intervals by placing each side of the evaporimeter over two 1.5 cm^2 model eyes for 30 seconds. Measurements were performed outside the environmental chamber at a sample rate of 4 times per

second with the right goggle placed over the right model eye (n=5), and then repeated with the goggle reversed so that the right goggle was positioned over the left model eye (n=5).

RESULTS: AREA: The 1 cm^2 model eye had a significantly lower evaporation rate than the 1.5 cm^2 model eye for all test durations between 4 to 16 seconds (all $p<0.005$). The evaporation rate of the 1 cm^2 model eye was significantly lower than the 2 cm^2 model eye when the slope duration was 2 to 16 seconds (all $p<0.001$), and the evaporation rate of the 1 cm^2 model eye was significantly lower than the 2.5 cm^2 model eye when the slope duration was 2 to 16 seconds (all $p<0.001$). In addition, the 1.5 cm^2 model eye had a significantly lower evaporation rate than the 2.5 cm^2 model eye when the slope duration was 2 to 8 seconds (all $p<0.007$). VOLUME: The evaporation rate for the 10 cm^3 volume was significantly lower than for the 13 cm^3 volume over a 2 to 6 seconds slope duration (all $p<0.005$), and lower than the 18.63 cm^3 volume when the slope duration was 6 to 16 seconds (all $p<0.003$). RIGHT VERSUS LEFT GOGGLE: Initial comparison of each side of the evaporimeter found a significantly higher evaporation rate in the right goggle compared to the left goggle for all model eyes and each duration of slope calculation (all $p<0.044$). Repeated testing following removal of the anti-fog coating from the left goggle showed the right goggle had significantly higher evaporation rates with the 1.5, 2, and 2.5 cm^2 model eye at a slope duration of 5 seconds (all $p<0.044$), and for the 1, 2, and 2.5 cm^2 model eye at a slope duration of 15 or 20 seconds (all $p<0.044$), and for all model eyes at a slope duration of 10, 25, or 30 seconds (all $p<0.044$). SIMULTANEOUS MEASUREMENTS: Simultaneous testing of the evaporimeter measured a significantly higher RH in the right goggle compared to the left goggle from 5 to 30 seconds when the right model eye was examined (all $p<0.004$).

CONCLUSIONS: Initial testing of the binocular evaporimeter demonstrated that the sensor in each goggle of the instrument is able to measure a change in RH over time. The evaporimeter:

- Detected a higher evaporation rate in model eyes with a larger surface area;
- Detected a higher evaporation rate for air volumes of 13 or 18.63 cm^3 compared to 10 cm^3;
- Produced a lower apparent evaporation rate in the presence of an anti-fogging coating within the goggle, which interfered with the diffusion of water vapor within the air volume;
- Detected significant differences in RH between the right and left goggles when tested over the same model eye.

5.2 Introduction

Following the design and manufacture of the new evaporimeter that allows full access to the output of the sensors, testing was required to determine whether the instrument has the potential to be used as a clinical tool. *In vitro* testing was performed because it is cost effective and eliminates the variation in tear evaporation that can occur amongst individuals [16, 102, 163].

The first step was to demonstrate that the instrument was able to detect a change in relative humidity (RH) within the goggle upon exposure to a source of evaporation. Since the placement of the sensor in the lens of the swimming goggle is different to previous binocular evaporimeters that either attached the sensor to the goggle via tubing [1, 102] or a cylinder [2], the rate at which the RH will change over time is unknown.

If the evaporimeter is able to measure a change in RH, additional testing would then be necessary to examine whether the instrument has the ability to detect changes in the rate of evaporation from different simulated ocular surface areas [151]. The instrument should also be tested for the effect of different air volumes inside the evaporimeter, in a manner similar to that used for the Eye-VapoMeter and described in Chapter 3, since the apparent rate of evaporation has been found to change as the volume varies [21].

In addition, a comparison of the rate of evaporation of the right and left side of the evaporimeter is needed due to the binocular design of the device in order to examine whether each goggle changes at a similar rate.

The results of this chapter will provide insight as to how the evaporimeter responds to a quickly evaporating source and whether further testing of the instrument is warranted.

The aims of the chapter are to:

- Test whether the RH changes after exposure to heated distilled water;
- Investigate whether the size of a model eye affects the rate of evaporation;
- Evaluate whether the air volume within the instrument affects the rate of evaporation;
- Compare the rate of evaporation in the right and left side of the evaporimeter;
- Compare the RH in the right and left side of the evaporimeter.

5.3 Testing the Right Goggle with Different Sizes of Model Eye and Volumes Within the Evaporimeter

5.3.1 Materials and Methods

A similar methodology was used as described in Section 3.5.1.2. Four aluminum model eyes, each with a different elliptically shaped hole with a surface area of 1, 1.5, 2, or 2.5 cm^2, respectively, were tested (Mr. Daniel Knappert, Greig City Academy, London, UK, Figure 3-14). Modelling clay (Sculpey®^{III}, Polyform Products Co. Inc., Elk Grove Village, Illinois, USA) was placed inside the right goggle to decrease the air volume from 16 cm^3 to either 13 cm^3 or 10 cm^3 (Figure 5-1). The air volume within the evaporimeter was increased to 18.63 cm^3 by placing aluminum inserts between the goggle and the model eye (Figure 3-15).

Figure 5-1: Modelling clay inserted into the right goggle to decrease the volume inside the evaporimeter.

The model eyes, inserts, and distilled water were heated to 34°C in an Isotemp oven (Fisher Scientific, Waltham, Massachusetts, USA). The internal temperature inside the oven was monitored with a thermometer (Model HH21, Omega Engineering, Norwalk, Connecticut, USA). Different volumes of distilled water were pipetted (Reference 100-1000 µl, Eppendorf, Hamburg, Germany; Fisherbrand® 5000DG 100-1000 µl, Fischer Scientific, Waltham, Massachusetts, USA) into each model eye, depending on the design of the model eye (Table 5-1).

Table 5-1: Amount of water pipetted into each model eye

Area of Model Eye (cm^2)	Volume of Water (µl)
1	800
1.5	1050
2	1350
2.5	1750

After the model eye was filled with water, it was transferred from the oven to a controlled environment chamber (Model 5503-11 Package E, electro-tech systems inc., Glenside, Pennsylvania, USA) set to 40% RH. The temperature and RH inside the chamber were monitored with a digital thermo-hygrometer (RH411, Omega, Norwalk, Connecticut, USA). The model eye was placed on a 34°C heating plate (Digital Heatblock, vwr, Radnor, Pennsylvania, USA). The "Goggle" computer software (Mr. Ehsan Zare-Bidaki, MLEO, Waterloo, Canada; Section 4.3.2) was used to record the RH and temperature within each goggle every second. The left goggle acted as a control by reporting the ambient RH and temperature. The right goggle was held over the model eye until an arbitrary value of 85% RH was reached. The goggle was then removed from the model eye and the air within the goggle allowed to ventilate and equilibrate with the environment, during which the recording continued as the RH in the right goggle returned to ambient levels. The recording stopped when the difference in RH between the two goggles was less than 1%. Following each measurement, the water in the model eye was discarded, and the model eye was dried with Kimwipes (KIMTECH SCIENCE, Roswell, GA, USA) and placed back into the oven. Measurements were taken at least an hour apart to allow sufficient time for the model eye to fully dry and re-heat up to 34°C.

A total of seven measurements were taken for each combination of area and volume. The evaporimeter volume was tested from the largest to smallest volume to ensure the modelling clay position was maintained in the same place within the goggle as the volume within the goggle was decreased. However, for each particular volume, the order of testing for each size of model eye was randomized.

5.3.1.1 Statistical Analysis

Statistical analysis was conducted using SPSS (IBM SPSS Statistics for Windows, Version 27.0, IBM Corp., Armonk, NY, USA). Data was tested for normality using a Shapiro-Wilk test with $\alpha = 0.05$. Differences between goggles were analyzed using a Mann-Whitney test. A post-hoc Games-Howell test was used to determine the duration of the initial homogenous subset of RH in the right goggle. Comparison of slope duration was analyzed with a Friedman test and a post-hoc Dunn's

pairwise comparison test with Bonferroni correction. Comparisons between different sizes of model eye or volumes inside the evaporimeter were analyzed with a Kruskal-Wallis test and post-hoc Dunn's pairwise comparison test with Bonferroni correction. A p-value <0.05 was considered statistically significant.

5.3.2 Results

5.3.2.1 Oven Temperature

The mean ± SD temperature inside the oven was 34.04 ± 0.06°C (range: 33.9 to 34.2°C).

5.3.2.2 Environmental Chamber Temperature and Relative Humidity

The mean temperature inside the environmental chamber was 25.96 ± 0.19°C (range: 25 to 26°C) and RH was 40.63 ± 0.74 (range: 39 to 43%).

5.3.2.3 Change in Relative Humidity over Time

A summary of the median (interquartile range, IQR) RH measured over 0 to 30 seconds for different model eyes tested with various evaporimeter volumes is shown in Table 5-2. An example of the change in RH in each goggle appears in Figure 5-2 for a right goggle volume of 18.63 cm^3. The left goggle was used as a control with a 16 cm^3 volume. The median RH recorded with the right goggle was significantly different to the left goggle for all sizes of model eye (all areas: p<0.001) and for all volumes within the evaporimeter (all volumes: p<0.001).

Table 5-2: Summary of median (IQR) relative humidity for different sizes of model eye and evaporimeter volumes over a period of 30 seconds

	Right Goggle Volume (cm^3)							
	10		13		16		18.63	
Area (cm^2)	Right	Left	Right	Left	Right	Left	Right	Left
1	64.39	39.61	67.76	39.75	65.14	39.89	63.93	40.41
	(20.35)	(0.83)	(20.68)	(1.23)	(19.69)	(0.62)	(20.65)	(1.00)
1.5	66.61	39.46	68.53	39.61	66.71	39.96	64.87	39.73
	(20.99)	(1.70)	(21.57)	(0.97)	(21.08)	(0.56)	(23.75)	(2.71)
2	67.35	39.56	69.91	40.25	68.17	40.35	67.25	40.13
	(20.69)	(1.91)	(21.44)	(0.83)	(21.69)	(0.98)	(22.65)	(2.57)
2.5	67.30	39.72	72.12	40.36	70.30	40.22	70.51	40.29
	(20.82)	(0.79)	(20.19)	(1.05)	(22.57)	(1.31)	(22.74)	(1.03)

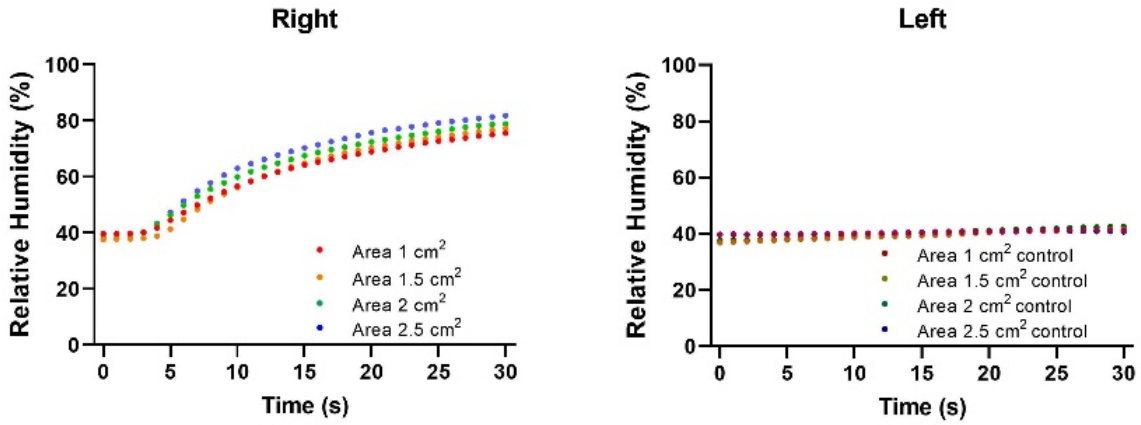

Figure 5-2: Median change in relative humidity over time in each goggle of the evaporimeter when the right evaporimeter volume was 18.63 cm^3. The right goggle was tested over 4 different sizes of model eye, while the left goggle served as a control.

Analysis of homogenous subsets for the right goggle found no significant difference in RH between the median values obtained between the initial 0 to 4 seconds. Therefore, the slope of the change in RH over time was only calculated from 4 seconds onwards.

5.3.2.4 Evaporation Rate and Area of the Model Eye

A summary of the evaporation rate of different sizes of model eye appears in Table 5-3 and Figure 5-3. To investigate the effect of the time period over which the RH measurements were analyzed on the measured evaporation rate, the evaporation rate was calculated over a range of time periods beginning with a 2 second time period, with the next time period increasing by 2 seconds up to a maximum of 16 seconds. A wide range of time intervals were tested because it was not known how well the sensors would be able to detect evaporation or how fast the RH would change over time. Knowledge of how the evaporimeter performs will help identify the optimal time period over which the evaporation rate should be assessed.

All model eyes had a significantly different evaporation rate depending on the length of time the slope was calculated (all $p<0.001$). Each individual duration of slope calculation was also able to detect a significant difference in evaporation rate between the different surface areas of each model eye (all $p<0.001$).

Table 5-3: Summary of median (IQR) evaporation rate for various sizes of model eye measured with the right goggle

	Duration of Slope Calculation (s)								
Area (cm^2)	2	4	6	8	10	12	14	16	p-value
1	2.74	2.60	2.43	2.24	2.08	1.92	1.79	1.67	**<0.001**
	(0.35)	(0.20)	(0.18)	(0.20)	(0.18)	(0.17)	(0.14)	(0.13)	
1.5	3.01	2.87	2.65	2.48	2.30	2.13	1.97	1.83	**<0.001**
	(0.28)	(0.32)	(0.31)	(0.26)	(0.24)	(0.24)	(0.24)	(0.22)	
2	3.26	3.04	2.81	2.55	2.33	2.15	1.98	1.85	**<0.001**
	(0.24)	(0.34)	(0.31)	(0.28)	(0.24)	(0.22)	(0.23)	(0.22)	
2.5	3.42	3.26	2.93	2.67	2.44	2.24	2.06	1.91	**<0.001**
	(0.44)	(0.43)	(0.37)	(0.34)	(0.32)	(0.29)	(0.26)	(0.26)	
p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	

Bold indicates statistical significance.

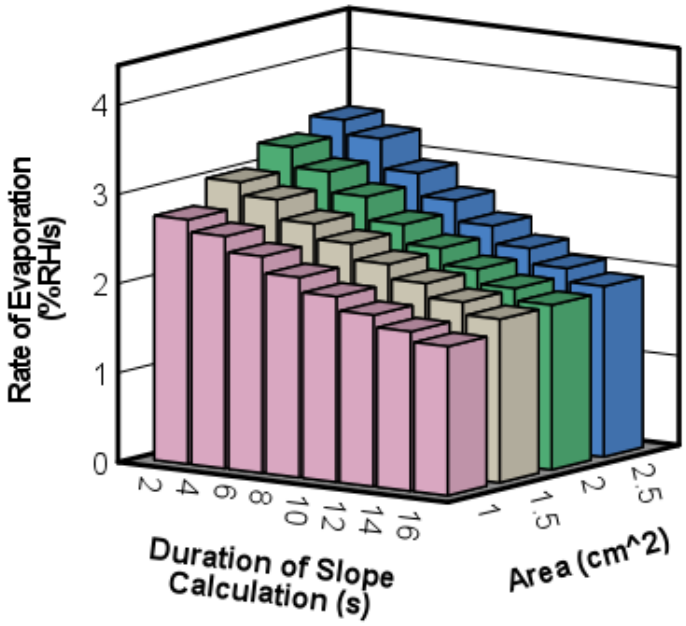

Figure 5-3: Relationship between median rate of evaporation, duration of slope, and surface area of the model eye. All measurements were recorded with the right goggle.

5.3.2.4.1 Difference in Evaporation Rate Between Slope Duration

A summary of pairwise comparisons for evaporation rate calculated over various time intervals for each size of model eye appears in Table 5-4. For all sizes of model eye, there were significant differences between the slope calculated over 2 or 4 seconds compared to a slope duration of 10 seconds (all $p<0.001$), the slope calculated over 2 to 6 seconds compared to 12 seconds (all $p<0.001$), the slope

calculated over 2 to 8 seconds compared to 14 s (all $p<0.001$), and the slope calculated over 2 to 10 seconds compared to 16 seconds (all $p<0.001$). Additionally, there were significant differences between the evaporation rate calculated over 2 versus 8 seconds for the 1, 2, and 2.5 cm^2 model eyes (all $p<0.001$), the evaporation rate calculated over 6 versus 10 seconds for the 1.5 and 2.5 cm^2 model eyes (all $p<0.0017$), the evaporation rate calculated over 8 versus 12 seconds for the 1 and 1.5 cm^2 model eyes (all $p<0.0017$), the evaporation rate calculated at 10 versus 14 seconds for the 1, 1.5, and 2 cm^2 model eyes (all $p<0.0018$), and for the slope calculated over 12 versus 16 seconds for the 2 cm^2 model eye ($p=0.0016$).

Table 5-4: Summary of statistical significance from pairwise comparisons of slope duration measured with the right goggle for different sizes of model eye

	Area of Model Eye (cm^2)			
Duration of Slope Calculation	1	1.5	2	2.5
2 s versus 4 s	0.643	0.870	0.623	0.585
2 s versus 6 s	0.038	0.326	0.046	0.050
2 s versus 8 s	**<0.001**	0.005	**<0.001**	**<0.001**
2 s versus 10 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
2 s versus 12 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
2 s versus 14 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
2 s versus 16 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
4 s versus 6 s	0.108	0.252	0.134	0.156
4 s versus 8 s	0.002	0.003	0.004	0.003
4 s versus 10 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
4 s versus 12 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
4 s versus 14 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
4 s versus 16 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
6 s versus 8 s	0.156	0.072	0.164	0.114
6 s versus 10 s	0.002	**<0.001**	0.004	**0.0016**
6 s versus 12 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
6 s versus 14 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
6 s versus 16 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
8 s versus 10 s	0.108	0.114	0.141	0.114
8 s versus 12 s	**0.0016**	**0.001**	0.002	0.002
8 s versus 14 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
8 s versus 16 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
10 s versus 12 s	0.120	0.102	0.120	0.127
10 s versus 14 s	**0.0017**	**0.0016**	**0.0017**	0.002
10 s versus 16 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
12 s versus 14 s	0.114	0.127	0.114	0.127
12 s versus 16 s	0.002	0.002	**0.0016**	0.002
14 s versus 16 s	0.127	0.127	0.114	0.114

Bold indicates statistical significance following a Bonferroni correction for multiple comparisons.

5.3.2.4.2 Comparison of Evaporation Rate Between Surface Areas

A summary of pairwise comparisons for the evaporation rate between different sizes of model eyes appears in Table 5-5. There was a significant difference in the evaporation rate at all different intervals of slope calculation between the 1 and 2 cm^2 model eyes (all $p<0.001$), and between the 1 and 2.5 cm^2 model eyes (all $p<0.001$). In addition, there were significant differences between the 1 and 1.5 cm^2 model eyes when the slope duration was between 4 to 16 seconds (all $p<0.005$), and between the 1.5 and 2.5 cm^2 model eyes when the slope was calculated between 2 to 8 seconds (all $p<0.007$).

Table 5-5: Summary of statistical significance from pairwise comparisons of evaporation rate measured with the right goggle for different sizes of model eye

	Duration of Slope Calculation (s)							
Area (cm^2)	2	4	6	8	10	12	14	16
1 versus 1.5	0.040	**0.004**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
1 versus 2	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
1 versus 2.5	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
1.5 versus 2	0.016	0.059	0.149	0.281	0.270	0.328	0.421	0.426
1.5 versus 2.5	**<0.001**	**<0.001**	**0.001**	**0.006**	0.015	0.040	0.076	0.098
2.0 versus 2.5	0.049	0.041	0.074	0.091	0.182	0.284	0.331	0.390

Bold indicates statistical significance following a Bonferroni correction for multiple comparisons.

5.3.2.5 Evaporation Rate and Volume Within the Evaporimeter

A summary of the evaporation rate of different volumes within the evaporimeter appears in Table 5-6 and Figure 5-4. The slope was calculated over a range of intervals because the rate of evaporation of the new evaporimeter had not been previously investigated.

The evaporation rate significantly changed for all of the different volumes inside the evaporimeter depending on the duration of the slope calculation (all $p<0.001$). Each separate slope calculated over 2 to 16 seconds was also able to detect a significant difference in evaporation rate between different volumes within the evaporimeter (all $p<0.015$).

Table 5-6: Summary of median (IQR) evaporation rate for different volumes within the evaporimeter measured with the right goggle

	Duration of Slope Calculation (s)								
Volume (cm^3)	2	4	6	8	10	12	14	16	p-value
10	3.06	2.84	2.60	2.38	2.23	2.04	1.88	1.74	**<0.001**
	(0.68)	(0.44)	(0.36)	(0.24)	(0.21)	(0.21)	(0.18)	(0.16)	
13	3.31	3.06	2.79	2.54	2.34	2.14	1.98	1.85	**<0.001**
	(0.27)	(0.31)	(0.31)	(0.28)	(0.25)	(0.22)	(0.20)	(0.18)	
16	3.04	2.84	2.60	2.40	2.21	2.03	1.87	1.75	**<0.001**
	(0.40)	(0.44)	(0.36)	(0.30)	(0.27)	(0.25)	(0.23)	(0.22)	
18.63	2.94	3.01	2.84	2.63	2.42	2.25	2.10	1.96	**<0.001**
	(0.85)	(0.66)	(0.58)	(0.44)	(0.33)	(0.31)	(0.28)	(0.26)	
p-value	**0.014**	**0.008**	**0.004**	**0.003**	**0.003**	**0.003**	**0.002**	**0.001**	

Bold indicates statistical significance.

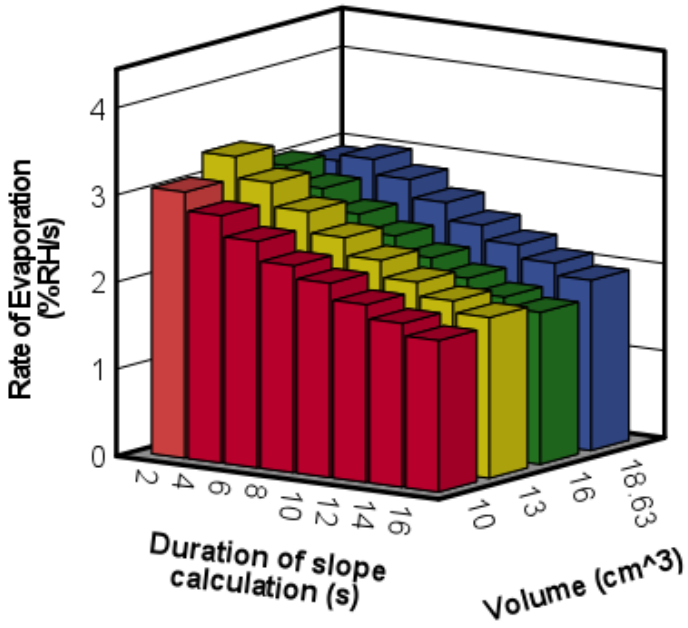

Figure 5-4: Relationship between median rate of evaporation, duration of slope, and volume within the evaporimeter. All measurements were recorded with the right goggle.

5.3.2.5.1 Difference in Evaporation Rate Between Slope Duration

A summary of pairwise comparisons of evaporation rate calculated over different time intervals for each volume inside the evaporimeter appears in Table 5-7. For all volumes, there were significant differences between the slope calculated over 2 or 4 seconds compared to a slope duration of 10 seconds (all $p<0.001$), the slope calculated over 2 to 6 seconds compared to 12 seconds (all $p<0.001$), the slope

calculated over 2 to 8 seconds compared to 14 seconds (all $p<0.001$), and the slope calculated over 2 to 10 seconds compared to 16 seconds (all $p<0.001$). Additionally, there were significant differences between the evaporation rate calculated over 2 versus 8 seconds for the 13 and 16 cm^3 volumes (all $p<0.001$), the evaporation rate calculated over 4 versus 8 seconds for the 18.63 cm^3 volume ($p<0.001$), the evaporation rate calculated over 6 versus 10 seconds for the 18.63 cm^3 volume ($p<0.001$), and the evaporation rate calculated at 12 versus 16 seconds for the 10 cm^3 volume ($p=0.001$).

Table 5-7: Summary of statistical significance from pairwise comparisons of slope duration measured with the right goggle for different sizes of model eye

	Volume Within the Evaporimeter (cm^3)			
Duration of Slope Calculation	10	13	16	18.63
2 s versus 4 s	0.785	0.156	0.478	0.287
2 s versus 6 s	0.173	0.003	0.036	0.585
2 s versus 8 s	0.007	**<0.001**	**<0.001**	0.022
2 s versus 10 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
2 s versus 12 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
2 s versus 14 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
2 s versus 16 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
4 s versus 6 s	0.275	0.114	0.164	0.108
4 s versus 8 s	0.015	0.002	0.003	**<0.001**
4 s versus 10 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
4 s versus 12 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
4 s versus 14 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
4 s versus 16 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
6 s versus 8 s	0.181	0.127	0.114	0.081
6 s versus 10 s	0.004	0.002	0.002	**<0.001**
6 s versus 12 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
6 s versus 14 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
6 s versus 16 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
8 s versus 10 s	0.114	0.127	0.114	0.120
8 s versus 12 s	0.002	0.002	0.002	0.002
8 s versus 14 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
8 s versus 16 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
10 s versus 12 s	0.120	0.127	0.114	0.108
10 s versus 14 s	0.001	0.002	0.002	0.002
10 s versus 16 s	**<0.001**	**<0.001**	**<0.001**	**<0.001**
12 s versus 14 s	0.102	0.127	0.127	0.127
12 s versus 16 s	**0.001**	0.002	0.002	0.002
14 s versus 16 s	0.114	0.127	0.127	0.114

Bold indicates statistical significance following a Bonferroni correction for multiple comparisons.

5.3.2.5.2 Comparison of Evaporation Rate Between Evaporimeter Volumes

A summary of pairwise comparisons of evaporation rate between different volumes within the evaporimeter appears in Table 5-8. There was a significant difference in the evaporation rate calculated

over the 2 to 6 seconds slope durations between the 10 and 13 cm^3 volumes (all $p<0.005$) and over the 6 to 16 seconds slope durations between the 10 and 18.63 cm^3 volumes (all $p<0.003$).

Table 5-8: Summary of statistical significance from pairwise comparisons of evaporation rate measured with the right goggle for different volumes inside the evaporimeter

	Duration of Slope Calculation (s)							
Volume (cm^3)	2	4	6	8	10	12	14	16
10 versus 13	**0.002**	**0.003**	**0.004**	0.009	0.017	0.022	0.024	0.019
10 versus 16	0.232	0.344	0.340	0.386	0.448	0.379	0.319	0.271
10 versus 18.63	0.340	0.009	**0.002**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
13 versus 16	0.049	0.044	0.059	0.083	0.103	0.160	0.210	0.212
13 versus 18.63	0.027	0.736	0.834	0.488	0.351	0.238	0.178	0.153
16 versus 18.63	0.810	0.094	0.036	0.015	0.010	0.010	0.009	0.007

Bold indicates statistical significance following a Bonferroni correction for multiple comparisons.

5.3.3 Discussion

Preliminary work demonstrated that the RH in the right goggle increased after placement over a source of evaporation, while the left goggle had significantly lower rates of RH when exposed to ambient conditions. A previously developed binocular evaporimeter was also able to utilize the instrument to detect a difference between each side of the instrument, with higher evaporation rates recorded in eyes wearing a contact lens compared to the fellow eye without a contact lens [16, 102].

5.3.3.1 Duration of Slope Calculation

The change in RH showed an initial 4 seconds period where the slope did not significantly change, and after which the RH began to increase. Nuutinen et al. [64] described a similar pattern when testing the VapoMeter for dermatological use with a quickly evaporating semipermeable membrane and petri dish. They excluded an initial stable period of RH that lasted approximately 2 seconds from their calculation of the slope of the skin evaporation rate. This represents the time for the water vapor evaporating from the surface to diffuse within the air volume not yet adjacent to the sensor. Once the RH distribution within the goggle begins to equalize, any additional increases in RH are detectable by the sensor.

A range of evaporimetry measurement times have been reported, ranging from less than 10 seconds [21] to 5 minutes [99]. Analysis of different model eye surface areas found that calculating the slope over a shorter duration of time yields a higher evaporation rate because the change in RH slows with prolonged periods of measurement as the air within the goggle saturates.

Significant differences in evaporation rate were detected between the smallest model eye (1 cm^2) compared to the other three model eyes, and between the 1.5 and the 2.5 cm^2 model eyes, when the slope was calculated over 4 to 8 seconds. Significant differences in evaporation rate were also found between the smallest volume (10 cm^3) and 13 cm^3 volume over a slope duration of 2 to 6 seconds, and between the smallest and largest volumes (18.63 cm^3), when the slope was calculated over 6 to 16 seconds. Combining the results of the area and volume analyses suggests that a slope duration of 6 seconds is the optimal time period to maximize the ability of the instrument to differentiate between different sizes of model eye and various volumes within the evaporimeter. Additional work will need to be done to determine if this is also observed *in vivo.*

5.3.3.2 Area

The smallest model eye (1 cm^2) had significantly lower evaporation rates than the other model eyes, as did the 1.5 cm^2 model eye in comparison to the largest model eye (2.5 cm^2). Tsubota and Nakamori [151] also measured faster rates of tear evaporation with larger ocular surfaces, and Rolando and Refojo [1] reported a greater change in RH over time as the palpebral aperture increased. However, Tomlinson et al. [80] were unable to find a correlation between tear evaporation and the size of the ocular surface, which could have been due to their small sample size (n=10).

Since the size of the ocular surface seems to affect the evaporation rate, researchers have included an assessment of the ocular surface area using photographs [21, 49, 99, 163], by measuring the palpebral aperture and photographing the eye [1, 101], or by estimating the size from the vertical palpebral aperture [2, 3]. Future evaporimetry work should include an assessment of the ocular surface area in order to evaluate its effect on the rate of tear evaporation.

5.3.3.3 Volume

Although there was no significant difference in evaporation rate between the 16 cm^3 goggle volume and the other volumes, the rate of evaporation for the 10 cm^3 volume was significantly lower than for the 13 cm^3 volume when the slope was calculated over 2 to 6 seconds. In comparison, no clear relationship was observed between comparisons of the 10 and 13 cm^3 volumes obtained with the same model eyes and the Eye-VapoMeter in Chapter 3 (1 cm^2: 241.8 versus 262.5 g/m^2h, 1.5 cm^2: 285.8 versus 286.0 g/m^2h, 2 cm^2: 305.6 versus 310.1 g/m^2h, 2.5 cm^2: 366.2 versus 378.7 g/m^2h). Testing of the new evaporimeter also found that the evaporation rate for the 10 cm^3 volume was significantly lower than for the 18.63 cm^3 volume over a slope duration of 8 to 16 seconds. In contrast, comparisons of the values obtained with the Eye-VapoMeter in Chapter 3 found higher rates of evaporation when

the evaporimeter volume was 10 cm^3 compared to 18.63 cm^3 (1 cm^2: 241.8 versus 206.7 g/m^2h, 1.5 cm^2: 285.8 versus 202.3 g/m^2h, 2 cm^2: 305.6 versus 296.9 g/m^2h, 2.5 cm^2: 366.2 versus 330.3 g/m^2h). Rohit et al. [21] also measured higher rates of evaporation as the internal volume of the evaporimeter decreased from 13 to 5 cm^3 during the validation of the Eye-VapoMeter. However, the potentially smaller goggle size could account for some the discrepancies between the new evaporimeter and the original one used during validation work. As reported in Chapter 3, testing of the commercially available Eye-VapoMeter showed non-linear relationships between the correction factors and air volume within the evaporimeter compared to the negative linear relationship described by Rohit et al. [21], which may be due to differences in how water vapor dispersed inside a larger goggle versus a smaller one.

Differences between the evaporation rate of the two instruments may be due to variations in the design of the instrument, with most other closed-chamber evaporimeters (including the Eye-VapoMeter) placing the sensor in a hollow cylinder behind the goggle [1, 2, 21, 99, 101]. This will alter how the water vapor diffuses within the air volume of the instrument. The new binocular evaporimeter has the sensor located much closer to the ocular surface and without any hindering effects on vapor diffusion from the hollow cylinder.

In addition, as demonstrated in this study, the measurement time period will affect the reported evaporation rate, as will the methodology used to calculate the slope. The specific time period over which the Eye-VapoMeter analyzes the RH is not known, instead the RH is analyzed until there is a >5% deviation from the calculated linear slope of RH, with a least squares method used to determine the slope [64].

As a preliminary investigation into the measurement capabilities of the new evaporimeter has been conducted, further work is needed to assess whether the tear evaporation rate is significantly affected by the air volume within the device.

5.4 Comparison of the Right and Left Goggle with Different Areas of Model Eye

5.4.1 Materials and Methods

The same equipment listed in Section 5.3.1 was used to separately test each goggle. Briefly, the same four model eyes were tested with a single evaporimeter volume of 16 cm^3. The right and left goggle were tested in a random order on alternate days, while the order of testing of each model eye was also randomized. After filling the model eye with warm water in the oven, the model eye was transferred to

the environmental chamber. The temperature and RH within each goggle were recorded every second for 30 seconds.

Each model eye was measured five times with each goggle. A 15-minute break was taken between each model eye measurement to allow the models eyes to re-heat to eye temperature.

5.4.1.1 Statistical Analysis

Statistical analysis was conducted using SPSS version 27.0. Data was tested for normality using a Shapiro-Wilk test with $\alpha = 0.05$. Differences between goggles were analyzed using a Wilcoxon signed-rank test. The slope was calculated in 5-second increments over the duration of the measurement. Comparison of the duration of the slope or the RH at different time points were analyzed with a Friedman test and a post-hoc Dunn's pairwise comparison test with Bonferroni correction. Comparisons between different sizes of model eye were analyzed with a Kruskal-Wallis test and post-hoc Dunn's pairwise comparison test with Bonferroni correction. A p-value <0.05 was considered statistically significant.

5.4.2 Results

5.4.2.1 Environmental Chamber Temperature and Relative Humidity

The mean ± SD temperature inside the environmental chamber was 25.12 ± 0.76°C (range: 24 to 26°C) and the RH was 41.03 ± 0.53% (range: 40 to 42%).

5.4.2.2 Comparison of Evaporation Rate with the Right and Left Goggle and Area of Model Eye

A summary of the evaporation rate of different sizes of model eye measured with the right and left goggle appears in Figure 5-5, Table 5-9, and Figure 5-6.

Each model eye had a significantly different evaporation rate depending on the duration of the slope calculation (all $p<0.001$). There was also a significant difference in the evaporation rate of the right goggle versus the left goggle at each duration of slope calculation (all $p<0.044$).

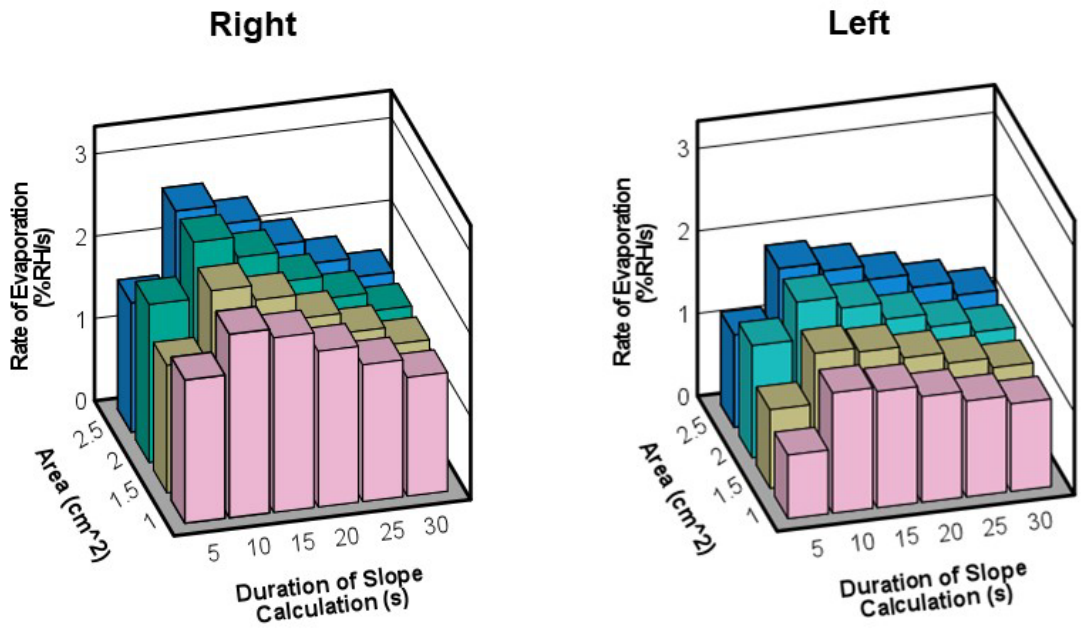

Figure 5-5: Three-dimensional plot of the median rate of evaporation, duration of slope calculation, and ocular surface area from initial testing of the right and left goggle.

Table 5-9: Summary of median (IQR) evaporation rate for different sizes of model eye measured with the right and left goggle (initial testing)

Duration of Slope Calculation (s)	Area 1 cm^2			Area 1.5 cm^2			Area 2 cm^2			Area 2.5 cm^2		
	Right Goggle	Left Goggle	p-value	Right Goggle	Left Goggle	p-value	Right Goggle	Left Goggle	p-value	Right Goggle	Left Goggle	p-value
5	1.74 (0.60)	0.77 (0.21)	**0.043**	1.55 (0.74)	0.95 (0.36)	**0.043**	1.92 (0.22)	1.36 (0.42)	**0.043**	1.57 (0.59)	1.12 (0.17)	**0.043**
10	2.23 (0.23)	1.46 (0.16)	**0.043**	2.39 (0.39)	1.57 (0.28)	**0.043**	2.61 (0.07)	1.82 (0.24)	**0.043**	2.62 (0.26)	1.86 (0.15)	**0.043**
15	2.12 (0.12)	1.41 (0.11)	**0.043**	2.21 (0.24)	1.52 (0.13)	**0.043**	2.36 (0.08)	1.68 (0.06)	**0.043**	2.39 (0.14)	1.76 (0.11)	**0.043**
20	1.89 (0.06)	1.28 (0.09)	**0.043**	1.92 (0.16)	1.38 (0.06)	**0.043**	2.00 (0.07)	1.48 (0.06)	**0.043**	2.06 (0.09)	1.59 (0.08)	**0.043**
25	1.66 (0.04)	1.16 (0.08)	**0.043**	1.68 (0.09)	1.25 (0.05)	**0.043**	1.73 (0.06)	1.32 (0.04)	**0.043**	1.78 (0.07)	1.43 (0.07)	**0.039**
30	1.44 (0.04)	1.06 (0.09)	**0.043**	1.47 (0.06)	1.13 (0.05)	**0.042**	1.51 (0.06)	1.19 (0.04)	**0.041**	1.55 (0.06)	1.27 (0.06)	**0.042**
p-value	**<0.001**	**<0.001**		**<0.001**	**<0.001**		**<0.001**	**<0.001**		**<0.001**	**<0.001**	

Bold indicates statistical significance following a Bonferroni correction for multiple comparisons.

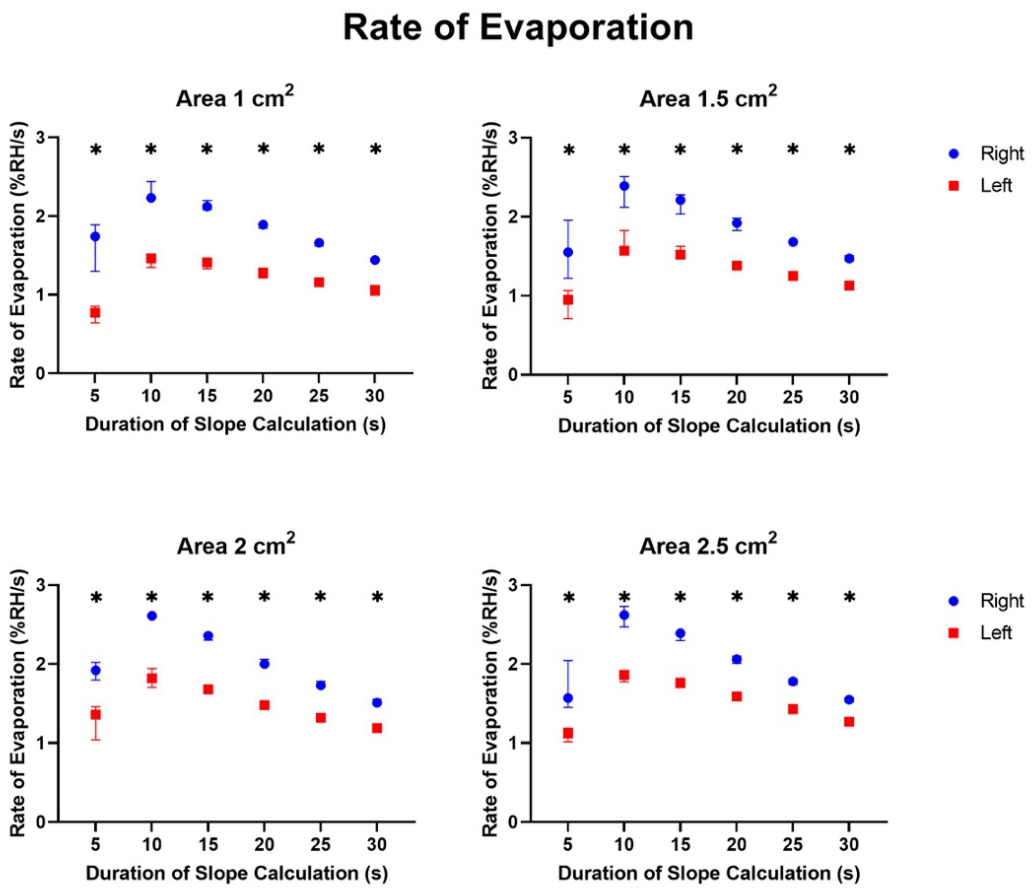

Figure 5-6: Change in tear evaporation of the right versus left goggle (initial testing). Error bars indicate IQR. Asterisks indicate a significant difference between the right and left goggle ($p<0.05$).

A summary of pairwise comparisons of evaporation rate calculated over the different time intervals for each size of model eye appears in Table 5-10. Post-hoc testing found a significant difference between the evaporation rate calculated at 10 seconds versus 30 seconds for all four sizes of model eye. There was also a significant difference between 5 seconds and 10 seconds for all sizes of the model eye measured with the left goggle, and for the 1, 1.5, and 2.5 cm^2 model eyes measured with the right goggle. The right goggle also had significant differences in the evaporation rate calculated at 10 seconds versus 25 seconds for the 1 and 2 cm^2 model eyes, and between 15 seconds and 30 seconds for all four model eyes. In addition, there were differences between the rate of evaporation at 5 seconds versus 15 seconds for all sizes of model eye measured with the left goggle.

Table 5-10: Summary of statistical significance from pairwise comparisons of slope duration for different sizes of model eye measured with each goggle (initial testing)

	Area 1 cm^2		Area 1.5 cm^2		Area 2 cm^2		Area 2.5 cm^2	
Duration of Slope Calculation	Right Goggle	Left Goggle	Right Goggle	Left Goggle	Right Goggle	Left Goggle	Right Goggle	Left Goggle
5 s versus 10 s	**0.003**	**<0.001**	**0.001**	**<0.001**	0.009	**<0.001**	**0.002**	**<0.001**
5 s versus 15 s	0.035	**<0.001**	0.018	**<0.001**	0.076	**<0.001**	0.028	**<0.001**
5 s versus 20 s	0.272	0.011	0.176	0.018	0.398	0.018	0.237	0.011
5 s versus 25 s	0.933	0.091	0.735	0.128	0.554	0.128	0.866	0.091
5 s versus 30 s	0.310	0.398	0.499	0.612	0.108	0.612	0.310	0.398
10 s versus 15 s	0.398	0.612	0.398	0.499	0.398	0.499	0.398	0.398
10 s versus 20 s	0.063	0.128	0.063	0.108	0.076	0.108	0.063	0.091
10 s versus 25 s	**0.002**	0.018	0.004	0.014	**0.001**	0.014	0.004	0.011
10 s versus 30 s	**<0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
15 s versus 20 s	0.310	0.310	0.310	0.353	0.353	0.353	0.310	0.398
15 s versus 25 s	0.028	0.063	0.043	0.076	0.018	0.076	0.043	0.091
15 s versus 30 s	**0.002**	0.007	**0.002**	0.005	**<0.001**	0.005	**<0.001**	0.011
20 s versus 25 s	0.237	0.398	0.310	0.398	0.151	0.398	0.310	0.398
20 s versus 30 s	0.035	0.091	0.043	0.063	0.014	0.063	0.028	0.091
25 s versus 30 s	0.353	0.398	0.310	0.310	0.310	0.310	0.237	0.398

Bold indicates statistical significance following a Bonferroni correction for multiple comparisons.

5.4.3 Discussion

As seen in Section 5.4.2.2, a slower rate of evaporation was observed over the first 5-seconds due to a period of time where the RH increased at a slower rate. Future work will need to consider whether to exclude this period from the calculation of the slope due to anticipated differences between the evaporation rate of water compared to a human tear film with an intact lipid layer.

Comparison of the two goggles found the right goggle measured significantly higher rates of evaporation than the left goggle. Examination of the right lens of the instrument showed a clear lens, while the entire back surface of the left lens was crazed. This is assumed to be due to the presence of a temporary [404] anti-fog coating [405] that had begun to deteriorate. The reason for the deterioration in the anti-fog coating is unknown because modelling clay was not added to the left goggle. However, anti-fog coatings degenerate over time [405, 406] and the swimming goggle may have been old.

Since the anti-fog coating of the right goggle may have inadvertently been taken off during the testing of different volumes inside the evaporimeter, the coating was removed from the left goggle using a combination of alcohol wipes, a cotton-tipped applicator and isopropyl alcohol [406], and a layer of modelling clay.

Additional testing of the left goggle was needed to determine whether the evaporation rate of the two goggles were more similar following removal of the anti-fog coating. The results appear in the following section.

5.5 Repeated Testing of the Left Goggle

5.5.1 Materials and Methods

The procedure in Section 5.4.1 was repeated on the left goggle and the results were compared to the original results obtained from the right goggle. Each model eye was tested once a day, with a 15-minute interval between each measurement, for a total of 5 measurements of each model eye.

5.5.1.1 Statistical Analysis

See Section 5.4.1.1. Differences within the left goggle were analyzed using a Wilcoxon signed-rank test.

5.5.2 Results

5.5.2.1 Environmental Chamber Temperature and Relative Humidity

The mean ± SD temperature inside the environmental chamber was 25.25 ± 0.44°C (range: 25 to 26°C) and the RH was 41.25 ± 0.64% (range: 40 to 43%).

5.5.2.2 Comparison of Evaporation Rate with the Right and Left Goggle and Area of Model Eye

A summary of the evaporation rate of different sizes of model eye measured with the right and left goggle appears Table 5-11, Figure 5-7, and Figure 5-8.

Each model eye had a significantly different evaporation rate depending on the duration of the slope calculation (all $p<0.001$). There was also a significant difference in the evaporation rate of all sizes of model eye when measured with the right goggle versus the left goggle when the duration of slope calculation was 10 seconds, 25 seconds, or 30 seconds (all $p<0.044$). There were also significant differences in the rate of evaporation between the right and left goggle when the slope duration was 5 seconds for the 1.5, 2, and 2.5 cm^2 model eyes (all $p<0.044$). The evaporation rate was also significantly different between the right and left goggle when the slope was calculated over 15 seconds or 20 seconds when the area of the model eye was 1, 2, and 2.5 cm^2 (all $p<0.044$).

Table 5-11: Summary of median (IQR) evaporation rate for different sizes of model eye measured with the right and left goggle (repeated testing)

Duration of Slope Calculation (s)	Area 1 cm^2			Area 1.5 cm^2			Area 2 cm^2			Area 2.5 cm^2		
	Right Goggle	Left Goggle	p-value	Right Goggle	Left Goggle	p-value	Right Goggle	Left Goggle	p-value	Right Goggle	Left Goggle	p-value
5	1.74 (0.60)	0.90 (0.27)	0.080	1.55 (0.74)	1.03 (0.32)	**0.043**	1.92 (0.22)	1.27 (0.44)	**0.043**	1.57 (0.59)	0.96 (0.32)	**0.043**
10	2.23 (0.23)	1.72 (0.18)	**0.043**	2.39 (0.39)	1.85 (0.18)	**0.043**	2.61 (0.07)	2.04 (0.22)	**0.043**	2.62 (0.26)	2.08 (0.13)	**0.042**
15	2.12 (0.12)	1.79 (0.10)	**0.041**	2.21 (0.24)	1.89 (0.14)	0.080	2.36 (0.08)	1.97 (0.13)	**0.043**	2.39 (0.14)	2.08 (0.07)	**0.043**
20	1.89 (0.06)	1.67 (0.08)	**0.043**	1.92 (0.16)	1.73 (0.11)	0.080	2.00 (0.07)	1.77 (0.08)	**0.042**	2.06 (0.09)	1.85 (0.06)	**0.043**
25	1.66 (0.04)	1.51 (0.06)	**0.043**	1.68 (0.09)	1.55 (0.08)	**0.042**	1.73 (0.06)	1.57 (0.06)	**0.043**	1.78 (0.07)	1.62 (0.04)	**0.042**
30	1.44 (0.04)	1.36 (0.05)	**0.043**	1.47 (0.06)	1.38 (0.07)	**0.043**	1.51 (0.06)	1.39 (0.06)	**0.043**	1.55 (0.06)	1.43 (0.03)	**0.043**
p-value	**<0.001**	**<0.001**		**<0.001**	**<0.001**		**<0.001**	**<0.001**		**<0.001**	**<0.001**	

Bold indicates statistical significance.

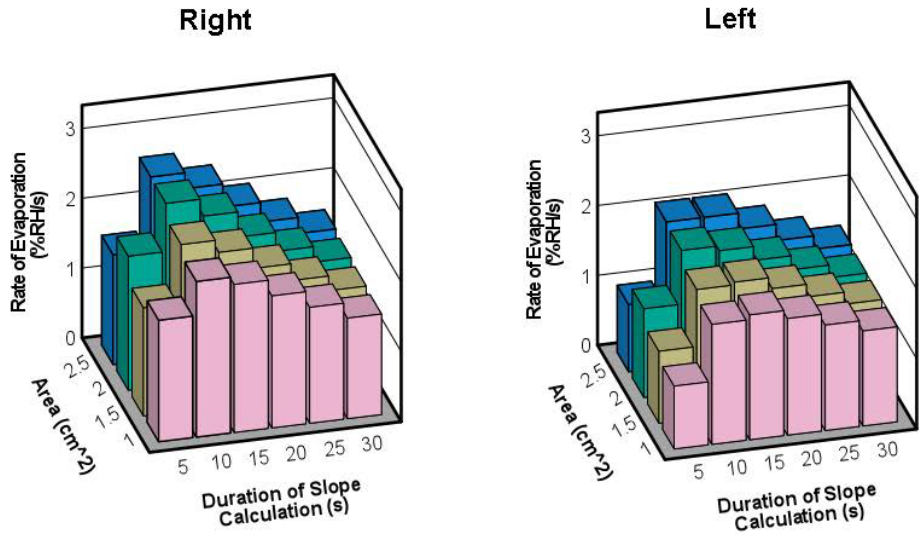

Figure 5-7: Three-dimensional plot of rate of evaporation, duration of slope calculation, and ocular surface area of the right and left goggle after removing the anti-fog coating from the left goggle.

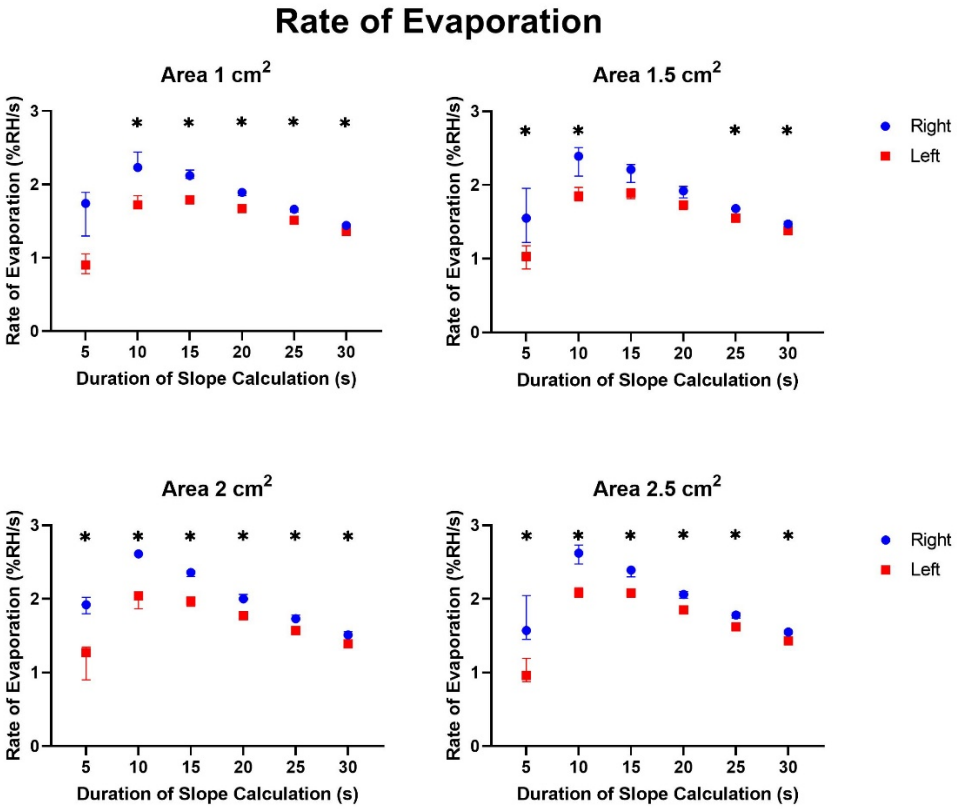

Figure 5-8: Change in evaporation of the right versus left goggle (repeated testing of the left goggle). Error bars indicate IQR. Asterisks indicate a significant difference between the right and left goggle ($p<0.05$).

A summary of pairwise comparisons of evaporation rate calculated over different time intervals for each model eye appears in Table 5-12. Post-hoc testing found a significant difference between 5 seconds and 10 seconds for the 1, 1.5, and 2.5 cm^2 model eyes measured with the right goggle (all $p<0.004$), and for all sizes of the model eye measured with the left goggle (all $p<0.001$). The right goggle also had significant differences in the evaporation rate calculated at 10 seconds versus 25 seconds for the 1 and 2 cm^2 model eyes (all $p<0.003$), and between 10 seconds versus 30 seconds (all $p<0.001$) and 15 seconds versus 30 seconds for all four model eyes (all $p<0.003$). In addition, there were differences between the rate of evaporation at 5 seconds versus 15 seconds for all sizes of model eye measured with the left goggle (all $p<0.001$), between 10 seconds versus 30 seconds for the 2 and 2.5 cm^2 model eyes (all $p<0.003$), and between 15 seconds and 30 seconds for the 1 and 1.5 cm^2 model eyes (all $p=0.001$).

Table 5-12: Summary of statistical significance from pairwise comparisons of slope duration for different sizes of model eye (repeated testing)

	Area 1 cm^2		**Area 1.5 cm^2**		**Area 2 cm^2**		**Area 2.5 cm^2**	
Duration of Slope Calculation	Right Goggle	Left Goggle	Right Goggle	Left Goggle	Right Goggle	Left Goggle	Right Goggle	Left Goggle
5 s versus 10 s	**0.003**	**<0.001**	**0.001**	**<0.001**	0.009	**<0.001**	**0.002**	**<0.001**
5 s versus 15 s	0.035	**<0.001**	0.018	**<0.001**	0.076	**<0.001**	0.028	**<0.001**
5 s versus 20 s	0.272	0.011	0.176	0.011	0.398	0.011	0.237	0.011
5 s versus 25 s	0.933	0.091	0.735	0.091	0.554	0.091	0.866	0.091
5 s versus 30 s	0.310	0.398	0.499	0.398	0.108	0.398	0.310	0.398
10 s versus 15 s	0.398	0.612	0.398	0.612	0.398	0.866	0.398	0.735
10 s versus 20 s	0.063	0.310	0.063	0.310	0.076	0.176	0.063	0.151
10 s versus 25 s	**0.002**	0.063	0.004	0.063	**0.001**	0.028	0.004	0.022
10 s versus 30 s	**<0.001**	0.007	**<0.001**	0.007	**<0.001**	**0.002**	**<0.001**	**0.002**
15 s versus 20 s	0.310	0.128	0.310	0.128	0.353	0.237	0.310	0.272
15 s versus 25 s	0.028	0.063	0.043	0.018	0.018	0.043	0.043	0.052
15 s versus 30 s	**0.002**	**0.001**	**0.002**	**0.001**	**<0.001**	0.004	**<0.001**	0.005
20 s versus 25 s	0.237	0.398	0.310	0.398	0.151	0.398	0.310	0.398
20 s versus 30 s	0.035	0.091	0.043	0.091	0.014	0.091	0.028	0.091
25 s versus 30 s	0.353	0.398	0.310	0.398	0.310	0.398	0.237	0.398

Bold indicates statistical significance following a Bonferroni correction for multiple comparisons.

5.5.2.3 Comparison of Evaporation Rate of the Left Goggle Before and After Removal of Anti-fog Coating

A summary of the evaporation rate of different sizes of model eye measured with the left goggle before and after removal of the anti-fog coating appears in Figure 5-9 and Table 5-13.

There was a significant difference in the evaporation rate of all sizes of model eye before and after the anti-fog coating was removed at a slope duration of 10 seconds for the 1, 2, and 2.5 cm^2 model eyes (all $p<0.044$), and for slopes calculated from 15 to 30 seconds (all $p<0.044$) in all model eyes.

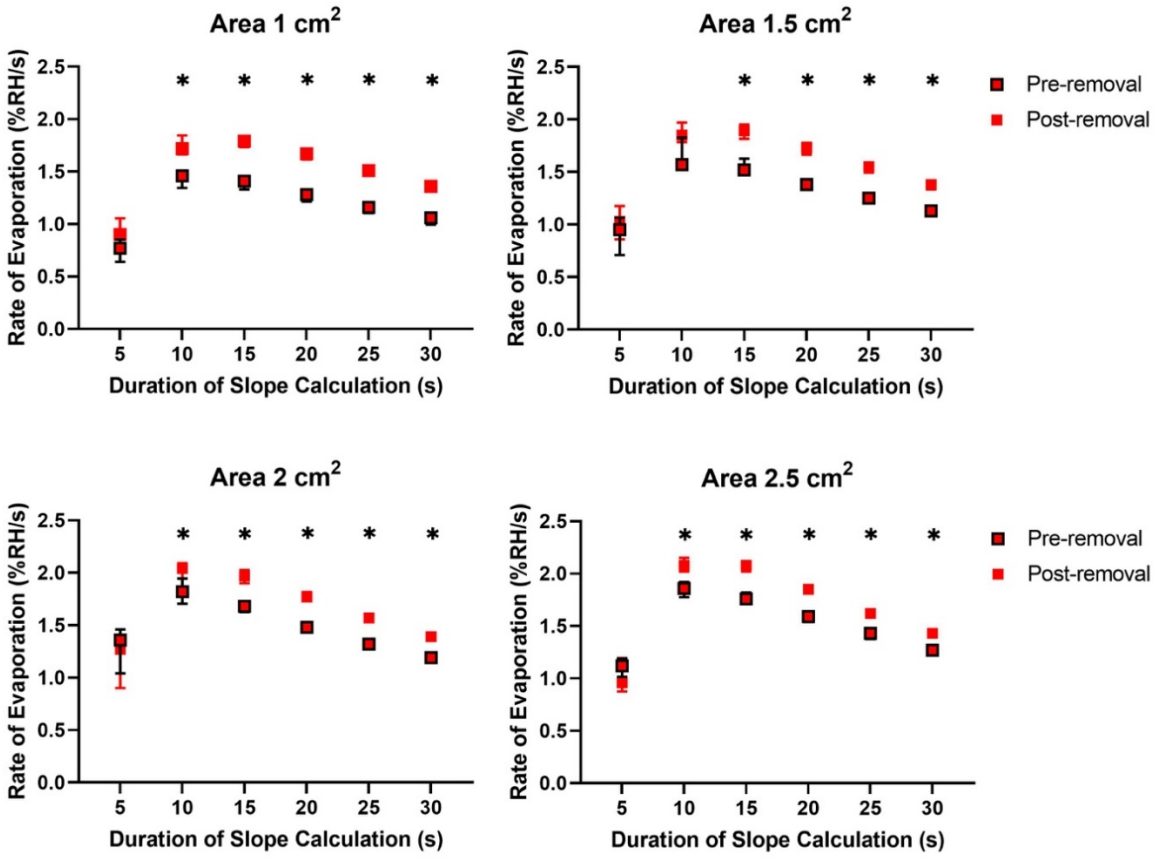

Figure 5-9: Change in evaporation rate in the left goggle before and after removal of the anti-fog coating. Error bars indicate IQR. Asterisks indicate a significant difference for the left goggle before and after removal of the anti-fog coating ($p<0.05$).

Table 5-13: Summary of evaporation rate for different sizes of model eye measured with the left goggle before and after removal of the anti-fog coating

Duration of Slope Calculation (s)	Area 1 cm^2			Area 1.5 cm^2			Area 2 cm^2			Area 2.5 cm^2		
	Pre-removal	Post-removal	p-value	Pre-removal	Post-removal	p-value	Pre-removal	Post-removal	p-value	Pre-removal	Post-removal	p-value
5	0.77 (0.21)	0.90 (0.27)	0.138	0.95 (0.36)	1.03 (0.32)	0.138	1.36 (0.42)	1.27 (0.44)	0.102	1.12 (0.17)	0.96 (0.32)	0.138
10	1.46 (0.16)	1.72 (0.18)	**0.043**	1.57 (0.28)	1.85 (0.18)	0.068	1.82 (0.24)	2.04 (0.22)	**0.042**	1.86 (0.15)	2.08 (0.13)	**0.042**
15	1.41 (0.11)	1.79 (0.10)	**0.042**	1.52 (0.13)	1.89 (0.14)	**0.043**	1.68 (0.06)	1.97 (0.13)	**0.043**	1.76 (0.11)	2.08 (0.07)	**0.043**
20	1.28 (0.09)	1.67 (0.08)	**0.043**	1.38 (0.06)	1.73 (0.11)	**0.043**	1.48 (0.06)	1.77 (0.08)	**0.043**	1.59 (0.08)	1.85 (0.06)	**0.043**
25	1.16 (0.08)	1.51 (0.06)	**0.043**	1.25 (0.05)	1.55 (0.08)	**0.043**	1.32 (0.04)	1.57 (0.06)	**0.043**	1.43 (0.07)	1.62 (0.04)	**0.043**
30	1.06 (0.09)	1.36 (0.05)	**0.043**	1.13 (0.05)	1.38 (0.07)	**0.043**	1.19 (0.04)	1.39 (0.06)	**0.042**	1.27 (0.06)	1.43 (0.03)	**0.043**

Bold indicates statistical significance.

5.5.3 Discussion

Although the majority of comparisons still showed a higher evaporation rate in the right goggle compared to the left goggle after removal of the anti-fog coating, there was no significant difference between the two goggles for the smallest model eye over a 5 second slope duration or between the 15 second or 20 second slope duration for the 1.5 cm^2 model eye.

The evaporation rate of the left goggle was significantly higher after removing the anti-fog coating for the majority of model eyes at a 10 second slope duration and for all model eyes when the slope calculated over 15 to 30 seconds. This provides evidence that the anti-fog coating was able to reduce evaporation inside the swimming goggle by hydrogen bonding water to the hydrophilic coating, which causes water molecules to spread out and prevent condensation [407]. Although there was no significant difference in evaporation rate when the slope duration was calculated over the initial 5 seconds, this is likely to be due to the slow rate of change in RH after placing the goggle on the model eye as the water vapor evaporating from the surface of the water disperses inside the goggle before reaching the sensor.

5.6 Simultaneous Comparison of the Right and Left Goggle

5.6.1 Materials and Methods

5.6.1.1 Methods

Two aluminum elliptical model eyes with a surface area of 1.5 cm^2 were used to simulate the right and left eye. All measurements were recorded outside the environmental chamber. Ambient temperature and RH were monitored with an Omega RH411 digital thermo-hygrometer.

The model eyes and distilled water were warmed to 34°C in an Isotemp oven. The model eyes were removed from the oven and placed on a heating plate. An Eppendorf Reference 100-1000 µl pipette was used to pipette 1000 µl of distilled water into each model eye. Both goggles were simultaneously placed on the model eyes and temperature/RH was recorded four times per second for 30 seconds. Upon completing each measurement, the model eyes were heated in the oven for 30 minutes.

Five recordings were taken with the right goggle placed on the right model eye and the left goggle on the left model eye. The following day an additional five measurements were taken with the goggle reversed to record from the opposite model eye (Figure 5-10).

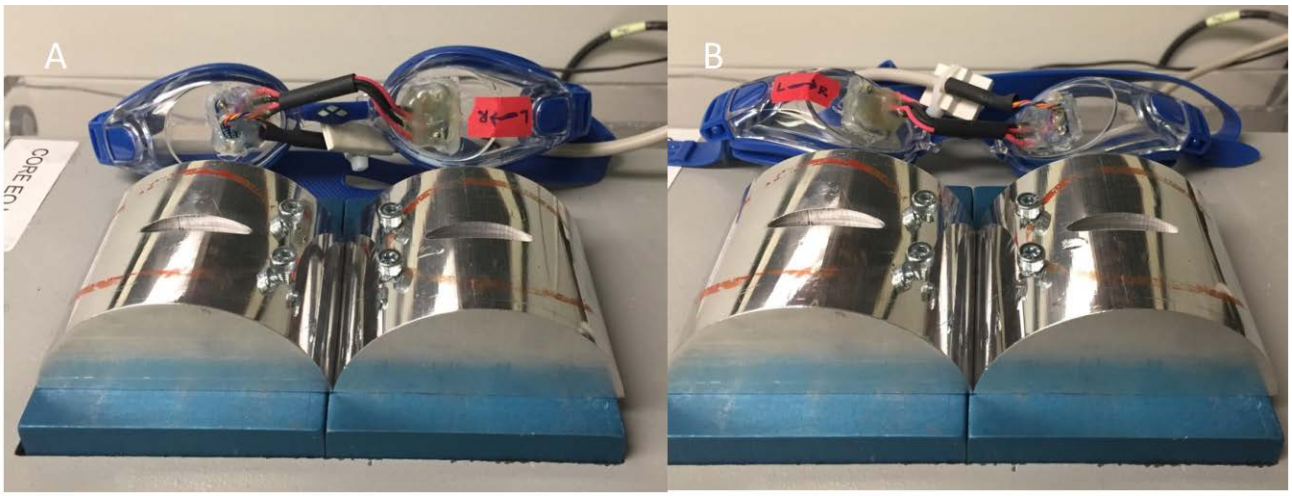

Figure 5-10: Set-up of the simultaneous goggle measurement for the right lens over the right model eye (A) and the right left over the model eye (B).

The orange outline on the model eye represents the placement of each goggle to ensure consistency between measurements.

5.6.1.2 Statistical Analysis

Statistical analysis was conducted using SPSS version 27.0. Data was tested for normality using a Shapiro-Wilk test with α=0.05. Comparison of the RH at different time points was analyzed in 5-second intervals with a Friedman test and a post-hoc Dunn's pairwise comparison test with Bonferroni correction. A p-value <0.05 was considered statistically significant.

5.6.2 Results

5.6.2.1 Ambient Temperature and Relative Humidity

The mean ± SD ambient temperature was 24.0 ± 0.0°C and RH was 35.6 ± 1.5% (range: 33 to 38%). The temperature remained stable at 24°C throughout the measurements.

5.6.2.2 Change in Relative Humidity over Time

The change in RH over time in the right and left goggle is shown in Figure 5-11. Analysis of the RH in 5-second intervals revealed a significant difference between the two goggles and model eyes (all p<0.034, Table 5-14).

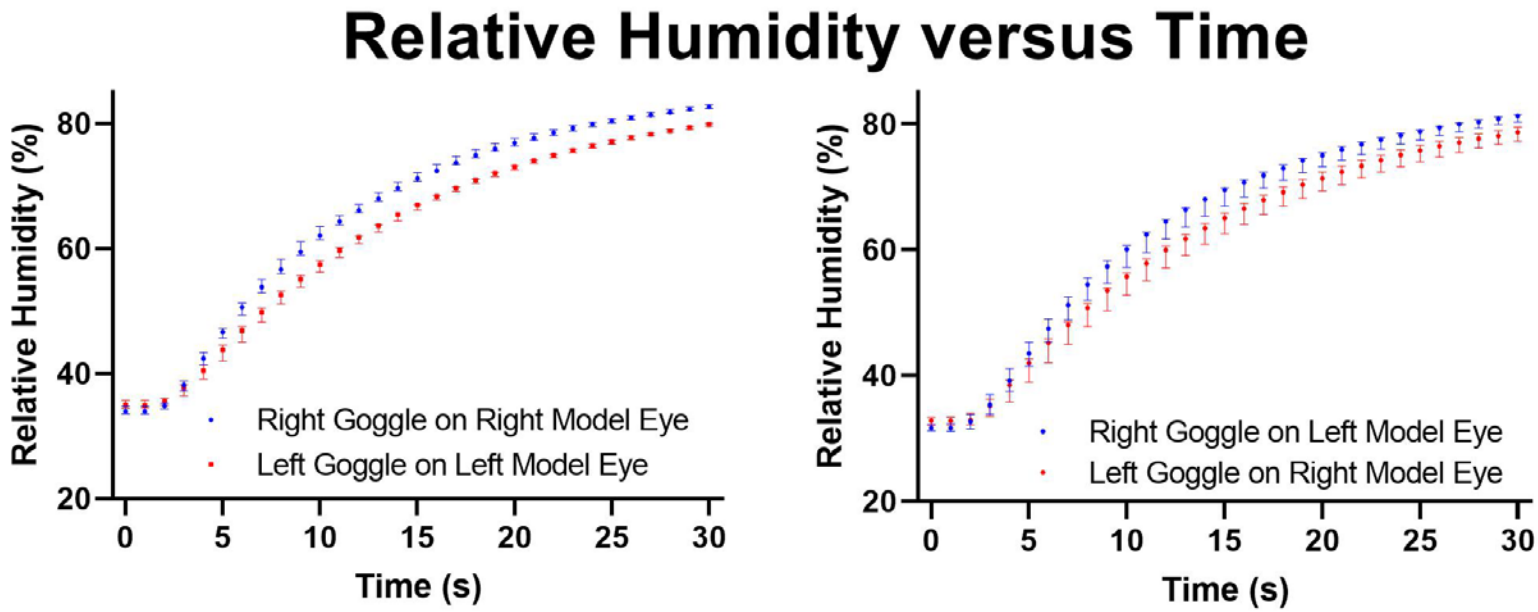

Figure 5-11: Median change in relative humidity over time in the right and left goggle with an evaporimeter volume of 16 cm^3. Both goggles were simultaneously held over the same size of model eye, then testing was repeated with each goggle held over the opposite model eye. Error bars indicate IQR. Data is shown in 1-second intervals for ease of visualization, although measurements were recorded every 0.25 seconds.

A summary of pairwise comparisons of evaporation rate calculated over different time intervals with the right and left goggle appears in Table 5-15. Post-hoc testing found a significant difference in the initial RH of the right and left goggle when the evaporimeter was placed over the left model eye ($p<0.001$), and between the RH of the two goggles between 5 seconds to 30 seconds when the right model eye was investigated (all $p<0.004$). No significant difference was found between the right and left goggle when the model eyes were simultaneously measured (all $p>0.015$).

Table 5-14: Summary of median (IQR) relative humidity in the right and left goggle

	Relative Humidity (%)						
	0 s	5 s	10 s	15 s	20 s	25 s	30 s
Right goggle/right model eye	33.97	46.68	62.07	71.22	76.90	80.35	82.66
	(1.26)	(1.57)	(2.16)	(1.41)	(1.23)	(0.66)	(0.63)
Left goggle/left model eye	34.98	43.81	57.43	66.92	73.04	77.07	79.81
	(1.26)	(2.48)	(1.79)	(0.88)	(0.65)	(0.59)	(0.47)
Right goggle/left model eye	31.68	43.53	60.04	69.53	75.01	78.82	81.30
	(1.09)	(3.80)	(3.39)	(2.83)	(2.25)	(1.57)	(1.22)
Left goggle/right model eye	32.85	42.00	55.70	65.06	71.35	75.75	78.70
	(1.27)	(3.73)	(3.51)	(3.24)	(2.99)	(2.59)	(2.17)
p-value	**0.002**	**0.033**	**0.005**	**0.002**	**0.002**	**0.002**	**0.002**

Bold indicates statistical significance.

Table 5-15: Summary of statistical significance from pairwise comparisons of relative humidity with various goggle combinations and the two 1.5 cm^2 model eyes

	0 s	5 s	10 s	15 s	20 s	25 s	30 s
Right goggle/right model eye versus left goggle/left model eye	0.221	0.221	0.050	0.014	0.014	0.014	0.014
Right goggle/right model eye versus right goggle/left model eye	0.014	0.221	0.327	0.221	0.221	0.221	0.221
Right goggle/right model eye versus left goggle/right model eye	0.221	**0.003**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Right goggle/left model eye versus left goggle/right model eye	0.221	0.086	0.014	0.014	0.014	0.014	0.014
Right goggle/left model eye versus left goggle/left model eye	**<0.001**	0.221	0.327	0.221	0.221	0.221	0.221
Left goggle/left model eye versus left goggle/right model eye	0.014	0.086	0.142	0.221	0.221	0.221	0.221

Bold indicates statistical significance following a Bonferroni correction for multiple comparisons.

5.6.3 Discussion

Both goggles were able to detect changes in RH over time for various sizes of model eye and air volumes inside the instrument. However, the optimal time period over which the slope should be analyzed was not calculated due to the difference in results between the right and left goggle. Therefore, further testing was performed to test the evaporimeter with two 1.5 cm^2 model eyes to examine whether there was a significant difference between the RH values obtained from the right and left goggle.

Although there was a significant difference in the baseline RH of each goggle, this is likely to be due to a fluctuation in the ambient conditions of the room, rather than a real difference between the sensors. Comparison of the median difference in baseline RH of measurements recorded at the same time showed a maximum difference of 1.17%, which is within the ±2% accuracy of the sensor [402]. Comparison of the other RH values that were recorded at the same time also found no significant difference between the right and left goggle, which best represents the anticipated *in vivo* testing procedure. However, there was also a significant difference between each goggle at the baseline measurement of the left model eye and significantly higher RHs in the right goggle from 5 to 30 seconds when the right model eye was tested. This could have implications for any study designs which use repeated measurements or contralateral testing. Investigators should ensure that the right goggle is always placed over the right eye in order to avoid a difference in results caused by measurements recorded with the opposite sensor.

5.7 Future Work and Limitations

5.7.1 Possible Sources of Error

Coatings designed to prevent fogging can deteriorate over time [405, 406]. The best choice of a swimming goggle would have been one that was manufactured by directly incorporating the anti-fog component into the lens matrix [405]. On this occasion, the X-Fit swimming goggle was chosen because it was the same one used in the commercially available Eye-VapoMeter.

The removal of the anti-fog coating from this evaporimeter could result in higher values than those obtained from a closed-chamber evaporimeter with an intact anti-fog coating. A wide range of normal and abnormal tear evaporation rates have been reported from different open-chamber and closed-chamber instruments [5]. The evaporation rate from a closed-chamber instrument may be expected to be lower than an open-chamber device because the goggle provides protection from exposure to the

external environment [5], including drafts from air-conditioning or movement within the room [64, 408]. *In vivo* dermatological testing of the trans-epidermal water loss reported higher rates of evaporation in open-chamber devices compared to closed-chamber instruments [62, 63], although *in vitro* work reported the opposite findings [60].

Since the evaporimeter is an unventilated type, it would have been ideal to record all of the measurements inside an environmental chamber to control the ambient RH. However, measurements of the two 1.5 cm^2 model eyes were conducted outside the environmental chamber because the large size of the chamber's gloves interfered with the ability to simultaneously place both goggles on the model eyes. In hindsight, the methodology of the testing should also have been randomized to alternate between testing the right goggle over the right model eye and the right goggle over the left model eye, rather than separating the testing into different days. If the testing had been randomized, this may have eliminated the difference in baseline RHs because the ambient RH was lower on the second day of testing in comparison to the first day.

5.7.2 Future Work

This chapter demonstrated that sensors in the right and left goggle are able to detect changes in RH when placed over a source of evaporation. Since water evaporates quickly [175], additional *in vitro* work can be conducted to examine the difference in the evaporation rate of more complex solutions containing lipids [409].

Faster rates of evaporation have been reported in rabbit [48, 50] and human [49] tear films when the lipid layer is missing. However, as a thin lipid layer is not always found in the presence of dry eye [410, 411], additional work is required to investigate the response of the evaporimeter to *in vivo* testing in a range of participants.

Further work should monitor the results to see if there is a consistently higher rate of evaporation in the right goggle. If there is a difference between the two goggles, a correction factor may need to be applied to the results.

5.8 Conclusions

Initial testing of the new binocular evaporimeter found that each goggle was able to detect a change in RH when placed over a model eye containing warm distilled water. The plot of the RH over time

shows three phases: (i) an initial stable period, (ii) a period of rapid increase in RH, followed by (iii) a slower increase in RH.

The evaporimeter was able to detect significantly lower rates of evaporation in the smallest model eye at a slope duration of 4 to 16 seconds. The instrument was also able to measure lower rates of evaporation when the evaporimeter volume was 10 cm^3 compared to the 13 and 18.63 cm^3 volumes, and when the slope was calculated over 6 seconds. Further work should be conducted to assess whether these values are within the range of typical ocular surface areas and air volumes encountered in human participants, and whether these changes are also observed with *in vivo* testing.

Although each side of the evaporimeter was able to demonstrate a change in RH over time, preliminary comparison of the two goggles found higher rates of evaporation in the right goggle. Removal of the crazed anti-fog coating from the left goggle caused a significant increase in evaporation rate for 3 out of 4 model eyes over a 10 second slope duration, and for all of the model eyes when the slope was calculated from 15 to 30 seconds. Simultaneous testing of the model eyes found no significant difference in the RH within each goggle. However, since a comparison of the RH measured over the same model eye found significant differences between the two goggles, further work should ensure that the right goggle is always tested with the right eye to maintain consistency between measurements.

Lastly, additional work is required to determine whether these *in vitro* results are replicated *in vivo*.

Chapter 6

Optimization of a Method for Testing Humans with a Novel Evaporimeter

6.1 Abstract

PURPOSE: To examine whether the new evaporimeter was able to measure a change in relative humidity (RH) when placed over an open or closed eye; to compare the RH of the right goggle to the left goggle; to investigate how the evaporation rate changes after the use of a liposomal spray or artificial lubricant; to examine how applying petroleum jelly to the skin surrounding the eye affects the evaporation rate; to investigate how fixation affects the change in RH inside the evaporimeter; to determine the best method of ventilating the instrument; and to establish the optimal blink rate.

METHODS: A series of *in vivo* studies was conducted at a sampling rate of 0.25 seconds. OPEN VERSUS CLOSED EYE: Comparison of open versus closed eye and right versus left eye was investigated in non-contact lens wearers (n=5) by measuring the change in RH over the closed eyes for 20 seconds with the novel evaporimeter, followed by the eyes held open for as long as possible for 20 seconds. Evaporimetry was tested at four time points: baseline, 5 minutes, 15 minutes, and 30 minutes. The overall change in RH was investigated in 0.5 second increments and comparisons over time and between goggles were analyzed in 5 second intervals. LIPOSOMAL SPRAY/ARTIFICIAL LUBRICANT: The same procedure was followed to examine the effect of 1 to 2 sprays of CALMO® Eye Spray (n=5) and a single application of Refresh Tears® (n=5), except volunteers were asked to blink as needed. Two baseline measurements were taken 15 minutes apart, followed by measurements immediately after use of the dry eye product and 15 minutes later. The evaporation rate was calculated as the change in RH between 5 to 15 seconds after the instrument was placed over the eyes. Tear evaporation rate (TER) from the ocular surface was calculated as the difference between the open eye and closed eye evaporation rate. PETROLEUM JELLY: To measure the effect of petroleum jelly on TER, a thin layer was applied to the skin surrounding each eye. Three consecutive 20 second open eye and then three subsequent closed eye measurements were taken at four time points separated by 10 minutes (n=5). The evaporation rate was calculated from slope of the change in RH between 5 and 15 seconds. FIXATION: Fixation was tested in a contact lens wearer (n=1) by comparing the change in RH that occurred with the eyes open and blinking normally over 20 seconds in downgaze, primary

gaze, and upgaze. VENTILATION: Ventilation of the evaporimeter was investigated by testing the total amount of time required to take three consecutive sets of 20 second open and closed eye measurements without ventilation, compared to reducing the RH with compressed air, a dust blower, a table fan, or tissues. BLINK RATE: The optimal blink rate during measurement was investigated by alternating between two rounds of 20 second open eye measurements at blink rates of either three or five seconds in volunteers with self-reported dry eye (n=3). Volunteers were asked to report reflex tearing and to choose which blink rate they preferred.

RESULTS: OPEN VERSUS CLOSED EYE: RH significantly changed in each side of the goggle when the evaporimeter was placed over the open eye (all $p<0.001$) and closed eye (all $p<0.001$). No significant differences in RH were detected between the right and left goggles when the eyes were open (all $p>0.137$) or closed (all $p>0.079$). LIPOSOMAL SPRAY: Application of the liposomal spray resulted in a significantly lower evaporation rate at the second baseline measurement in the closed left eye compared to immediately after application of the liposomal spray ($p=0.001$). TER from the ocular surface of the left eye was significantly lower immediately after application of the liposomal spray compared to the second baseline measurement ($p=0.001$) and 15 minutes after the spray was applied ($p=0.003$). ARTIFICIAL LUBRICANT: Use of an artificial lubricant found significantly higher evaporation rates in both eyes when they were closed immediately after instillation compared to the first baseline measurement (right eye: $p=0.007$, left eye: $p=0.007$) and 15 minutes post-instillation (right eye: $p=0.003$, left eye: $p=0.003$). This resulted in significantly lower TERs in each eye (right eye: $p=0.007$, left eye: $p=0.003$) following instillation of Refresh Tears® compared to the second baseline measurement and 15 minutes post-drop in the left eye ($p=0.003$). PETROLEUM JELLY: Petroleum jelly applied to the skin resulted in a lower evaporation rate at baseline compared to measurements taken 20 minutes ($p=0.020$) and 30 minutes ($p=0.010$) later in the closed right eye. However, no significant change in TER occurred from the ocular surface over time (right eye: $p=0.564$, left eye: $p=0.564$). FIXATION: Comparison of different positions of gaze revealed less of a change in RH over time in downgaze compared to primary gaze ($p=0.04$) and upgaze ($p=0.02$). VENTILATION: The fastest methods of ventilating the evaporimeter were with long bursts of compressed air (5 minutes and 33 seconds) and a table fan (6 minutes 12 seconds). BLINK RATE: Comparison of blink rate resulted in no reports of reflex tearing, with two out of three participants preferring a five second blink rate over a three second frequency.

CONCLUSIONS:

The new evaporimeter:

- Detected changes in RH over time when placed over open and closed eyes;
- Provided similar RH values between the right and left goggles when the eyes were open and closed;
- Detected a lower TER in the left eye immediately after application of CALMO® Eye Spray;
- Detected lower TERs in both eyes immediately after application of Refresh Tears®;
- Provided repeatable TERs in each eye following the use of petroleum jelly;
- Measured a smaller change in RH over time in downgaze compared to primary gaze and upgaze;
- Was quickly and safely ventilated with a table fan;
- Did not induce reflex tearing at a 3 or 5 second blink rate.

Preliminary testing has shown that the evaporimeter can provide repeatable baseline measurements of TER. Further work will need to be conducted using the finalized evaporimetry method on larger sample sizes.

6.2 Introduction

Following confirmation that each side of the novel evaporimeter was able to detect changes in relative humidity (RH) after exposure to model eyes filled with heated water, additional testing was required to examine whether these changes can also be observed when the instrument is used on humans.

If the device is able to detect changes in RH, further work is required to determine the optimal method of conducting evaporimetry. No consistent protocol has been developed between researchers to measure evaporimetry due to differences between instruments. Researchers have asked participants to blink as frequently as every three seconds [7, 109] or as infrequently as once per minute [1], with measurement durations ranging from less than 10 seconds [21] up to 5 minutes [99]. Others have only taken measurements when the eyes are open [73, 74], whereas others have measured both the open and closed eye [1-3, 21, 49, 99, 101]. Petroleum jelly has also been used by some researchers to prevent air from leaking around the edge of the swimming goggle [102] or to minimize water loss from the skin

[1, 3, 21]. Due to variations between different evaporimetry techniques, additional testing is needed to determine the optimal method of testing with the new device.

The aims of the chapter are to:

- Test whether the RH changes after exposure to closed and open eyes;
- Compare the RH in the right and left goggle;
- Examine the effect of a liposomal spray and artificial lubricant on TER;
- Investigate the effect of petroleum jelly on the rate of skin and ocular surface evaporation;
- Examine the how different positions of gaze affect the change in RH;
- Evaluate the best method of ventilating the evaporimeter;
- Determine the preferred blink rate of people with self-reported dry eye.

6.3 Initial Testing

6.3.1 Materials and Methods

Volunteers adapted to the room environment for a minimum of 15 minutes prior to undergoing any measurements. Room conditions were monitored using a digital thermo-hygrometer (RH411, Omega, Norwalk, Connecticut, USA). Volunteers were given a 5-second countdown prior to being asked to place the evaporimeter over the closed eyes for 20 seconds (Figure 6-1). The change in temperature and RH was recorded in 0.25 second increments with the “Goggle” software (Mr. Ehsan Zare-Bidaki, MLEO, Waterloo, Ontario, Canada, Section 4.3.2). After completing the closed eye measurement, the instrument was placed on a table facing away from the volunteer until the RH returned to ambient levels. Another 5-second warning was given prior to another 20 second measurement with the eyes held open for as long as possible. A single closed eye and open eye measurement was recorded at four time points: baseline, 5 minutes, 15 minutes, and 30 minutes. Testing was also conducted with the evaporimeter reversed so that the right goggle was held over the left eye and the left goggle was held over the right eye, but this data has not been included in the thesis.

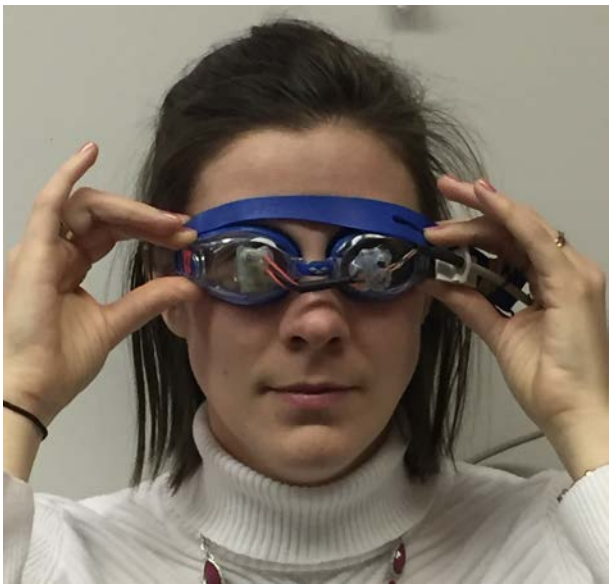

Figure 6-1: Use of the evaporimeter with the swimming goggle strap attached.

6.3.1.1 Statistical Analysis

Statistical analysis was performed with IBM® SPSS® Statistics version 27 (IBM Corporation, Armonk, New York, USA). Data was tested for a normal distribution using a Shapiro-Wilk test. Overall changes over time were analyzed with a Friedman test in 0.5 second increments, while comparisons between different time points were analyzed in 5 second intervals. Comparisons between each goggle were analyzed in 5 second intervals and differences between the open and closed eye were investigated with a Wilcoxon signed-ranks test. A post-hoc Tukey Honestly Significant Difference test was used to identify homogenous subsets at the start of each measurement over which there was no significant change in RH (INITIAL SUBSET) and at the end of each measurement (END SUBSET). The last value of the initial subset (INITIAL VALUE) and the first value of the end subset (END VALUE) were recorded. A p-value <0.05 was considered statistically significant.

6.3.2 Results

Five non-contact lens wearers (2 males, 3 females) were examined.

6.3.2.1 Ambient Temperature and Relative Humidity

The mean ambient temperature was 24°C and the mean ± SD RH was 21.65 ± 3.23% (range: 18 to 27%). The temperature was stable at 24°C throughout the duration of measurements.

6.3.2.2 Change in Relative Humidity over Time

Significant changes in RH occurred within each goggle over time when the eyes were open (right eye: all $p<0.001$, left eye: all $p<0.001$) and closed (right eye: all $p<0.001$, left eye: all $p<0.001$). The median RH of the open eye was higher than the closed eye at all four time points in the right goggle (all $p<0.001$) and left goggle (all $p<0.001$). The median interquartile range (IQR) change in RH over the 20 second measurement period for the open and closed eye appears in Figure 6-2.

A summary of the median (IQR) RH measured in 5 second intervals for the open and closed eye appears in Table 6-1 and Table 6-2, respectively. No significant change in RH over time occurred between the four time points when the eyes were open (right eye: all $p>0.094$, left eye: all $p>0.265$) or closed (right eye: all $p>0.094$, left eye: all $p>0.061$). In addition, no significant difference in RH was found between the right eye compared to the left eye when the eyes were open (all $p>0.137$) or closed (all $p>0.079$).

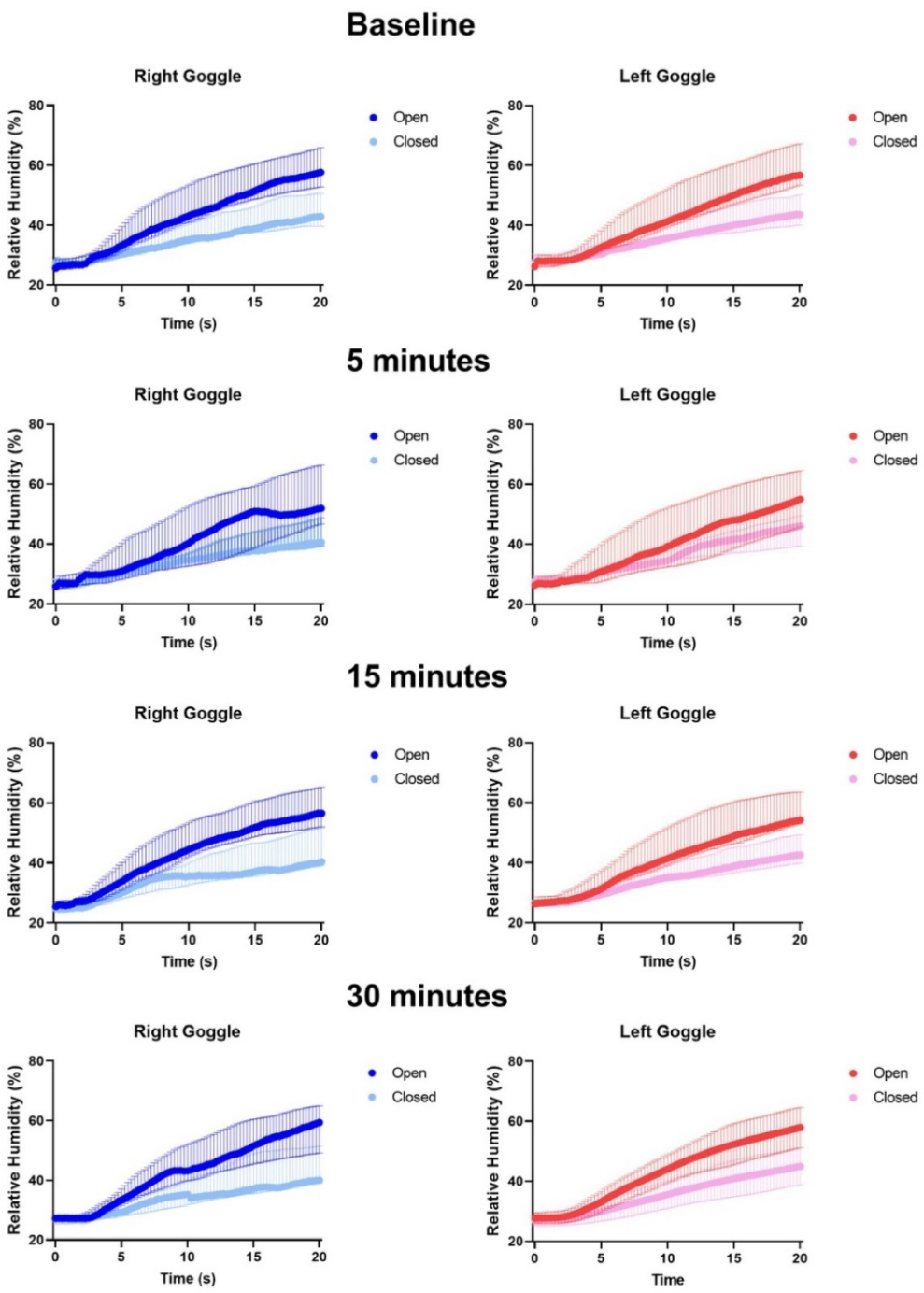

Figure 6-2: Median change in relative humidity over time in each goggle with the eyes open and closed (n=5). Error bars indicate interquartile range (IQR).

Table 6-1: Summary of median (IQR) relative humidity in five second intervals for the right and left goggle with eyes open

	Relative Humidity (%) at														
	0 s			5 s			10 s			15 s			20 s		
Time (min)	Right Goggle	Left Goggle	p-value	Right Goggle	Left Goggle	p-value	Right Goggle	Left Goggle	p-value	Right Goggle	Left Goggle	p-value	Right Goggle	Left Goggle	p-value
0	26.43 (3.70)	28.11 (3.87)	0.138	33.32 (8.51)	33.13 (7.36)	0.345	43.01 (12.24)	41.62 (12.93)	0.345	51.39 (11.70)	50.44 (13.76)	0.686	57.88 (12.99)	56.97 (13.95)	0.686
5	26.83 (4.06)	26.95 (3.01)	0.686	31.44 (12.53)	30.92 (14.56)	0.686	40.24 (19.89)	39.34 (19.67)	0.345	50.86 (20.51)	48.05 (20.78)	0.686	52.16 (19.49)	54.98 (18.73)	0.686
15	26.02 (1.98)	26.51 (2.88)	0.225	33.93 (9.09)	31.79 (6.67)	0.345	44.47 (11.32)	41.44 (12.52)	0.225	52.92 (11.69)	48.88 (13.83)	0.345	56.54 (13.13)	54.27 (10.69)	0.686
30	27.25 (2.00)	27.82 (3.15)	0.345	33.42 (5.36)	32.90 (5.20)	0.225	43.18 (12.20)	43.20 (9.39)	0.345	51.52 (14.99)	50.32 (12.99)	0.893	59.38 (15.84)	55.15 (13.00)	0.893
p-value	0.145	0.668		0.668	0.782		0.392	0.948		0.095	0.782		0.392	0.266	

Table 6-2: Summary of median (IQR) relative humidity in five second intervals for the right and left goggle with eyes closed

	Relative Humidity (%) at														
	0 s			5 s			10 s			15 s			20 s		
Time (min)	Right Goggle	Left Goggle	p-value	Right Goggle	Left Goggle	p-value	Right Goggle	Left Goggle	p-value	Right Goggle	Left Goggle	p-value	Right Goggle	Left Goggle	p-value
0	27.45 (2.77)	27.77 (3.65)	0.080	30.26 (3.64)	30.38 (3.66)	0.225	35.04 (6.34)	35.60 (5.91)	0.500	38.80 (8.45)	40.08 (8.84)	0.500	42.88 (11.03)	43.59 (10.20)	0.893
5	26.18 (2.91)	27.64 (3.13)	0.225	30.13 (1.32)	30.58 (2.53)	0.225	34.60 (2.69)	34.53 (3.88)	0.345	41.59 (7.31)	41.59 (7.31)	0.500	40.52 (9.45)	46.07 (10.14)	0.345
15	24.74 (2.84)	26.67 (1.18)	0.138	30.58 (4.77)	29.39 (3.50)	0.893	35.63 (7.37)	35.18 (4.98)	0.893	37.11 (11.07)	38.86 (8.52)	0.893	40.37 (12.61)	42.59 (9.40)	0.893
30	26.98 (2.80)	26.86 (2.84)	0.893	29.26 (4.44)	29.23 (5.01)	0.500	35.27 (8.22)	33.08 (7.67)	0.893	37.11 (13.33)	37.56 (10.94)	0.893	40.08 (12.46)	41.95 (11.85)	0.686
p-value	0.095	0.356		0.323	0.062		0.668	0.145		0.204	0.323		0.908	0.323	

6.3.2.3 Initial and End Values

The INITIAL VALUES and END VALUES for the right and left eye are shown in Table 6-3. The homogenous subsets in each eye were similar for the open eye and closed eye.

Table 6-3: Initial and end values

		OPEN EYE		CLOSED EYE	
Eye	**n**	**Initial Value (s)**	**End Value (s)**	**Initial Value (s)**	**End Value (s)**
Right	20	5.50	12.50	5.50	13.50
Left	20	5.50	14.00	5.50	14.50
Mean	40	5.50	13.25	5.50	14.00

6.3.3 Discussion

Each goggle was able to detect an increase in RH after being placed over the open and closed eye. As previously observed with *in vitro* testing (Section 5.3.2.3), *in vivo* results also demonstrated an initial time period where the RH did not significantly change, followed by a faster increase in RH. A graph of the change in RH over time obtained with the Mathers evaporimeter shows a similar pattern, with a prolonged initial period of stability lasting approximately 15 seconds [3]. However, graphs of the Yamada-Tsubota evaporimeter show a rapid increase in RH over time immediately after the goggles were placed over the open and closed eye [2, 104], although the difference in results is likely to be caused by the 10 second [2] measurement interval.

The median RH of the open eye was higher than the closed eye, which was expected because evaporation occurs from both the ocular surface and peri-ocular skin when the eye is open. Previous researchers have determined the rate of evaporation from the ocular surface by calculating the difference between the evaporation rate of the open eye and closed eye [1-3, 21, 49, 98, 99, 101].

Rolando and Refojo [1] reported a mean increase in RH of 3.38% over 1 minute when the eyes were closed. The mean increase in RH with the novel evaporimeter was 17.62% in the right eye and 17.36% in the left eye over 20 seconds with the eyes closed, and 30.33% in the right eye and 29.13% in the left eye with the eyes open. A faster rate of RH change may have been obtained with the new evaporimeter because the ambient starting RH was lower than the baseline RH of 29.5% used in the ventilated Rolando-Refojo evaporimeter [1].

In vitro testing with two different model eyes and *in vivo* work demonstrated that the evaporimeter provides similar RH values in each side of the goggle, with no significant difference between the two goggles when the eyes are open and closed.

A potential source of error could arise from the strap of the swimming goggle interfering with placement of the evaporimeter against the face. The strap was not placed around the back of the head due to the short measurement time and because the volunteers were able to judge when the goggles had formed a sufficient seal around the eye. Having to adjust the strap after the instrument was placed over the eyes could result in jostling the device and changing the position of the sensors. Or a gap could occur between the face and goggle as the strap is adjusted, that would allow ambient air to enter the device, which could affect the resulting tear evaporation rate (TER). In order to allow volunteers to better handle the evaporimeter, the strap was removed from the device for subsequent measurements.

The change in temperature inside the evaporimeter was also examined, but the data has not been included in the thesis due to the small change in temperature over the measurement period.

6.4 Effect of a Liposomal Spray

6.4.1 Methods and Materials

Volunteers adapted to the room environment for at least 15 minutes prior to the first measurement. An Omega RH411 Thermo-Hygrometer was used to monitor the temperature and RH within the room. Evaporimetry consisted of one measurement of 20 seconds duration with the eyes closed, followed by a measurement with the eyes open. There was approximately a 1-minute interval between the closed and open eye measurements as the RH in the goggle returned to baseline levels. Volunteers were given a 3-second countdown prior to placing the evaporimeter over the eyes and were asked to blink normally when the eyes were open. Measurements were taken at four time points: baseline 1, baseline 2 (15 minutes after the first measurement), immediately after applying a liposomal spray, and 15 minutes post-spray. As per the manufacturer's instructions, 1 to 2 pumps of CALMO® Eye Spray, (Optima Medical Swiss AG, Zug, Switzerland, Table 6-4) were administered 10 cm away from each eye. The product was always applied to the right eye first, followed by the left eye.

Table 6-4: CALMO® Eye Spray details

Liposomal spray	CALMO® Eye Spray
Manufacturer	Optima Medical Swiss AG
Active ingredients	Liposomes, Dexpanthenol
Preservative	None
Health Canada License #	97465
Device class	2

Calculation of the rate of evaporation was based on the initial and end values of homogenous subsets reported in Section 6.3.2.3. Since volunteers were asked to blink normally during these measurements, rather than hold their eyes open, the RH was expected to change at a slower rate because there was less of a risk of inducing reflex tearing. Therefore, the slope of the change in RH was calculated over a slightly longer interval, from 5 to 15 seconds.

6.4.1.1 Statistical Analysis

Statistical analysis was conducted using SPSS version 27.0. Data was tested for normality using a Shapiro-Wilk test with α=0.05. Comparison of the evaporation rate over time was analyzed with a Friedman test and a post-hoc Dunn's pairwise comparison test with Bonferroni correction. A p-value <0.05 was considered statistically significant.

6.4.2 Results

The same five non-contact lens wearing volunteers that participated in Section 6.3 were tested.

6.4.2.1 Ambient Temperature and Relative Humidity

The mean ± SD ambient temperature was 23.95 ± 0.22°C (range: 23 to 24°C) and the RH was 11.05 ± 1.54% (range: 9 to 14%).

6.4.2.2 Change in Evaporation Rate over Time

A summary of median (IQR) evaporation rate from the open eye, closed eye, and ocular surface appears in Table 6-5 and Figure 6-3. No significant change in evaporation rate occurred over time when the eyes were open (right eye: p=0.724, left eye: p=0.095) or in the right eye when it was closed (p=0.178). However, there was a significant difference between the evaporation rate of the left eye when it was closed (p=0.011), with a significantly lower evaporation rate at the second baseline measurement compared to immediately after application of the liposomal spray (p=0.001).

No significant change occurred in the TER from the ocular surface of the right eye over time (p=0.266), although there was a significant difference over time in the left eye (p=0.004). Post-hoc testing revealed a significantly lower TER immediately after application of the liposomal spray compared to the second baseline measurement (p=0.001) and 15 minutes after the spray was applied (p=0.003).

Table 6-5: Summary of median (IQR) evaporation rate pre- and post-liposomal spray

	Evaporation Rate (%RH/s)					
	Open Eye		**Closed Eye**		**Ocular Surface**	
	Right Eye	Left Eye	Right Eye	Left Eye	Right Eye	Left Eye
Baseline 1	1.53 (1.00)	1.63 (0.49)	1.23 (0.59)	1.05 (0.41)	0.30 (0.63)	0.49 (0.35)
Baseline 2	1.60 (0.80)	1.64 (0.83)	0.88 (0.66)	0.83 (0.37)	0.72 (1.17)	0.92 (0.35)
Post-spray	1.63 (1.00)	1.61 (0.42)	2.08 (1.86)	1.78 (1.89)	–0.36 (2.54)	–0.21 (1.99)
15 minutes post-spray	1.64 (0.65)	1.72 (0.56)	1.06 (0.47)	1.00 (0.42)	0.54 (0.86)	0.55 (0.81)
p-value	0.724	0.095	0.178	**0.011**	0.266	**0.004**

Bold indicates significant differences.

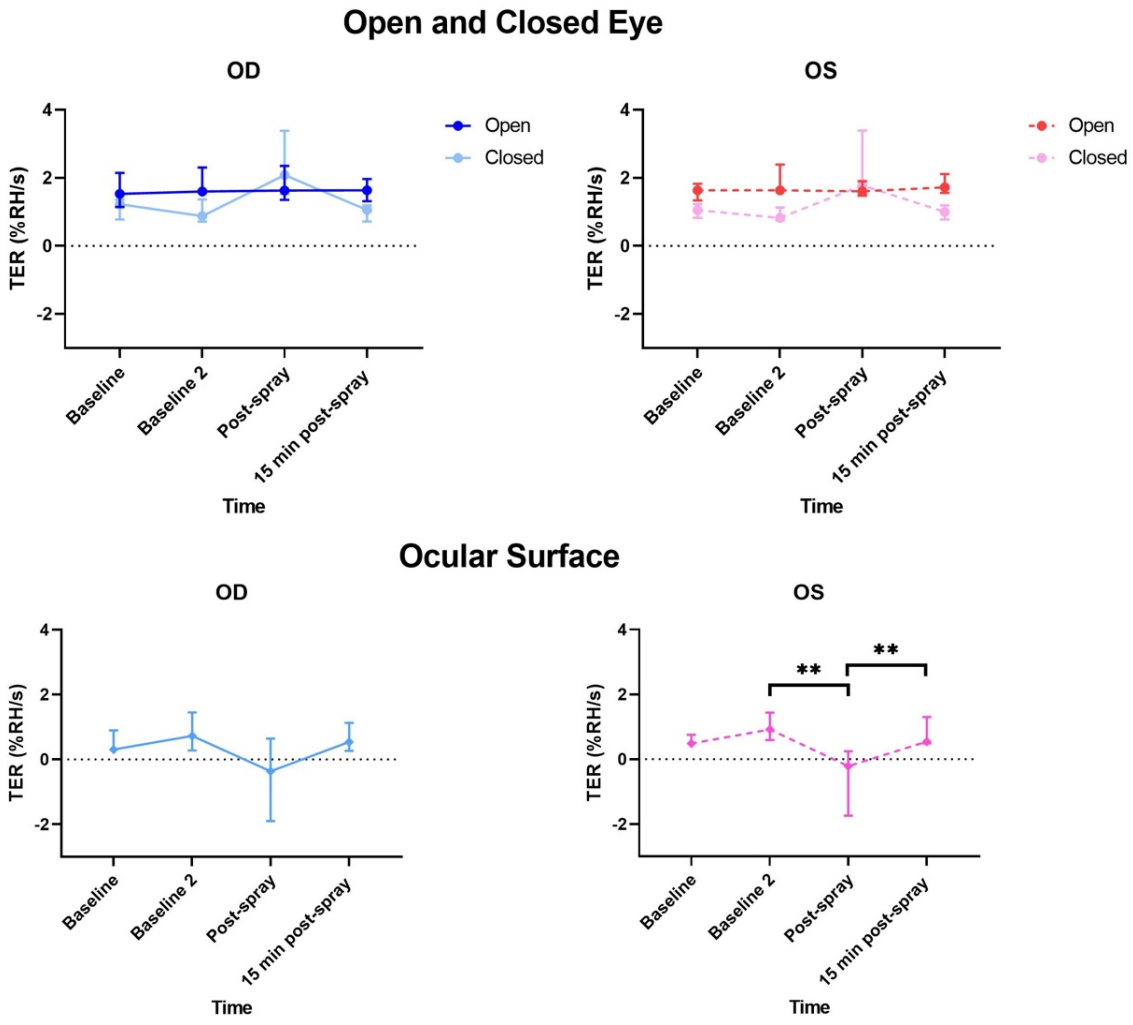

Figure 6-3: Median evaporation rate of the open eye, closed eye, and ocular surface for the right and left eye after instillation of a liposomal spray. Error bars indicate IQR. There was no significant difference in the evaporation rate of the right eye over time (open eye: p=0.724, closed eye: p=0.178, ocular surface: p=0.266) or in the left eye when it was open (p=0.095). Significant changes over time occurred in the left eye when it was closed (p=0.011) and from the ocular surface (p=0.004).

The change in TER from the ocular surface of each participant is shown in Figure 6-4. Immediately after the spray was applied, negative TERs were observed in the right eye of three volunteers and in the left eye of four volunteers.

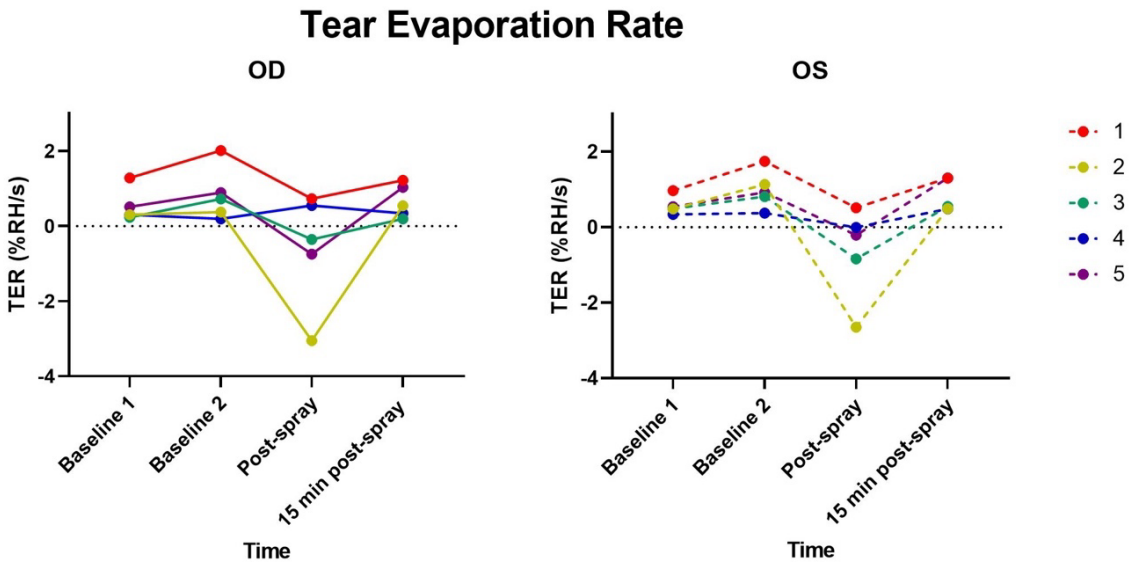

Figure 6-4: Individual plots of the mean tear evaporation rate over time of each eye before and after instillation of a liposomal spray.

Additional analysis of the TER from the ocular surface excluding the time point immediately after application of the spray did not find a significant difference in TER over time in the right eye ($p=0.549$). A significant difference in TER over time was still present in the left eye ($p=0.022$), with the first baseline measurement significantly lower than the second baseline measurement ($p=0.011$).

6.4.3 Discussion

The closed eye was investigated prior to the open eye to allow a short period of time for the liposomes to migrate into the tear film [412] while the RH returned to ambient levels. However, negative median TERs were observed in each eye immediately after use of the liposomal spray due to faster rates of evaporation occurring with the eyes closed. This is believed to be caused by the closed eye measurement being performed directly after the spray was applied to the surface of the skin. Droplets expelled from the spray will have been trapped on the skin underneath each goggle and will have been detected by the sensors as additional moisture, leading to an increase in RH over time. The significant decrease in TER that occurred after use of the spray (OS: 0.92 to −0.21 %RH/s, Figure 6-3) should not be interpreted as in improvement in the quality of the tear film or an increase in skin perspiration. Instead, it is an unintended consequence of introducing additional moisture to the skin and evaporimeter. This is likely to be why

previous researchers have waited to perform evaporimetry 10 minutes [82], 15 minutes [98], or a minimum of 30 minutes [20] after use of a liposomal spray.

No significant changes in TER were observed between baseline and 15 minutes after use of the spray. Other researchers were also unable to detect a difference in TER following a single application of TearsAgain® liposomal spray [82, 98] or with a liposomal spray and moisture chamber goggles [95].

Difficulties were encountered in trying to dispense a consistent amount of product to each volunteer, which may be why all volunteers did not have a negative TER after the spray was applied. A full pump of spray also may not have been given to each person, with some volunteers receiving two sprays when it was seemed like an inadequate amount of product had been dispensed. The direction of the nozzle was also difficult to control, which occasionally resulted in liposomal spray being applied more nasal or temporal than anticipated. In future, use of an eye drop should allow a more consistent volume to be dispensed to each individual, especially if a specific volume is pipetted into the eye.

6.5 Effect of an Artificial Lubricant

6.5.1 Materials and Methods

The same procedure as described in Section 6.4.1 was followed, with the use of Refresh Tears® (Allergan Inc., Irvine, California, USA, Table 6-6) as an ocular lubricant. The slope was calculated as the change in RH over 5 to 15 seconds.

Table 6-6: Refresh Tears® details

Ocular lubricant	Refresh Tears®
Manufacturer	Allergan Inc.
Active ingredient	Carboxymethylcellulose sodium 0.5%
Preservative	PURITE®
Health Canada Drug Identification Number #	02231008

6.5.1.1 Statistical Analysis

Statistical analysis was conducted using SPSS version 27.0. Data was tested for normality using a Shapiro-Wilk test with α=0.05. Comparison of the evaporation rate over time was analyzed with a Friedman test and a post-hoc Dunn's pairwise comparison test with Bonferroni correction or a Wilcoxon signed-ranks test. A p-value <0.05 was considered statistically significant.

6.5.2 Results

The same five non-contact lens wearers that participated in Sections 6.3 and 6.4 were examined.

6.5.2.1 Ambient Temperature and Relative Humidity

The mean ± SD ambient temperature was 23.75 ± 0.44°C (range: 23 to 24°C) and the RH was 7.10 ± 1.55% (range: 6 to 10%).

6.5.2.2 Change in Evaporation Rate over Time

A summary of median (IQR) evaporation rate from the open eye, closed eye, and ocular surface appears in Table 6-7. There was no significant change in evaporation rate over time with the eyes open (right eye: p=0.069, left eye: p=0.516, Figure 6-5). However, a significant difference in TER occurred in both eyes over time when the eye was closed (right eye: p=0.014, left eye: p=0.014). Post-hoc testing of the closed eyes revealed a significantly lower evaporation rate at the first baseline measurement compared to after instillation of the ocular lubricant (right eye: p=0.007, left eye: p=0.007), and a significantly higher evaporation rate immediately after the drop was instilled compared to 15 minutes later (right eye: p=0.003, left eye: p=0.003).

A significant change in the TER from the ocular surface occurred in both eyes over time (right eye: p=0.041, left eye: p=0.008). Post-hoc testing of both eyes showed a significantly higher TER at the second baseline measurement versus immediately after instillation of the drop (right eye: p=0.007, left eye: p=0.003), and a significantly lower TER after the drop was instilled compared to 15 minutes later in the left eye (p=0.003).

Table 6-7: Summary of median (IQR) tear evaporation pre- and post-artificial lubricant instillation

	Evaporation Rate (%RH/s)					
	Open Eye		**Closed Eye**		**Ocular Surface**	
	Right Eye	Left Eye	Right Eye	Left Eye	Right Eye	Left Eye
Baseline 1	1.40 (0.68)	1.59 (0.53)	1.11 (0.42)	1.12 (0.37)	0.15 (1.04)	0.52 (0.41)
Baseline 2	1.56 (0.90)	1.78 (0.99)	0.94 (0.58)	1.14 (0.37)	0.69 (1.07)	0.64 (0.62)
Post-drop	1.97 (0.70)	1.75 (0.90)	1.86 (0.74)	1.80 (0.74)	–0.04 (0.54)	–0.08 (0.44)
15 minutes post-drop	1.64 (0.67)	1.55 (0.32)	0.82 (0.43)	1.04 (0.48)	0.45 (0.53)	0.81 (0.55)
p-value	0.069	0.516	**0.014**	**0.014**	**0.041**	**0.008**

Bold indicates significant differences.

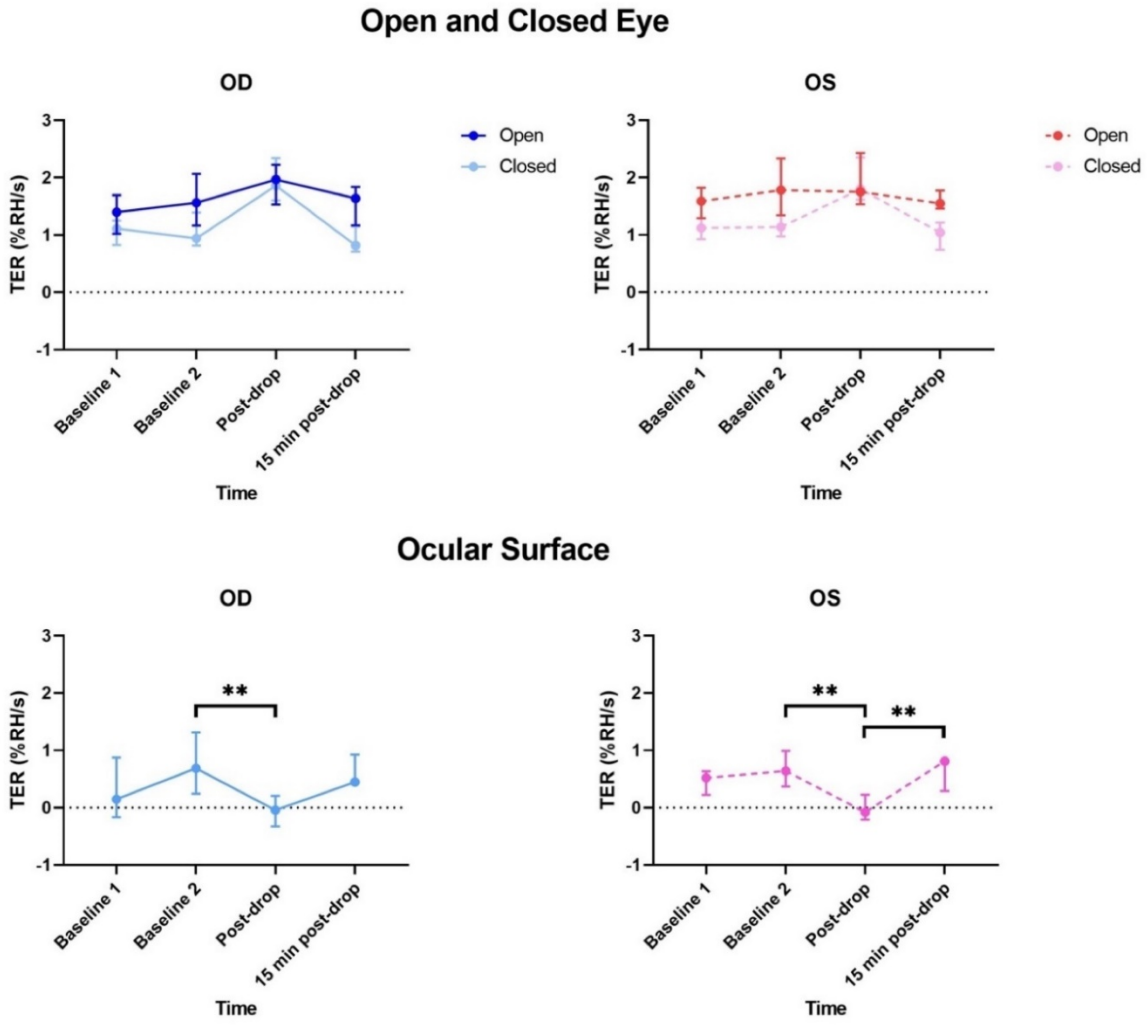

Figure 6-5: Median evaporation rate of the open eye, closed eye, and ocular surface for the right and left eye. Error bars indicate IQR. There was no significant difference in the evaporation rate of the right or left eye when it was open (right eye: p=0.069, left eye: p=0.516). Significant changes over time occurred in both eyes when they were closed (right eye: p=0.014, left eye: p=0.014) and from the ocular surface (right eye: p=0.041, left eye: p=0.008).

The change in TER from the ocular surface of each participant appears in Figure 6-6. At the first baseline measurement, negative TERs were found in two right eyes and one left eye. Negative TERs were also found in the right and left eye of four individuals immediately after the ocular lubricant was instilled.

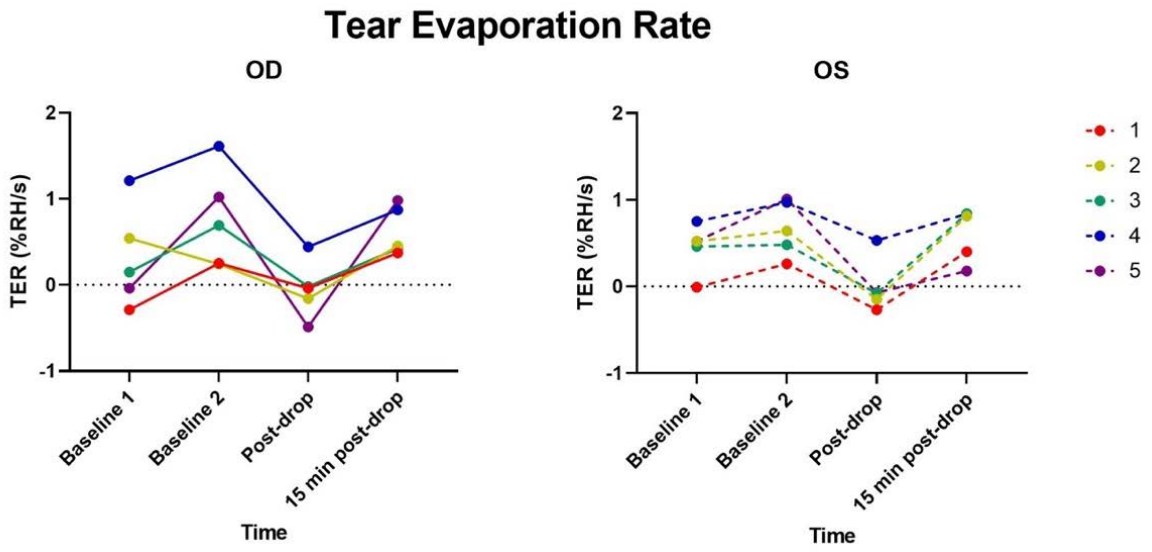

Figure 6-6: Individual plots of the mean tear evaporation rate of each eye before and after instillation of an ocular lubricant.

To exclude the negative TERs and to compare the effect of the artificial lubricant, additional analysis was conducted to compare the second baseline measurement with values obtained 15 minutes after the drop was instilled. No significant difference was found between the TER before or after the eye drop in the right eye (p=0.500) or left eye (p=0.686).

6.5.3 Discussion

Similar to the TER results reported after application of a liposomal spray (Section 6.4.2.2), some negative TERs were also obtained following a single instillation of artificial lubricants. This may be because the average size of a drop of ocular lubricant ranges from 24.5 to 53.9 µl when dispensed from a bottle held at 45° [413]. The droplet, in combination with an average tear volume of 6.2 to 7.0 µl [414], will be larger than the maximum volume of 30 µl [414] that can be retained within the conjunctival sac. Following instillation of the drop, volunteers were asked to gently dab their eyes with a tissue to remove any excess fluid prior to evaporimetry. However, not all of the liquid may have been absorbed, which could have resulted in an increased rate in evaporation during the closed eye measurement. In addition, three negative TERs also occurred at the first baseline measurement. Previous work conducted with a ServoMed Evaporimeter reported four individuals with negative TERs [415]. This was thought to be caused by a lack

of concentration during the open eye measurement and the goggle not being held in close contact to the eye, which prevented evaporation from the tear film from reaching the sensors.

The decision on whether to test the closed eye or open eye first could affect the TER if measurements are performed close to the time a product is instilled. If the open eye is initially examined following instillation of an eye drop, this could lead to faster rates of evaporation due to an increase in volume of fluid within the eye and/or a destabilized tear film. Conversely, choosing to test the closed eye first could result in negative evaporation rates, as found following the use of a liposomal spray and artificial lubricant. Therefore, evaporimetry measurements should be taken after allowing for a period of time for the tear film to stabilize and to allow excess product to be removed from and around the eye.

Tsubota and Yamada [2] reported higher TERs one minute after ocular lubricants were instilled, which returned to baseline levels at 5 or 10 minutes. Although the evaporation rate from the ocular surface was also calculated from the difference between the open and closed eye, it was not specifically stated in which order the testing was performed. Differences in the results could be due to the 10 seconds sampling rate conducted over 110 seconds, which ignored the first and last data points [2]. Rolando and Refojo [1] also reported increased TERs following a single drop of proparacaine when the closed eye was measured before the open eye. Different results could have been obtained because participants were asked not to blink when the drop was instilled, which could have maintained more of the drop within the eye, and because the eyes were held open for the entirety of the minute long open eye measurements [1]. In addition, the evaporimeter may have been insensitive to detecting the faster changes in RH that occur soon after the evaporimeter is placed over the eyes because the RH was only recorded before and after the one-minute measurement period.

The results will have been affected by the negative TERs which were included in the analysis. The values were not removed from the analysis due to the small sample size and because the aim of the chapter was to optimize the evaporimetry testing method, rather than to determine the efficacy of an ocular lubricant.

6.6 Effect of Petroleum Jelly

6.6.1 Materials and Methods

A 15-minute adaptation period to the room environment was required prior to any measurements being taken. Ambient temperature and RH were monitored with an Omega RH411 digital thermo-hygrometer. A small amount of petroleum jelly (Vaseline®, Unilever, Toronto, Ontario, Canada) was given to each

volunteer in a paper cup. Each volunteer applied a thin layer of petroleum jelly to the skin surrounding the eye using a sterile wooden cotton-tipped applicator prior to taking any measurements. Applying petroleum jelly near the lid margins was avoided to prevent contaminating the tear film.

Four sets of evaporimetry measurements were recorded at 10-minute intervals. Evaporimetry was recorded for 20 seconds with eyes open and blinking normally (OPEN EYE). Volunteers were asked rest their elbows on a 13.1 cm high computer stand (AmazonBasics, Seattle, Washington, USA, Figure 6-7A) and told to either fixate on the sensor inside the goggle or at the center of a cross-shaped fixation target (Figure 6-7B). The evaporimeter was ventilated by the investigator using a dust blower (Tronixpro, Littlehampton, West Sussex, UK) between open and closed eye measurements. After the RH returned to ambient levels, a 20 second measurement was taken with the eyes closed (CLOSED EYE). Three repeated measurements were taken at each time point and the difference in the slope between the OPEN EYE and CLOSED EYE measurements from 5 to 15 seconds were used to calculate TER of the ocular surface. The mean of three ocular surface TERs at each time point was calculated for the right and left eye.

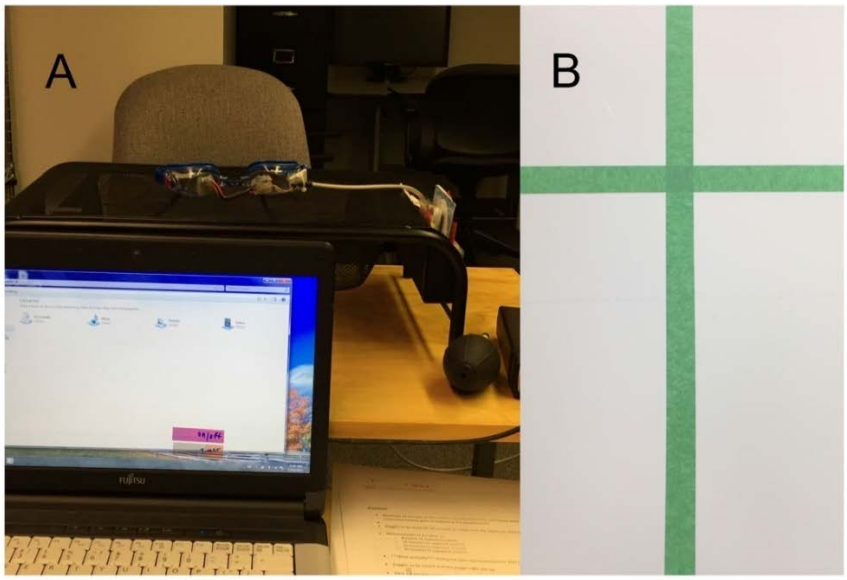

Figure 6-7: Experimental set-up for evaporimetry measurements.

Evaporimetry equipment included a computer stand and a dust blower to ventilate the evaporimeter (A). A fixation target (B) was placed between the volunteer and the laptop.

6.6.2 Statistical Analysis

Statistical analysis was performed using SPSS version 27.0. Data was tested for normality using a Shapiro-Wilk test with α=0.05. Comparison over time was analyzed with a Friedman test and an uncorrected Dunn's pairwise comparison test. A p-value <0.05 was considered statistically significant.

6.6.3 Results

The same five volunteers examined in Sections 6.3, 6.4, and 6.5 participated.

6.6.3.1 Ambient Temperature and Relative Humidity

The mean ambient temperature was 24°C and the mean ± SD RH was 10.25 ± 3.17% (range: 7 to 15%). The temperature remained stable at 24°C throughout the measurements.

6.6.3.2 Tear Evaporation Rate

A summary of the evaporation rate from the open eye, closed eye, and ocular surface appears in Table 6-8 and Figure 6-8. There was no significant difference in the evaporation rate of the open eye over time (right eye: p=0.050, left eye: p=0.668) or for the closed left eye (p=0.266). However, the evaporation rate of the closed right eye significantly changed over time (p=0.031). Post-hoc uncorrected Dunn's test showed a significantly lower evaporation rate at baseline compared to measurements taken 20 minutes (p=0.020) and 30 minutes (p=0.010) later. No significant difference in TER occurred over time from the ocular surface (right eye: p=0.564, left eye: p=0.564).

Table 6-8: Summary of median (IQR) evaporation rate from the open eye, closed eye, and ocular surface using petroleum jelly

	Evaporation Rate (%RH/s)					
	Open Eye		**Closed Eye**		**Ocular Surface**	
	Right Eye	Left Eye	Right Eye	Left Eye	Right Eye	Left Eye
Baseline	1.42 (1.04)	1.35 (0.75)	0.59 (0.31)	0.61 (0.33)	0.57 (0.99)	0.83 (0.75)
10 minutes	1.50 (1.06)	1.26 (1.00)	0.58 (0.47)	0.72 (0.35)	0.59 (0.85)	0.77 (1.06)
20 minutes	1.51 (0.97)	1.54 (0.31)	0.62 (0.48)	0.67 (0.31)	0.70 (0.85)	0.87 (0.46)
30 minutes	1.66 (0.80)	1.54 (0.65)	0.65 (0.38)	0.66 (0.29)	0.85 (0.99)	0.98 (0.66)
p-value	0.050	0.668	**0.031**	0.266	0.564	0.564

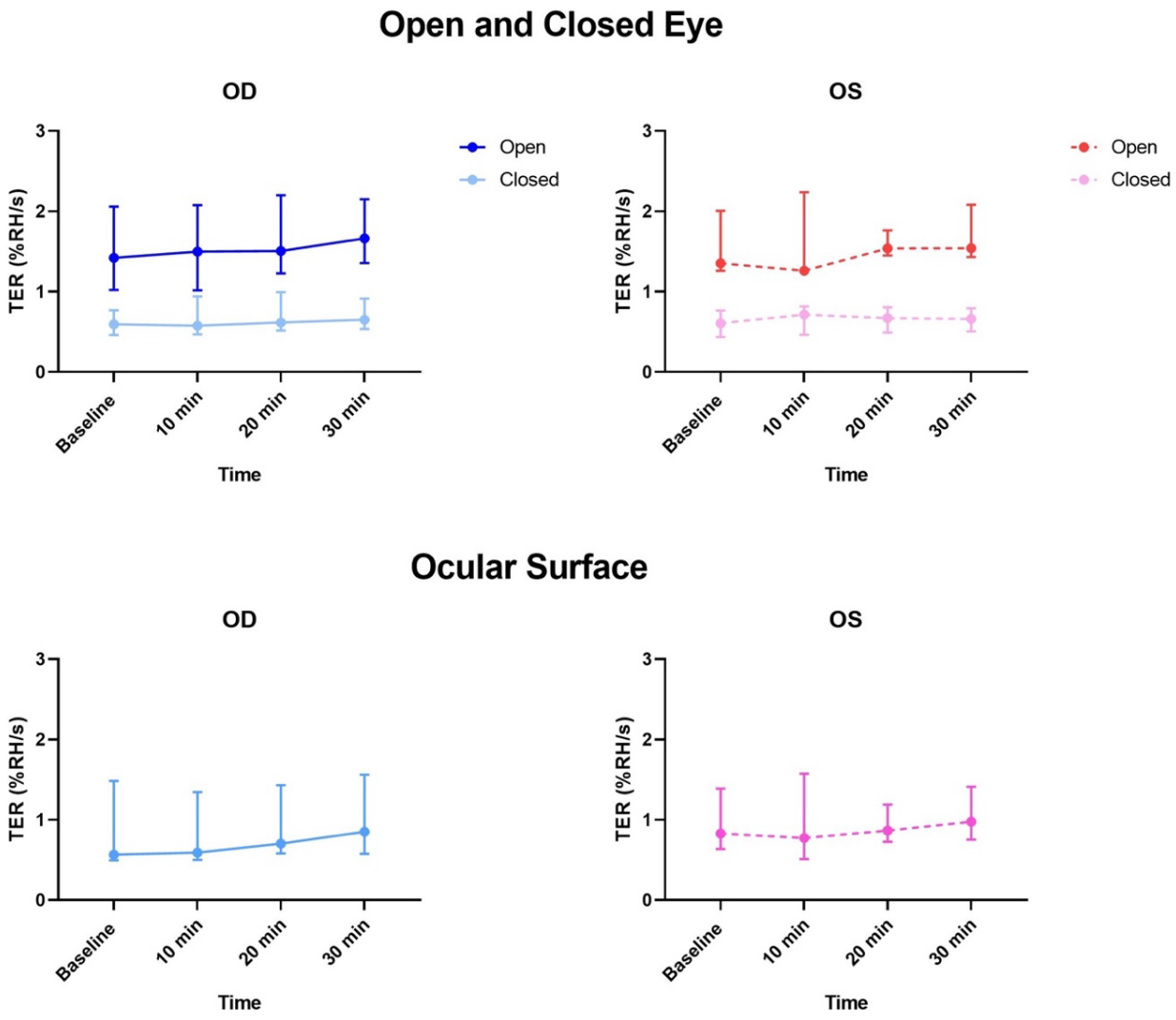

Figure 6-8: Median tear evaporation rate for each eye with the eyes open and closed (top), and from the ocular surface (bottom) after applying petroleum jelly to the skin. Error bars indicate IQR. There was no significant difference in the evaporation rate of the right or left eye when it was open (right eye: p=0.050, left eye: p=0.668). Significant changes over time occurred in right eye when it was closed (right eye: p=0.031), but not the left eye (p=0.326). No significant change occurred from the ocular surface of either eye (right eye: p=0.564, left eye: p=0.564).

The change in the ocular surface TER for each volunteer is shown in Figure 6-9.

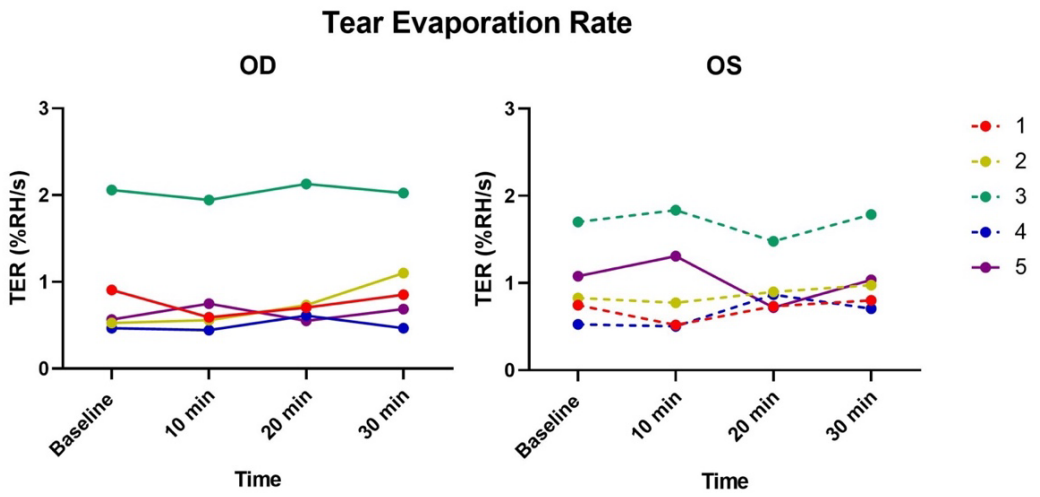

Figure 6-9: Individual plots of the mean tear evaporation rate over time for each eye after application of petroleum jelly.

6.6.4 Discussion

Repeatable evaporation rates were observed for the open eye and ocular surface of each eye, and for the left eye when it was closed. Although there was a statistically significant difference between repeated measurements recorded with the closed right eye, these changes were most likely to be due to normal variation between measurements and are unlikely to be clinically significant because the maximum difference between measurements was 0.07 %RH/s.

Temperatures below 22°C are required to prevent activation of the sweat glands [62] and petroleum jelly reduces water loss from the skin [21, 416] by up to 87.6% when placed around the eye [1]. The evaporation rate from the closed eye when covered with a thin layer of petroleum jelly was significantly lower than previous closed eye measurements taken on the same volunteers (Sections 6.4 and 6.5), even when the time point measured directly after application of the liposomal spray or artificial lubricant was excluded (CALMO® Eye Spray: right eye: $p=0.006$, left eye: $p=0.003$; Refresh Tears®: right eye: $p=0.001$, left eye: $p<0.001$).

Mathers et al. [3] reported the skin surrounding the eye accounted for 18% and 9% of the ocular surface evaporation in normal and dry eye participants, respectively. In contrast to the some of the negative baseline TERs obtained with Refresh Tears® (Section 6.5), petroleum jelly proved effective at minimizing the rate of skin evaporation [21] and all volunteers exhibited positive ocular surface TERs at each time point. Use

of petroleum jelly also made visualizing differences between individuals with high and lower TERs easier, which may improve the detection differences between non-dry eye and dry eye participants in the future. Therefore, in agreement with previous researchers [1, 3, 21], the use of petroleum jelly, in combination with measurements taken with the eyes open and closed, was determined to be the best method to measure the TER from the ocular surface.

It should be noted that a conflicting methodology was reported by Mathers. The original description of the evaporimeter in 1993 stated that petroleum jelly was used to reduce evaporation from the skin [3], whereas a 2004 review reported that petroleum jelly was not applied to the skin because it increased evaporation [175]. A typographical error could have been made in the review by reporting that petroleum jelly caused an increase in evaporation from the skin. Since detailed explanations of the methodology were provided in the original paper [3], this is believed to be the accurate description of how evaporimetry was performed.

6.7 Changes in Fixation

6.7.1 Methods

The same set-up as in Section 6.6.1 was used. Ambient temperature and RH were monitored with an Omega RH411 thermo-hygrometer. A volunteer was asked to blink normally and look downwards at the top of a laptop, straight ahead at the fixation cross, and upwards at a target on a wall. A single measurement was taken for 20 seconds with the eyes open at a sampling rate of four times per second. The percentage change in RH for each eye was calculated as the difference between the RH after 20 seconds and the initial RH when the evaporimeter was placed over the eyes. Petroleum jelly was not applied to the skin.

6.7.2 Statistical Analysis

Data was analyzed with GraphPad Prism v.8.3.0 (GraphPad Software, LLC, San Diego, California, USA). A one-way repeated measures analysis of variance was used to examine differences in fixation. Post-hoc testing was conducted with a Tukey's multiple comparisons test.

6.7.3 Results

One female soft contact lens wearing volunteer was examined with lenses *in situ.*

6.7.3.1 Ambient Temperature and Relative Humidity

The ambient temperature was 24°C and RH was 15%.

6.7.3.2 Change in Relative Humidity with Fixation

A summary of the percentage change of RH with different positions of gaze is shown in Table 6-9 and a graph of the entire measurement period appears in Figure 6-10. The change in RH was significantly different depending on the position of gaze (p=0.017, Figure 6-11). Post-hoc testing revealed a significant difference between downgaze and primary gaze (p=0.04), and downgaze and upgaze (p=0.02).

Table 6-9: Change in relative humidity with position of gaze

	Change in Relative Humidity (%)		
	Right Eye	**Left Eye**	**Mean ± SD**
Downgaze	34.76	36.10	35.43 ± 0.95
Primary gaze	40.93	41.84	41.39 ± 0.64
Upgaze	44.91	43.96	44.44 ± 0.67

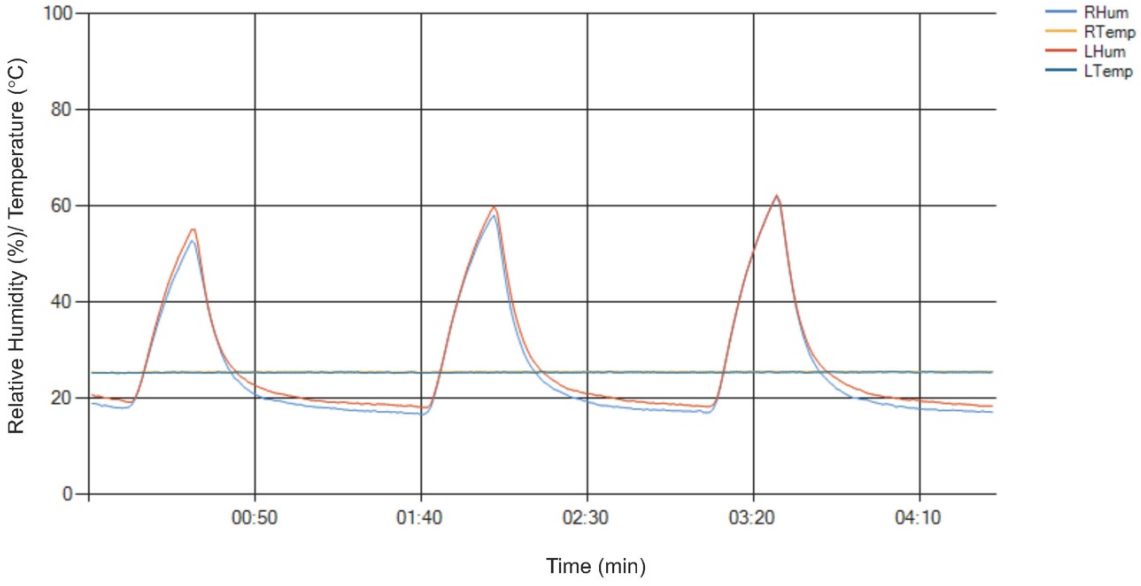

Figure 6-10: Change in relative humidity and temperature of each eye over time in downgaze (left), primary gaze (center), and upgaze (right).

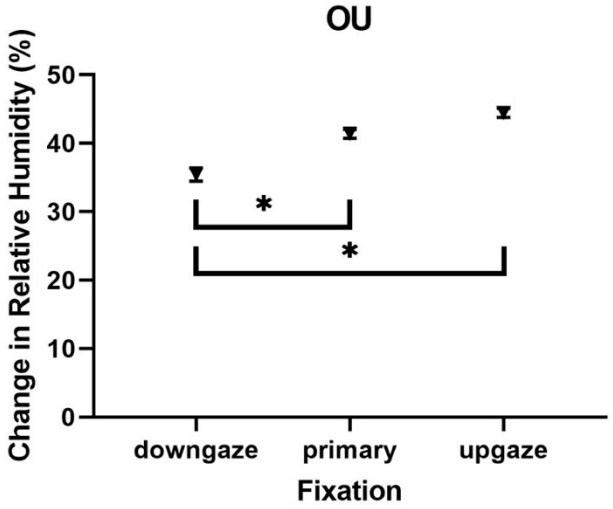

Figure 6-11: Mean change in relative humidity with different positions of gaze. Downgaze was significantly lower than primary gaze (p=0.04) and upgaze (p=0.02).

6.7.4 Discussion

The amount of change in RH between the two eyes was similar in each position of gaze, with a difference of less than the 2% accuracy of the sensors [402]. Strong positive correlations have been reported between the change in RH over 1-minute and the size of the palpebral aperture [1]. Additionally, moderate and strong positive correlations have also been described between the palpebral aperture and the TER [151], with *in vivo* testing measuring higher rates of tear evaporation as the size of the ocular surface increased [21]. However, one group was unable to find a correlation between the palpebral aperture and TER measured with and without the instillation of fluorescein [80], which could be due to a small sample size and individual variations in TER.

The results demonstrate that fixation must be controlled during evaporimetry to avoid changes in RH caused by variations in the size of the palpebral aperture.

6.8 Comparison of Different Methods of Ventilation

6.8.1 Methods and Materials

Various methods of ventilating the new evaporimeter were tested at ambient temperature and RH. An Omega RH411 digital thermo-hygrometer was used to monitor the temperature and RH inside the room.

The different ventilation methods were: (i) short or long bursts of compressed air (Gust Premium Easy Duster, Stoner, Quarryville, Pennsylvania, USA), (ii) gentle or strong bursts of air from a small Tronixpro dust blower, (iii) the highest setting on a 27 cm table fan (Sunbeam, Boca Raton, Florida, USA), and (iv) wiping each goggle with a tissue (Kimwipes, KIMTECH SCIENCE, Roswell, GA, USA, Figure 6-12). Measurements taken without the use of ventilation served as a control. The outcome measure was the length of time displayed in the "Goggle" software program required to record three consecutive 20 second measurements with the eyes open and then closed, including the time required for the RH to return to baseline levels following the final closed eye measurement. The cross-shaped target (Section 6.6.1) was used to control fixation and measurements were conducted without the addition of petroleum jelly.

Figure 6-12: Different methods of ventilating the evaporimeter.

The evaporimeter was ventilated using a dust blower, tissues, compressed air, and a table fan (right to left).

6.8.2 Results

One female contact lens wearing volunteer was examined with soft contact lenses *in situ*.

6.8.2.1 Ambient Temperature and Relative Humidity

The mean ambient temperature was 24°C and the mean ± SD RH was 13.89 ± 0.78% (range: 13 to 15%). The temperature remained stable at 24°C during the measurements.

6.8.2.2 Duration of Measurements

A summary of the measurement duration using different techniques of ventilating the evaporimeter appears in in Table 6-10. Initial testing found that long bursts of compressed air (5 minutes and 33 seconds) was faster at ventilating the instrument compared to strong (9 minutes and 8 seconds) or gentle (9 minutes and 58 seconds) bursts of air from a small dust blower. Additional testing conducted on a separate day found that a table fan was faster (6 minutes and 12 seconds) than short bursts of compressed air (7 minutes and 27 seconds) or forceful bursts of air from a dust blower (8 minutes and 6 seconds). Repeated testing with the fan was performed on a different day, where it took a similar amount of time (6 minutes 13 seconds) to ventilate the instrument. Use of the fan was faster than not ventilating the instrument (10 minutes and 57 seconds) or wiping the back surface with a tissue (12 minutes and 8 seconds).

Table 6-10: Duration of measurements using different methods of ventilation

	Time Taken to Record 3 Open and 3 Closed Eye Measurements (Including the Return to Baseline)
Compressed air[a]	5 minutes and 33 seconds
Dust blower[b]	9 minutes and 8 seconds
Dust blower[c]	9 minutes and 58 seconds
Fan	6 minutes and 12 seconds
Compressed air[d]	7 minutes and 27 seconds
Dust blower[b]	8 minutes and 6 seconds
Fan	6 minutes and 13 seconds
No ventilation	10 minutes and 57 seconds
Kimwipes	12 minutes and 8 seconds

[a] = long bursts of air; [b] = gentle bursts of air; [c] = strong bursts of air; [d] = short bursts of air.

6.8.3 Discussion

Although using long bursts of compressed air was the fastest method of ventilating the evaporimeter, the canister became too cold to touch with bare hands and did not sufficiently warm up enough to handle over the remainder of the day, which would make it an unsuitable choice for taking repeated measurements. Additional testing of the compressed air canister using short bursts of air caused small droplets of liquid to be expelled onto the lens surrounding the sensor and the canister temperature again became too cold to

touch. Therefore, due to safety reasons and because the evaporimeter can detect the additional moisture in the instrument, compressed air was eliminated as a possible method of ventilation.

The table fan was the second fastest method of ventilation and showed consistent measurement times over two days. The change in temperature over the entire measurement period with the fan was 0.54°C compared to 1.07°C when ventilation was not used; hence, the fan was a more efficient method of ventilating and controlling the temperature within the evaporimeter. Forced ventilation of the instrument also allows the investigator to ensure that the RH has returned to baseline prior to starting the next measurement, which was a criticism of the VapoMeter when exposed to conditions of high water loss because it automatically allows a new measurement to be taken after 2 minutes [60].

6.9 Effect of Blink Rate

6.9.1 Materials and Methods

The same experimental set-up as described in Section 6.6.1 was used. Ambient room conditions were measured with an Omega RH411 digital thermo-hygrometer. Volunteers fixated on the center of a cross-shaped target and were asked to blink every three (https://www.youtube.com/watch?v=9ypeNJJeKIs) or five seconds (https://www.youtube.com/watch?v=TD4EIr85A5A) in response to the sound of a metronome. Two rounds of 20 second measurements were performed with the eyes open, with testing alternating between the three and five second blink rate. The RH inside the evaporimeter returned to ambient levels prior to starting the next measurement without the use of any ventilation. Petroleum jelly was not applied to skin. Volunteers were asked to report any reflex tearing and were asked to choose which blink rate was preferable.

6.9.2 Results

Three female volunteers (1 habitual soft contact lens wearer, 1 occasional soft contact lens wearer, 1 non-contact lens wearer) with self-reported dry eye were tested. Measurements were performed on the habitual lens wearer while wearing contact lenses.

6.9.2.1 Ambient Temperature and Relative Humidity

The mean ± SD ambient temperature was 23.33 ± 0.58°C (range: 23 to 24°C) and RH was 41.33 ± 0.58% (range: 41 to 42%).

6.9.2.2 Preferred Blink Rate

None of the volunteers reported reflex tearing during the measurements. One volunteer preferred the three second blink rate, while the remaining two volunteers preferred blinking at a five second interval. Although reflex tearing did not occur, one person reported needing to partially blink during the five second blink rate measurements and another said that extra blinks were needed following the five second blink rate in order to try and restore the tear film.

6.9.3 Discussion

People suffering from dry eye have an average inter-blink rate of 1.5 [417] to 2.56 [418] seconds, whereas those without dry eye have a longer interval of 4.0 [417] to 5.97 [418] seconds. Rolando and Refojo [1] reported that a one minute blink interval was an acceptable amount of time for a participant not to blink when shielded from the external environment by a goggle. However, Tsubota and Nakamori [151] found that blinking less than every 6 seconds could induce reflex tearing and volunteers in this study required significantly shorter inter-blink intervals than one minute to avoid discomfort.

Despite the majority of participants preferring a five second blink interval, the blink interval for further testing will be set at three seconds because all volunteers did not feel comfortable keeping their eyes open for longer periods of time. Choosing the shorter blink interval should hopefully permit people with moderate to severe dry eye to be tested in low RH conditions without inducing reflex tearing. This may also allow for comparisons to be made with previous evaporimetry work [7, 13, 109, 151] that was conducted at a three second blink rate.

Although the testing the blink rate interval in a random order would have been ideal, this was not chosen in case the longer blink interval resulted in reflex tearing, which would have affected any subsequent measurements.

6.10 Future Work

6.10.1 Possible Sources of Error

The sample sizes and number of measurements conducted in this chapter were small, in particular when only a single participant was tested with one set of measurements. In addition, although the reported typical tolerance level of the sensor is a consistent ±2% from 0 to 100% RH, low ambient RH conditions were encountered during testing, which were outside the manufacturer's suggested optimal range of 20 to 80%

RH [402]. A humidifier was not used to increase the ambient RH within the testing room because it would be ineffective at significantly changing the RH within the large room. Future work could be conducted in a smaller room with the addition of a humidifier, although the placement would need to be far enough away from the participant and evaporimeter to prevent any excess moisture from being detected by the instrument.

6.10.2 Future Work

Additional testing of the optimized methodology is required on a larger sample size to assess the ability of the instrument to detect changes in tear evaporation. A range of participants should be investigated to determine if the instrument can be used on adults of different ages, including male and females. In order to investigate the ability of the instrument to detect evaporative dry eye, contact lens wearers and participants with dry eye should also be examined.

6.11 Conclusions

Preliminary testing of the novel evaporimeter confirmed that each goggle was able to detect a change in RH when placed over an open and closed eye. The plot of the change in RH over time showed an initial period of approximately 5 seconds where the RH remained stable, before the RH changed at a faster rate.

Testing of the liposomal spray and artificial lubricant revealed negative TERs following application of the dry eye products. This is believed to be due to the decision to measure the closed eye evaporation rate immediately after use of the dry eye product. Since a sufficient period of time was not permitted to allow excess moisture to evaporate from the skin, the evaporation rate of the skin increased at a faster rate than the ocular surface. Future evaporimetry measurements will need to wait for the tear film to stabilize and the skin evaporation rate to recover following use of a dry eye product.

Some negative TERs were also encountered during baseline testing of the artificial lubricant, which may be due to faster rates of evaporation from the skin or poor fixation. To reduce the evaporation rate of the skin, a thin layer of petroleum jelly was applied to the skin surrounding the eye. This method provided repeatable results and eliminated negative rates of tear evaporation. Testing the change in RH over time in different positions of gaze resulted in significantly slower changes in RH in downgaze compared to primary gaze or upgaze. Further work will ensure fixation is controlled during open eye measurements. The evaporimeter will also be ventilated with a fan to control the temperature within the device and ensure that excess moisture has been eliminated from the system prior to the next measurement. Future work will also

calculate the TER from the ocular surface as the difference between the open eye and closed eye measurement, with petroleum jelly used to decrease the skin evaporation rate.

Chapter 7

Comparison of Tear Evaporation Rate with Systane® Complete in Dry Eye and Non-Dry Eye

7.1 Overview

PURPOSE: To investigate the ability of a novel binocular evaporimeter to produce a tear evaporation rate (TER) that can: provide repeatable results; differentiate between dry eye and non-dry eye; and detect changes following the instillation of an artificial lubricant.

METHODS: This was a prospective, bilateral eye, non-dispensing, pilot study. Thirty-six non-contact lens wearers were enrolled and screened for inclusion at a screening visit. Twenty-one participants were suitable and classified using the Ocular Surface Disease Index (OSDI) and non-invasive break-up time as either non-dry eye or dry eye (10 non-dry eye, 11 dry eye). At the test visit, two baseline TERs were taken, 20 minutes apart. A single dose (15 µl) of Systane® Complete was then instilled, and the TER assessed at 3 further time points post-instillation: 10 minutes, 30 minutes, and 60 minutes. Three repeated evaporation measurements were recorded for 20 seconds with the eyes open and closed to produce an average TER at baseline and at each time point. The primary outcome variable was TER, calculated from the slope of the change in relative humidity over time while the evaporimeter was placed over the eyes (open eye: 7 to 17.5 seconds; closed eye: 10 to 17.5 seconds). TER from the ocular surface was calculated as the difference between the closed eye and open eye TER.

RESULTS: Twenty people (10 non-dry eye, 10 dry eye) completed the study (non-dry eye: median age: 25.2 years, 70% female; dry eye: median age: 45 years, 90% female). Baseline TER measurements were repeatable in each eye of the non-dry eye group (right eye: p=0.216, left eye: p=0.260) and dry eye group (right eye: p=0.537, left eye: p=0.358). The right eye of the dry eye group had a significantly higher TER than the non-dry eye group at the second baseline measurement (p=0.022). Changes in TER over time were detected for both eyes. The TER of the left eye of the non-dry eye group was significantly higher 10 minutes after the eye drop was instilled compared to the second baseline measurement (p=0.048). The TER of the non-dry eye group was also significantly higher at 10 minutes compared to 30 minutes after the drop was instilled (right eye: p=0.022, left eye: p=0.045). The right eye of the dry eye group had a significantly higher TER at the second baseline measurement compared to 30 minutes after the eye drop was instilled (p=0.038).

CONCLUSIONS: The binocular evaporimeter was able to:

- Provide repeatable baseline TER measurements for each eye;
- Detect a higher TER in dry eye participants compared to non-dry eye participants;
- Detect a change in TER in each side of the evaporimeter after instillation of Systane® Complete;
- The dry eye group had a statistically significant decrease in TER 30 minutes after instillation of Systane® Complete, which suggests that a short-term improvement (reduction) in tear evaporation can occur following instillation of the artificial lubricant.

Preliminary testing of the evaporimeter shows that the instrument can be used to detect dry eye and monitor changes in response to dry eye treatment.

7.2 Introduction

As a result of the multifactorial nature of the disease, dry eye can be diagnosed using various clinical tests including tear break-up time (TBUT), ocular surface staining, or osmolarity [143]. No single test can diagnose dry eye disease and its sub-type; therefore, a variety of supplemental tests are available for a practitioner to use. Following *in vitro* testing of the evaporimeter and optimization of the method used to record evaporimetry measurements, additional testing of the instrument was required to investigate whether the device can be used to diagnose dry eye disease and whether it can be reliably used to test the efficacy of a treatment for dry eye.

Due to the fact that *in vitro* testing was conducted using only distilled water and that an absent lipid layer leads to a higher tear evaporation rate (TER) [48, 49], the rate of evaporation measured from an intact human tear film is expected to be different because of its complex nature and its ability to be replenished with each blink. Because of these anticipated differences between *in vitro* and *in vivo* work, the optimal time period for calculating the slope using the finalized *in vivo* evaporimetry methodology must be determined.

Following calculation of the *in vivo* TER, the potential application of the novel evaporimeter to the field of dry eye can be examined. Although many clinical tests for dry eye have either poor or fair repeatability [419], the evaporimeter should be able to provide repeatable results if it is to be used in a clinical setting. In addition, it must also be able to distinguish between people with and without dry eye disease to be used as a screening tool, and it must be able to detect changes in TER in response to a dry eye treatment.

The 2017 TFOS DEWS II Management and Therapy report [140] recommended ocular lubricants as one of the first step treatments for the management of dry eye. Systane® Complete Lubricant Eye Drops (Alcon,

Fort Worth, Texas, USA) is a propylene glycol-hydroxypropyl-guar nano-emulsion [420] released in 2018 and is designed to treat all three sub-types of dry eye [420, 421]. To date, none of the peer-reviewed literature on the *in vivo* efficacy of Systane® Complete [391, 420-422] have included evaporimetry as one of the clinical tests. Therefore, a further objective of this study is to investigate whether the novel binocular evaporimeter can assist in the diagnosis of dry eye disease, and to assess its ability to evaluate the efficacy of a drop-based dry eye treatment.

The aims of the study are summarized as to:

- Evaluate whether the instrument can provide repeatable baseline results;
- Test whether the instrument can differentiate dry eye and non-dry eye;
- Evaluate whether a change in TER occurs following instillation of Systane® Complete.

7.3 Materials and Methods

This study was a prospective, bilateral eye, non-dispensing, non-randomized pilot study, involving one screening and one test visit.

7.3.1 Participant Recruitment

The study was designed to follow the ethical principles in the Declaration of Helsinki, along with the International Council for Harmonization: Good Clinical Practice, the University of Waterloo Guidelines for Research with Human Participants, and the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans, 2nd edition. The study received ethics clearance from the Office of Research Ethics at the University of Waterloo, Waterloo, Ontario (ORE #41327) and was registered with ClinicalTrials.gov (ID: NCT04091581). The study was advertised using the recruitment system at the Centre for Ocular Research & Education (CORE) at the University of Waterloo.

7.3.2 Inclusion and Exclusion Criteria

Participants were eligible for inclusion in the study if they: were at least 18 years of age and had the full legal capacity to volunteer; had read and signed the information consent letter; were willing and able to follow instructions and maintain the appointment schedule; were willing to be awake for at least 2 hours before to the test visit; were willing to not wear eye make-up on the day of the test visit; were willing to not use eye drops or artificial lubricants on the day of the screening visit or test visit, and if they met the group specific criteria at the screening visit for the:

- **Dry eye group**: Ocular Surface Disease Index (OSDI) ≥13 and non-invasive Keratograph break-up time (NIKBUT) ≤5 seconds in the worst eye;
- **Non-eye group**: OSDI <13 and NIKBUT ≥10 seconds in the worst eye.

Participants were excluded from the study if they: were participating in any concurrent clinical or research study; had any known active ocular disease and/or infection[1]; had a systemic condition that in the opinion of the investigator may affect a study outcome variable; were using any systemic or topical medications that in the opinion of the investigator may affect a study outcome variable; were pregnant, lactating, or planning a pregnancy at the time of the enrolment; were aphakic; had undergone refractive error surgery; had undergone ocular surgery in the last 6 months; had punctal plugs; had a known sensitivity to sodium fluorescein dye; had a known sensitivity to any one of the range of Systane® eye drops; had a known sensitivity to petroleum jelly (Vaseline®); had epilepsy and/or sensitivity to flashing lights; had worn contact lenses within the past month or were planning to wear contact lenses during the study; had any physical impairment that would interfere with holding the evaporimeter, or had taken part in another clinical research study involving ocular drops or treatments within the past 14 days.

7.3.3 Study Design

Informed consent was obtained from all participants prior to enrolment. Two scheduled visits were conducted on separate days. A summary of the clinical tests that were conducted is included in Table 7-1. Briefly, symptomology was assessed at the screening visit using the OSDI score. Participants were classified as asymptomatic if the OSDI score was <13 and symptomatic if the score was ≥13, as per the TFOS DEWS II screening guidelines for dry eye [143]. The median NIKBUT of the worse eye was used to assess homeostasis markers. Participants who did not meet the criteria in Section 7.3.2 to be classified as either dry eye or non-dry eye based on a modified TFOS DEWS II definition of dry eye [143] were discharged from the study. Participants who were eligible to continue underwent additional tests including slit-lamp biomicroscopy, and assessment of ocular surface area and air volume within the evaporimeter.

[1] For the purposes of this study, active ocular disease was defined as infection or inflammation which requires therapeutic treatment. Mild (i.e. not considered clinically relevant) lid abnormalities (blepharitis, meibomian gland dysfunction, papillae), corneal and conjunctival staining and dry eye are not considered active ocular disease. Neovascularization and corneal scars are the result of previous hypoxia, infection, or inflammation and are therefore not active.

The test visit was scheduled to occur between 1 to 14 days after the screening visit. Subjective comfort ratings, tear meniscus height, evaporimetry, NIKBUT, and objective lipid layer thickness (LLT) assessments were measured at 5 time points before and after the eye drop was instilled: two baseline measurements were taken 20 minutes apart, followed by measurements at 10, 30, and 60 minutes after Systane Complete® was instilled (Figure 7-1). All measurements were recorded for both eyes.

Table 7-1: Summary of procedures and instruments

Visit	Testing order	Procedure	Instrument
Screening	1	Informed consent	N/A
	2	Demographics	N/A
	3	Medical history	N/A
	4	Symptoms assessment	OSDI
	5	Entrance visual acuity	Electronic logMAR chart
	6	Tear meniscus height	Keratograph® 5M
	7	Non-invasive break-up time	Keratograph® 5M
	8	Lipid layer thickness	EASYTEAR®view+
	9	Meibomian gland expression	Meibomian Gland Evaluator
	10	Lid margin assessment	Slit-lamp
	11	Biomicroscopy, including corneal and conjunctival staining	Slit-lamp and fluorescein
	12	Meibography	Keratograph® 5M
	13	Anterior eye photographs	Slit-lamp, Canon EOS 60D
	14	Evaporimetry	Evaporimeter
	15	Goggle volume measurement	Modified swimming goggles
	16	Biomicroscopy safety check, including corneal and conjunctival staining	Slit-lamp and fluorescein
	17	Exit visual acuity	Electronic logMAR chart
Test visit	1	Entrance visual acuity	Electronic logMAR chart
	2	Biomicroscopy (without staining)	Slit-lamp
	3[a]	Subjective comfort rating	N/A
	4[a]	Tear meniscus height	Keratograph® 5M
	5[a]	Evaporimetry	Evaporimeter
	6[a]	Non-invasive break-up time	Keratograph® 5M
	7[a]	Lipid layer thickness	LipiView® II
	8	Biomicroscopy, including corneal and conjunctival staining	Slit-lamp and fluorescein
	9	Exit visual acuity	Electronic logMAR chart

[a]Two baseline measurements, followed by measurements at 10, 30, and 60 minutes after the eye drop was instilled.

N/A: not applicable.

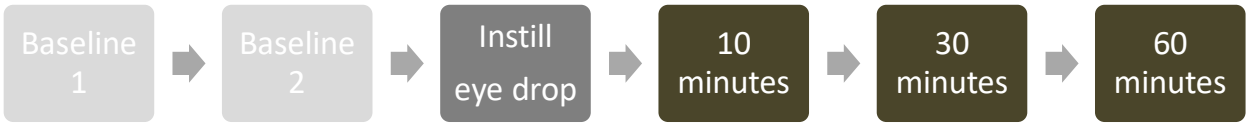

Figure 7-1: Measurement time points at the test visit.

7.3.4 Study Procedures

Subjective and objective clinical tests were performed at the study visits by a single investigator (SW). Data was manually recorded on clinical record forms. A description of the procedures that were conducted is provided below.

7.3.4.1 Demographics

Demographic information was recorded for each participant, including age and sex.

7.3.4.2 Medical History

Medical history was obtained at the screening visit to record current medications, dry eye treatments, allergies, and other relevant medical conditions. At the test visit, participants were asked about any changes in their medication or medical condition.

7.3.4.3 Visual Acuity

Distance visual acuity was measured using a computerized high-contrast logMAR chart (Clinical Trial Suite, M&S® Technologies, Niles, Illinois, USA) under high ambient room illumination. Visual acuity was tested with the participant's habitual spectacle correction (if required).

7.3.4.4 Ocular Surface Disease Index

All participants completed the OSDI questionnaire [423] at the screening visit to assess how often various symptoms (e.g. light sensitivity, gritty eyes, etc.) were experienced while performing different tasks (e.g. reading, driving at night) or in different environments (e.g. windy conditions, places with low humidity, etc.) over the previous week. Symptoms were rated on a scale of 0 ("none of the time") to 4 ("all of the time").

7.3.4.5 Subjective Comfort Ratings

Participants were asked to fill out a numerical analog scale regarding their comfort, dryness, and burning or stinging for each eye on a 0–100 scale. A score of 0 indicated the eyes were uncomfortable, dry, or stinging or burning, while 100 represented an eye that was comfortable, not dry, or not experiencing any stinging or burning.

7.3.4.6 Tear Meniscus Height

Tear meniscus height (TMH) was measured from a photograph of the tear meniscus taken with the OCULUS Keratograph® 5M (K5M) (OCULUS Optikgeräte GmbH, Wetzlar, Germany) using the proprietary software. The TMH for each eye was calculated from the average of a single measurement of the central and peripheral TMH.

7.3.4.7 Tear Film Stability

Tear film stability was assessed using the NIKBUT measurement of the K5M. Participants were asked to blink twice and then to keep their eyes open for as long as they could. The K5M software automatically detected the location of the first distortion in the reflection of the instrument's Placido rings from the tear film, and if no break-up was detected would stop measuring after ~25 seconds. Three measurements of the break-up were taken for each eye and the median value used.

7.3.4.8 Grading of Tear Film Lipid Layer Thickness

Illumination from the EASYTEAR®view+ (EASYTEAR s.r.l., Trento, Italy) was used to evaluate the LLT. The observed lipid pattern was graded using Guillon and Guillon's classification system [424] (1: open meshwork, 2: closed meshwork, 3: wave, 4: amorphous, 5: colour fringe, and 6: other).

7.3.4.9 Meibomian Gland Expression

Patency of the lower eyelid meibomian glands was assessed using the Meibomian Gland Evaluator (MGE) (Johnson & Johnson Vision Care, Inc., Jacksonville, Florida, USA) [425]. The MGE was depressed halfway to apply pressure to the lower eyelid just below the lash line. The MGE was applied to three separate areas (nasal, central, temporal) on the lid and five consecutive glands in each area were assessed for the quality and quantity of expression. The quality of expression was graded using a modified 0-4 scale based on Bron and Snibson [426] (0: clear fluid, 1: cloudy fluid, 2: cloudy particulate fluid, 3: inspissated, like toothpaste, 4: waxy, inexpressible). The total expression for each eye was summed. The number of

meibomian glands yielding lipid secretion was also graded for each eye ((0: >75% (almost all), 1: 50–75% (more than half), 2: 25–50% (less than half), 3: <25% (only a few), and 4: ~0% (close to none)).

7.3.4.10 Lid Margin Assessment

The lid margin was assessed for vascularity (erythema), amount of lash loss, edema, telangiectasia, and tear film debris (Appendix B). The vascularity of the lid margin and telangiectasia were graded on a 0–4 scale. The presence or absence of lid margin edema and tear film debris were also recorded.

7.3.4.11 Slit-lamp Biomicroscopy

A slit-lamp biomicroscopy examination was conducted to assess anterior segment ocular health. Ocular findings, including external adnexa anomalies, bulbar and limbal hyperemia, the presence of scars or infiltrates, endothelium abnormalities, and anterior chamber cells and flare were recorded. Corneal and conjunctival staining were assessed using a DIOFLUOR sodium fluorescein strip (DIOPTIC Pharmaceuticals Inc, Toronto, Ontario, Canada). The fluorescein strip was wetted with a few drops of Sensitive Eyes® Saline Plus Solution (Bausch + Lomb, Rochester, NY, USA) and applied to the superior-temporal bulbar conjunctiva of both eyes. Staining was illuminated using cobalt blue light and viewed through a yellow Wratten #12 filter (Appendix B). Palpebral conjunctiva hyperemia was assessed under white light and the presence of papillae was assessed using either white light or blue light with a yellow Wratten #12 filter, depending on whether fluorescein was to be instilled in the eye, and graded with a 0–4 scale (Appendix B).

7.3.4.12 Meibography

The K5M was used to image the meibomian glands of the upper and lower eyelids of each eye. The lower lid was everted with the investigator's thumb to expose as much of the palpebral conjunctiva as possible and the upper eyelid was everted with a cotton-tipped applicator. The amount of missing glandular tissue was graded using the 0–3 scale by Arita et al. [210] (Appendix B). The grade of the lower eyelid and upper eyelid was summed for each eye to calculate the total meiboscore.

7.3.4.13 Ambient Temperature and Relative Humidity

The ambient temperature and relative humidity (RH) inside the room where the clinical tests were performed was monitored using an EXTECH RHM15 mini hygro-thermometer (FLIR Commercial

Systems Inc., Nashua, New Hampshire, USA). The temperature and RH were recorded at each time point after the participant completed their subjective comfort ratings.

7.3.4.14 Application and Removal of Petroleum Jelly

Petroleum jelly (Vaseline®, Unilever, Toronto, Ontario, Canada) was removed from its original container at the test visit using a cotton-tipped applicator and placed in a paper cup. A new cotton-tipped applicator and mirror were given to each participant to assist them in applying a thin layer of petroleum jelly to the skin surrounding the eye. Participants were advised to take care when applying the petroleum jelly to avoid the eyelid margin area, which might lead to contamination of the tear film. Petroleum jelly was applied prior to the first set of baseline measurements and before the 10 minutes post-eye drop measurements. To remove the petroleum jelly, the participant was asked to close their eyes and wipe with a tissue in a nasal to temporal direction. Participants were advised to be careful when wiping near the lid margin to avoid contaminating the tear film. Petroleum jelly was removed after the second set of baseline measurements and after the 60 minutes post-eye drop measurements.

7.3.4.15 Evaporimeter Measurements

7.3.4.15.1 Evaporimetry

The height of a table was adjusted so that the participant could comfortably rest their elbows on top of a table (Figure 7-2). The participant initially held the pair of modified swimming goggles (Figure 7-3) over their eyes for 20 seconds while both eyes were open (OPEN EYE). The participant was asked to fixate on the center of a cross-shaped target and was prompted to blink every three seconds under the guidance of a metronome (https://www.youtube.com/watch?v=9ypeNJJeKIs). After 20 seconds, the goggle was removed and ventilated by the investigator in front of a 7-inch foldable fan (MAINSTAYS, China) until the RH returned to ambient baseline levels. The fan was enclosed in a box to prevent drafts of air from affecting the participant's tear film. The evaporimeter was then placed over the closed eyes for 20 seconds to measure evaporation rate from the skin (CLOSED EYE). Three consecutive series of both OPEN EYE and CLOSED EYE measurements were taken at each study time point. The temperature and RH were recorded in 0.25 second increments with the "Goggle" software program "Goggle" software (Mr. Ehsan Zare-Bidaki, MLEO, Waterloo, Ontario, Canada, Section 4.3.2) and saved as a word document. The rate of tear evaporation from the OPEN EYE was calculated from the slope of the change in RH from 7 to 17.5 seconds (Appendices C and D). The TER from the CLOSED EYE was calculated from the change in RH for the

period 10 to 17.5 seconds after the evaporimeter was held over the eyes (Appendices C and D). The TER from the ocular surface was calculated by subtracting the TER of the CLOSED EYE from the OPEN EYE TER. The average of three ocular surface evaporation rates was calculated for each time point for the right and left eye.

Figure 7-2: Experimental set-up for evaporimetry measurements.

Evaporimetry instrumentation included an adjustable height table (A), hygro-thermometer (B), a fixation target (C), the evaporimeter (D), a laptop computer (E), and a box containing a fan (F).

Figure 7-3: *In vivo* use of the evaporimeter.

The evaporimeter was held over the eyes to create a tight seal during the measurement.

7.3.4.15.2 Volume Inside the Evaporimeter

The participant was seated in an upright consulting chair and asked to rest their head against a headrest. Participants placed a pair of modified Arena Zoom X-Fit (Arena Distribution SA, Lugano, Switzerland) swimming goggles over their eyes and adjusted the strap to fit their head. The modified swimming goggles had a small hole in the top of each eyepiece to allow liquid to be added into the eyepiece chamber (Figure 7-4). Sensitive Eyes Saline Plus® Solution was withdrawn into a 30 ml plastic syringe (BD, Franklin Lakes, New Jersey, USA) using a 200 µl pipette tip and the starting volume of saline in the syringe was recorded in ml. Participants were asked to close their eyes, and saline was then added into the eyepiece chamber until it was filled with saline. The difference between the starting and ending volume of saline in the syringe was used to determine the volume inside each eyepiece. The ending volume of saline in the syringe was recorded to the nearest ml.

Figure 7-4: View of the top of the modified swimming goggles.

The arrows indicate the location of the small holes drilled into eyepiece chamber through which saline was added to allow the chamber volume to be measured.

7.3.4.16 Anterior Eye Photographs

The anterior eye was photographed using a Canon EOS 60D (Canon, Tokyo, Japan) digital camera attached to a Zeiss slit-lamp. A photograph of each eye was taken at 5x magnification with additional external illumination provided by a Canon Macro Twin Lite flash. The participant held a ruler underneath each eye while the photographs were taken to provide a calibration reference. The images were analysed with ImageJ (ImageJ 1.52k, National Institutes of Health, USA) using the Polygon tool to trace along the boundary of the upper and lower eyelids. The area enclosed within the upper and lower eyelids was considered to be the size of the exposed ocular surface in cm^2.

7.3.4.17 Objective Lipid Layer Thickness

The LipiView® II Ocular Surface Interferometer (Johnson & Johnson Vision Care, Inc., Jacksonville, Florida, USA) was used to assess tear film LLT. The software automatically computes the average, minimum, and maximum LLT. Participants were asked to blink freely during the approximately 30 seconds recording.

7.3.4.18 Instillation of Lubricating Drops

A single bottle of Systane® Complete (Alcon, Fort Worth, Texas, USA) (Table 7-2) was used as the source for all instilled drops in the study. The bottle was shaken and 2 drops were transferred to a 0.5 ml microtube (Eppendorf, Hamburg, Germany). The participant was asked to sit in a consulting chair with their head against the headrest and to tilt their chin upwards. While looking up and towards their nose, the investigator pulled down the lower lid to pipette 15 µl of Systane® Complete into the inferior-temporal fornix using a 2–20 µl micropipette (Eppendorf, Hamburg, Germany). The participant was asked to blink their eyes after the eye drop was instilled. Drops were first instilled in the right eye, followed by the left eye.

Table 7-2: Systane® Complete details

Lubricating drop	Systane® Complete
Manufacturer	Alcon
Active ingredient	Propylene glycol 0.6%
Preservative	POLYQUAD® 0.001%
Health Canada License #	100469
Device class	2

7.3.5 Statistical Analysis

A sample size calculation could not be determined for this pilot study because this was the first time the evaporimeter was tested on a cohort of non-contact lens wearers. Statistical analyses were conducted using GraphPad Prism v.8.3.0 (GraphPad Software, LLC, San Diego, California, USA). Data was tested for normal distribution using the Shapiro-Wilk test with α=0.05. Differences between baseline measurements were analyzed with a paired t-test. A three-way mixed analysis of variance (ANOVA) was used to determine the interaction between time, eye, and group. Two-way repeated measures ANOVAs were used to examine the interaction between time and eye for each group. Differences between groups were analyzed with a Mann-Whitney test for non-parametric data or an unpaired t-test for normally distributed data. Differences between eyes were analyzed with a Wilcoxon signed-rank test for non-parametric data or a paired t-test for normally distributed data. Changes over time were analyzed with a Friedman test and a post-hoc Dunn's test for non-normally distributed data, or with a one-way RM ANOVA for normally distributed data and a post-hoc Tukey's multiple comparisons test. Pearson's correlation coefficient (r) or Spearman's rank correlation (r_s) were used to determine the linear correlation between the TER and the other clinical tests. A p-value <0.05 was considered statistically significant. Post-hoc power (α=0.05) and

a priori sample size calculation (effect size=0.59, α=0.05, power=0.8) was conducted using G*Power v. 3.1.9.4 (Heinrich Heine Universität Düsseldorf, Düsseldorf, Germany) [145].

7.4 Results

7.4.1 Demographics

Thirty-six participants were screened between November 2019 and February 2020. Twenty-one participants (10 non-dry eye, 11 dry eye) were found to be suitable and fifteen were discharged for not meeting the classification criteria as either dry eye or non-dry eye. One dry eye participant was lost to follow-up after the screening visit. Data from ineligible participants and the person who did not complete the study were excluded from the analysis.

A total of twenty people (4 males, 16 females) completed the study. The majority of participants were female (non-dry eye: 70%, dry eye: 90%). The median age (interquartile range, IQR) of the non-dry eye group was 25.5 (21.50) and 45 (41.75) years old in the dry eye group (p=0.09, Figure 7-5). The age of the participants ranged from 21 to 83 years old.

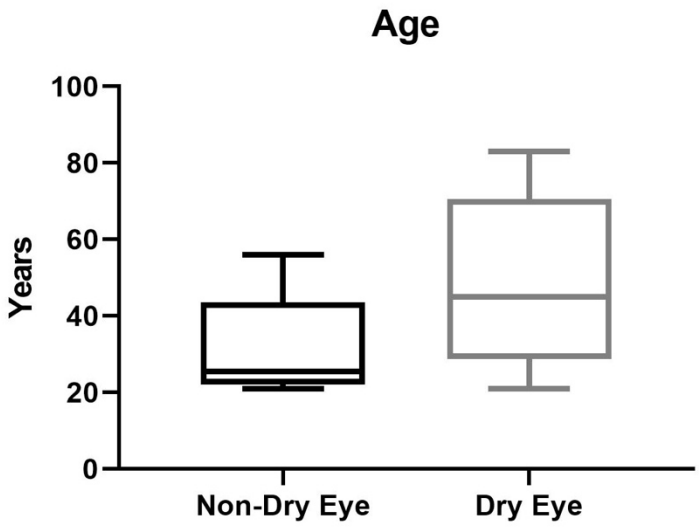

Figure 7-5: Distribution of participant age by study group (p=0.09).

None of the non-dry eye participants used artificial lubricants, whilst six of the dry eye participants reported habitually using eye drops between five times per day to once a week. Participants did not use habitual eye

drops on the day of the screening visit or on the day of the test visit, and reported that the drops were last used between one day to two weeks prior to the screening visit.

Fifty percent of non-dry eye participants and ten percent of dry eye participants reported suffering from allergies. One participant in the non-dry eye group reported that their allergy could affect their eyes; however, none of the participants reported any active episodes of ocular allergies.

7.4.2 Baseline Clinical Characteristics

A summary of the baseline clinical findings for each study group is included in Table 7-3. Data is presented as the median (IQR) or as mean ± standard deviation (SD). Significant differences between the study groups (all $p<0.04$, Figure 7-6, Figure 7-8, Figure 7-9, Figure 7-10, Figure 7-11) or between the eyes are graphed below (all $p<0.03$, Figure 7-7, Figure 7-8, Figure 7-12).

Table 7-3: Baseline demographics and characteristics by study group

	Non-Dry Eye Group (n = 10)	**Dry Eye Group (n = 10)**	**p-value**
Age	25.50 (21.50)	45.00 (41.75)	0.09
Dry eye symptomology			
OSDI (0-100)	1.04 (5.73)	35.56 (28.02)	**<0.01**
Visual acuity			
OD	–0.06 (0.10)	–0.06 (0.11)	0.86
OS	–0.10 (0.11)	–0.04 (0.19)	0.18
OU	–0.13 (0.10)	–0.10 (0.17)	0.49
Tear film quality			
Tear meniscus height (mm) (OD)	0.23 ± 0.06	0.27 ± 0.09	0.23
Tear meniscus height (mm) (OS)	0.27 ± 0.10	0.24 ± 0.13	0.55
NIKBUT (s) (OD)	15.87 (7.12)	6.34 (4.83)	0.92
NIKBUT (s) (OS)	12.72 (9.71)	3.28 (1.53)	**0.03**
NIKBUT (s) (worst eye)	11.22 (4.33)	3.00 (1.38)	**<0.01**
Subjective lipid layer thickness (OD)	3.00 (0.50)	3.00 (1.25)	0.26
Subjective lipid layer thickness (OS)	3.50 (2.00)	3.00 (2.50)	0.16
Meibomian gland assessment			
Quality of expression (OD)	21.50 (34.00)	52.00 (19.25)	**0.02**
Quality of expression (OS)	21.00 (26.00)	57.00 (14.25)	**<0.01**
MGYLS (OD)	1.00 (3.00)	3.50 (2.25)	**0.03**
MGYLS (OS)	1.00 (2.00)	4.00 (3.25)	0.08
Meiboscore (OD)	2.50 (2.00)	3.50 (2.00)	0.14
Meiboscore (OS)	3.00 (2.00)	4.00 (3.25)	0.35

	Non-Dry Eye Group (n = 10)	Dry Eye Group (n = 10)	p-value
Ocular surface staining			
Corneal staining (OD)	2.00 ± 2.06	2.25 ± 2.40	0.81
Corneal staining (OS)	2.55 ± 1.83	3.00 ± 1.78	0.58
Conjunctival staining (OD)	1.15 ± 0.94	1.45 ± 1.30	0.56
Conjunctival staining (OS)	1.25 ± 1.21	1.45 ± 1.42	0.74
Ocular surface/evaporimeter measurements			
Ocular surface area (cm^2) (OD)	1.61 ± 0.32	1.60 ± 0.43	0.95
Ocular surface area (cm^2) (OS)	1.87 ± 0.19	1.65 ± 0.48	0.19
Evaporimeter volume (cm^3) (OD)	16.20 ± 1.81	16.40 ± 2.01	0.82
Evaporimeter volume (cm^3) (OS)	15.60 ± 1.78	16.20 ± 2.20	0.51

OSDI: Ocular Surface Disease Index; OD: right eye; OS: left eye; OU: both eyes; NIKBUT: Non-Invasive Keratograph Break-Up Time; MGYLS: Meibomian Glands Yielding Liquid Secretion.

Data are presented as mean ± standard deviation (SD) or median (interquartile range) (IQR). **Bold** indicates significant differences.

7.4.2.1 Ocular Surface Disease Index (OSDI)

The median (IQR) OSDI score in the non-dry eye group was 1.04 (5.73) and 35.56 (28.02) in the dry eye group (Figure 7-6). The difference in OSDI scores between the groups was statistically significant ($p<0.0001$).

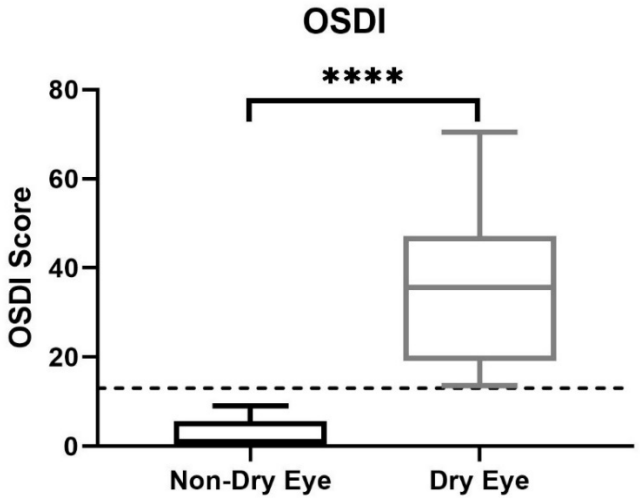

Figure 7-6: Box and whisker plot of OSDI scores for the study groups ($p<0.0001$).

The dashed line indicates the threshold value (13). Participants with OSDI scores ≥ 13 were considered symptomatic.

7.4.2.2 Visual Acuity

The median (IQR) visual acuity of the non-dry eye group was –0.06 (0.10) and –0.10 (0.11) for the right eye and left eye, respectively. The median (IQR) visual acuity of the dry eye group was –0.06 (0.11) and –0.04 (0.19) for the right eye and left eye, respectively. The median (IQR) visual acuity of both eyes was –0.13 (10) in the non-dry eye group and –0.10 (0.17) in the dry eye group. There was no significant difference in the visual acuity between the groups (right eye: p=0.86; left eye: p=0.18; both eyes: p=0.49). However, the visual acuity of the left eye was significantly better than the right eye in the non-dry eye group (p=0.008, Figure 7-7), but not in the dry eye group (p=0.344).

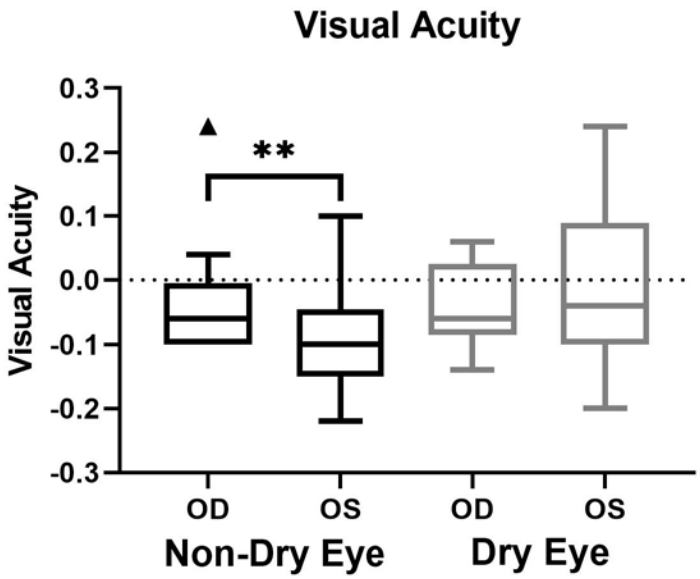

Figure 7-7: Box and whisker plot of the visual acuity for each eye of the study groups (non-dry eye right eye (OD) versus left eye (OS): p=0.008; dry eye right eye versus left eye: p=0.344). An outlier (>75th percentile + (1.5*IQR)) is denoted by a triangle.

7.4.2.3 Tear Meniscus Height

The mean ± SD TMH of the non-dry eye group was 0.23 ± 0.06 mm and 0.27 ± 0.10 mm for the right eye and left eye, respectively. The mean ± SD TMH of the dry eye group was 0.27 ± 0.09 mm and 0.24 ± 0.13 mm for the right eye and left eye, respectively. There was no significant difference in the TMH between the groups (right eye: p=0.23; left eye: p=0.55), or between the eyes (non-dry eye: p=0.11; dry eye: p=0.20).

7.4.2.4 Non-Invasive Keratograph Break-up Time

The median (IQR) NIKBUT of the non-dry eye group was 15.87 (7.12) seconds in the right eye and 12.72 (9.71) seconds in the left eye (Figure 7-8). There was no significant difference in the NIKBUT between the two eyes in the non-dry eye group (p=0.922). The median (IQR) NIKBUT of the dry eye group was 6.34 (4.83) seconds in the right eye and 3.28 (1.53) seconds in the left eye. The NIKBUT of the right eye of the dry eye group was significantly longer than the left eye (p=0.029). The NIKBUT in both the right eye and left eye of the non-dry eye group was also significantly different from the dry eye group (right eye: p=0.005; left eye: p<0.0001).

As per the inclusion criteria, the median (IQR) NIKBUT in the worst eye was 11.22 (4.33) seconds and 3.00 (1.38) seconds in the non-dry eye and dry eye group, respectively (Figure 7-9). The difference in NIKBUT between the groups was statistically significant (p<0.0001).

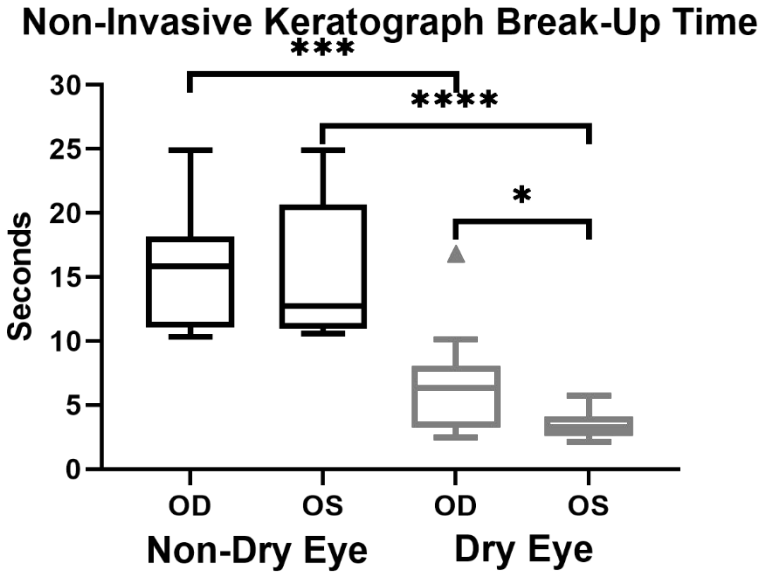

Figure 7-8: Box and whisker plot of NIKBUT for each eye of the study groups (right eye non-dry eye versus dry eye: p=0.005; left eye non-dry eye versus dry eye: p<0.0001). A significant difference was also found between each eye of the dry eye group (p=0.029), but not the non-dry eye group (p=0.922). An outlier (>75th percentile + (1.5*IQR)) is denoted by a triangle.

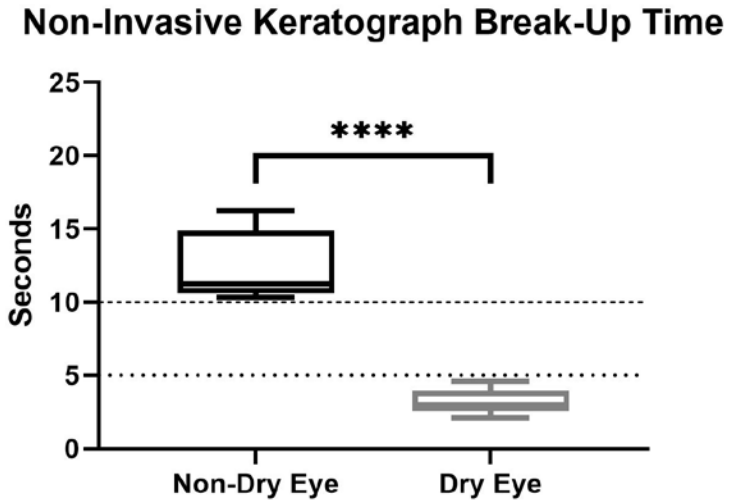

Figure 7-9: Box and whisker plot of NIKBUT of the worst eye for the study groups (p<0.0001).
The dashed line indicates the threshold value (10), with a break-up time ≥10 seconds considered non-dry eye. The dotted line indicates the threshold value (5), with a break-up time ≤5 seconds classified as dry eye.

7.4.2.5 Subjective Lipid Layer Thickness

The median (IQR) subjective LLT of the non-dry eye group was 3.00 (0.50) and 3.50 (2.00) for the right eye and left eye, respectively, indicating the median LLT was a wave pattern. The median (IQR) subjective LLT of the dry eye group was 3.00 (1.25) and 3.00 (2.50) for the right eye and left eye, respectively. There was no significant difference in LLT between the groups (right eye: p=0.26; left eye: p=0.16), or between the eyes (non-dry eye: p=0.25; dry eye: p=0.99).

7.4.2.6 Meibum Quality of Expression

The median (IQR) meibum score for the non-dry eye group was 21.50 (34) and 21.00 (26) for the right eye and left eye, respectively (Figure 7-10). The median (IQR) meibum score for the dry eye group was 52.00 (19.25) and 57.00 (14.25) for the right eye and left eye, respectively. The meibum quality score of the dry eye group was significantly higher than that of the non-dry eye group in each eye (right eye: p=0.02; left eye: p=0.005), indicating that the dry eye group had greater amounts of cloudy, inspissated, or waxy,

inexpressible glands. There was no significant difference in the quality of expression between the eyes of the non-dry eye group (p=0.999), or the dry eye group (p=0.389).

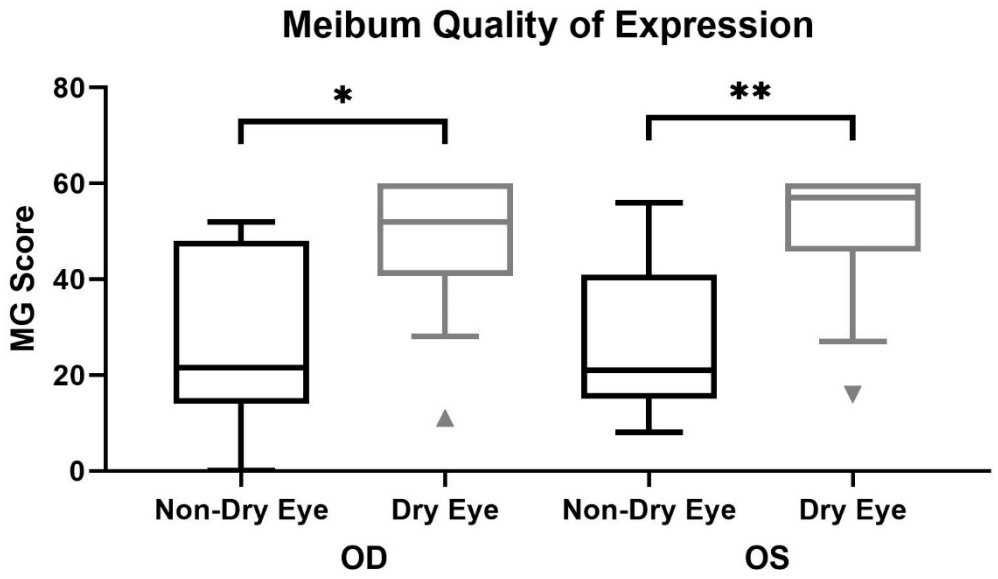

Figure 7-10: Box and whisker plot of the meibum quality for each eye of the study groups(right eye non-dry eye versus dry eye: p=0.02; left eye non-dry eye versus dry eye: p=0.005). Outliers (<25th percentile - (1.5*IQR)) are denoted by triangles.

7.4.2.7 Meibomian Glands Yielding Liquid Secretion

The median (IQR) meibomian glands yielding liquid secretion (MGYLS) scores in the non-dry eye group were 1.00 (3.00) and 1.00 (2.00) for the right eye and left eye, respectively (Figure 7-11). The median (IQR) MGYLS scores in the dry eye group were 3.50 (2.25) and 4.00 (3.25) for the right eye and left eye, respectively, indicating that the majority of glands were not able to be expressed. The difference in MGYLS score between the two groups was statistically significantly different for the right eye (p=0.03), but not the left eye (p=0.08). There was no significant difference in MGYLS between the two eyes of the non-dry eye group (p=0.625), or the dry eye group (p=0.625).

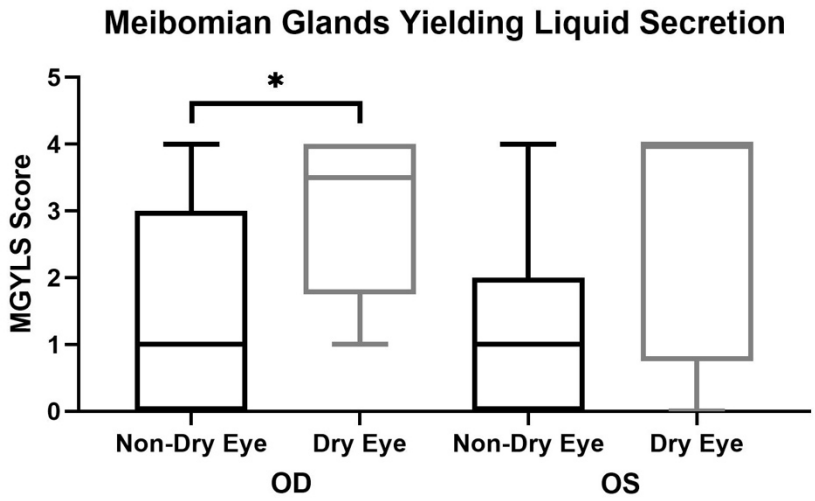

Figure 7-11: Box and whisker plot of the MGYLS score for each eye of the study groups (right eye non-dry eye versus dry eye: p=0.03; left eye non-dry eye versus dry eye: p=0.08).

7.4.2.8 Meiboscore

The median (IQR) total meiboscore of the non-dry eye group was 2.50 (2.00) and 3.00 (2.00) for the right eye and left eye, respectively, with larger values indicating greater amounts of glandular loss. The median (IQR) total meiboscore of the dry eye group was 3.50 (2.00) and 4.00 (3.25) for the right eye and left eye, respectively. There was no significant difference in meibsoscore between the groups (right eye: p=0.14; left eye: p=0.35), or between the eyes (non-dry eye: p=0.38; dry eye: p=0.81).

7.4.2.9 Lid Margin Assessment

The median (IQR, range) erythema non-dry eye group was 0 (0, 0–1) in the right and left eye of the non-dry eye group. The median (IQR, range) erythema in the dry group was 0 (0.25, 0–1) in the right and left eye of the dry eye group. There was no significant difference in erythema between the groups (right eye: p>0.99; left eye: p>0.99).

The median (IQR) amount of telangiectasia in the non-dry eye group was 0 (2) and 0 (1.25) in the right eye and left eye, respectively. The median (IQR) amount of telangiectasia in the dry eye group was 2 (2.25) in both the right eye and left eye. There was no significant difference in the amount of telangiectasia between the groups (right eye: p=0.19; left eye: p=0.12) or between the eyes (non-dry eye: p>0.99). No lash loss, edema, or tear film debris was noted in either eye of the two groups.

7.4.2.10 Corneal Staining

The mean ± SD amount of corneal staining in the non-dry eye group was 2.00 ± 2.06 and 2.55 ± 1.83 for the right eye and left eye, respectively, with larger values indicating greater amounts of staining. The mean ± SD amount of corneal staining in the dry eye group was 2.25 ± 2.40 and 3.00 ± 1.78 for the right eye and left eye, respectively. There was no significant difference in amount of corneal staining between the groups (right eye: p=0.81; left eye: p=0.58) or between the eyes (non-dry eye: p=0.28; dry eye: p=0.09).

7.4.2.11 Conjunctival Staining

The mean ± SD amount of conjunctival staining in the non-dry eye group was 1.15 ± 0.94 and 1.25 ± 1.21 for the right eye and left eye, respectively, with larger values indicating greater amounts of staining. The mean ± SD amount of corneal staining in the dry eye group was 1.45 ± 1.30 and 1.45 ± 1.42 for the right eye and left eye, respectively. There was no significant difference in amount of corneal staining between the groups (right eye: p=0.56; left eye: p=0.74) or between the eyes (non-dry eye: p=0.19; dry eye: p=0.21).

7.4.2.12 Ocular Surface Area

The mean ± SD area of the ocular surface of the non-dry eye group was 1.61 ± 0.32 cm^2 and 1.87 ± 0.19 cm^2 for the right eye and left eye, respectively. The mean ± SD area of the ocular surface of the dry eye group was 1.60 ± 0.43 cm^2 and 1.65 ± 0.48 cm^2 for the right eye and left eye, respectively. There was no significant difference in the ocular surface area between the groups (right eye: p=0.95; left eye: p=0.19). However, the ocular surface area of the left eye was significantly larger than the right eye in the non-dry eye group (p=0.01, Figure 7-12), but not in the dry eye group (p=0.44).

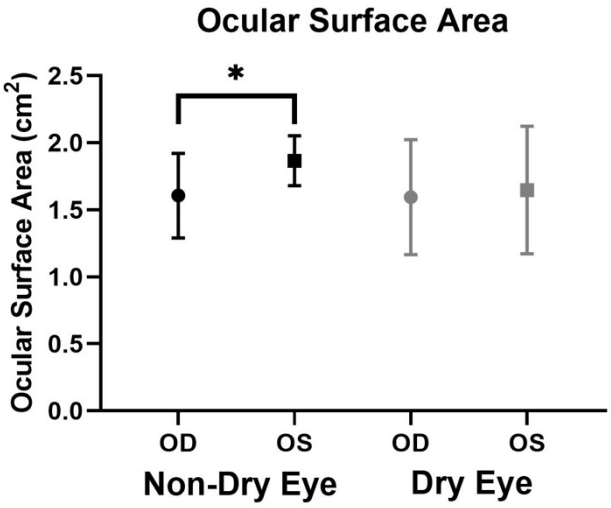

Figure 7-12: Mean and standard deviation (SD) ocular surface area for each eye of the study groups(non-dry eye right eye versus left eye: p=0.01; dry eye right eye versus left eye: p=0.44).

7.4.2.13 Evaporimeter Volume

The mean ± SD air volume within goggle of the non-dry eye group was 16.20 ± 1.81 cm^3 and 15.60 ± 1.78 cm^3 for the right eye and left eye, respectively. The mean ± SD air volume within goggle of the dry eye group was 16.40 ± 2.01 cm^3 and 16.20 ± 2.20 cm^3 for the right eye and left eye, respectively. There was no significant difference in the evaporimeter goggle volume between the groups (right eye: p=0.82; left eye: p=0.51), or between the eyes of each group (non-dry eye group: p=0.14; dry eye group: p=0.34).

7.4.3 Test Visit Results

The test visit occurred between 1 to 15 days after the screening visit. One participant attended one day out-of-range following the resolution of a non-ocular, non-serious adverse event. The six habitual eye drop users reported lubricating drops were last instilled between one day to two weeks prior to the test visit, with two participants having used drops within one to two days prior to the visit. A summary of the clinical findings from the test visit appears in Table 7-6. Data is presented as the median (IQR) or as mean ± SD.

7.4.3.1 Ambient Temperature and Relative Humidity

The mean ± SD ambient room temperature on the day of the test visit was 71.9 ± 1.1°F (22.2 ± 0.6°C) and the RH was 30.7 ± 1.1%. The range of ambient temperature was 69 to 74°F (20.6 to 23.3°C) and RH varied between 29 and 34%.

7.4.3.2 Tear Evaporation Rate (TER)

7.4.3.2.1 TER by Individual

The change in TER for each participant in the non-dry eye and dry eye group is shown in Figure 7-13.

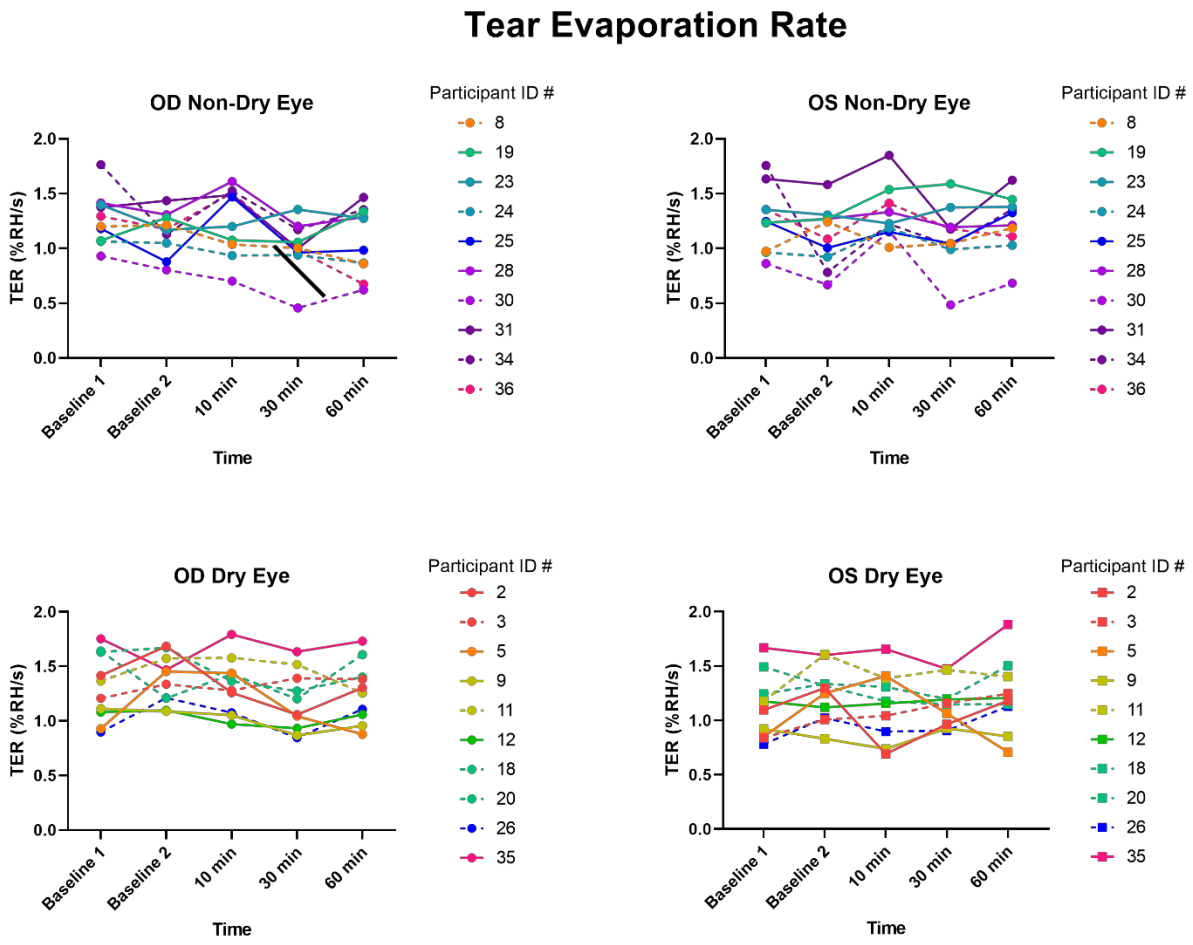

Figure 7-13: Individual plots of the mean change in tear evaporation rate over time for each eye of all the participants.

7.4.3.2.2 Baseline TER

Due to the binocular nature of the evaporimeter, the mean ± SD change in TER for each eye was investigated (Table 7-4). There was no significant difference in the repeatability of the two baseline measurements of the non-dry eye group (right eye: p=0.122; left eye: p=0.199, Figure 7-14), or the dry eye group (right eye: p=0.417; left eye: p=0.117). Rather than include all the time points in the subsequent analysis (Appendix E), only the second baseline measurement was used since all of the eyes had a smaller SD at this time point in comparison to the first baseline measurement.

Table 7-4: Summary of mean ± SD tear evaporation rate over time

	Tear Evaporation Rate (%RH/s)			
	Non-Dry Eye		**Dry Eye**	
	Right Eye (n = 10)	**Left Eye (n = 10)**	**Right Eye (n = 10)**	**Left Eye (n = 10)**
Baseline 1	1.27 ± 0.24	1.26 ± 0.29	1.30 ± 0.30	1.13 ± 0.29
Baseline 2	1.15 ± 0.19	1.11 ± 0.27	1.38 ± 0.22	1.24 ± 0.25
10 minutes post-drop	1.26 ± 0.31	1.31 ± 0.24	1.32 ± 0.25	1.15 ± 0.31
30 minutes post-drop	1.01 ± 0.24	1.11 ± 0.28	1.18 ± 0.27	1.15 ± 0.20
60 minutes post-drop	1.07 ± 0.31	1.24 ± 0.26	1.27 ± 0.27	1.23 ± 0.33

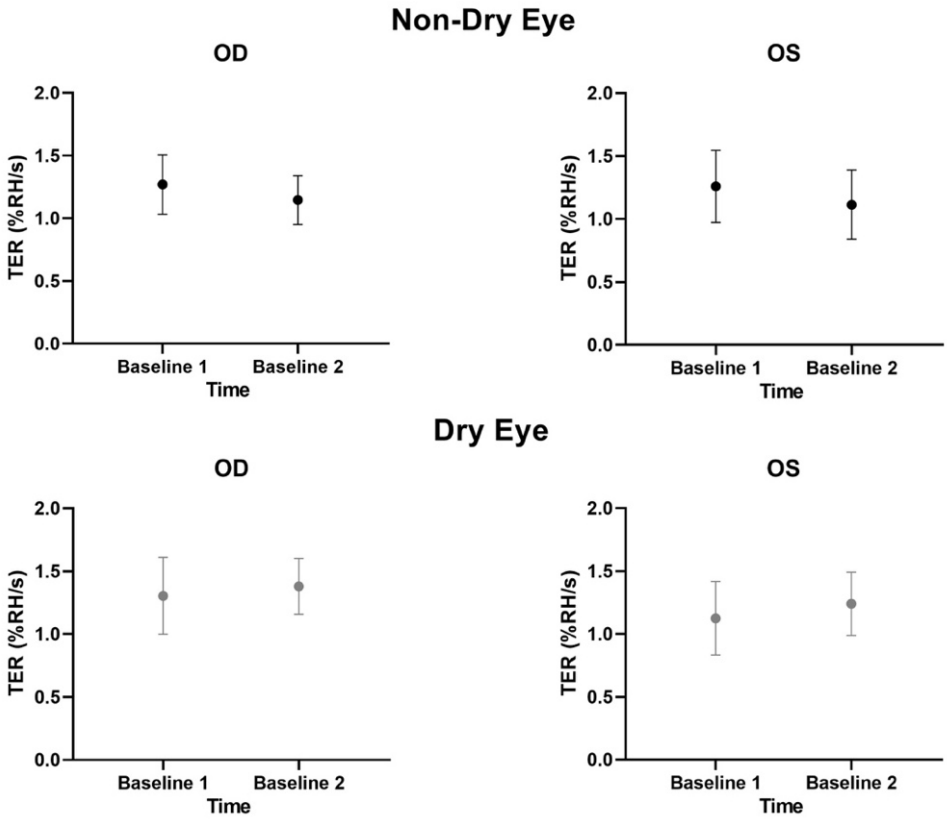

Figure 7-14: Average baseline tear evaporation rate for the right and left eye of each group. Error bars indicate SD. There was no significant difference in the baseline tear evaporation rate of the non-dry eye group (right eye: p=0.122; left eye: p=0.199) or the dry eye group (right eye: p=0.417; left eye: p=0.117).

7.4.3.2.3 Relationship Between Time, Group, and Eye

There was no statistically significant three-way interaction between time, group, and eye on TER (p=0.567, Table 7-5, Figure 7-15) or two-way interaction between time and group (p=0.100). However, there was a statistically significant two-way interaction between time and eye (p=0.012). The simple two-way interaction between time and eye for non-dry eye participants was not statistically significant (p=0.154); however, the interaction was significant for dry eye participants (p=0.034). There was a statistically significant simple main effect of time on the right eye of dry eye participants (p=0.049), but not the left eye (p=0.436).

Table 7-5: Summary of two-way and three-way interactions between time, eye, and group

Interactions		p-value
time*group*eye		0.567
time*group		0.100
time*eye		**0.012**
	non-dry eye	0.154
	dry eye	**0.034**

Bold indicates significance.

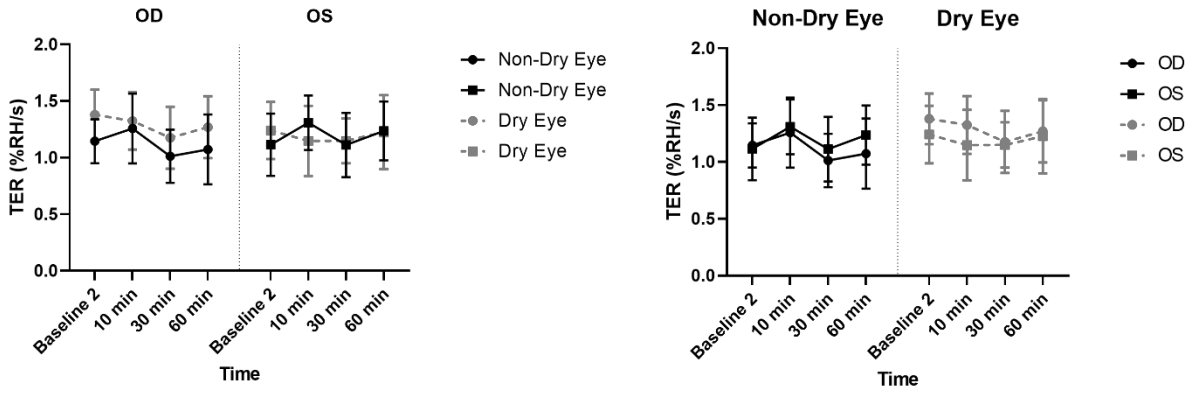

Figure 7-15: Change in mean tear evaporation rate for the right and left eye of each group. There was no significant interaction between time and group (p=0.100). There was a significant interaction between time and eye (p=0.012) for the dry eye group (p=0.034), but not the non-dry eye group (p=0.154). Error bars indicate SD.

7.4.3.2.4 Change in TER over Time

The change in TER over time was significantly different for the right eye (p=0.028) and the left eye (p=0.023) of the non-dry eye group (Figure 7-16). The change in TER over time was also significantly different for the right eye of the dry eye group (p=0.049), but not the left eye (p=0.436). The TER of the left eye of the non-dry eye group was significantly higher 10 minutes after the eye drop was instilled compared to the second baseline measurement (p=0.048). The TER of the non-dry eye group was also significantly lower 30 minutes after the drop was instilled compared to 10 minutes in both eyes (right eye:

p=0.022; left eye: p=0.045). The dry eye group had a significantly lower TER measured 30 minutes after the eye drop was instilled in the right eye compared to the baseline measurement (p=0.038).

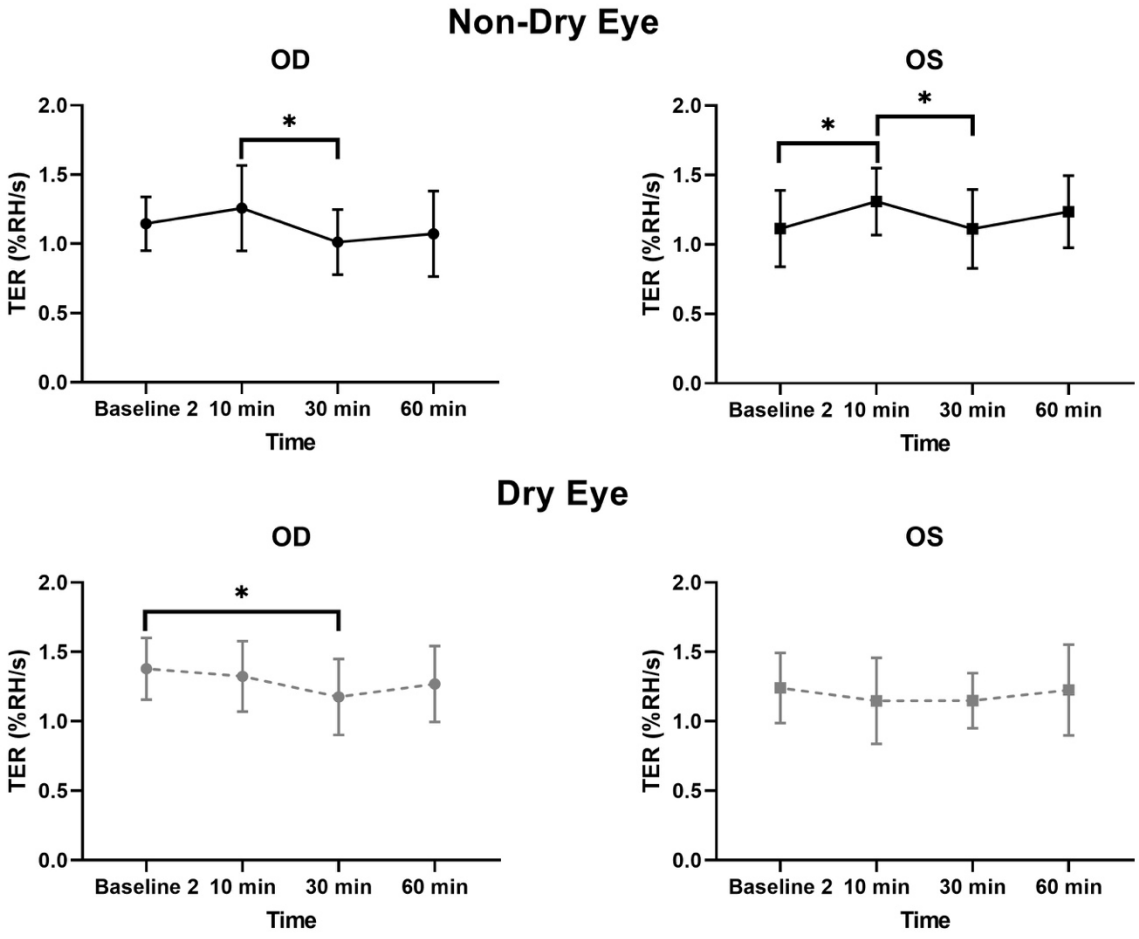

Figure 7-16: Average change in tear evaporation rate over time for each eye of the non-dry eye and dry eye group. Error bars indicate SD. The change in tear evaporation rate was significant for both eyes of the non-dry eye group (right eye: p=0.028; left eye: p=0.023) and the right eye of the dry eye group (p=0.049). No significant change was observed for the left eye of the dry eye group (p=0.436).

Since the change in TER was only statistically significant for the right eye of both groups, the remaining statistical analyses for changes over time (Table 7-6), differences between groups (Table 7-6), and correlations (Table 7-7, Table 7-8, Table 7-9) were conducted only on the right eye. Significant changes over time (all $p<0.05$, Figure 7-17, Figure 7-20, Figure 7-22) and between the study groups are plotted below (all $p<0.023$, Figure 7-18, Figure 7-19, Figure 7-21).

Table 7-6: Summary of subjective comfort and clinical measurements for the right eye

	Non-Dry Eye Group (n = 10 eyes)	Dry Eye Group (n = 10 eyes)	p-value
Comfort Rating			
Baseline 2	100.00 (6.25)	70.00 (27.50)	**<0.001**
10 minutes post-drop	100.00 (6.25)	72.50 (22.50)	**<0.001**
30 minutes post-drop	100.00 (11.25)	67.50 (28.75)	**<0.001**
60 minutes post-drop	100.00 (11.25)	73.00 (12.50)	**<0.001**
p-value	0.572	0.583	
Dryness Rating			
Baseline 2	100.00 (2.50)	57.50 (23.75)	**<0.001**
10 minutes post-drop	100.00 (6.25)	70.00 (26.25)	**<0.001**
30 minutes post-drop	100.00 (5.00)	63.00 (30.00)	**<0.001**
60 minutes post-drop	100.00 (5.00)	60.00 (23.75)	**<0.001**
p-value	0.392	0.753	
Stinging/Burning Rating			
Baseline 2	100.00 (1.25)	90.00 (56.25)	0.071
10 minutes post-drop	100.00 (0.00)	94.50 (82.50)	**0.026**
30 minutes post-drop	100.00 (0.00)	97.50 (90.00)	0.076
60 minutes post-drop	100.00 (0.00)	100.00 (81.25)	0.087
p-value	0.572	0.769	
Tear Meniscus Height			
Baseline 2	0.29 (0.12)	0.28 (0.12)	0.810
10 minutes post-drop	0.27 (0.08)	0.28 (0.13)	0.566
30 minutes post-drop	0.33 (0.12)	0.25 (0.11)	0.137
60 minutes post-drop	0.28 (0.12)	0.22 (0.19)	0.423
p-value	**0.017**	0.116	
Tear Evaporation Rate			
Baseline 2	1.15 ± 0.19	1.38 ± 0.22	**0.022**
10 minutes post-drop	1.26 ± 0.31	1.32 ± 0.25	0.602
30 minutes post-drop	1.01 ± 0.24	1.18 ± 0.27	0.170
60 minutes post-drop	1.07 ± 0.31	1.27 ± 0.27	0.148
p-value	**0.028**	**0.049**	
Non-Invasive Keratograph Break-up Time			
Baseline 2	12.04 (16.37)	4.05 (3.76)	**0.002**
10 minutes post-drop	10.29 (10.60)	3.63 (3.16)	**0.004**
30 minutes post-drop	14.21 (13.81)	3.63 (1.81)	**<0.001**
60 minutes post-drop	7.91 (19.85)	3.92 (3.92)	**0.011**
p-value	0.236	0.784	

	Non-Dry Eye Group (n = 10 eyes)	Dry Eye Group (n = 10 eyes)	p-value
Objective Lipid Layer Thickness (mean)			
Baseline 2	61.10 ± 13.56	69.40 ± 18.08	0.261
10 minutes post-drop	68.70 ± 20.21	75.00 ± 18.32	0.475
30 minutes post-drop	63.30 ± 17.84	73.00 ± 20.17	0.270
60 minutes post-drop	67.00 ± 17.83	70.20 ± 16.52	0.682
	0.401	0.396	
Objective Lipid Layer Thickness (maximum)			
Baseline 2	81.50 (36.00)	80.00 (27.00)	0.776
10 minutes post-drop	84.50 (39.00)	93.50 (20.50)	0.526
30 minutes post-drop	89.50 (32.50)	85.00 (27.50)	0.665
60 minutes post-drop	87.50 (29.75)	81.50 (27.00)	0.837
p-value	0.869	**0.031**	
Objective Lipid Layer Thickness (minimum)			
Baseline 2	53.50 (15.00)	56.50 (14.00)	0.402
10 minutes post-drop	57.00 (34.25)	59.50 (12.50)	0.424
30 minutes post-drop	52.50 (31.75)	57.50 (13.75)	0.362
60 minutes post-drop	55.50 (29.00)	60.50 (14.00)	0.541
p-value	0.392	0.896	

Data are presented as mean ± SD or median (IQR). **Bold** indicates significant differences.

7.4.3.2.5 Change in TER over Time for the Right Eye

As reported in Section 7.4.3.2.4, the change in TER over time for was significantly different for both groups (non-dry eye: p=0.028; dry eye p=0.049, Figure 7-17).

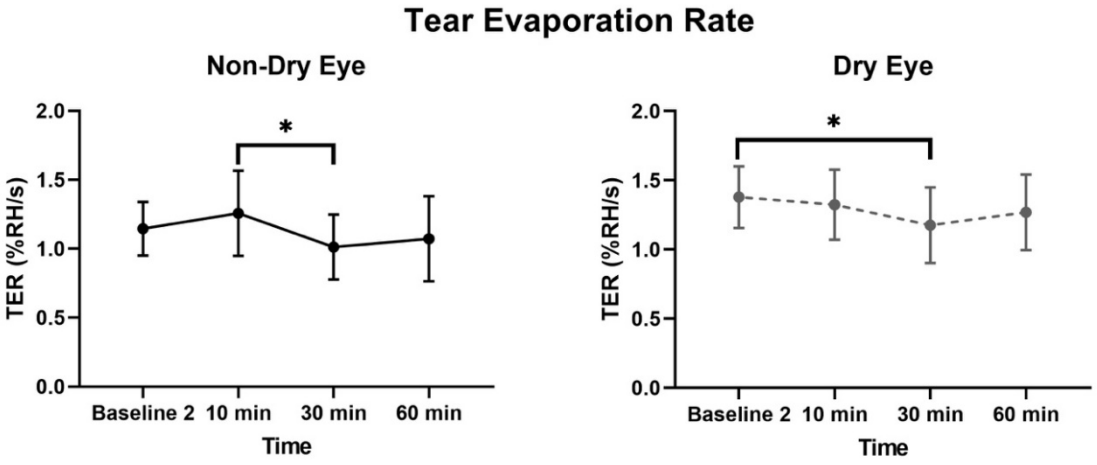

Figure 7-17: Average tear evaporation rate for right eye of the non-dry eye (p=0.028) and the dry eye (p=0.049) group. Error bars indicate SD.

7.4.3.2.6 Difference in TER Between Groups

The dry eye group had a significantly higher TER than the non-dry eye group at the second baseline measurement (p=0.022, Figure 7-18), but not at any of the other time points (all p>0.14).

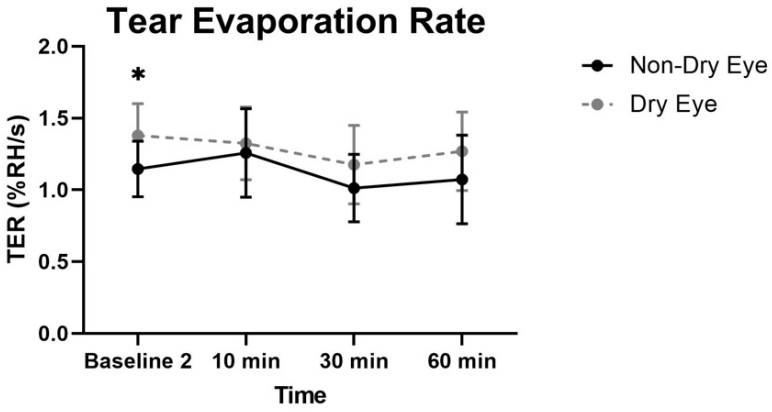

Figure 7-18: Difference in mean tear evaporation rate for right eye between the two groups. The TER of the dry eye group was higher at the second baseline measurement (p=0.022) than the non-dry eye group. Error bars indicate SD.

7.4.3.3 Subjective Comfort Ratings

7.4.3.3.1 Change in Subjective Comfort over Time

There was no significant change in subjective comfort, dryness, or stinging/burning over time for either group (non-dry eye: all $p>0.391$; dry eye: all $p>0.582$).

7.4.3.3.2 Difference in Subjective Comfort Between Groups

The right eye of the dry eye group experienced greater amounts of discomfort and dryness compared to the non-dry eye group at all time points (all $p<0.001$, Figure 7-19). The dry eye group also felt more stinging/burning 10 minutes after the drop was instilled ($p=0.026$).

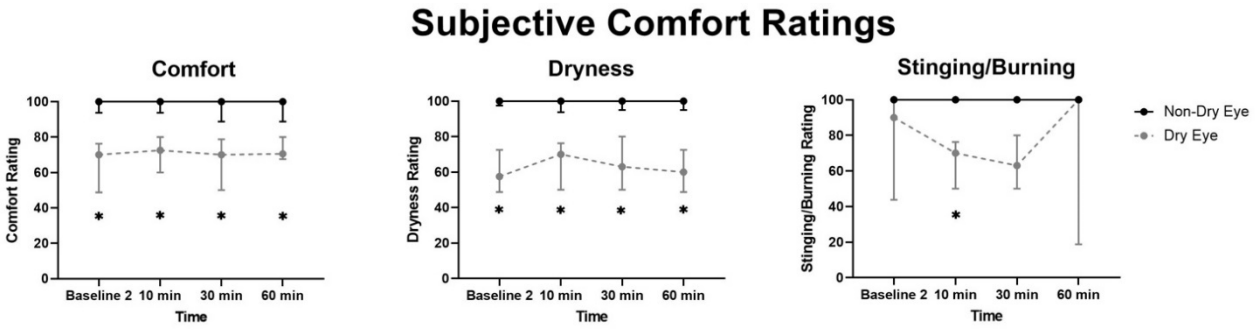

Figure 7-19: Difference in subjective comfort over time between the groups. The dry eye group had lower comfort and dryness scores at all time points (comfort: all $p<0.001$, dryness: all $p<0.001$), and 10 minutes after instillation of the eye drop ($p=0.026$). Error bars indicate IQR.

7.4.3.4 Tear Meniscus Height

7.4.3.4.1 Change in Tear Meniscus Height over Time

The change in TMH over time was significantly different for the right eye of the non-dry eye group ($p=0.017$), but not in the dry eye group ($p=0.116$). Post-hoc multiple comparison Dunn's test for the right eye of the non-dry eye group revealed a significantly higher TMH 30 minutes after the eye drop was instilled compared to the second baseline measurement ($p=0.026$, Figure 7-20).

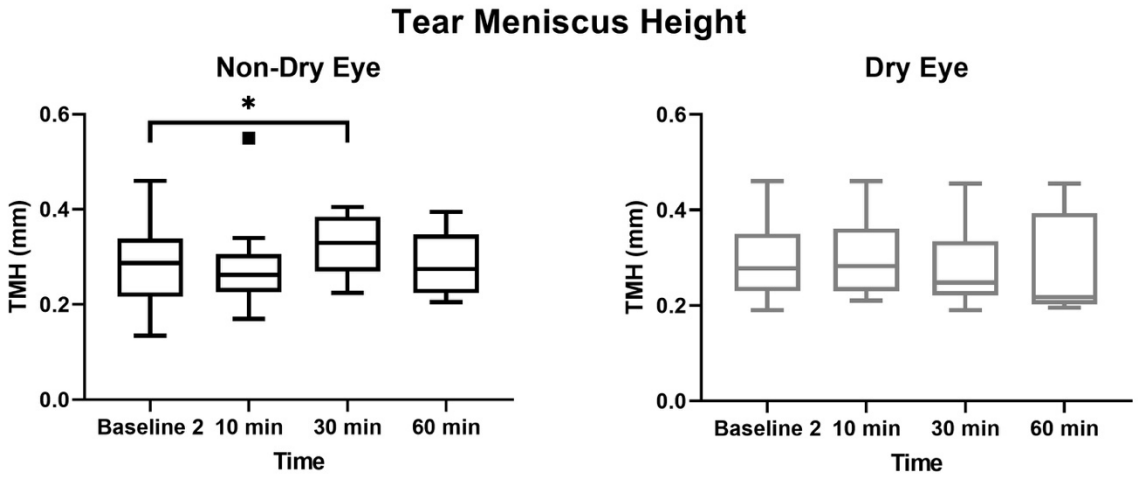

Figure 7-20: Box and whisker plot of the change in tear meniscus height over time for the non-dry eye (p=0.017) and dry eye (p=0.116) group. An outlier (>75th percentile + (1.5*IQR)) is denoted by a square.

7.4.3.4.2 Difference in Tear Meniscus Height Between Groups

There was no significant difference in the TMH between the two groups at any of the time points (all p>0.136).

7.4.3.5 Non-Invasive Keratograph Break-Up Time

7.4.3.5.1 Change in Non-Invasive Keratograph Break-Up Time over Time

Neither group experienced a significant change in NIKBUT during the duration of the measurements (non-dry eye: p=0.236; dry eye: p=0.784).

7.4.3.5.2 Difference in Non-Invasive Keratograph Break-Up Time Between Groups

The dry eye group had significantly lower NIKBUTs than the non-dry eye group at all of the time points (all p<0.012, Figure 7-21).

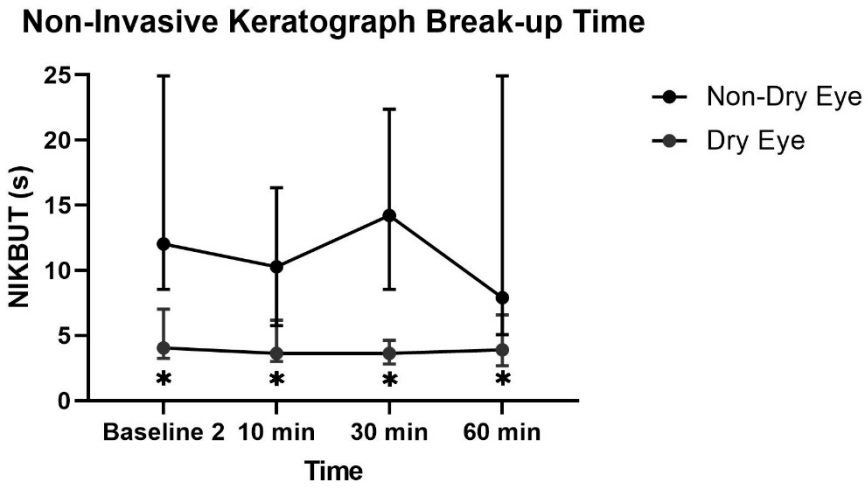

Figure 7-21: Difference in median non-invasive break-up time between the groups. The dry eye group had faster break-up times at all time points (all p<0.012). Error bars indicate IQR.

7.4.3.6 Lipid Layer Thickness

7.4.3.6.1 Change in Lipid Layer Thickness over Time

There was no significant difference in mean LLT (non-dry eye: p=0.401; dry eye: mean p=0.396) or minimum LLT over time for either group (non-dry eye: p=0.392; dry eye: p=0.896). Although the change in maximum LLT over time was not significantly different in the non-dry eye group (p=0.869), it was significantly different for the dry eye group (p=0.031). Post-hoc testing with an uncorrected Dunn's test revealed a significantly higher maximum LLT 10 minutes after the eye drop was instilled compared to the second baseline measurement (p=0.030), and a lower LLT 60 minutes after the eye drop was instilled compared to 10 minutes (p=0.046, Figure 7-22).

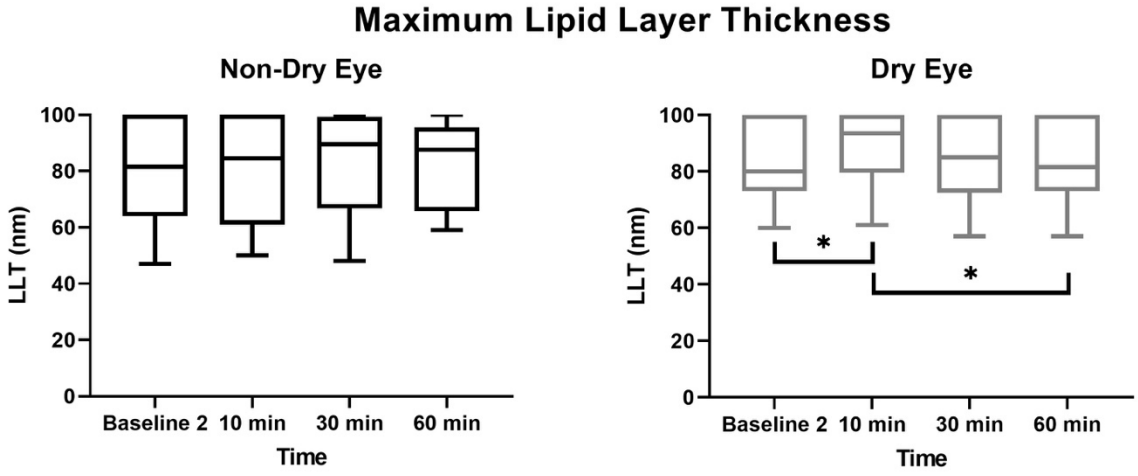

Figure 7-22: Box and whisker plot of the change in maximum lipid layer thickness over time for the non-dry eye (p=0.869) and dry eye (p=0.031) group.

7.4.3.6.2 Difference in Lipid Layer Thickness Between Groups

There was no significant difference in the mean, maximum, or minimum LLT between the two groups at any time point (mean: all p>0.260; maximum: all p>0.525; minimum: all p>0.361).

7.4.4 Correlations Between Tear Evaporation Rate and Clinical Measurements by Group

7.4.4.1 Ocular Surface Area and Evaporimeter Volume

A summary of correlations between the TER of the right eye and the ocular surface area and volume within the evaporimeter are shown in Table 7-7. The non-dry eye group had a significant positive correlation with the ocular surface area at 10 minutes (r=0.754, p=0.012, Figure 7-23) and 60 minutes (r=0.708, p=0.022) after instillation of Systane Complete. The ocular surface area of the dry eye group did not have a significant correlation with the TER (all r<0.418, all p>0.230).

There was no significant correlation between the TER and the volume inside the evaporimeter (non-dry eye: all r<–0.044, all p>0.129; dry eye: all r<–0.081, all p>0.070, Figure 7-24).

There was also no significant correlation between ocular surface area and evaporimeter volume of the non-dry eye group (right eye: r=–0.158, p=0.664; left eye: r=–0.548, p=0.101), or the dry eye group (right eye: r=–0.221, p=0.539; left eye: r=–0.306, p=0.391).

Table 7-7: Correlations between tear evaporation rate of the right eye and characteristics of the ocular surface and evaporimeter

		Non-Dry Eye Group (n = 10 eyes)	p-value	Dry Eye Group (n = 10 eyes)	p-value
Ocular surface area					
	Baseline 2	0.552	0.098	0.230	0.523
	10 minutes	**0.754**	**0.012**	0.417	0.231
	30 minutes	0.618	0.057	0.223	0.535
	60 minutes	**0.708**	**0.022**	–0.128	0.725
Evaporimeter volume					
	Baseline 2	–0.275	0.441	–0.593	0.071
	10 minutes	–0.045	0.903	–0.380	0.279
	30 minutes	–0.512	0.130	–0.427	0.218
	60 minutes	–0.289	0.418	–0.082	0.822

Bold indicates significant correlations.

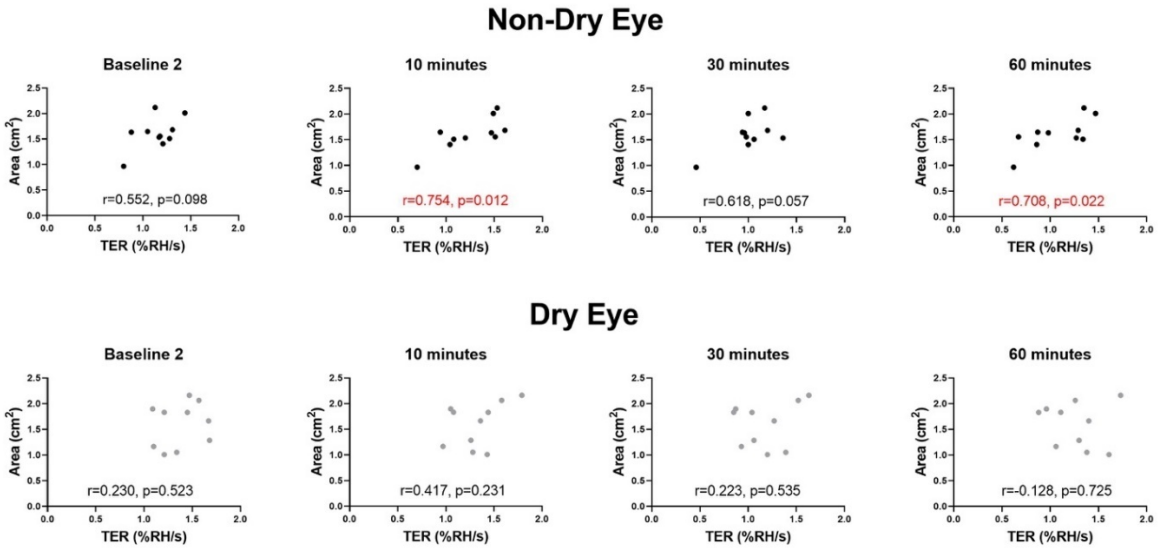

Figure 7-23: Correlations between the tear evaporation rate and the ocular surface area of the right eye. Significant Pearson correlations for non-dry eye participants are shown in red (10 minutes: r=0.754, p=0.012, 60 minutes: r=0.708, p=0.022, all other r>0.551, all other p>0.056; dry eye: all r<0.418, all p>0.230).

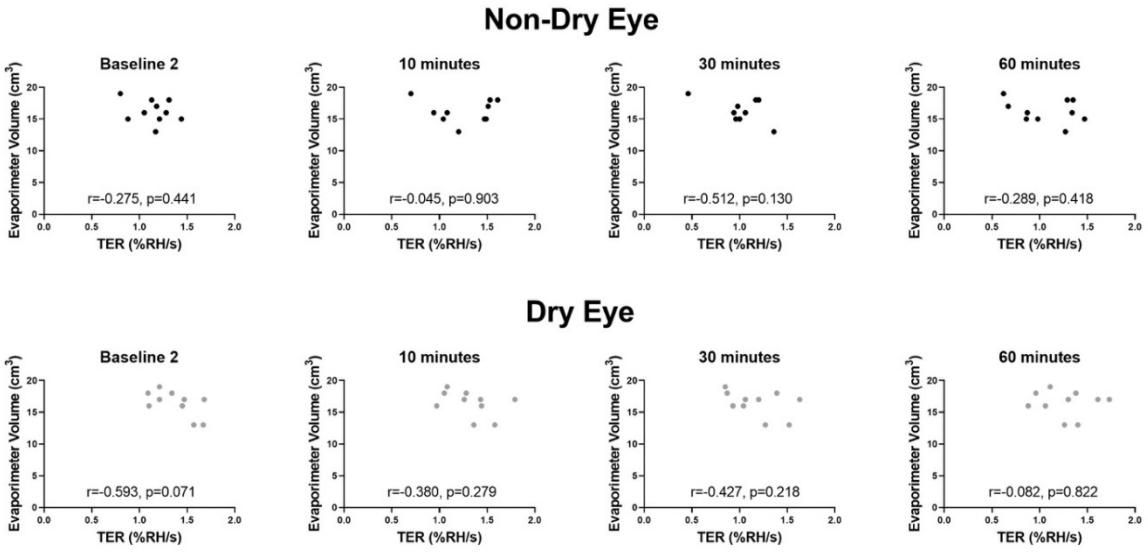

Figure 7-24: Correlations between the tear evaporation rate and the volume inside the evaporimeter of the right eye (non-dry eye: all r<–0.044, all p>0.129; dry eye: all r<–0.081, all p>0.070).

7.4.4.2 Symptomology

A summary of correlations between the TER of the right eye and symptomology are shown in Table 7-8. There was no significant correlation between the TER and symptomology (OSDI or any of the subjective comfort ratings, Figure 7-25, Figure 7-26, Figure 7-27, Figure 7-28) of either group (non-dry eye: all r_s<0.411, all p>0.104; dry eye: all r_s<0.286, all p>0.065).

Table 7-8: Correlations between tear evaporation rate of the right eye and symptomology

		Non-Dry Eye Group (n = 10 eyes)	p-value	Dry Eye Group (n = 10 eyes)	p-value
OSDI					
	Baseline 2	0.149[a]	0.688	–0.097[a]	0.792
	10 minutes	–0.278[a]	0.437	0.285[a]	0.427
	30 minutes	–0.545[a]	0.105	0.115[a]	0.759
	60 minutes	–0.239[a]	0.506	0.091[a]	0.811
Comfort rating					
	Baseline 2	–0.440[a]	0.214	–0.074[a]	0.842
	10 minutes	0.410[a]	0.240	–0.348[a]	0.321
	30 minutes	0.223[a]	0.527	–0.466[a]	0.175
	60 minutes	0.007[a]	0.984	–0.230[a]	0.528

		Non-Dry Eye Group (n = 10 eyes)	p-value	Dry Eye Group (n = 10 eyes)	p-value
Dryness rating					
	Baseline 2	–0.435[a]	0.267	–0.152[a]	0.671
	10 minutes	–0.539[a]	0.117	–0.598[a]	0.073
	30 minutes	0.117[a]	0.800	–0.006[a]	0.994
	60 minutes	–0.087[a]	0.889	–0.135[a]	0.712
Stinging/burning rating					
	Baseline 2	–0.156[a]	0.667	0.072[a]	0.846
	10 minutes	–0.174[a]	0.800	–0.525[a]	0.122
	30 minutes	0.000[a]	1.000	–0.610[a]	0.066
	60 minutes	–0.522[a]	0.200	–0.608[a]	0.067

[a] = Spearman rank correlation.

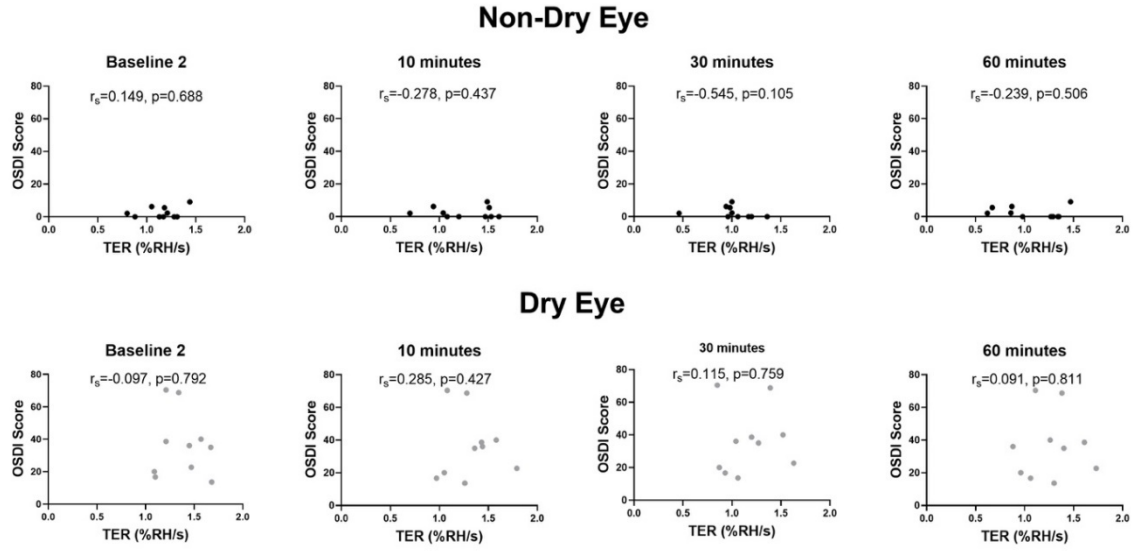

Figure 7-25: Correlations between the tear evaporation rate and OSDI score of the right eye(non-dry eye: all r_s<0.150, all p>0.104; dry eye: all r_s<0.286, all p>0.426).

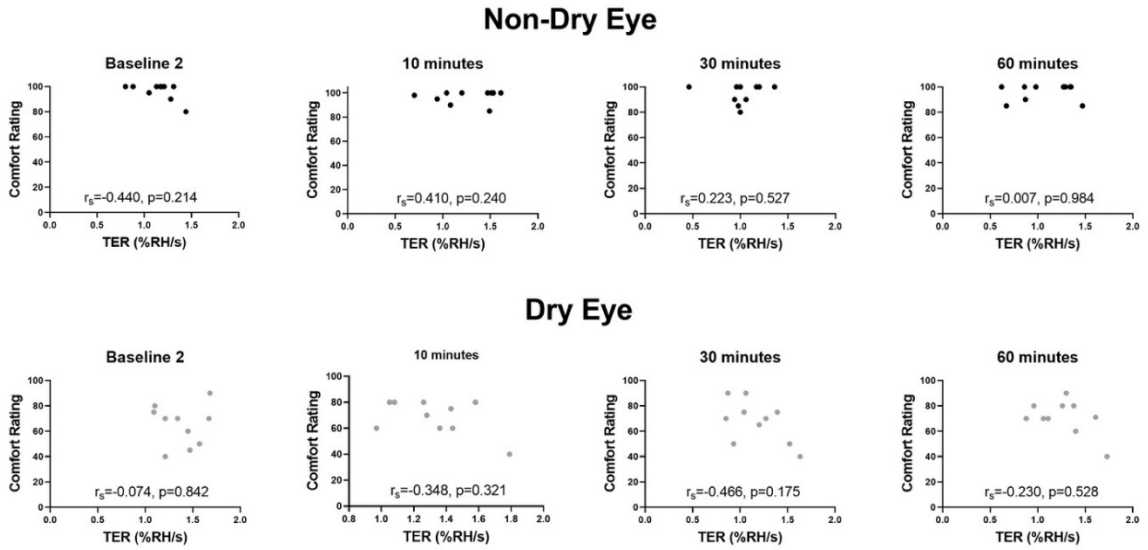

Figure 7-26: Correlations between the tear evaporation rate and the comfort rating of the right eye (non-dry eye: all r_s<0.411, all p>0.213; dry eye: all r_s<–0.073, all p>0.174).

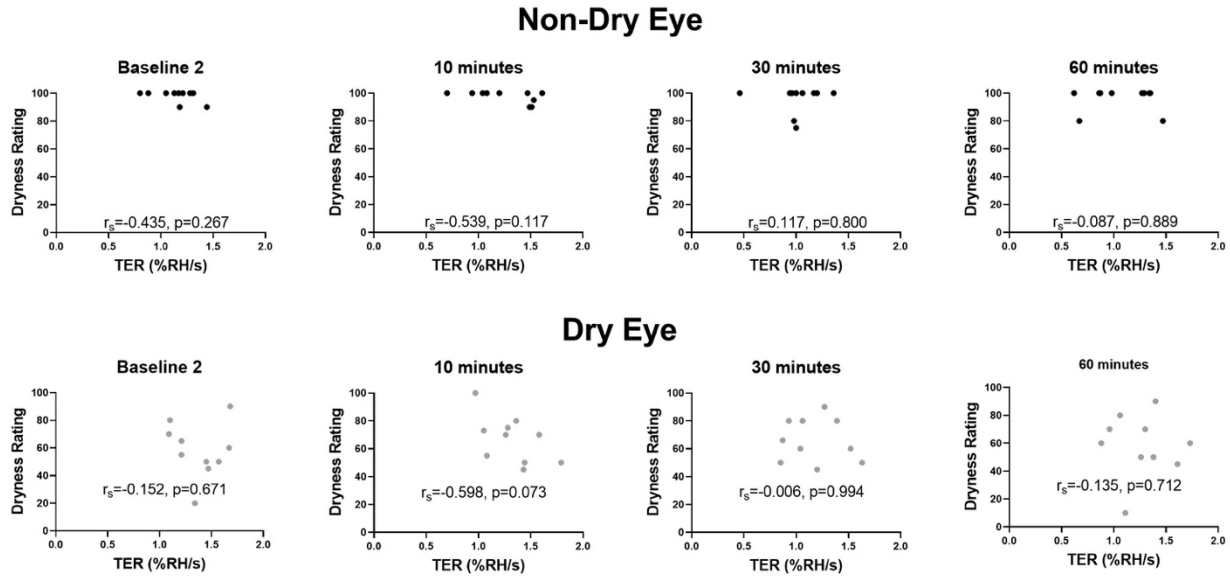

Figure 7-27: Correlations between the tear evaporation rate and the dryness rating of the right eye (non-dry eye: all r_s<0.118, all p>0.116; dry eye: all r_s<–0.005, all p>0.072).

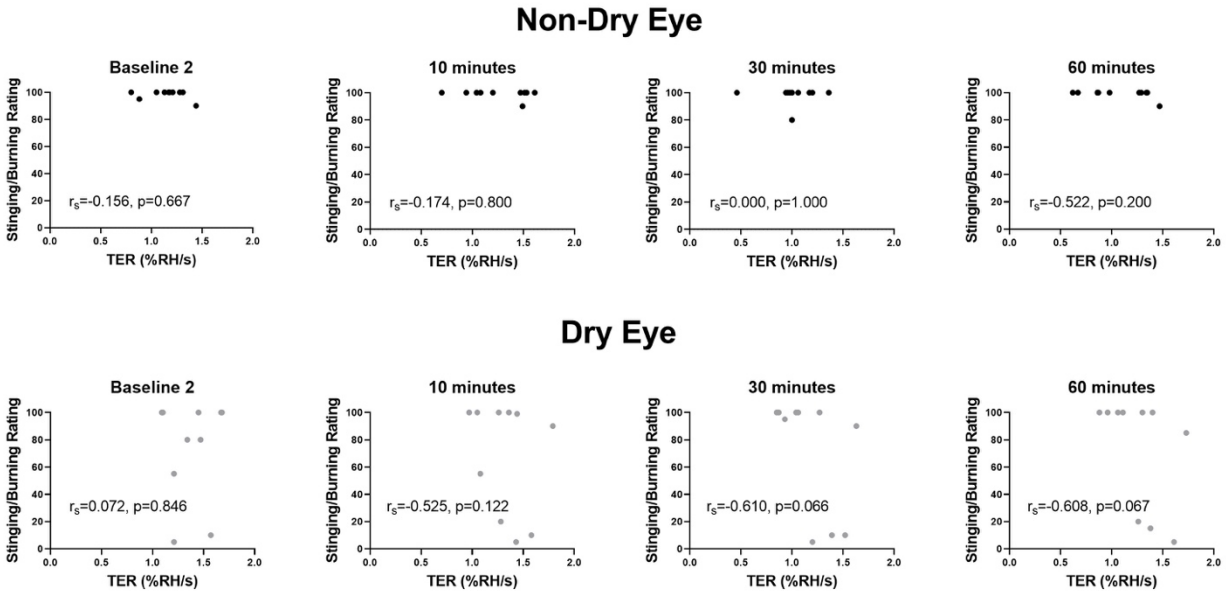

Figure 7-28: Correlations between the tear evaporation rate and the stinging/burning rating of the right eye(non-dry eye: all r_s<0.001, all p>0.199; dry eye: all r_s<0.073, all p>0.065).

7.4.4.3 Clinical Tests

A summary of correlations between the TER of the right eye and other clinical tests are shown in Table 7-9. The non-dry eye group had a significant negative correlation with the mean, maximum, and minimum LLT values obtained with the LipiView II at the second baseline measurement (mean: r=–0.720, p=0.019; maximum: r_s=–0.803, p=0.008; minimum: r_s=–0.768, p=0.012, Table 7-9, Figure 7-31, Figure 7-32, Figure 7-33). The dry eye group also had a significant negative correlation with the maximum and minimum LLT (maximum: r_s=–0.714, p=0.025; minimum: r_s=–0.835, p=0.004) at the second baseline measurement.

There was no significant correlation between the TER and the TMH (Figure 7-29) or the NIKBUT (Figure 7-30) of either group (non-dry eye: all r_s<0.324, all p>0.357; dry eye: all r_s<–0.042, all p>0.217).

Table 7-9: Correlations between tear evaporation rate of the right eye and other clinical tests

		Non-Dry Eye Group (n = 10 eyes)	p-value	Dry Eye Group (n = 10 eyes)	p-value
Tear meniscus height					
	Baseline 2	0.212[a]	0.560	–0.238[a]	0.503
	10 minutes	–0.212[a]	0.560	–0.430[a]	0.218
	30 minutes	0.323[a]	0.358	–0.043[a]	0.911
	60 minutes	–0.018[a]	0.973	–0.256[a]	0.471
Non-Invasive Keratograph break-up time					
	Baseline 2	–0.264[a]	0.463	–0.262[a]	0.459
	10 minutes	–0.139[a]	0.707	–0.292[a]	0.411
	30 minutes	–0.061[a]	0.872	–0.358[a]	0.313
	60 minutes	–0.239[a]	0.508	–0.371[a]	0.289
Lipid layer thickness (mean)					
	Baseline 2	**–0.720**	**0.019**	–0.457	0.184
	10 minutes	–0.043	0.906	–0.071	0.845
	30 minutes	–0.378	0.281	0.127	0.727
	60 minutes	0.126	0.728	–0.126	0.728
Lipid layer thickness (maximum)					
	Baseline 2	**–0.803**[a]	**0.008**	**–0.714**[a]	**0.025**
	10 minutes	–0.188[a]	0.599	–0.288[a]	0.416
	30 minutes	–0.320[a]	0.361	0.031[a]	0.937
	60 minutes	0.442[a]	0.204	–0.485[a]	0.160
Lipid layer thickness (minimum)					
	Baseline 2	**–0.768**[a]	**0.012**	**–0.835**[a]	**0.004**
	10 minutes	–0.139[a]	0.707	–0.085[a]	0.818
	30 minutes	–0.058[a]	0.873	–0.030[a]	0.939
	60 minutes	0.176[a]	0.624	–0.333[a]	0.349

[a] = Spearman rank correlation. **Bold** indicates significant correlations.

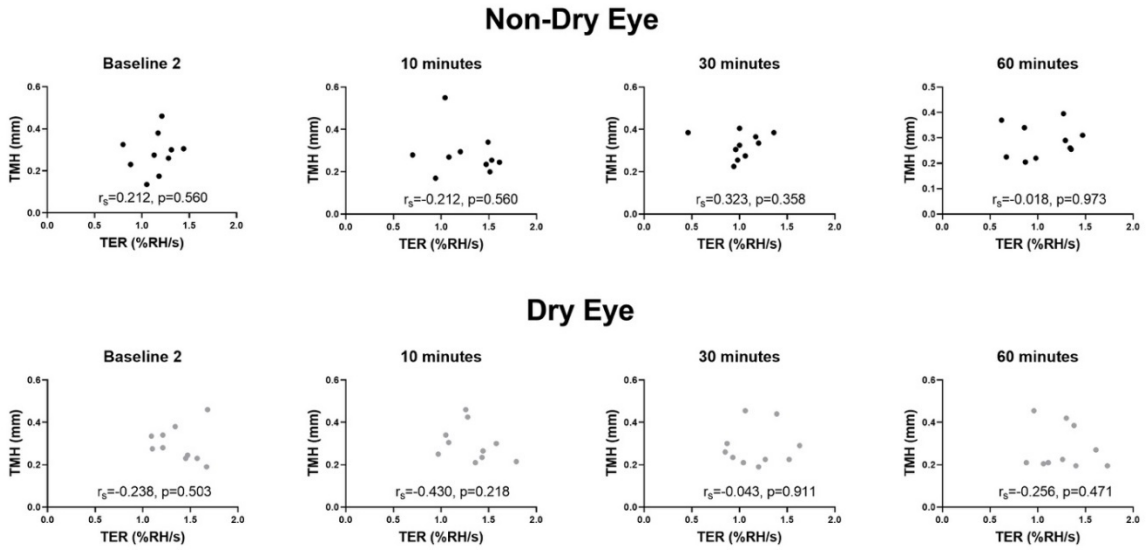

Figure 7-29: Correlations between the tear evaporation rate and the tear meniscus height of the right eye (non-dry eye: all r_s<0.324, all p>0.357; dry eye: all r_s<–0.042, all p>0.217).

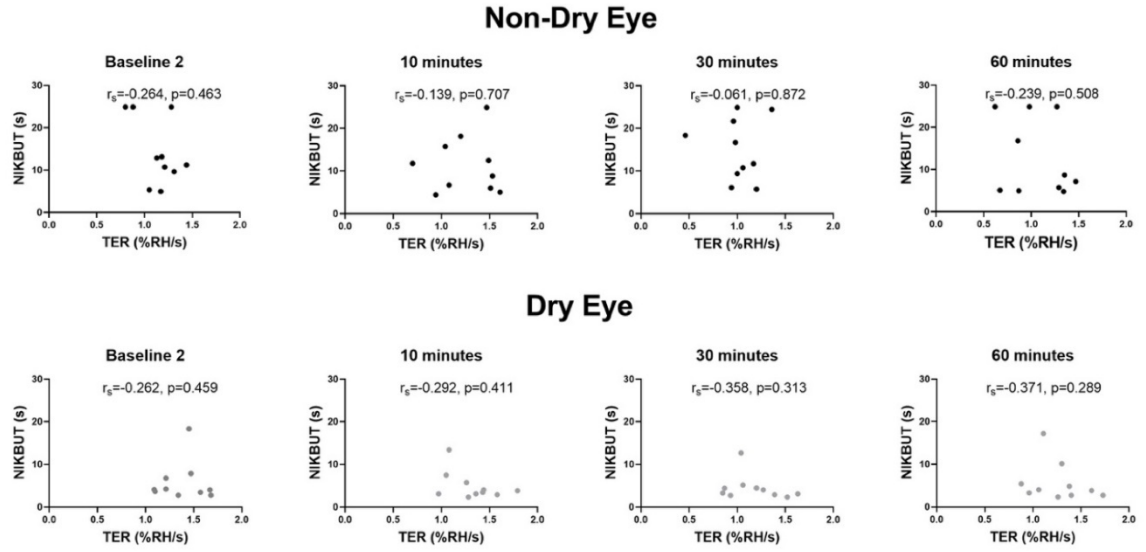

Figure 7-30: Correlations between the tear evaporation rate and the NIKBUT of the right eye(non-dry eye: all r_s<–0.060, all p>0.462; dry eye: all r_s<–0.261, all p>0.288).

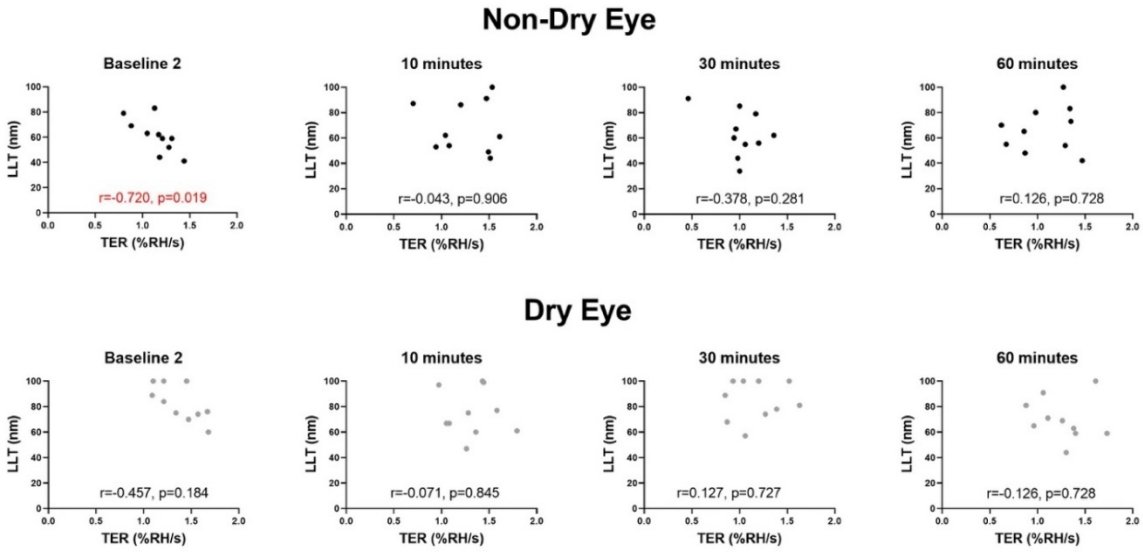

Figure 7-31: Correlations between tear evaporation rate and the mean lipid layer thickness of the right eye. A significant Pearson correlation is shown in red (non-dry eye: baseline 2 r=–0.720, p=0.019, all other r<0.127, all other p>0.280; dry eye: all r<0.128, all p>0.183).

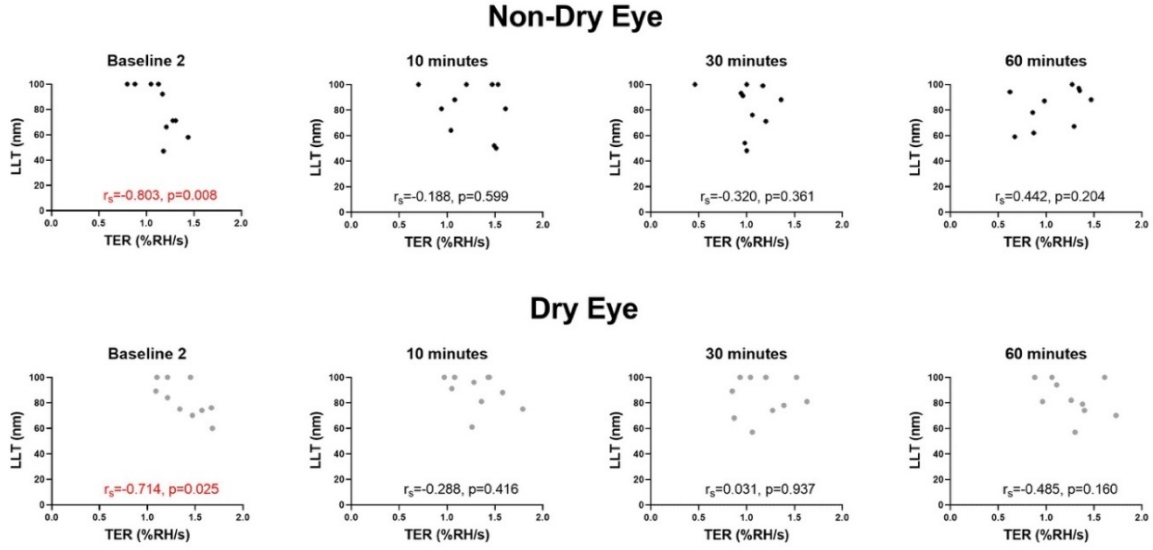

Figure 7-32: Correlations between the tear evaporation rate and the maximum lipid layer thickness of the right eye. Significant Spearman correlations are shown in red (non-dry eye: baseline 2 r_s=–0.803, p=0.008, other r_s<0.443, other p>0.203; dry eye: baseline 2: r_s=–0.714, p=0.025; other r_s<0.032, other p>0.159).

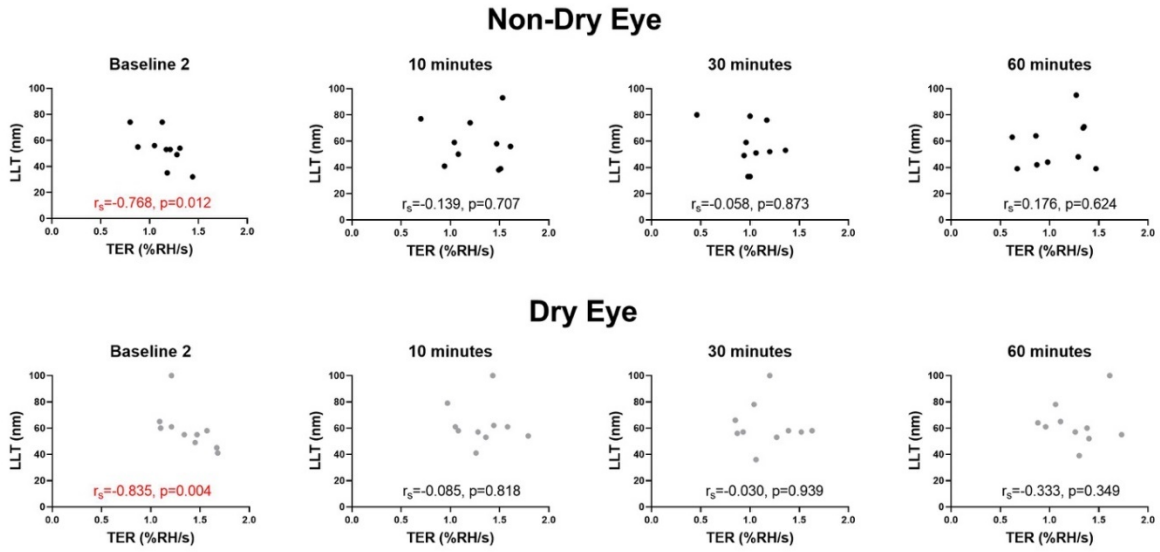

Figure 7-33: Correlations between the tear evaporation rate and the minimum lipid layer thickness of the right eye. Significant Spearman correlations are shown in red (non-dry eye: baseline 2 r_s=–0.768, p=0.012 other r_s<0.177, other p>0.623; dry eye: baseline 2 r_s=–0.835, p=0.004, other r_s<–0.029, other p>0.348).

7.5 Discussion

Following *in vitro* testing of the evaporimeter, further testing of the instrument was required to investigate whether the device was able to differentiate between dry eye and non-dry eye, and whether it could detect *in vivo* changes in TER following the instillation of Systane® Complete. Validation of the new device against a "gold standard" [427] was not possible because a "gold standard" test for evaporimetry does not currently exist. Construct validity [428] was examined by comparing dry eye participants to non-dry eye participants, and by investigating the effect of the ocular lubricant on the TER. The evaporimeter demonstrated the ability to detect higher rates of TER in the dry eye participants, and a lower TER in the dry eye population 30 minutes after the eye drop was instilled. External validity [429] was tested by comparing the results to previous research. Although no specific findings have been published regarding evaporimetry and Systane® Complete, other research has measured higher TERs in people suffering from dry eye [5, 11, 150] and a short-term improvement in other clinical tests [391, 422] after instillation of an artificial lubricant. Convergent validity [427, 428, 430] was demonstrated with strong correlations found

between TER and LLT (all r or r_s>−0.713), with all of the significant correlations greater than the recommended value of r=0.70 [430].

7.5.1 Changes over Time

Since the novel evaporimeter simultaneously measures both eyes, the results from each side of the goggles were initially examined separately. Each side of the evaporimeter provided repeatable baseline measurements for both the dry eye and non-dry eye participants.

The evaporimeter also demonstrated the ability to detect significant changes in TER over time in both groups. A significant increase in TER occurred in the left eye of the non-dry eye group 10 minutes after the eye drop was instilled, which may be due to the eye drop causing disruption of the lipid layer or an increase in tear volume [75]. Both eyes of the non-dry eye group had a significant decrease in TER between 10 minutes and 30 minutes after the eye drop was instilled.

The TER of the right eye of the dry eye group significantly decreased between the second baseline measurement and 30 minutes after instillation of the eye drop. Measurements from the left side of the evaporimeter for the dry eye group may not have had a statistically significant change in TER due to a lack of power (observed power=0.39) required to detect a difference between the second baseline measurement and 30 minutes post-instillation of the drop, with a sample size of 25 required to reach 0.80 power. In addition, the baseline measurements found no significant difference in the amount of MGYLS between the two groups, and the right eye had the worst median NIKBUT at all time points during the test visit (baseline 1: 80%, baseline 2: 60%, 10 minutes: 70%, 30 minutes: 70%, 60 minutes: 70%). Despite this, a similar trend was observed for both sides of the evaporimeter, with a lower TER observed 30 minutes after the drop was instilled and followed by a subsequent increase at 60 minutes, by which time the effect of the drop had presumably decreased.

Due to the lack of peer-reviewed literature examining the effect of Systane® Complete and evaporimetry, a direct comparison of our results is not possible. Trees and Tomlinson [15, 75] found an increase in TER following a 50 µl instillation of artificial tears or saline in participants without dry eye. A prolonged increase in TER was observed in some participants, with the TER remaining higher than the baseline measurement throughout the entire measurement period of approximately 38 minutes following instillation of the drops. A binocular evaporimeter used by Tsubota and Yamada [2] found a significant increase in TER lasting between 1 to 5 minutes after instillation of artificial tears or sodium hyaluronate, which was followed by a

subsequent return to baseline levels by 10 minutes. Additional work by the same group found increased TER at 10 minutes and 30 minutes after instillation of an eye drop containing hydroxypropyl methylcellulose, with no significant difference in TER at 45 minutes, compared to the baseline measurement [193]. However, no significant change in TER was observed following the use of sodium hyaluronate or saline. Other work investigating a single 40 μl application of Systane® found a significant decrease in TER 30 minutes after instillation of the eye drop, with a non-significant difference in TER between the baseline measurement and 60 minutes after the drop was applied [12]. However, additional work done by the same group were unable to find a significant difference in TER between baseline and 30 minutes after instillation of Optive or a different formulation of Systane [13], nor was any change in TER detected with the Eye-VapoMeter 15 minutes after a single application of Systane® Balance or Tears Again® [98].

Previous work investigating Systane® Complete found no significant change in clinical signs after two weeks of use in symptomatic contact lens wearers [421]. Muntz et al. [391] reported improvements in the LLT and NIKBUT of dry eye participants 10 minutes after the artificial lubricant was instilled, which were sustained following 2.5 minutes of exposure to a fan in order to create an adverse environment. Although this current study used the same instrumentation to measure the NIKBUT, approximately twice the amount of eye drop was instilled (~30 μl) by Muntz and colleagues [391]. Therefore, a significant change in NIKBUT may not have been found in this study due to the smaller, controlled volume of eye drop inserted into each participant's eyes. Muntz et al. [391] did not observe a change in TMH, although this study found a statistically significant increase in TMH in the non-dry eye group 30 minutes after the eye drop was instilled. This change in TMH was not clinically significant if the minimal clinically important difference is 0.1 mm [143].

A short-term improvement in the maximum LLT of dry eye participants was also observed in this study, with a significant increase in maximum LLT 10 minutes after Systane® Complete was instilled, which was significantly lower by one hour after the drop was instilled. Weisenberger et al. [422] were unable to find a significant change in LLT in dry eye subjects with a LLT <75 nm. However, a sub-group of participants with thinner LLTs <50 nm experienced a short-term increase in LLT 15 minutes after the artificial lubricant was instilled. Although this increase in LLT was not present between 1 and 6 hours after instillation of the drop, an improvement in symptoms was observed for up to 6 hours after use of the drop. No significant long-term change in LLT was detected after 1 month of eye drop use.

Use of different instrumentation could be one reason why varying LLT results were obtained between studies, with other researchers finding that the LLT results from the Tearscope Plus cannot be inter-changed with the LipiView maximum and minimum LLT [431]. This could be due to the fact that the LipiView II examines a smaller area of the inferior cornea approximately 2.5 mm in height by 5 mm in width [410, 432], whereas the K5M used by Muntz et al. [391] visualizes the tear film covering the superior, central, and inferior cornea. Weisenberger et al. [422] used a stroboscopic video color microscope to view the overall LLT covering a 6 mm area, which was further sub-divided into 2 mm sections of the overall area.

Yeu et al. [420] studied the use of Systane® Complete for 28 days and found an increase in fluorescein TBUT after 14 and 28 days of use, and an improvement in ocular discomfort after 14 days of use. Although short-term [391, 422] and long-term [420, 421] improvements in symptoms have been reported with Systane® Complete, a change in subjective symptoms was not observed in this study. This could be due to the small volume of product instilled, the small sample size, a lack of symptoms in the non-dry eye group, and variability in the amount of discomfort reported by the dry eye group.

7.5.2 Baseline Differences Between Eyes

Differences between the eyes were examined at the baseline visit to investigate whether discrepancies between the eyes of each group might explain why a significant difference in TER over time was not found in all the eyes of the two groups. Although there was a statistically significant difference in visual acuity between eyes of the non-dry eye group, this was not clinically significant because the acuity only differed by two letters. The non-dry eye group also had a larger ocular surface area in the left eye than the right eye, which may be due to anatomical variations between the eyes. A difference in the length of the horizontal palpebral aperture between the two eyes occurs in thirty percent of individuals and there may also be asymmetry in the height of the palpebral aperture [433]. The area of the ocular surface found in this study falls within the previously reported values that range from $144.46 \pm 30.14\ mm^2$ [434] to $240 \pm 58\ mm^2$ [97].

The only difference noted in the dry eye group between the two eyes at the baseline visit was in the longer NIKBUT of the right eye of the dry eye group. Although there was a significant difference, the median NIKBUT of 6.34 seconds meets the TFOS DEWS II dry eye criteria of <10 seconds [143].

7.5.3 Differences Between Groups

At the baseline visit, the OSDI scores of the dry eye participants were significantly higher than the non-dry eye participants, with the severity ranging from mild to severe [435]. Dry eye participants also had

shorter NIKBUTs, with poor quality of meibomian gland expression in both eyes, and fewer MGYLS in the right eye, indicating an evaporative component to their dry eye disease. No significant difference in TMH was found at the screening visit between the groups, which implies that the impact of tear volume on evaporation should be similar from each group. Participants were required to not use ocular lubricants on the day of their visits. This was assumed to be a sufficient washout period because the maximum residency time of different artificial lubricants is reported to be 41 minutes [436].

At the test visit, the decreased levels of comfort, dryness, and stinging/burning experienced by the dry eye group during the test visit reflects the higher OSDI [423] scores required in the inclusion criteria. NIKBUT was the only objective clinical test which consistently measured significantly higher values for the non-dry eye group compared to the dry eye group. Nevertheless, the evaporimeter was able to detect a significantly higher TER in dry eye participants compared to non-dry eye participants at the second baseline measurement. Other evaporimeters have also measured higher TERs have in dry eye participants at RHs of 5% [92], 10–15% [101, 177], 20% [91], 25–35% [109] and 35–45% [74], although others have also reported no difference in TER between non-dry eye and dry eye participants at 10–15% RH [177] and between 20–25% RH [6, 107].

7.5.4 Correlations Between Tear Evaporation Rate and Other Tests

No significant correlations between TER and different measurements of symptomology provide further evidence of a poor relationship between clinical signs and symptoms of dry eye [437-439] due to the complex, multifactorial nature of the disease.

Significant negative correlations were found between the TER and minimum/maximum LLT of both groups (all r or r_s>–0.713, all p<0.026) and between the TER and the average LLT of the non-dry eye group (r=–0.720, p=0.019) at the second baseline measurement. Craig and Tomlinson [182] reported a negative correlation between TER and LLT, with significantly higher TERs associated with either an absent lipid layer or an abnormal colored fringe pattern [49]. However, others have reported a positive correlation between TER and LLT under different ambient temperatures [115].

A significant correlation between TER and NIKBUT may not have been found in this study due to participants blinking every three seconds during evaporimetry. Despite the fact a frequent blink rate may have replenished the lipid layer prior to or just after a tear film break-up, a short blink interval was chosen to prevent reflex tearing. Since blinking less than every 6 seconds can induce reflex tearing in people

without dry eye [151], a more frequent blink interval was chosen to minimize reflex tearing in the dry eye group.

Jeon et al. [74] reported a moderate negative correlation between TER and fluorescein TBUT in people with evaporative dry eye. However, Cedarstaff and Tomlinson [163] were unable to find a correlation between TER and fluorescein TBUT. This lack of correlation was attributed to TBUT measuring the break-up of the entire tear film, whereas evaporimetry can detect loss of the lipid layer, which may precede total rupture of the tear film [15]. Another reason why a correlation may not have been found is because TBUT measured with different techniques, including OCULUS Keratograph 4, can have poor agreement [21] between visits [440]. Some of the non-dry eye participants exhibited poor agreement between the two visits, suggesting that some of the participants may be asymptomatic and predisposed to dry eye [119], and this could have affected the ability to distinguish between the two groups at the test visit. Although NIKBUTs are significantly shorter than fluorescein TBUTs [440, 441], a different cut-off value for non-invasive techniques to diagnose dry eye has not been specified. However, the NIKBUT values of the participants fell within the range estimated by Cox et al. [440] whereby a person with a first NIKBUT of 10 seconds at one visit could have a NIKBUT ranging between 2.12 to 44.85 seconds one week later. The difference in NIKBUTs observed in the non-dry eye group may also be because longer TBUTs exhibit greater amounts of variability than short TBUTs [442].

No significant correlation was found between TER and the air volume within the goggle. However, in Chapter 5, *in vitro* testing of the evaporimeter showed a variation in TER as the range of volumes changed between 10 to 18.63 cm^3. Although previous equations have accounted for the volume inside the evaporimeter in their TER calculation [1, 3, 21, 161], the *in vivo* results from this study suggest that evaporimeter volume does not have a significant impact on TER. It should be noted that initial work to validate the VapoMeter measured a range of participant volumes inside the evaporimeter of 5 to 11 cm^3 [21], with further work using a standardized goggle volume of 7 cm^3 for Asians and 9 cm^3 for non-Asians to calculate the absolute TER [161]. The participants in this study had larger volumes that ranged from 13 to 19 cm^3, which may be due to the use of a different goggle on the commercially available instrument.

Significant positive correlations were found between the TER and the ocular surface area of the non-dry eye group at 10 minutes ($r=0.754$, $p=0.012$) and 60 minutes ($r=0.708$, $p=0.022$) after insertion of the eye drop. In Chapter 5, *in vitro* work demonstrated higher TERs as the size of the ocular surface increased. However, as a consistent correlation was not observed at all of the time points in the non-dry eye group and

there was no significant correlation in the dry eye group, the complexity of the tear film means that TER cannot be predicted by a single factor.

7.6 Future Work

7.6.1 Possible Sources of Error

Despite the study being a small, single-arm study with variations in individual TER, the evaporimeter was able to detect a difference in the baseline TER between the two groups and in response to an ocular lubricant. Ideally all of the clinical measurements would have been recorded inside an environmental chamber to control the effect of the ambient temperature [115] and RH [91, 92, 182]. However, as this was not possible, all of the measurements were taken during the same season and all clinical tests were performed within the same room. Fluctuations in the ambient RH are not expected have affected the TER in this study since the change in RH during the study was confined to a 5% range.

Some of the clinical measurements were limited by the maximum values of the instrument. The maximum LLT of the LipiView II is 100+ nm, which was converted to 100 nm for statistical analysis. The K5M also had a pre-determined upper limit NIKBUT of 24.92 seconds. Although some have advocated remeasuring suspicious results [431], in cases where the software incorrectly detected a first break due to hippus or eyelash artefacts, the video of the NIKBUT was reviewed by the investigator and the area of the first break was manually selected.

Although there was not a statistically significant difference between the ages of the participants, the median older age of the dry eye group may have contributed to the higher TER [111]. In addition, despite the baseline measurements being repeatable, the TER of the non-dry eye group showed a trend of decreased TER between the two measurements, while the TER of the dry eye group showed a trend of increased TER. This variation may be due to insufficient adaptation time to the room environment or disruption of the tear film prior to the first baseline measurement. A safety check was performed at the beginning of the test visit to record a baseline measurement of the eyes prior to instillation of the eye drop, which included recording visual acuities and slit-lamp biomicroscopy with lid eversion. Participants were then required to adapt for 15 minutes to the room where the clinical tests were performed, prior to any further clinical measurements being taken. Although longer adaptation times have been used [7, 13], a 15 minute adaptation period was chosen based on the findings that non-dry eye and mild dry eye participants tested with an open-chamber

evaporimeter required 10 minutes of adaptation to a 40% RH environmental chamber before the TER stabilized [92].

7.6.2 Refining the Evaporimetry Technique

Environmental factors should be controlled as much as possible in order to minimize their effect on the TER. Inclusion criteria can also include matching participants by age and sex to see if this reduces the amount of the individual variation in the TER observed in this study.

Since the second baseline measurement showed less variability than the first baseline measurement, additional testing should avoid the use of any test which might disrupt the tear film prior to evaporimetry. Multiple measurements should also be taken over an extended period of time without the use of an intervention, in order to determine the amount of normal variation expected to occur in TER over time. Extra testing will also need to be done to determine the test-retest reliability of the evaporimeter between different visits.

Although this study controlled the blink rate in order to control as many extraneous variables as possible, additional work should also include testing the TER with the eyes held open for longer periods of time, such as until they begin to feel uncomfortable, to see if a better correlation can be found between TER and NIKBUT.

Despite three repeated measurements of the open and closed eye being taken on this study, further work should aim to discover whether fewer measurements can be taken to speed up total testing time.

Future work should also investigate the optimal formula to calculate the TER. Other researchers have calculated TER or absolute TER using different formulae based on the area of ocular surface [1, 3, 21, 75, 101, 102, 161], the area of the eye chamber [3, 21, 75, 101, 102, 161], and the volume within the evaporimeter [1, 3, 21, 161]. Since the ocular surface area was occasionally correlated with the TER in this study, preliminary attempts to correct the TER by dividing by the ocular surface area resulted in the same post-hoc results for the non-dry eye group as the uncorrected TER. To improve the technique for recording the ocular surface area, both eyes should be photographed simultaneously ensure that differences between the eyes are not due to a change in fixation.

Once the optimal TER formula has been determined, the computer software can be upgraded to make it more user-friendly. After inputting any additional information that is required, such as the ocular surface

area or evaporimeter volume, the software should be able to record the temperature and RHs over a desired time interval, and this data can be used to automatically calculate the TER.

7.6.3 Future Dry Eye Work

This study demonstrated that the evaporimeter can detect a difference between people with and without dry eye, and that both sides of the evaporimeter can detect a change in TER following the use of Systane® Complete. Future dry eye work with evaporimetry can include testing different dry eye treatments, such as other lubricating drops, liposomal sprays, warm compresses, or thermal pulsation, and testing people suffering from different types of dry eye.

7.7 Conclusions

Pilot testing of the novel binocular evaporimeter validated its use for measuring the TER of non-contact lens wearers. The instrument provided repeatable baseline measurements in both eyes of the non-dry eye and dry eye group. NIKBUT and evaporimetry were the only objective clinical tests able to differentiate between dry eye and non-dry eye. Although dry eye participants had shorter NIKBUTs at all time points, and the evaporimeter was able to detect a higher baseline TER in dry eye participants compared to those without dry eye. The evaporimeter also measured changes in the TER of both groups over time, with the dry eye participants experiencing a lower TER 30 minutes after instillation of an eye drop designed to treat all types of dry eye.

Since the evaporimeter was the only objective clinic test that could detect a higher rate of TER in dry eye participants and also measure a change over time, this instrument seems to be a sensitive method of evaluating changes in the tear film.

The results of the study show that additional testing of the evaporimeter for dry eye is worthwhile. Its ability to measure differences between groups may be improved by ensuring the non-dry eye group has a consistent break-up time >10 seconds at each visit or by recruiting dry eye participants with a thin lipid layer. Future applications of the instrument could include contralateral testing of different eye drops, including ones specifically designed to treat evaporative dry eye, or other treatments for dry eye, such as thermal pulsation.

Chapter 8

Comparison of Tear Evaporation Rate with Delefilcon A and Nesofilcon A

8.1 Overview

PURPOSE: To examine whether a novel binocular evaporimeter can produce a tear evaporation rate (TER) that is able to: provide repeatable results; detect a change in response to contact lens (CL) wear; differentiate between asymptomatic and symptomatic CL wearers; and detect a difference between two types of CL after 6 hours of wear.

METHODS: This was a prospective, double-masked, contralateral eye, dispensing, randomized pilot study. Twenty CL wearers were enrolled and screened for inclusion at a screening visit. All participants were suitable and classified as asymptomatic or symptomatic (10 asymptomatic, 10 symptomatic) using the Contact Lens Dry Eye Questionnaire (CLDEQ-8) and trialed with both study lenses to ensure an acceptable fit and comfort. At the dispensing visit, two baseline TERs were recorded after a 15 minute interval. Participants were randomly assigned to wear delefilcon A in one eye and nesofilcon A in the other eye. TER was assessed after CLs had settled for 15 minutes. A follow-up visit was conducted on the same day to measure the TER after CLs were worn for 6 hours. Evaporimetry consisted of three repeated 20 second measurements with the eyes open and closed. Ocular surface TER was determined by subtracting closed eye TER from open eye TER. The primary outcome variable was the mean TER at each time point. TER was calculated as the slope of the change in relative humidity over time when the evaporimeter was held over the eyes (open eye: 7 to 17.5 seconds, closed eye: 10 to 17.5 seconds).

RESULTS: Twenty people (10 asymptomatic, 10 symptomatic) completed the study (asymptomatic: median age: 22 years, 90% female; symptomatic: median age: 23 years, 100% female). Baseline TER measurements were repeatable in both eyes of the symptomatic group (right eye: p=0.49; left eye: p=0.08) and the right eye of the asymptomatic group (p=0.38), but not in the left eye of the asymptomatic group (p=0.04). Changes in TER over time were detected in both eyes with the groups combined (right eye: p=0.037; left eye: p=0.008). The TER of the right eye was significantly higher after 6 hours of CL wear compared to the second baseline measurement (p=0.043). The TER of the left eye was significantly higher after 15 minutes (p=0.002) and 6 hours of CL wear (p=0.004) compared to the second baseline measurement. Analysis of each eye of the two groups found a significantly higher TER at 6 hours of CL

wear compared to the second baseline measurement (p=0.002) in the left eye of the symptomatic group, but no significant change over time was found in either eye of the asymptomatic group (right eye: p=0.057; left eye: p=0.062) or the right eye of the symptomatic group (p=0.062). No significant difference in TER was detected between the two groups (second baseline measurement: p=0.451, 15 minutes: p=0.211, 6 hours: p=0.434), or between delefilcon A and nesofilcon A after 15 minutes (p=0.268) or 6 hours of wear (p=0.436).

CONCLUSIONS: The novel binocular evaporimeter:

- Provided repeatable baseline results for both eyes of the symptomatic group and the right eye of the asymptomatic group;
- Detected a change in TER in each side of the evaporimeter with CL wear;
- Detected a significantly higher TER in the left eye after 15 minutes of CL wear and in both eyes after 6 hours of wear;
- Measured a significantly higher TER after 6 hours of wear in the left eye of the symptomatic group;
- Did not detect a difference in TER between asymptomatic and symptomatic CL wearers;
- Did not detect a difference in TER between delefilcon A and nesofilcon A after 15 minutes or 6 hours of wear.

Preliminary testing of the evaporimeter demonstrates that the instrument was able to detect changes in TER when a CL was worn. However, further testing is needed to determine whether it can be used to differentiate between asymptomatic and symptomatic CL wearers or between different types of CLs.

8.2 Introduction

Multiple clinical tests have been associated with contact lens (CL) discomfort, including corneal and conjunctival staining, conjunctival indentation, palpebral conjunctival staining, non-invasive break-up time, and tear meniscus height [443]. Although CL discomfort cannot be diagnosed with a single clinical test, Young et al. [444] reported that an assessment of the stability of the pre-lens tear film was the most appropriate test to identify symptomatic CL wearers.

Siddireddy et al. [161] included evaporimetry as one of the clinical tests and determined that tear evaporation rate (TER) measured with and a without CL was an acceptable test for predicting CL discomfort. Other researchers have also reported that evaporimetry can detect significant differences in TER between various types of CLs [16, 102, 155, 368]. Therefore, *in vivo* testing of the novel evaporimeter was warranted to determine whether it could be used to diagnose CL discomfort and to test its ability to detect changes in TER with CL wear.

Although the tear film is altered by CL wear [30, 36, 165] and results in higher rates of evaporation [16, 21, 73, 102, 110], the tear film remains present on the front surface of a CL [30, 288, 444, 445]. As mentioned in the previous chapter, because *in vitro* testing of the evaporimeter was conducted with heated water, the rate of evaporation from the human tear film is expected to be different. Thus, the first step in investigating the potential application of the novel evaporimeter to future CL work will be to determine the time period over which the TER slope should be calculated.

Chapter 7 also demonstrated that the novel evaporimeter can take repeatable baseline measurements in non-CL wearers. However, since disruption to the tear film and TER is present the day after CLs are worn [110], the repeatability of two consecutive baseline measurements must also be separately examined in CL wearers prior to insertion of CLs. The CLs selected for *in vivo* testing were based on the *in vitro* results from Chapter 2, which demonstrated that the Eye-VapoMeter measured significantly different rates of evaporation with delefilcon A and nesofilcon A. None of the current *in vivo* peer-reviewed literature comparing delefilcon A to nesofilcon A [245, 253, 294, 359, 446] has included evaporimetry as a clinical test. Therefore, an additional aim of this study was to examine whether the novel evaporimeter could detect a difference in TER between two different CLs.

The aims of the study were to:

- Evaluate whether the evaporimeter can provide repeatable baseline results;
- Evaluate whether the evaporimeter can detect a change in TER with CL wear;
- Test whether the evaporimeter can differentiate between asymptomatic and symptomatic CL wearers;
- Test whether the instrument can detect a difference in TER between delefilcon A and nesofilcon A after 6 hours of wear.

8.3 Materials and Methods

This study was a prospective, double-masked, contralateral eye, dispensing, randomized pilot study, involving one screening and two test visits.

8.3.1 Participant Recruitment

The study was designed to follow the ethical principles of the Declaration of Helsinki, along with the International Council for Harmonization: Good Clinical Practice, the University of Waterloo Guidelines for Research with Human Participants, and the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans, 2nd edition. The study received ethics clearance from the Office of Research Ethics at the University of Waterloo, Waterloo, Ontario (ORE #41195) and was registered with ClinicalTrials.gov (ID: NCT04037969). The study was advertised using the recruitment system at the Centre for Ocular Research & Education (CORE) at the University of Waterloo.

8.3.2 Inclusion and Exclusion Criteria

Participants were eligible for inclusion in the study if they: were at least 17 years of age and had the full legal capacity to volunteer; had read and signed the information consent letter; were willing and able to follow instructions and maintain the appointment schedule; had worn soft CLs for a minimum of 6 months; currently wore soft CLs at least 4 days per week and 8 hours per day; had an acceptable fit and comfort with both study CLs in the powers available; had ≤1.00DS difference between eyes in their habitual CLs; were willing to be awake for at least 2 hours before the test visit; were willing not to wear eye makeup on the day of visits 2 and 3; were willing not to use eye drops or ocular lubricants on the day of the screening visit or test visits, and had a wearable pair of spectacles.

Participants were excluded from the study if they: were participating in any concurrent clinical or research study; had any known active ocular disease and/or infection[2]; had a systemic condition that in the opinion of the investigator may affect a study outcome variable; were using any systemic or topical medications that in the opinion of the investigator may affect a study outcome variable; were pregnant, lactating, or planning a pregnancy at the time of the enrolment; were aphakic; had undergone refractive error surgery;

[2] For the purposes of this study, active ocular disease was defined as infection or inflammation which requires therapeutic treatment. Mild (i.e. not considered clinically relevant) lid abnormalities (blepharitis, meibomian gland dysfunction, papillae), corneal and conjunctival staining and dry eye are not considered active ocular disease. Neovascularization and corneal scars are the result of previous hypoxia, infection, or inflammation and are therefore not active.

had a known sensitivity to sodium fluorescein dye; had a known sensitivity to petroleum jelly (Vaseline®); had epilepsy and/or sensitivity to flashing lights; wore toric CLs; had any physical impairment that would interfere with holding the evaporimeter, or had taken part in another clinical research study involving ocular drops or treatments within the past 14 days.

8.3.3 Study Design

Informed consent was obtained from all participants prior to enrolment. The screening visit occurred on a separate day from the two test visits. A summary of the clinical tests that were undertaken is included in Table 8-1. In brief, symptomology was assessed using the Contact Lens Dry Eye Questionnaire [447]. Participants were classified as asymptomatic if the score was <12 and symptomatic if the score was ≥12 [448]. Following slit-lamp biomicroscopy, the eligibility criteria were assessed to ensure participants were suitable to continue with the screening visit. Suitable participants were fitted with both study CLs to ensure an acceptable fit. Afterwards, an assessment of the ocular surface area, volume inside the evaporimeter, and a final review of all the inclusion and exclusion criteria were conducted.

The dispensing visit and follow-up visit were both scheduled to occur on the same day, between 1 to 14 days after the screening visit. Evaporimetry, non-invasive break-up time, subjective lipid layer thickness (LLT), and objective LLT were assessed at 4 time points prior to and after CLs were worn: two baseline measurements were taken 15 minutes apart, followed by measurements at 15 minutes and 6 hours of CL wear (Figure 8-1). Subjective comfort ratings were recorded prior to the first baseline measurement, 15 minutes post-CL insertion, and after 6 hours of CL wear. CL fit was assessed after 15 minutes and 6 hours of CL wear. All measurements were recorded for both eyes.

Table 8-1: Summary of procedures and instruments

Visit	Testing order	Procedure	Instrument
Screening	1	Informed consent	N/A
	2	Demographics	N/A
	3	Medical history	N/A
	4	Contact lens history	N/A
	5	Symptoms assessment	CLDEQ-8
	6	Entrance visual acuity	Electronic logMAR chart
	7	Meibomian gland expression	Meibomian Gland Evaluator
	8	Lid margin assessment	Slit-lamp
	9	Biomicroscopy, including corneal and conjunctival staining	Slit-lamp and fluorescein

Visit	Testing order	Procedure	Instrument
Screening	10[a]	Insert study contact lenses	N/A
	11[a]	Visual acuity	Electronic logMAR chart
	12[a]	Spherical over-refraction	Phoropter
	13[a]	Best-corrected visual acuity	Electronic logMAR chart
	14[a]	Contact lens fit	Slit-lamp
	15	Remove study contact lenses #1	N/A
	16	Anterior eye photographs	Slit-lamp, Canon EOS 60D
	17	Evaporimetry	Evaporimeter
	18	Remove study contact lenses #2	N/A
	19	Goggle volume measurement	Modified swimming goggles
	20	Biomicroscopy safety check, including corneal and conjunctival staining	Slit-lamp and fluorescein
Dispense	1	Entrance visual acuity	Electronic logMAR chart
	2	Biomicroscopy (without staining)	Slit-lamp
	3	Randomize participant	Randomization
	4[b]	Subjective comfort rating	N/A
	5[c]	Evaporimetry	Evaporimeter
	6[c]	Non-invasive break-up time	Tearscope Plus
	7[c]	Lipid layer thickness	Tearscope Plus
	8[c]	Lipid layer thickness	LipiView® II
	9	Insert study contact lenses	N/A
	10	Subjective comfort rating	N/A
	11	Evaporimetry	Evaporimeter
	12	Non-invasive break-up time	Tearscope Plus
	13	Lipid layer thickness	Tearscope Plus
	14	Lipid layer thickness	LipiView® II
	15	Contact lens fit	Slit-lamp
	16	Exit visual acuity	Electronic logMAR chart
Follow-up	1	Entrance visual acuity	Electronic logMAR chart
	2	Contact lens fit	Slit-lamp
	3	Subjective comfort rating	N/A
	4	Evaporimetry	Evaporimeter
	5	Non-invasive break-up time	Tearscope Plus
	6	Lipid layer thickness	Tearscope Plus
	7	Lipid layer thickness	LipiView® II
	8	Contact lens removal	N/A
	9	Biomicroscopy, including corneal and conjunctival staining	Slit-lamp and fluorescein
	10	Exit visual acuity	Electronic logMAR chart

[a]: Contact lens 1 = nesofilcon A; Contact lens 2 = delefilcon A.
[b]: First baseline measurement only.
[c]: Two baseline measurements (15 minutes apart).
N/A: not applicable.

Figure 8-1: Summary of the measurement time points at the test visits.

8.3.4 Study Procedures

Subjective and objective clinical tests were performed at the study visits by a single investigator (SW). Data was manually recorded on clinical record forms. A description of the procedures that were performed is provided below.

8.3.4.1 Demographics

Demographic information was recorded for each participant, including age and sex.

8.3.4.2 Contact Lens History

Information regarding the brand of CL, CL solution, and frequency of CL wear was recorded.

8.3.4.3 Medical History

Medical history was obtained at the screening visit to record current medications, dry eye treatments, allergies, and other relevant medical conditions. Participants were asked about any changes in their medication or medical condition at each subsequent visit.

8.3.4.4 Visual Acuity

Distance visual acuity was measured using a computerized high-contrast logMAR chart (VACUITY MkII v3.0, CORE, Waterloo, ON, Canada or Clinical Trial Suite, M&S® Technologies, Niles, IL, USA) under high ambient room illumination. Visual acuity was tested with the participant's habitual spectacle correction/spectacle refraction or the study CLs.

8.3.4.5 Contact Lens Dry Eye Questionnaire (CLDEQ-8)

All participants completed the Contact Lens Dry Eye Questionnaire (CLDEQ-8) [447] at the screening visit. The CLDEQ-8 assesses how often various symptoms (e.g. discomfort, dryness, variable vision, etc.) were experienced over the previous two weeks and how intense the symptoms felt. Symptoms were rated on a scale of 0 (“never”) to 4 (“constantly”), except for frequency of how often it felt like the lenses needed to be removed which was rated from 1 (“never”) to 6 (“several times a day”). The intensity of the symptoms ranged from 0 (“never have it”) to 5 (“very intense”). Participants were classified as asymptomatic if the CLDEQ-8 score was <12 and symptomatic if the score was ≥12 [448].

8.3.4.6 Subjective Comfort Ratings

Participants were asked to complete a numerical analog scale regarding their comfort, dryness, and burning or stinging for each eye on a 0–100 scale. A score of 0 indicated the eyes were uncomfortable, dry, or stinging/burning while 100 represented an eye that was comfortable, not dry, or not experiencing any stinging/burning.

8.3.4.7 Meibomian Gland Expression

Patency of the lower eyelid meibomian glands was assessed using the Meibomian Gland Evaluator (MGE) (Johnson & Johnson Vision Care, Inc., Jacksonville, Florida, USA) [425]. The MGE was depressed halfway to apply pressure to the lower eyelid just below the lash line. The MGE was applied to the nasal, central, and temporal area of each lid. Five consecutive glands in each area were assessed for the quality of expression. The quality of expression was graded using a modified 0–3 scale ((0: no secretion (including capped orifices), 1: inspissated (semi-solid, toothpaste like), 2: colored/cloudy liquid, 3: clear, liquid oil)) [449]. The results from each eye were summed to create the Meibomian Gland Score.

8.3.4.8 Lid Margin Assessment

The lid margin was assessed for vascularity (erythema), amount of lash loss, edema, telangiectasia, and tear film debris (Appendix F). The vascularity of the lid margin and telangiectasia were graded on a 0–4 scale. The presence or absence of lid margin edema and tear film debris were also recorded.

8.3.4.9 Slit-lamp Biomicroscopy

See Section 7.3.4.11 and Appendix F. Conjunctival indentation was assessed using a DIOFLUOR sodium fluorescein strip (DIOPTIC Pharmaceuticals Inc, Toronto, Ontario, Canada) and the number of mucin balls was assessed.

8.3.4.10 Contact Lens Fitting

Details of the study CL parameters are provided in Table 8-2. The fit of the study CLs was assessed at the screening visit after the CLs had settled for a minimum of 10 minutes, and at the two test visits. The fit was examined using a slit-lamp biomicroscope set to 16x magnification. CL centration, movement, limbal coverage, tightness/push-up test, and overall fit were graded as acceptable or unacceptable. If the initial CL fit was deemed acceptable, participants were asked if the CLs felt comfortable. Participants who reported that the CLs felt uncomfortable or that had an unacceptable lens fit were not permitted to continue with the study. The presence or absence of lens deposits was also recorded.

Table 8-2: Contact lens parameters

Material	**Delefilcon A**	**Nesofilcon A**
Manufacturer	Alcon Canada Inc.	Bausch + Lomb Inc.
Brand Name	DAILIES TOTAL1®	Biotrue® ONEday
Equilibrium Water Content	core 33%, surface ≥80%	78%
Dk/t at -3.00D	156	42
Sphere power (D)	+0.50 to +6.00 (in 0.25 steps) –0.50 to –6.00 (in 0.25 steps) –6.50 to –9.00 (in 0.50 steps)	+0.50 to +6.00 (in 0.25 steps) –0.50 to –6.00 (in 0.25 steps) –6.50 to –9.00 (in 0.50 steps)
Base curve (mm)	8.5	8.6
Diameter (mm)	14.1	14.2
Centre thickness at -3.00D (mm)	0.09	0.10
Health Canada licence #	87774	89630

8.3.4.11 Contact Lens Dispensing

CLs were inserted directly from the blister pack at the screening visit. At the dispensing visit, a research assistant transferred the CLs and blister pack solution to a CL cup prior to the participant inserting the CLs in order to mask the participant and investigator.

8.3.4.12 Ambient Temperature and Relative Humidity

The ambient temperature and relative humidity (RH) inside the room where the clinical tests were performed was monitored using an EXTECH RHM15 mini hygro-thermometer (FLIR Commercial

Systems Inc., Nashua, New Hampshire, USA). The temperature and RH were recorded at each time point before performing evaporimetry.

8.3.4.13 Application and Removal of Petroleum Jelly

See Section 7.3.4.14. Petroleum jelly was applied prior to the first set of baseline measurements, before the measurements taken 15 minutes after the CLs were inserted, and before the measurements taken 6 hours after CLs were worn. Petroleum jelly was removed after the second set of baseline measurements, after the visual acuity check 15 minutes after CLs were worn, and after the measurements taken after 6 hours of CL wear.

8.3.4.14 Evaporimeter Measurements

See Section 7.3.4.15.1 regarding evaporimetry and Section 7.3.4.15.2 for details of the measurement of air volume inside the evaporimeter.

8.3.4.15 Anterior Aye Photographs

See Section 7.3.4.16.

8.3.4.16 Tear Film Stability

A slit-lamp and illumination from the Tearscope Plus (Keeler, Windsor, Berkshire, UK) [424] were used to evaluate the Non-Invasive Tear Film Break-Up Time (NITBUT) with and without CLs. The participant was asked to blink a few times and then to keep their eyes open for as long as possible. The NITBUT was recorded as the time taken after a blink until the appearance of the first dark spot [424] or the time until the next blink occurred if a break was not observed. Three consecutive measurements of the NITBUT were taken for each eye and the mean value was calculated for each eye.

8.3.4.17 Subjective Lipid Layer Thickness

A slit-lamp and illumination from the Tearscope Plus were used to evaluate the LLT. The observed lipid pattern was graded using Guillon and Guillon's classification system [424] (1: open meshwork, 2: closed meshwork, 3: wave, 4: amorphous, 5: colour fringe, and 6: other).

8.3.4.18 Objective Lipid Layer Thickness

The LipiView® II Ocular Surface Interferometer (Johnson & Johnson Vision Care, Inc., Jacksonville, Florida, USA) was used to assess LLT of the tear film. The software automatically computes the average,

minimum, and maximum LLT. Participants were asked to blink as needed during the approximately 30 seconds recording.

8.3.5 Statistical Analysis

A sample size calculation could not be determined for this pilot study because this was the first time the evaporimeter was tested on a cohort of CL wearing participants. Although a sample size was calculated after the first 20 participants were seen, additional participants were not able to be recruited because of clinical research being halted due to COVID-19.

Statistical analyses were conducted using GraphPad Prism v.8.3.0 (GraphPad Software, LLC, San Diego, California, USA). Data was tested for normality using the Shapiro-Wilk test with α=0.05. A three-way mixed ANOVA was used to determine the interaction between time, group, and eye. Differences between groups were analyzed with a Mann-Whitney test for non-parametric data or an unpaired t-test for normally distributed data. Differences between eyes were analyzed with a Wilcoxon signed-rank test for non-parametric data or a paired t-test for normally distributed data. Repeatability of the baseline TER measurements were compared using a Wilcoxon matched-pairs signed rank test. Changes over time were analyzed with a Friedman test and a post-hoc Dunn's test for non-normally distributed data, or with a one-way repeated measures analysis of variance (ANOVA) for normally distributed data with a Greenhouse-Geisser correction and a post-hoc Tukey's multiple comparisons test or Dunnett's multiple comparisons test. Differences between types of CL were analyzed using an unpaired t-test. Pearson's correlation coefficient (r) or Spearman's rank correlation (r_s) were used to determine the linear correlation between the TER and the other tests. A p-value <0.05 was considered statistically significant. Post-hoc power (α=0.05) and *a priori* sample size calculation (α=0.05, power=0.8) was conducted using G*Power v. 3.1.9.4 (Heinrich Heine Universität Düsseldorf, Düsseldorf, Germany) [145].

8.4 Results

8.4.1 Demographics

Twenty participants were screened between August 2019 and November 2019. All participants (10 asymptomatic, 10 symptomatic) were found to be suitable, and all participants (1 male, 19 females) completed the study.

The majority of participants were female (90% of the asymptomatic group, 100% of the symptomatic group). The median age (interquartile range, IQR) of the asymptomatic group was 22.00 (7.75) and 23.00 (14.25) years old in the symptomatic group (p=0.96, Figure 8-2). The age of the participants ranged from 18 to 71 years old.

Twenty percent of asymptomatic CL wearers used ocular lubricants between two to four times per week. Thirty percent of symptomatic CL wearers used ocular lubricants once or twice a week.

Ten percent of asymptomatic and forty percent of symptomatic CL wearers reported suffering from allergies, with none of the participants reporting any ocular allergies.

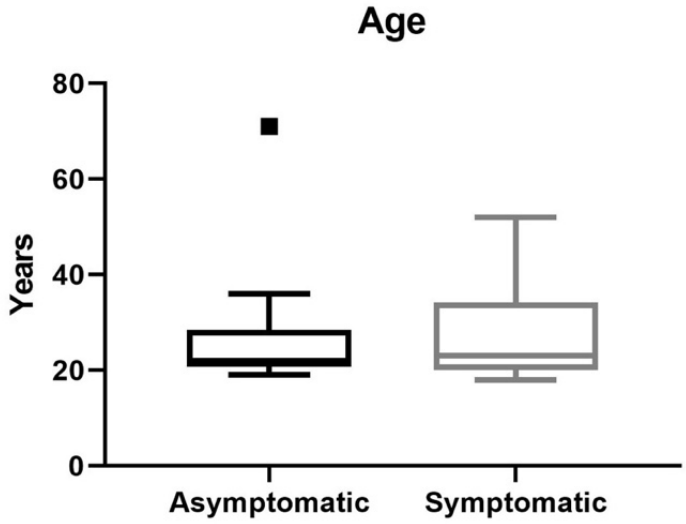

Figure 8-2: Distribution of participant age by study group (p=0.96). An outlier (>75th percentile + (1.5*IQR)) is denoted by a square.

8.4.2 Baseline Clinical Characteristics

A summary of the baseline clinical findings for the two study groups recorded at the screening visit is included in Table 8-3. Data is presented as the median (IQR) or as mean ± standard deviation (SD). Significant differences between the study groups (all p<0.05, Figure 8-4 and Figure 8-5) or between the eyes are graphed below all p<0.05, Figure 8-3, Figure 8-5, and Figure 8-6).

Table 8-3: Baseline demographics and characteristics by study group

	Asymptomatic Group (n = 10)	Symptomatic Group (n = 10)	p-value
Age	22.00 (7.75)	23.00 (14.25)	0.96
Spectacle visual acuity			
OD	–0.02 ± 0.11	–0.01 ± 0.10	0.86
OS	–0.03 ± 0.14	–0.01 ± 0.12	0.73
OU	–0.08 ± 0.10	–0.08 ± 0.10	0.93
Contact lens information			
Duration of contact lens wear (months)	96.00 (90)	96.00 (144)	0.64
Wearing time (days/week)	6.50 (2.00)	6.00 (1.25)	0.83
Contact lens information			
Wearing time (hours/day)	11.40 ± 3.17	12.00 ± 1.89	0.14
Power (OD)	–3.65 ± 2.82	–3.30 ± 3.29	0.80
Power (OS)	–3.73 ± 2.77	–3.05 ± 3.24	0.62
Contact lens symptomology			
CLDEQ-8 (1-37)	5.90 ± 3.00	17.00 ± 3.65	**<0.01**
Meibomian gland assessment			
Meibomian gland score (OD)	27.00 (19.50)	7.50 (23.50)	**0.03**
Meibomian gland score (OS)	13.50 (30.75)	1.50 (8.25)	**0.04**
Biomicroscopy			
Corneal staining (OD)	1.50 (2.63)	0.50 (2.00)	0.45
Corneal staining (OS)	0.00 (4.00)	0.00 (2.50)	0.93
Conjunctival staining (OD)	0.00 (2.00)	0.00 (1.25)	0.74
Conjunctival staining (OS)	1.25 (2.00)	0.50 (2.00)	0.62
Conjunctival indentation (OD)	0.00 (0.00)	0.00 (0.50)	0.74
Conjunctival indentation (OS)	0.00 (0.25)	0.00 (2.50)	0.58
Papillae (OD upper lid)	1.00 (1.00)	1.50 (1.00)	0.07
Papillae (OS upper lid)	1.00 (1.00)	1.50 (1.00)	0.07
Papillae (OD lower lid)	1.25 (0.75)	1.00 (1.00)	0.67
Papillae (OS lower lid)	1.25 (1.00)	1.00 (1.00)	0.99
Ocular surface/evaporimeter measurements			
Ocular surface area (cm^2) (OD)	1.56 (0.50)	1.59 (0.42)	0.53
Ocular surface area (cm^2) (OS)	1.87 (0.62)	1.90 (0.40)	0.85
Evaporimeter volume (cm^3) (OD)	18.00 (4.25)	16.00 (2.50)	0.51
Evaporimeter volume (cm^3) (OS)	18.00 (2.75)	15.50 (3.25)	0.14

CLDEQ-8: Contact Lens Dry Eye Questionnaire; OD: right eye; OS: left eye; OU: both eyes.

Data are presented as mean ± SD or median (IQR). **Bold** indicates significant differences.

8.4.2.1 Spectacle Visual Acuity

The mean ± SD spectacle visual acuity of the asymptomatic group was –0.02 ± 0.11 and –0.03 ± 0.14 for the right eye and left eye, respectively. The mean ± SD spectacle visual acuity of the symptomatic group was –0.01 ± 0.10 and –0.01 ± 0.12 for the right eye and left eye, respectively. The mean ± SD spectacle visual acuity of both eyes was –0.08 ± 0.10 in both groups. There was no significant difference in visual acuity between the groups (right eye: p=0.86; left eye: p=0.73, both eyes: p=0.93) or between the eyes (asymptomatic: p=0.64; symptomatic: p=0.95).

8.4.2.2 Habitual Wearing Time

The median (IQR) duration of CL wear was 96.00 (90.00) months in the asymptomatic group and 96.00 (144.00) months in the symptomatic group. The median (IQR) wearing time of the asymptomatic group was 6.50 (2.00) days per week and 6.00 (1.25) days per week in the symptomatic group. The mean ± SD wearing time in the asymptomatic group was 11.40 ± 3.17 hours per day and 12.00 ± 1.89 hours per day in the symptomatic group. There was no significant difference between the groups in the length of time they have been a CL wearer (p=0.64), nor was there a difference in the number of days per week (p=0.83) or in the hours per day that the CLs were worn (p=0.14).

8.4.2.3 Habitual Contact Lens Type

Seventy percent of asymptomatic CL wearers wore a daily disposable lens (20% hydrogel, 50% silicone hydrogel) and thirty percent wore a frequent replacement silicone hydrogel lens (10% bi-weekly, 20% monthly). A summary of the habitual CL brands worn by the participants is shown in Table 8-4.

Twenty percent of symptomatic CL wearers wore a daily disposable silicone hydrogel lens, while the remaining eighty percent wore a frequent replacement lens (10% monthly hydrogel, 20% bi-weekly silicone hydrogel, 50% monthly silicone hydrogel).

Table 8-4: Habitual contact lenses by study group

	Asymptomatic Group Number of Participants	Symptomatic Group Number of Participants
Daily Disposable		
Delefilcon A	3	2
Etafilcon A	1	0
Nelfilcon A	1	0
Somofilcon A	2	0
Frequent Replacement		
Comfilcon A	1	2
Hilafilcon B	0	1
Lotrafilcon B	1	3
Senofilcon A	1	2

8.4.2.4 Habitual Contact Lens Power

The mean ± SD power of the habitual CLs of the asymptomatic group was –3.65 ± 2.82 DS and –3.73 ± 2.77 DS in the right eye and left eye, respectively. The mean ± SD power of the habitual CLs of the symptomatic group was –3.30 ± 3.29 DS and –3.05 ± 3.24 DS in the right eye and left eye, respectively. There was no significant difference in the habitual CL power between the two groups (right eye: p=0.80; left eye: p=0.62) or between the eyes in the asymptomatic group (p>0.99). However, there was a significant difference between the habitual CL power of the right and left eye in the symptomatic group (p=0.03, Figure 8-3).

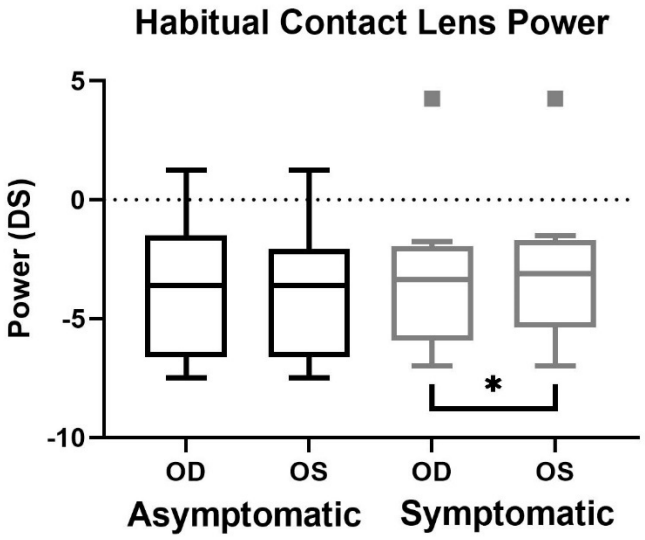

Figure 8-3: Box and whisker plots of the habitual contact lens power for each eye of the study groups (asymptomatic right eye versus left eye: p>0.99; symptomatic right eye versus left eye: p=0.03). Outliers (>75th percentile + (1.5*IQR)) are denoted by a square.

8.4.2.5 Habitual Contact Lens Solution

Twenty percent of asymptomatic CL wearers and ten percent of symptomatic CL wearers used a hydrogen peroxide solution (Table 8-5). Ten percent of asymptomatic CL wearers and seventy percent of symptomatic CL wearers used a multipurpose solution. The remaining participants did not use a solution.

Table 8-5: Habitual contact lens solution by study group

	Asymptomatic Group Percentage of Participants	Symptomatic Group Percentage of Participants
No care system	70	20
Biotrue	0	30
Clear Care	20	10
Opti-Free Puremoist	0	20
Opti-Free Replenish	0	10
Renu Fresh	10	10

8.4.2.6 CLDEQ-8

The mean ± SD CLDEQ-8 score was significantly lower for asymptomatic CL wearers at 5.90 ± 3.00 compared to 17.00 ± 3.65 for symptomatic CL wearers (p<0.0001, Figure 8-4).

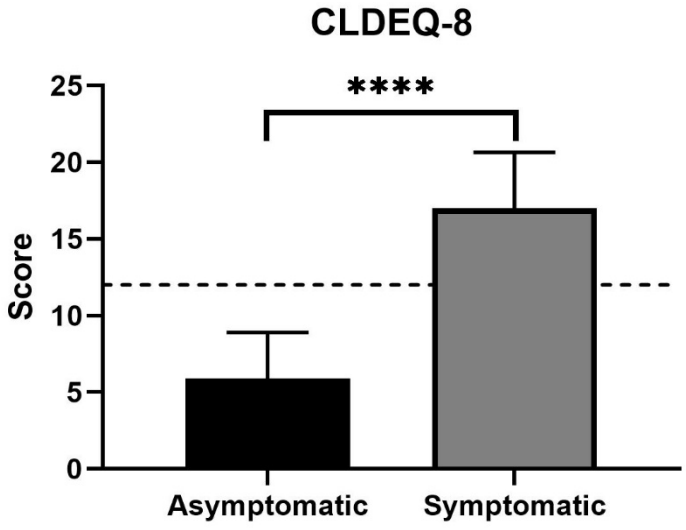

Figure 8-4: Mean CLDEQ-8 scores of the study groups. The dashed line indicates the threshold value (12), above which participants were considered symptomatic. There was a significant difference in CLDEQ-8 scores between the asymptomatic and symptomatic groups (p<0.0001). Error bars indicate standard deviation (SD).

8.4.2.7 Meibum Quality of Expression

The median (IQR) meibum score for the asymptomatic group was 27.00 (19.50) and 13.50 (30.75) for the right eye and left eye, respectively (Figure 8-5), with smaller values indicating poorer expression. The median (IQR) meibum score for the symptomatic group was 7.50 (23.50) and 1.50 (8.25) for the right eye and left eye, respectively. The meibum quality in both the right eye and left eye of the asymptomatic group was significantly different from the symptomatic group (right eye: p=0.03; left eye: p=0.04). There was also a significant difference in meibum quality of expression between the eyes of each group (asymptomatic: p=0.04; symptomatic: p=0.03).

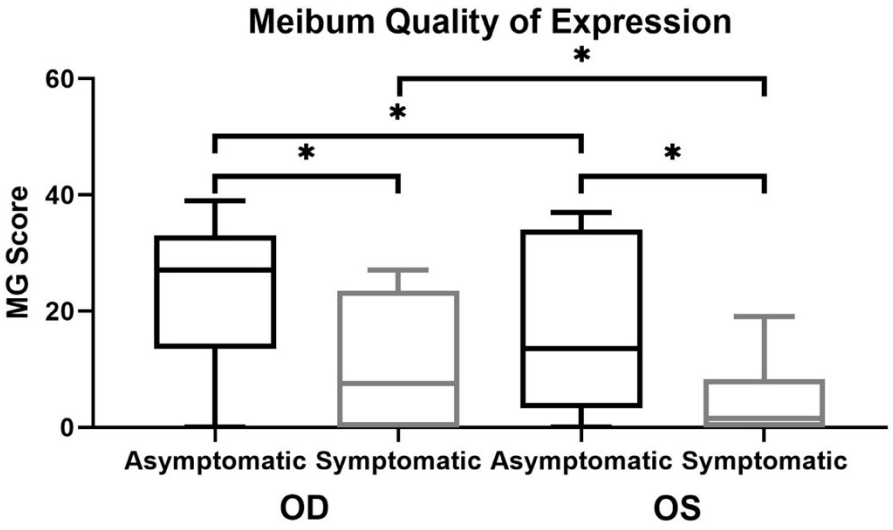

Figure 8-5: Box and whisker plot of the meibum quality for each eye of the study groups (right eye asymptomatic versus symptomatic: p=0.03; left eye asymptomatic versus symptomatic: p=0.04). A significant difference was also found between each eye of the study groups (asymptomatic: p=0.04; symptomatic: p=0.03).

8.4.2.8 Lid Margin Assessment

The median (IQR) amount of telangiectasia was 0.00 (0.25) in the right and left eye of the asymptomatic group. No erythema, eyelash loss, lid margin edema, or tear film debris was noted in either eye of the asymptomatic group.

The median (IQR) amount of telangiectasia was 0.00 (0.50) in the right and left eye of the symptomatic group. The median amount of erythema in both eyes and amount of tear film debris in the left eye was 0.00 (0.00) (range 0–1) in the symptomatic group. No eyelash loss or lid margin edema was present in either eye, nor was any tear film debris was noted in the right eye of the symptomatic group.

There was no significant difference in any of the lid margin assessments between the groups (right eye: all $p>0.99$; left eye: all $p>0.99$) or between eyes (symptomatic: tear film debris $p>0.99$, other values: p-value could not be calculated).

8.4.2.9 Corneal Staining

The median (IQR) amount of corneal staining in the asymptomatic group was 1.50 (2.63) and 0.00 (4.00) in the right eye and left eye, respectively, with larger values indicating larger amounts of staining. The median (IQR) amount of corneal staining in the symptomatic group was 0.50 (2.00) and 0.00 (2.50) in the right eye and left eye, respectively. There was no significant difference in the amount of corneal staining between the groups (right eye: $p=0.45$; left eye: $p=0.93$) or between eyes (asymptomatic: $p=0.56$; symptomatic: $p=0.95$).

8.4.2.10 Conjunctival Staining

The median (IQR) amount of conjunctival staining in the asymptomatic group was 0.00 (2.00) and 1.25 (2.00) in the right eye and left eye, respectively, with larger values indicating larger amounts of staining. The median (IQR) amount of conjunctival staining in the symptomatic group was 0.00 (1.25) and 0.50 (2.00) in the right eye and left eye, respectively.

There was no significant difference in the amount of conjunctival staining between the groups (right eye: $p=0.74$; left eye: $p=0.62$) or between eyes (asymptomatic: $p=0.50$; symptomatic: $p=0.81$).

8.4.2.11 Conjunctival Indentation

The median (IQR) amount of conjunctival indentation in the asymptomatic group was 0.00 (0.00) and 0.00 (0.25) in the right eye and left eye, respectively, with larger values indicating greater amounts of

staining. The median (IQR) amount of conjunctival indentation in the symptomatic group was 0.00 (0.50) and 0.00 (2.50) in the right eye and left eye, respectively.

There was no significant difference in the amount of conjunctival indentation between the groups (right eye: p=0.74; left eye: p=0.58) or between eyes (asymptomatic: p=0.50; symptomatic: p=0.50).

8.4.2.12 Papillae

The median (IQR) amount of papillae underneath each upper eyelid of the asymptomatic and symptomatic group was 1.00 (1.00) and 1.50 (1.00) for the right eye and left eye, respectively. The median (IQR) amount of papillae underneath the lower lid of the asymptomatic group was 1.25 (0.75) in the right eye and 1.25 (1.00) in the left eye. The median (IQR) amount of papillae underneath each lower eyelid of the symptomatic group was 1.00 (1.00).

There was no significant difference in the amount of papillae between the groups (right eye: upper lid p=0.07, lower lid p=0.67; left eye: upper lid: p=0.07, lower lid: p>0.99), or between the eyes of either group (asymptomatic: lower lid p=0.50; other values: p-value could not be calculated).

8.4.2.13 Ocular Surface Area

The median (IQR) area of the ocular surface of the asymptomatic group was 1.56 (0.50) cm^2 and 1.87 (0.62) cm^2 for the right eye and left eye, respectively. The median (IQR) ocular surface area of the symptomatic group was 1.59 (0.42) cm^2 and 1.90 (0.40) cm^2 for the right eye and left eye, respectively. There was no significant difference in the ocular surface area between the groups (right eye: p=0.53; left eye: p=0.85). However, the ocular surface area of the left eye was significantly larger than the right eye in the asymptomatic group (p=0.0098, Figure 8-6), but not in the symptomatic group (p=0.19).

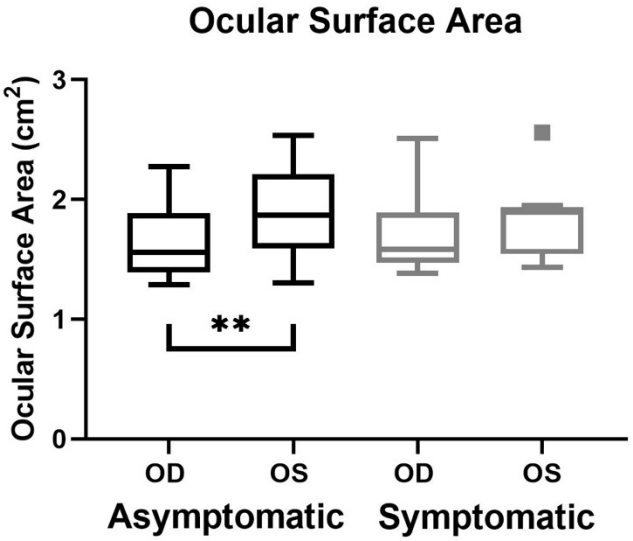

Figure 8-6: Ocular surface area for each eye of the study groups (asymptomatic right eye versus left eye: p=0.0098; symptomatic right eye versus left eye: p=0.19). An outlier (>75th percentile + (1.5*IQR)) is denoted by a square.

8.4.2.14 Evaporimeter Volume

The median (IQR) volume within the evaporimeter goggle of the asymptomatic group was 18.00 (4.25) cm^3 and 18.00 (2.75) cm^3 for the right eye and left eye, respectively. The median (IQR) volume within the goggle of the symptomatic group was 16.00 (2.50) cm^3 and 15.50 (3.25) cm^3 for the right eye and left eye, respectively. There was no significant difference in the evaporimeter volume between the groups (right eye: p=0.51; left eye: p=0.14), or between the eyes of each group (asymptomatic: p=0.40; symptomatic: p=0.77).

8.4.3 Dispensing Visit

The dispensing and follow-up visits occurred between 1 to 10 days after the screening visit. Participants attended the dispensing visit having been awake for an average of 2 hours and 43 minutes in the asymptomatic group (range 120 to 300 minutes) and 2 hours and 10 minutes in the symptomatic group (range 120 to 161 minutes). There was no significant difference in the amount of time the two groups had been awake prior to attending the visit (p=0.16).

Habitual users of ocular lubricants reported that the drops were last used between 1 day to 1 week prior to the test visit, with one participant having last instilled them one day prior to the visit. A summary of the

clinical findings from the dispensing and follow-up visits appears in Table 8-10. Data is presented as the median (IQR) or as mean ± SD.

8.4.3.1 Ambient Temperature and Relative Humidity

The mean ± SD ambient room temperature at the dispensing visit was 73.8 ± 2.2°F (23.2 ± 1.2°C) and the RH was 44.3 ± 7.7%. The range of ambient temperature was 70 to 79°F (21.1 to 26.1°C) and RH varied between 33 and 58%.

8.4.3.2 Dispensed Contact Lens Power

A summary of the mean ± SD power of the CLs dispensed appears in Table 8-6. The dispensed power of the CLs in the asymptomatic group ranged between –7.50 and +1.25 DS and –7.00 and +4.25 DS in the symptomatic group.

There was no significant difference in CL power between the groups (delefilcon A: right eye: p=0.68, left eye: p=0.96; nesofilcon A: right eye: p=0.89, left eye: p=0.62), or between the eyes of each group (delefilcon A: asymptomatic: p=0.84, symptomatic: p=0.60; nesofilcon A: asymptomatic: p=0.88, symptomatic: p=0.48).

Table 8-6: Dispensed mean ± standard deviation (SD) contact lens power by eye and study group

	Asymptomatic Group (n = 5 eyes)	**Symptomatic Group (n = 5 eyes)**	**p-value**
Delefilcon A (OD)	–3.40 ± 2.63	–2.45 ± 4.29	0.68
Delefilcon A (OS)	–3.90 ± 3.28	–3.80 ± 2.00	0.96
p-value	0.84	0.60	
Nesofilcon A (OD)	–3.90 ± 3.28	–4.15 ± 2.04	0.89
Nesofilcon A (OS)	–3.55 ± 2.53	–2.40 ± 4.30	0.62
p-value	0.88	0.48	

8.4.4 Follow-up Visit

Participants attended the follow-up visit wearing the study CLs for an average of 6 hours and 19 minutes in the asymptomatic group (range 360 to 419 minutes) and 6 hours and 23 minutes in the symptomatic group (range 360 to 419 minutes). There was no significant difference in the wearing time of the two groups (p=0.54).

8.4.4.1 Ambient Temperature and Relative Humidity

The mean ± SD ambient room temperature at the follow-up visit was 73.5 ± 1.4°F (23.1 ± 0.8°C) and the RH was 43.0 ± 8.1%. The range of ambient temperature was 72 to 76°F (22.2 to 24.4°C) and RH varied between 32 and 56%.

8.4.4.2 Tear Evaporation Rate (TER)

8.4.4.2.1 TER by Individual

The change in TER for each participant in the asymptomatic group and symptomatic group is shown in Figure 8-7.

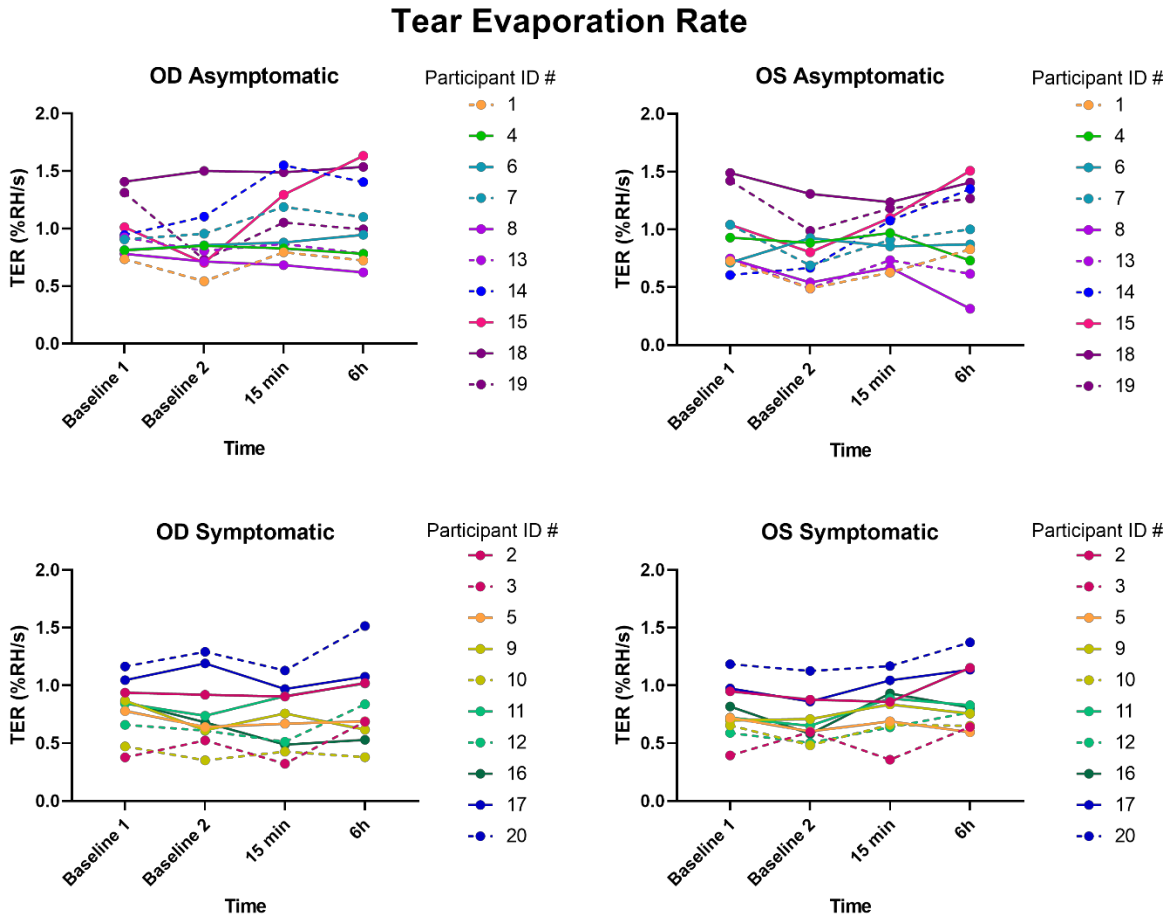

Figure 8-7: Individual plots of the mean change in tear evaporation rate over time for each eye of all the participants.

8.4.4.2.2 Baseline TER

The median (IQR) change in TER between baseline measurements was investigated for each eye (Table 8-7) because the instrument simultaneously measures from the two eyes. There was no significant difference in the repeatability of the two baseline measurements of the right eye in the asymptomatic group (p=0.38) or either eye of the symptomatic group (right eye: p=0.49; left eye: p=0.08, Figure 8-8). Due to the significant decrease in the TER of the left eye of the asymptomatic group between the first and second baseline measurement (p=0.04), only the second baseline measurement was included in subsequent analyses because this measurement best represents the TER of the eyes prior to insertion of the CLs.

Table 8-7: Summary of median interquartile range (IQR) tear evaporation rate over time

	Asymptomatic		**Symptomatic**	
	Right Eye (n = 10)	**Left Eye (n = 10)**	**Right Eye (n = 10)**	**Left Eye (n = 10)**
Baseline 1	0.91 (0.28)	0.84 (0.41)	0.85 (0.35)	0.72 (0.32)
Baseline 2	0.83 (0.28)	0.75 (0.41)	0.66 (0.40)	0.63 (0.30)
15 minutes post-lens	0.97 (0.52)	0.94 (0.40)	0.71 (0.45)	0.85 (0.31)
6 hours post-lens	0.97 (0.67)	0.94 (0.66)	0.76 (0.44)	0.79 (0.49)

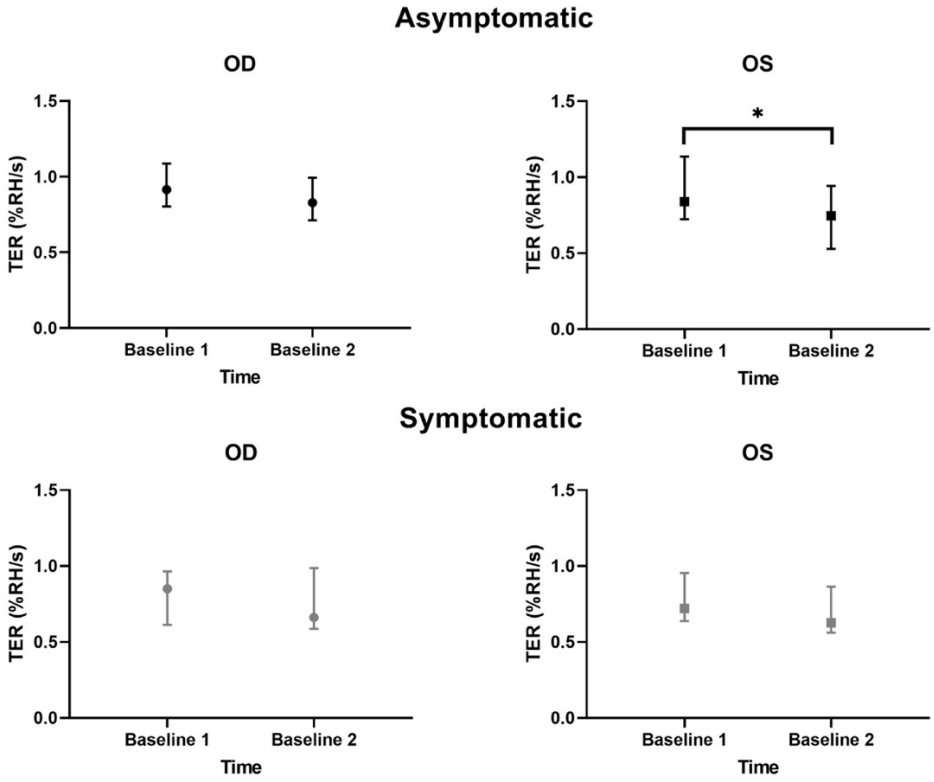

Figure 8-8: Median baseline tear evaporation rate for the right and left eye of each group. The tear evaporation rate of the left eye of the asymptomatic group significantly decreased between the two baseline measurements (p=0.04). There was no significant difference in the baseline measurements of the right eye in the asymptomatic group (right eye: p=0.38) or either eye of the symptomatic group (right eye: p=0.49; left eye: p=0.08). Error bars indicate IQR.

8.4.4.2.3 Relationship Between Time, Group, and Eye

There was no statistically significant three-way interaction between time, group, and eye on TER (p=0.07, Table 8-8). There was also no statistically significant two-way interaction between time and group (p=0.22, Figure 8-9) or time and eye (p=0.20, Figure 8-9).

Table 8-8: Summary of two-way and three-way interactions between time, eye, and group

Interactions	p-value
time*group*eye	0.07
time*group	0.22
time*eye	0.20

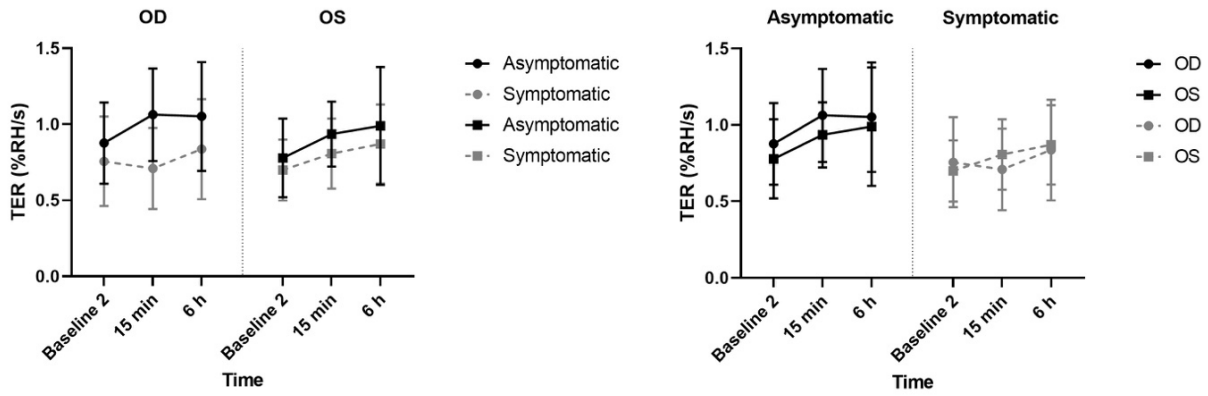

Figure 8-9: Change in mean tear evaporation rate for the right and left eye of each group. There was no significant interaction between time and group (p=0.22) or time and eye (p=0.20). Error bars indicate SD.

8.4.4.2.4 Overall Change in TER over Time

The change in TER over time was statistically significantly for both sides of the evaporimeter (right eye: p=0.037; left eye: p=0.008, Table 8-9, Figure 8-10). Post-hoc testing with a Dunnett's multiple comparisons test found the change in TER over time for the right eye was significantly lower at the second baseline measurement compared to 6 hours after CL wear (p=0.043). Post-hoc testing of the left eye with a Tukey's multiple comparisons test found the TER was significantly higher after 15 minutes (p=0.002) and 6 hours (p=0.004) of CL wear compared to the second baseline measurement.

Table 8-9: Summary of mean ± SD tear evaporation rate over time

	Tear Evaporation Rate (%RH/s)	
	Right Eye (n = 20)	**Left Eye (n = 20)**
Baseline 2	0.82 ± 0.28	0.74 ± 0.23
15 minutes post-lens	0.89 ± 0.33	0.87 ± 0.23
6 hours post-lens	0.94 ± 0.35	0.93 ± 0.33
p-value	**0.037**	**0.008**

Bold indicates significant differences.

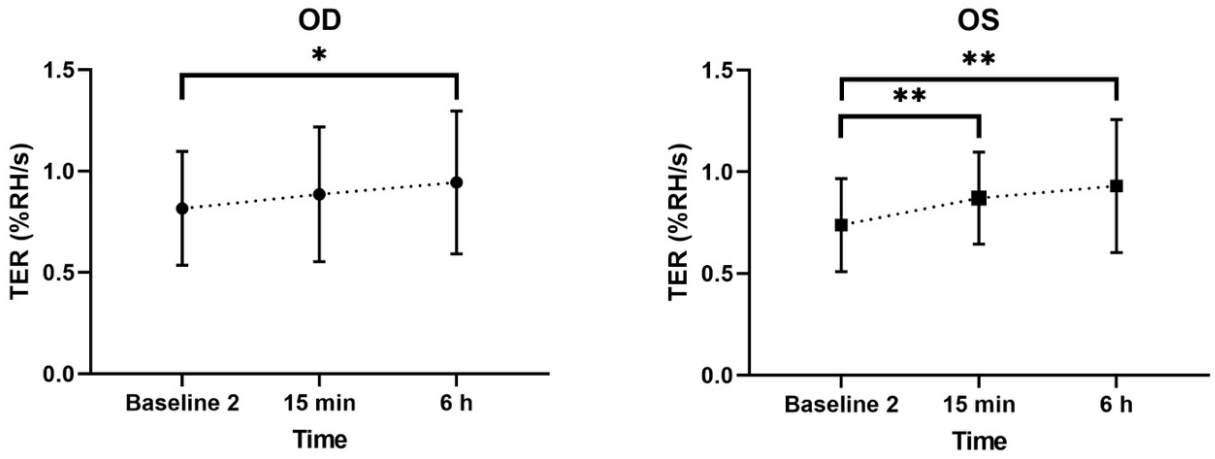

Figure 8-10: Mean change in tear evaporation rate over time for each eye of all participants. The change in tear evaporation rate over time was significant for both eyes (right eye: p=0.037; left eye: p=0.008). Error bars indicate SD.

8.4.4.2.5 Change in TER over Time by Eye and Group

The change in TER over time was not statistically significantly for either eye of the asymptomatic group (right eye: p=0.057; left eye: p=0.062) or the right eye of the symptomatic group (p=0.062). However, the change in TER over time was significantly different for the left eye of the symptomatic group (p=0.008, Figure 8-11). Post-hoc testing with a Tukey's multiple comparisons test found a significantly higher TER 6 hours after the CLs were instilled compared to the second baseline measurement (p=0.002).

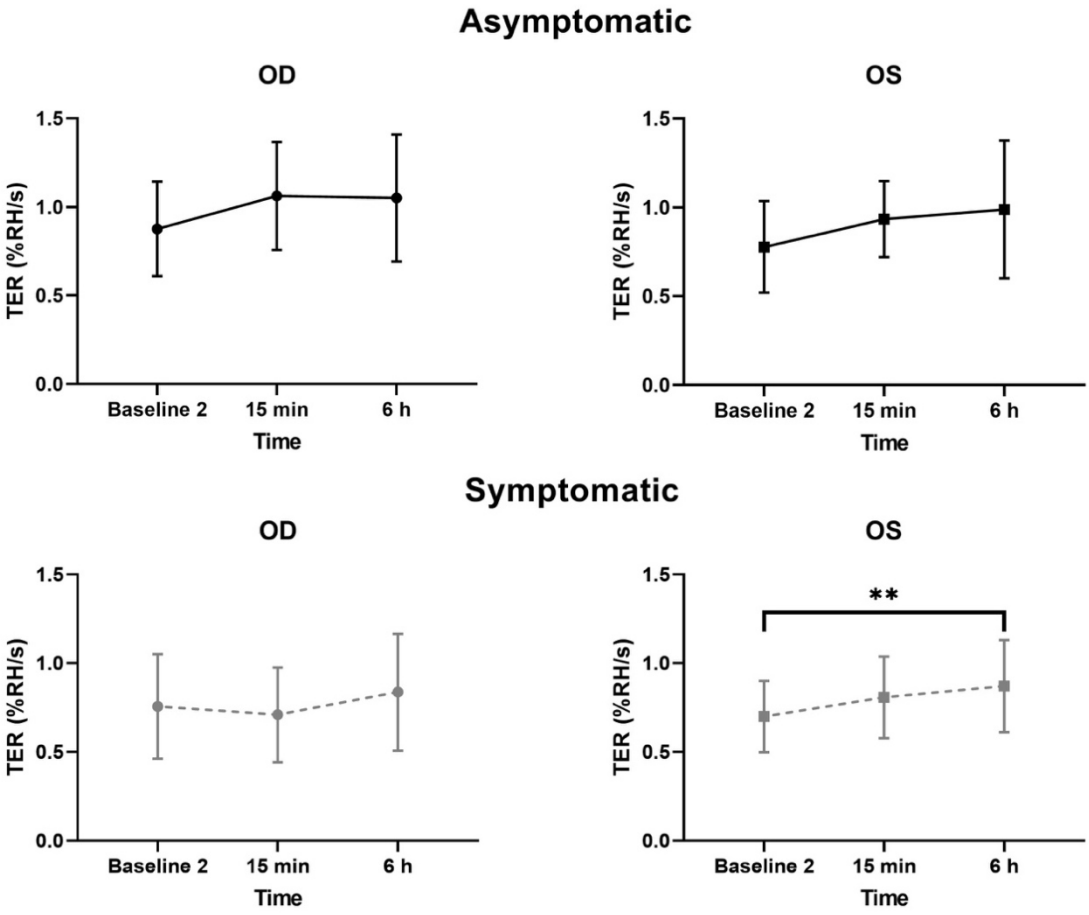

Figure 8-11: Mean change in tear evaporation rate over time for each eye of the asymptomatic and symptomatic group. The change in tear evaporation rate was significant for the left eye of the symptomatic group (p=0.008). No significant change was observed for either eye of the asymptomatic group (right eye: p=0.057; left eye p=0.062) or the right eye of the symptomatic group (p=0.062). Error bars indicate SD.

Since the change in TER was only statistically significant for the left eye of the symptomatic group, the remaining statistical analyses for changes over time (Table 8-10), differences between groups (Table 8-10), and correlations (Table 8-11, Table 8-12, and Table 8-13) were only conducted on the left eye. Significant changes over time (all $p<0.047$, Figure 8-12, Figure 8-15, Figure 8-16, Figure 8-17, Figure 8-18, and Figure 8-19) are plotted below. Graphs of differences in TER between groups (Figure 8-13, $p>0.210$) and CLs (Figure 8-14, $p>0.267$) have also been included.

Table 8-10: Summary of subjective comfort and clinical measurements for the left eye

	Asymptomatic Group (n = 10 eyes)	Symptomatic Group (n = 10 eyes)	p-value
Comfort Rating			
Baseline 1	87.50 (27.50)	92.00 (16.25)	0.485
15 minutes post-lens	90.00 (22.50)	90.00 (9.75)	0.624
6 hours post-lens	80.00 (22.50)	87.50 (18.50)	0.734
p-value	0.778	0.543	
Dryness Rating			
Baseline 1	95.00 (22.50)	92.50 (31.25)	0.616
15 minutes post-lens	97.50 (6.25)	90.00 (21.00)	0.139
6 hours post-lens	95.00 (20.00)	87.50 (12.50)	0.396
p-value	0.503	0.826	
Stinging/Burning Rating			
Baseline 1	100.00 (1.25)	100.00 (5.00)	0.861
15 minutes post-lens	100.00 (1.25)	100.00 (0.00)	0.999
6 hours post-lens	100.00 (1.25)	100.00 (0.00)	0.474
p-value	0.999	0.333	
Tear Evaporation Rate			
Baseline 2	0.78 ± 0.26	0.70 ± 0.20	0.451
15 minutes post-lens	0.94 ± 0.21	0.81 ± 0.23	0.211
6 hours post-lens	0.99 ± 0.39	0.87 ± 0.26	0.434
p-value	0.062	**0.008**	
Non-Invasive Break-up Time			
Baseline 2	6.47 (8.39)	8.82 (3.94)	0.314
15 minutes post-lens	6.99 (6.78)	7.62 (10.16)	0.684
6 hours post-lens	6.10 (3.15)	5.19 (5.03)	0.529
p-value	0.316	**0.026**	
Subjective Lipid Layer Thickness			
Baseline 2	5.00 (2.00)	5.00 (1.25)	0.880
15 minutes post-lens	3.00 (2.25)	3.00 (2.50)	0.903
6 hours post-lens	1.00 (2.00)	2.00 (2.00)	0.650
p-value	**<0.0001**	**0.0003**	

	Asymptomatic Group (n = 10 eyes)	Symptomatic Group (n = 10 eyes)	p-value
Objective Lipid Layer Thickness (mean)			
Baseline 2	76.50 (42.50)	93.50 (25.50)	0.391
15 minutes post-lens	59.50 (31.25)	70.50 (22.00)	0.382
6 hours post-lens	58.50 (15.75)	63.50 (12.75)	0.240
p-value	**0.046**	**0.036**	
Objective Lipid Layer Thickness (maximum)			
Baseline 2	94.50 (27.50)	100.00 (4.00)	0.183
15 minutes post-lens	71.50 (39.50)	86.00 (17.50)	0.191
6 hours post-lens	74.00 (32.00)	82.50 (17.75)	0.211
p-value	0.114	**0.042**	
Objective Lipid Layer Thickness (minimum)			
Baseline 2	60.50 (34.75)	82.50 (26.00)	0.065
15 minutes post-lens	50.00 (26.00)	59.00 (20.50)	0.224
6 hours post-lens	43.00 (19.00)	53.50 (21.50)	0.270
p-value	0.314	**0.004**	

Data are presented as mean ± SD or median (IQR). **Bold** indicates significant differences.

8.4.4.2.6 Change in TER over Time for the Left Eye

As reported in Section 8.4.4.2.5, the change in TER over time was significantly different for the left eye of the symptomatic group (p=0.008, Figure 8-12), but not for the asymptomatic group (p=0.062). Post-hoc testing with a Tukey's multiple comparisons test found a significantly higher TER 6 hours after the CLs were instilled compared to the second baseline measurement (p=0.002).

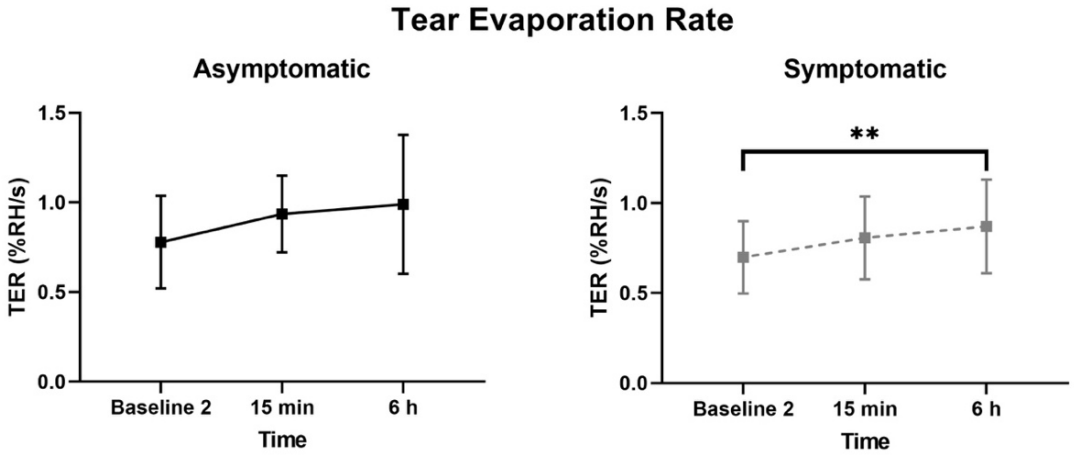

Figure 8-12: Mean change in tear evaporation rate over time for the left eye of the asymptomatic and symptomatic group. Error bars indicate SD. No significant change was observed for the left eye of the asymptomatic group (p=0.062), but it was significant for left eye of the symptomatic group (p=0.008).

8.4.4.2.7 Difference in TER Between Groups

There was no significant difference in TER between the two groups at any of the time points (baseline 2: p=0.451, 15 minutes: p=0.211, 6 hours: p=0.434, Figure 8-13).

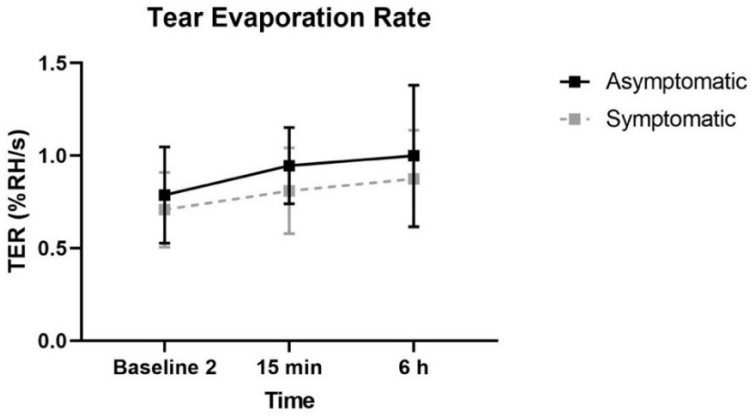

Figure 8-13: Difference in mean tear evaporation rate between the left eye of the two groups. There was no significant difference at any of the time points (baseline 2: p=0.451, 15 minutes: p=0.211, 6 hours: p=0.434). Error bars indicate SD.

8.4.4.2.8 Difference in TER Between Lens Types

Due to the fact that there was no significant difference in TER between the two groups, the values were combined to investigate whether there was a difference between delefilcon A and nesofilcon A. The mean ± SD TER of the left eye was 0.93 ± 0.47 %RH/s after 15 minutes and 1.00 ± 0.31%RH/s after 6 hours of delefilcon A wear. The mean ± SD of the left eye was 0.82 ± 0.24 %RH/s after 15 minutes and 0.88 ± 0.34 %RH/s after 6 hours of nesofilcon A wear. There was no significant difference in TER between the two types of CLs after 15 minutes (p=0.268, Figure 8-14) or 6 hours of wear (p=0.436), nor was there a significant difference in TER between the two groups at any of the time points (all p>0.210).

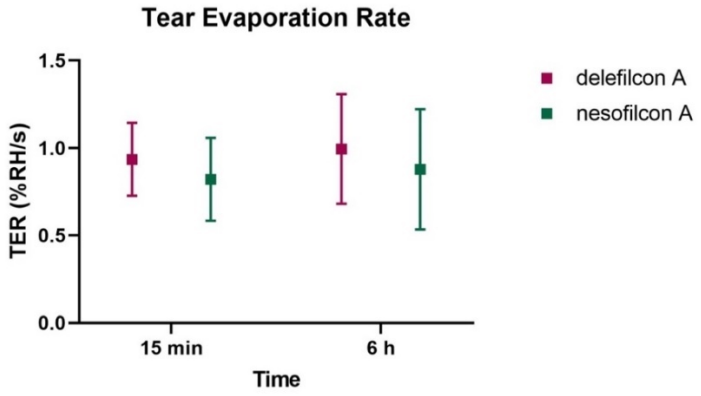

Figure 8-14: Mean tear evaporation rate of the two types of contact lenses in the left eye. There was no significant difference in TER after 15 minutes (p=0.268) or 6 hours of wear (p=0.436). Error bars indicate SD.

8.4.4.3 Non-Invasive Tear Film Break-Up Time

8.4.4.3.1 Change in Non-Invasive Tear Film Break-Up Time over Time

The NITBUT of the asymptomatic group did not change over time (p=0.316, Figure 8-15). However, the symptomatic group had a significant change in NITBUT over time (p=0.026, Figure 8-15). Post-hoc testing with a Dunn's multiple comparisons test found a significantly higher NITBUT at the second baseline measurement compared to 6 hours of CL wear (p=0.042).

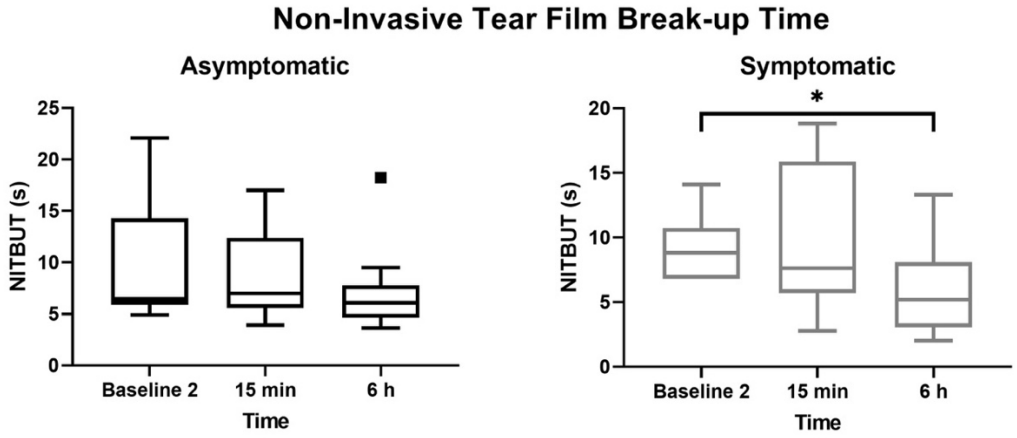

Figure 8-15: Box and whisker plot of the change in non-invasive break-up time for the asymptomatic (p=0.316) and symptomatic (p=0.026) group. An outlier (>75th percentile + (1.5*IQR)) is denoted by a square.

8.4.4.3.2 Difference in Non-Invasive Tear Film Break-Up Time Between Groups

There was no significant difference in NITBUT between the two groups at any of the time points (all p>0.313).

8.4.4.4 Subjective Lipid Layer Thickness

8.4.4.4.1 Change in Subjective Lipid Layer Thickness over Time

There was a significant difference in subjective LLT over time in the asymptomatic (p<0.0001, Figure 8-16) and symptomatic group (p=0.0003, Figure 8-16). Post-hoc testing with a Dunn's multiple comparisons test found a significantly higher LLT at the second baseline measurement compared to 6 hours of CL wear in both groups (asymptomatic: p=0.0024; symptomatic: p=0.0016).

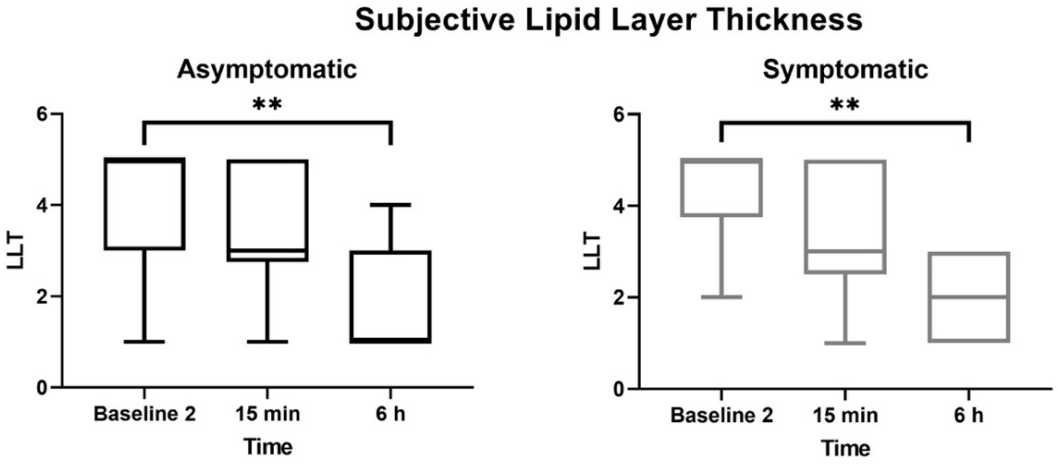

Figure 8-16: Box and whisker plot of the change in subjective lipid layer thickness for the asymptomatic (p<0.0001) and symptomatic (p=0.0003) group.

8.4.4.4.2 Difference in Subjective Lipid Layer Thickness Between Groups

There was no significant difference in subjective LLT between the two groups at any of the time points (all p>0.649).

8.4.4.5 Objective Lipid Layer Thickness

8.4.4.5.1 Change in Objective Lipid Layer Thickness over Time

The mean objective LLT of the asymptomatic group and symptomatic group changed over time (asymptomatic: p=0.046; symptomatic: p=0.036, Figure 8-17). Post-hoc testing of the asymptomatic group with a Dunn's multiple comparisons test found a significantly higher mean LLT at the second baseline measurement compared to 6 hours of CL wear (p=0.042). Post-hoc testing of the symptomatic group with an uncorrected Dunn's test revealed a significantly higher TER at the second baseline measurement compared to 15 minutes (p=0.034) and 6 hours (p=0.025) of CL wear.

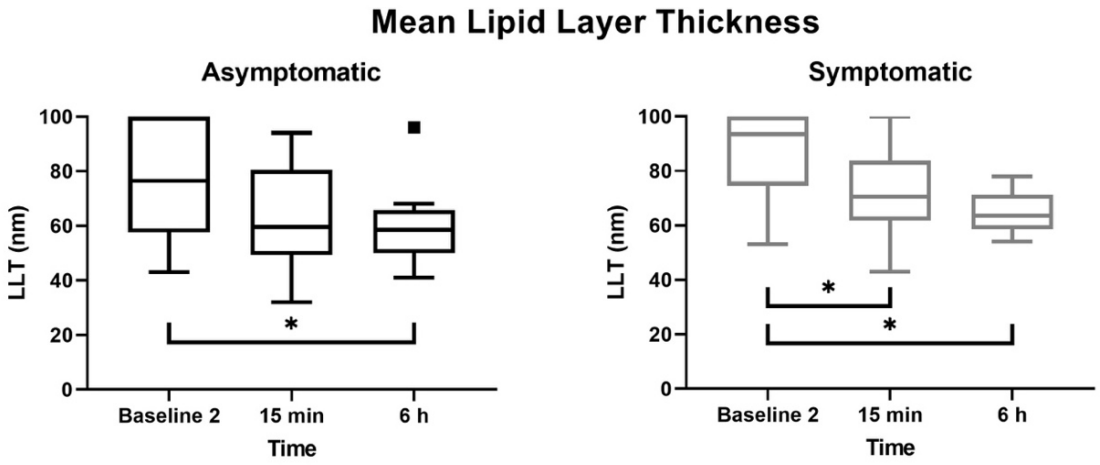

Figure 8-17: Box and whisker plot of the change in mean objective lipid layer thickness for the asymptomatic (p=0.046) and symptomatic (p=0.036) group. An outlier (>75th percentile + (1.5*IQR)) is denoted by a square.

The maximum LLT of the asymptomatic group did not change over time (p=0.114, Figure 8-18), although the symptomatic group had a significant change in LLT over time (p=0.042, Figure 8-18). Post-hoc testing with an uncorrected Dunn's test revealed a significantly higher TER at the second baseline measurement compared to 6 hours (p=0.025) of CL wear.

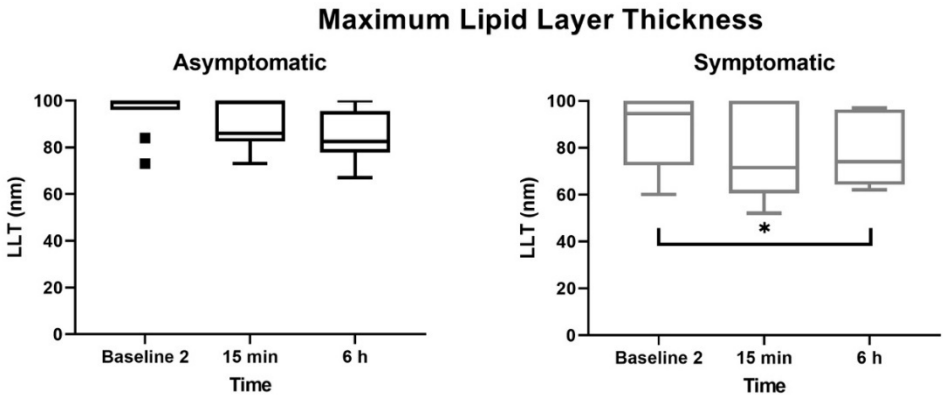

Figure 8-18: Box and whisker plot of the change in maximum objective lipid layer thickness for the asymptomatic (p=0.114) and symptomatic (p=0.042) group. Outliers (<25th percentile - (1.5*IQR)) are denoted by a square.

The minimum LLT of the asymptomatic group did not change over time (p=0.314, Figure 8-19), although the symptomatic group had a significant change in LLT over time (p=0.004, Figure 8-19). Post-hoc testing with a Dunn's multiple comparisons test revealed a significantly higher LLT at the second baseline measurement compared to 15 minutes (p=0.022) and 6 hours (p=0.022) of CL wear.

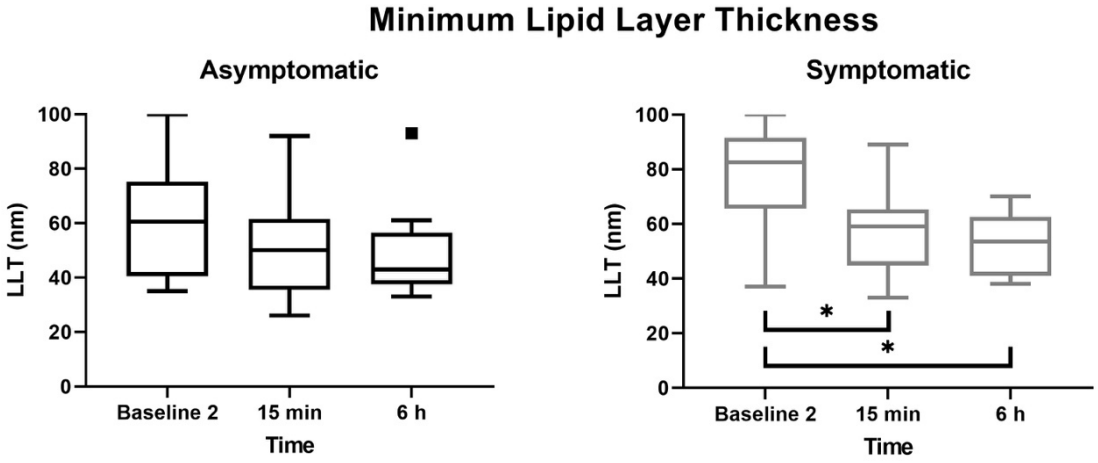

Figure 8-19: Box and whisker plot of the change in minimum objective lipid layer thickness for the asymptomatic (p=0.314) and symptomatic (p=0.004) group. An outlier (>75th percentile + (1.5*IQR)) is denoted by a square.

8.4.4.5.2 Difference in Objective Lipid Layer Thickness Between Groups

There was no significant difference in the objective LLT between the two groups at any of the time points (mean: all p>0.239; maximum: all p>0.182; minimum: all p>0.064).

8.4.5 Correlations Between TER and Clinical Measurements by Group

8.4.5.1 Ocular Surface Area and Evaporimeter Volume

A summary of correlations between the TER of the left eye and the ocular surface area and volume within the evaporimeter is shown in Table 8-11. There was no significant correlation between the TER and the ocular surface area (asymptomatic: all r<0.341, all p>0.336; symptomatic: all r<–0.066, all p>0.295, Figure 8-20) or the volume inside the evaporimeter (asymptomatic: all r_s<0.602, all p>0.070; symptomatic: all r_s<0.457, all p>0.184, Figure 8-21).

There was no significant correlation between the ocular surface area and volume within the evaporimeter of the asymptomatic group (right eye: r_s=<–0.265, p=0.456; left eye: r_s=<–0.135, p=0.710) or the symptomatic group (right eye: r_s=<–0.482, p=0.160; left eye: r_s=<–0.202, p=0.572).

Table 8-11: Correlations between tear evaporation rate of the left eye and characteristics of the ocular surface and evaporimeter

		Asymptomatic Group (n = 10 eyes)	p-value	Symptomatic Group (n = 10 eyes)	p-value
Ocular surface area					
	Baseline 2	–0.112	0.758	–0.367	0.298
	15 minutes	0.188	0.604	–0.067	0.855
	6 hours	0.340	0.337	–0.368	0.296
Evaporimeter volume					
	Baseline 2	0.601[a]	0.071	0.456[a]	0.185
	15 minutes	0.301[a]	0.397	0.345[a]	0.326
	6 hours	0.350[a]	0.321	0.072[a]	0.849

[a]: Spearman rank correlation.

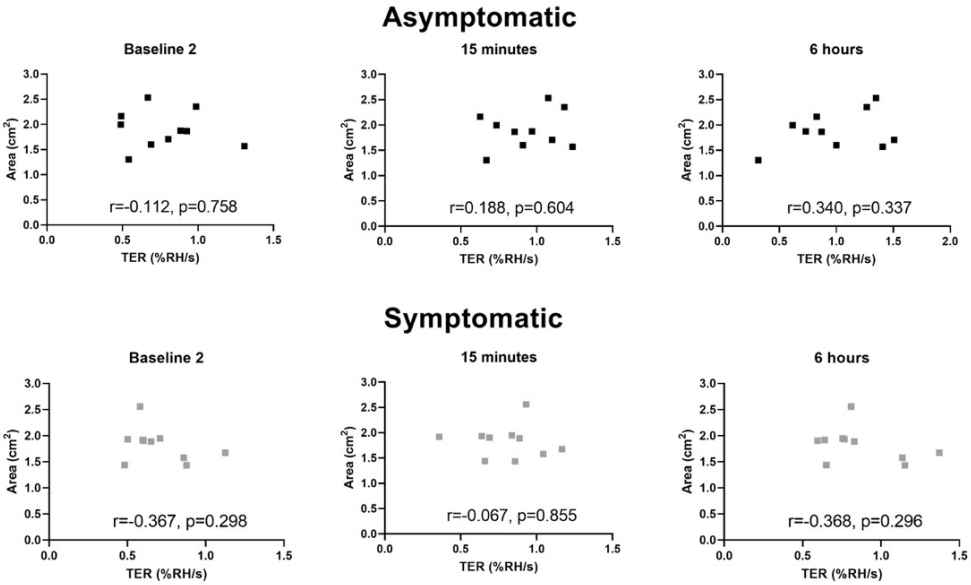

Figure 8-20: Correlations between the tear evaporation rate and the ocular surface area of the left eye (asymptomatic: all r<0.341, all p>0.336; symptomatic: all r<–0.666, all p>0.295).

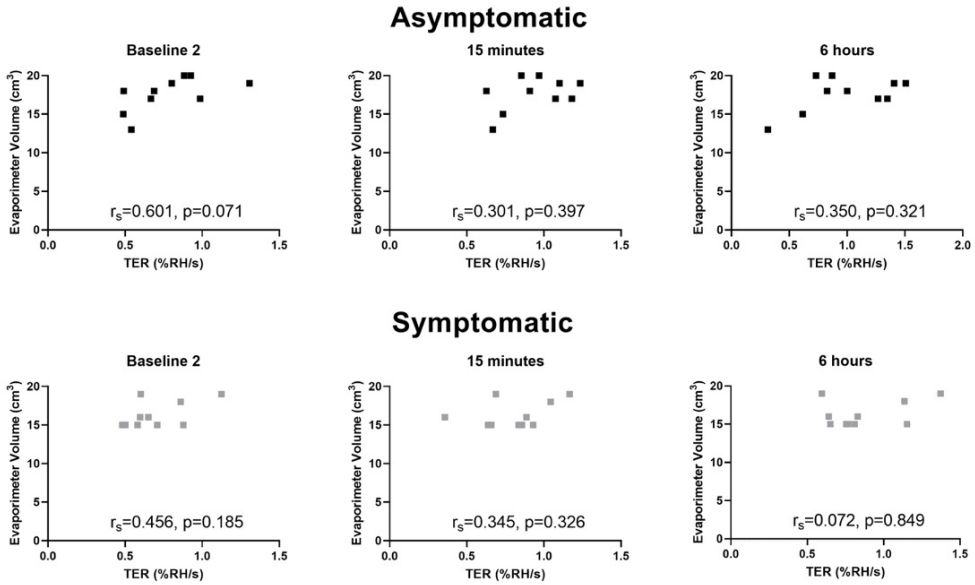

Figure 8-21: Correlations between the tear evaporation rate and volume within the evaporimeter of the left eye (asymptomatic: all r_s<0.602, all p>0.070; symptomatic: all r_s<0.457, all p>0.184).

8.4.5.2 Symptomology

A summary of correlations between the TER of the left eye and symptomology is shown in Table 8-12. There was no significant correlation between the TER and the CLDEQ-8 or the subjective comfort ratings (asymptomatic: all r or r_s<0.624, all p>0.066; symptomatic: all r or r_s<0.547, all p>0.108 except for the stinging/burning rating at 6 hours which could not be calculated, Figure 8-22, Figure 8-23, Figure 8-24, and Figure 8-25).

Table 8-12: Correlations between tear evaporation rate of the left eye and symptomology

		Asymptomatic Group (n = 10 eyes)	p-value	Symptomatic Group (n = 10 eyes)	p-value
CLDEQ-8					
	Baseline 2	–0.367	0.296	–0.159	0.662
	15 minutes	–0.394	0.260	–0.048	0.896
	6 hours	–0.393	0.262	–0.122	0.737
Comfort rating					
	Baseline 2*	–0.104[a]	0.778	0.314[a]	0.372
	15 minutes	0.121[a]	0.739	0.351[a]	0.321
	6 hours	0.130[a]	0.720	0.232[a]	0.515
Dryness rating					
	Baseline 2*	0.151[a]	0.675	0.546[a]	0.109
	15 minutes	–0.348[a]	0.328	0.522[a]	0.127
	6 hours	0.162[a]	0.654	0.025[a]	0.952
Stinging/burning rating					
	Baseline 2*	0.311[a]	0.400	0.510[a]	0.156
	15 minutes	0.623[a]	0.067	–0.290[a]	0.600
	6 hours	0.415[a]	0.244	-	--

*: Baseline 1 subjective comfort ratings versus Baseline 2 TER; [a]: Spearman rank correlation; -: horizontal line; --: no p-value calculated.

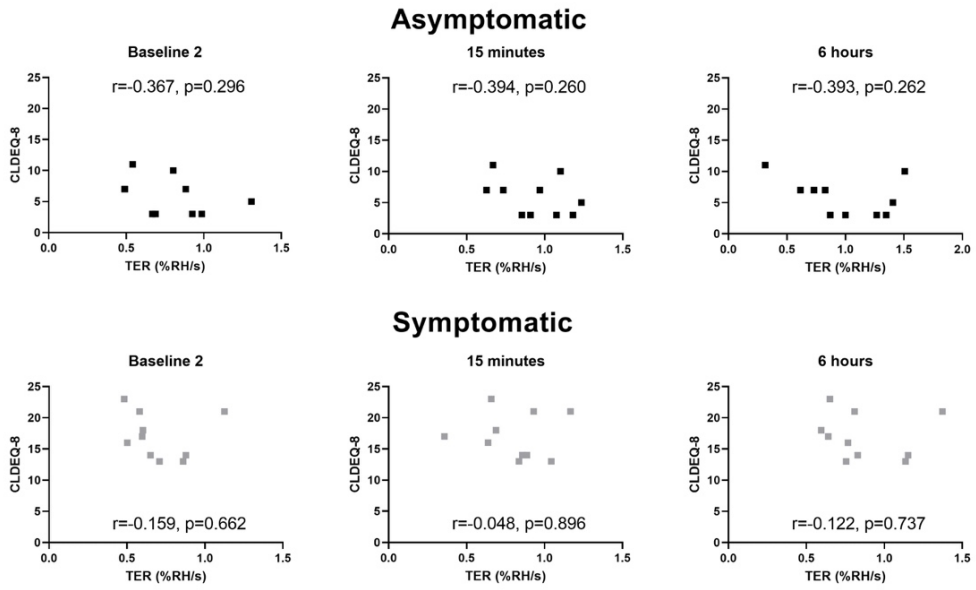

Figure 8-22: Correlations between the tear evaporation rate and CLDEQ-8 score of the left eye (asymptomatic: all r<–0.366, all p>0.259; symptomatic: all r<–0.047, all p>0.661).

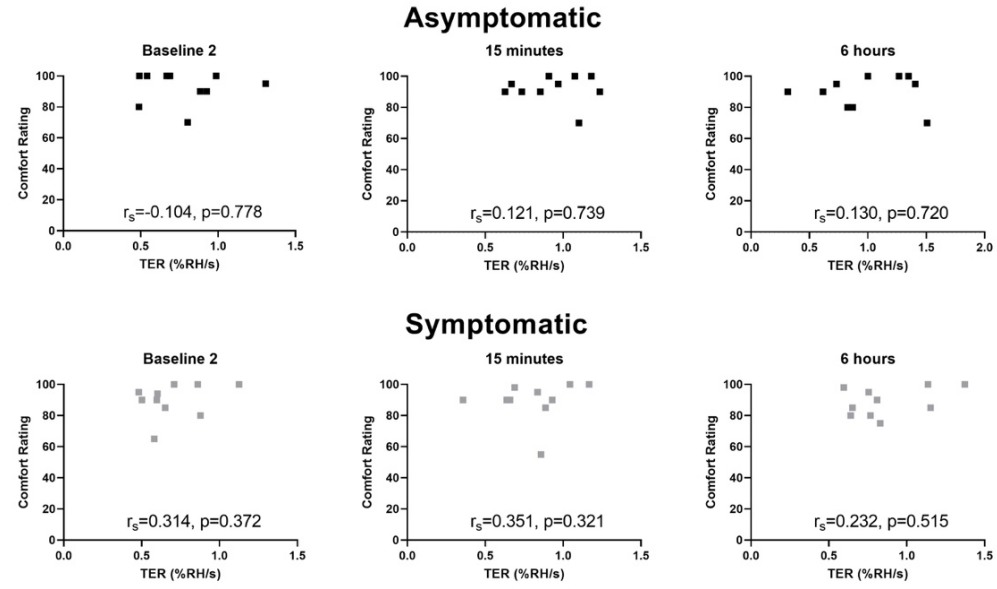

Figure 8-23: Correlations between the tear evaporation rate and comfort rating of the left eye (asymptomatic: all r_s<0.131, all p>0.719; symptomatic: all r_s<0.352, all p>0.320).

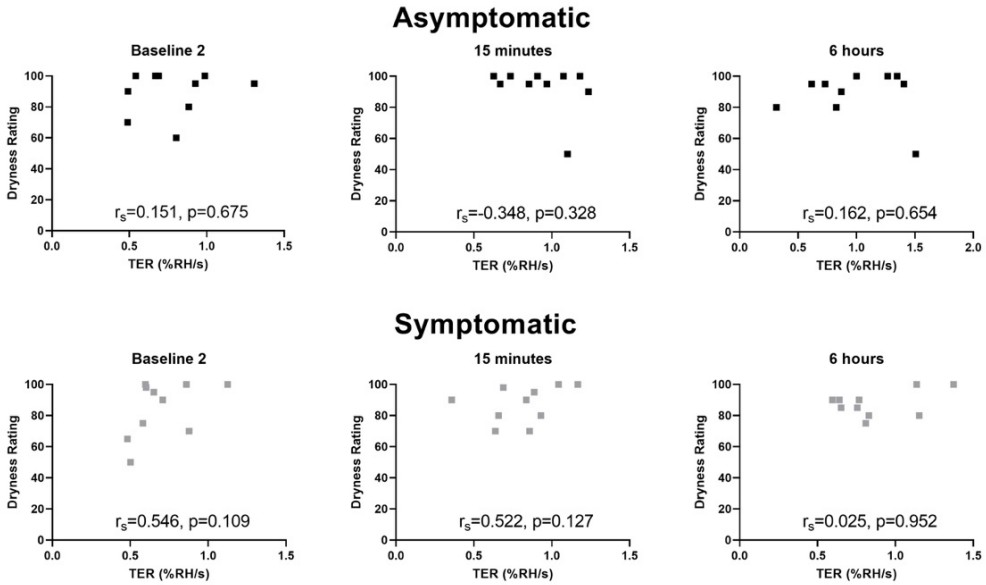

Figure 8-24: Correlations between the tear evaporation rate and dryness rating of the left eye (asymptomatic: all r_s<0.163, all p>0.327; symptomatic: all r_s<0.547, all p>0.108).

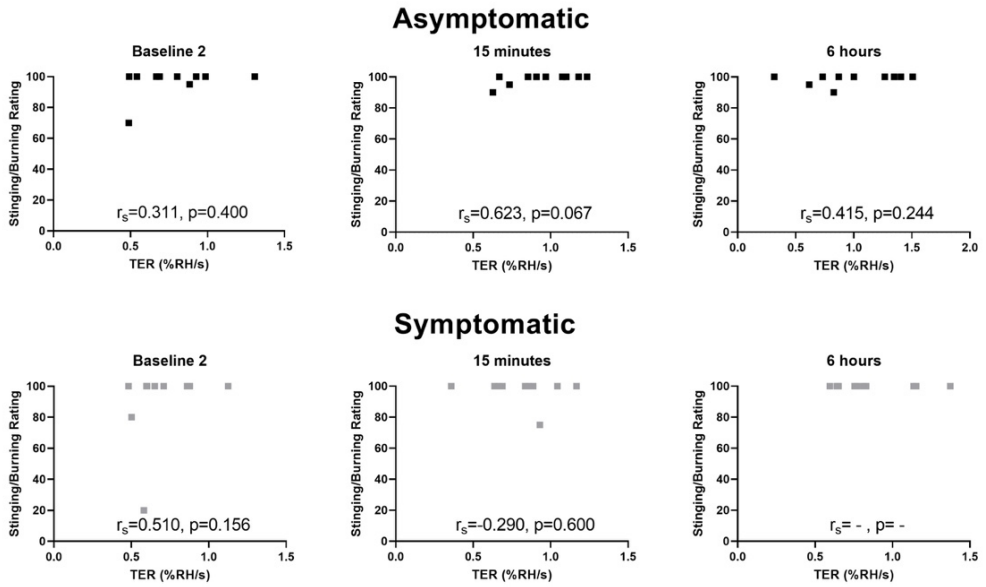

Figure 8-25: Correlations between the tear evaporation rate and stinging/burning rating of the left eye (asymptomatic: all r_s<0.624, all p>0.066; symptomatic: all r_s<0.511, all p>0.155 except for at 6 hours where r_s could not be calculated).

8.4.5.3 Clinical Tests

A summary of correlations between the TER of the left eye and symptomology is shown in Table 8-13. There was no significant correlation between the TER and the NITBUT (asymptomatic: all r_s<0.286, all p>0.426; symptomatic: all r_s<0.128, all p>0.183, Figure 8-26) or the mean objective LLT of either group (asymptomatic: all r_s<–0.199, all p>0.077; symptomatic: all r_s<0.533, all p>0.116, Figure 8-28).

The asymptomatic group had a significant negative correlation between the TER and the subjective LLT (r_s=–0.706, p=0.029, Figure 8-27) and the maximum LLT (r_s=–0.653, p=0.048, Figure 8-29) at the second baseline measurement. However, the symptomatic group had a significant positive correlation between TER and the minimum LLT at the second baseline measurement (r_s=0.758, p=0.015, Figure 8-30).

Table 8-13: Correlations between tear evaporation rate of the left eye and other clinical tests

	Asymptomatic Group (n = 10 eyes)	**p-value**	**Symptomatic Group (n = 10 eyes)**	**p-value**
Non-invasive tear film break-up time				
Baseline 2	0.212[a]	0.560	–0.460[a]	0.184
15 minutes	0.285[a]	0.427	–0.442[a]	0.204
6 hours	–0.079[a]	0.838	0.127[a]	0.733
Lipid layer thickness (subjective)				
Baseline 2	**–0.706[a]**	**0.029**	0.254[a]	0.479
15 minutes	–0.505[a]	0.144	–0.415[a]	0.236
6 hours	0.315[a]	0.394	–0.035[a]	0.999
Lipid layer thickness (mean)				
Baseline 2	–0.591[a]	0.078	0.532[a]	0.117
15 minutes	–0.200[a]	0.584	–0.219[a]	0.542
6 hours	–0.244[a]	0.495	–0.127[a]	0.733
Lipid layer thickness (maximum)				
Baseline 2	**–0.653[a]**	**0.048**	0.147[a]	0.689
15 minutes	–0.295[a]	0.404	0.259[a]	0.468
6 hours	–0.171[a]	0.635	0.061[a]	0.871
Lipid layer thickness (minimum)				
Baseline 2	–0.503[a]	0.144	**0.758[a]**	**0.015**
15 minutes	0.055[a]	0.892	–0.244[a]	0.495
6 hours	0.389[a]	0.266	–0.316[a]	0.371

[a]: Spearman rank correlation.
Bold indicates significant correlations.

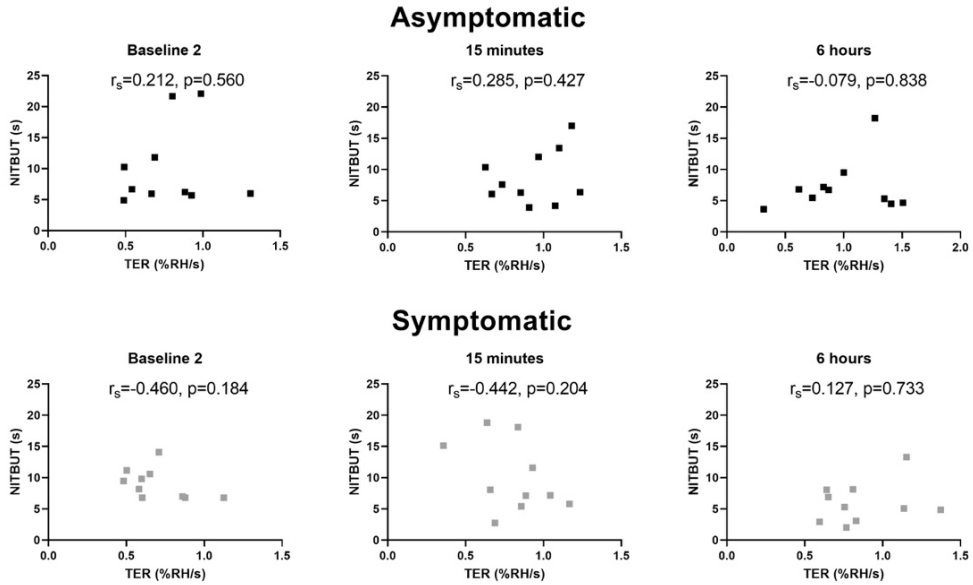

Figure 8-26: Correlations between the tear evaporation rate and non-invasive tear film break-up time of the left eye (asymptomatic: all r_s<0.286, all p>0.426; symptomatic: all r_s<0.128, all p>0.183).

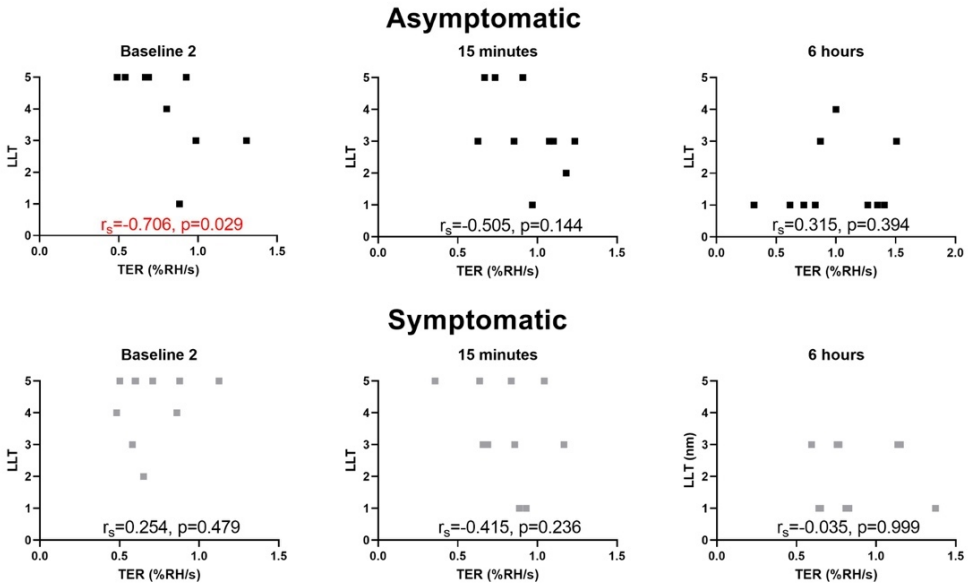

Figure 8-27: Correlations between the tear evaporation rate and subjective lipid layer thickness of the left eye. A significant Spearman correlation is shown in red (asymptomatic: baseline 2 r_s=-0.706, p=0.029, all other r_s<0.316, all other p>0.143; symptomatic: all r_s<0.255, all p>0.235).

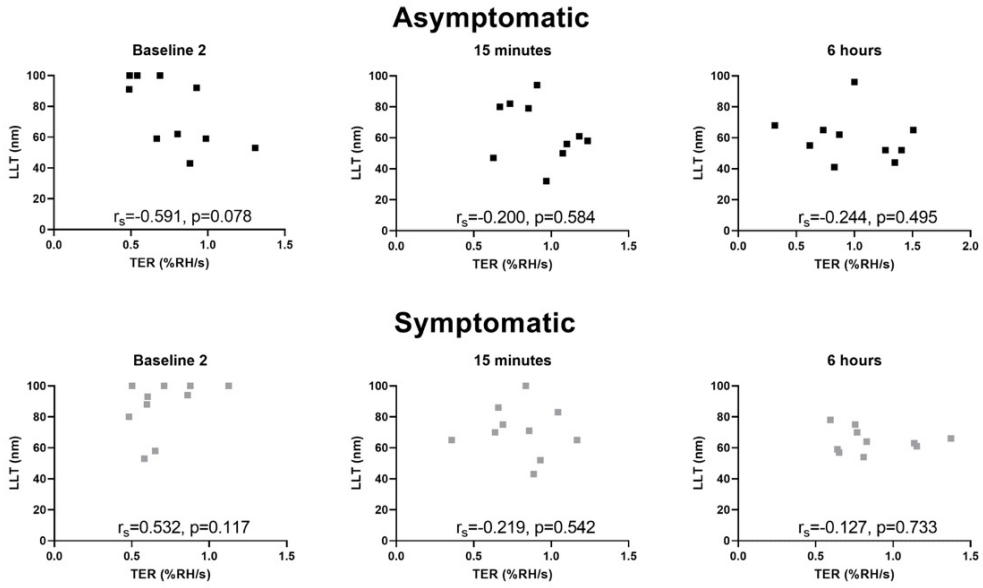

Figure 8-28: Correlations between the tear evaporation rate and mean objective lipid layer thickness of the left eye (asymptomatic: all r_s<–0.199, all p>0.077; symptomatic: all r_s<0.533, all p>0.116).

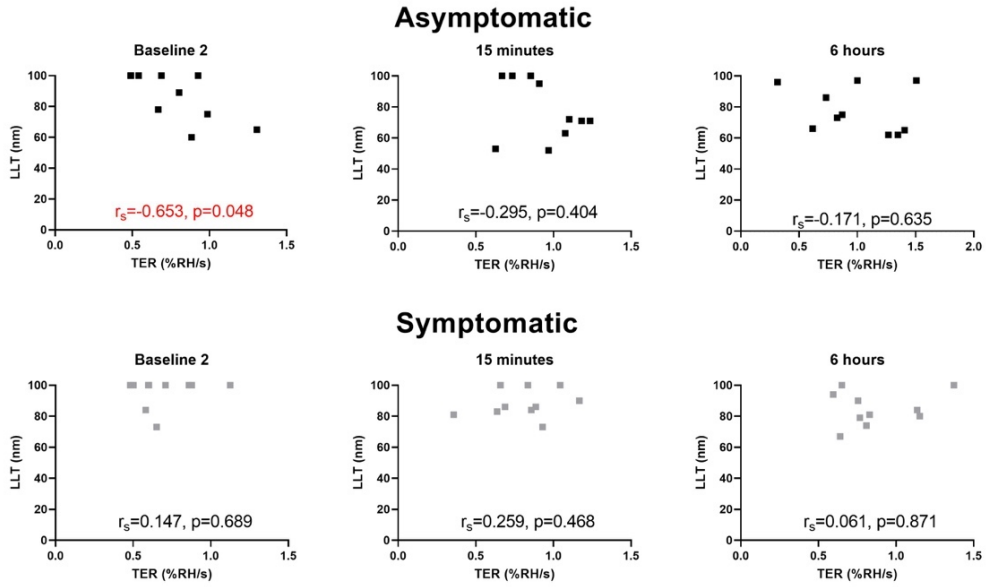

Figure 8-29: Correlations between the tear evaporation rate and maximum objective lipid layer thickness of the left eye. A significant Spearman correlation is shown in red (asymptomatic:

baseline 2 r_s=–0.653, p=0.048, all other r_s<0.170, all other p>0.403; symptomatic: all r_s<0.260, all p>0.467).

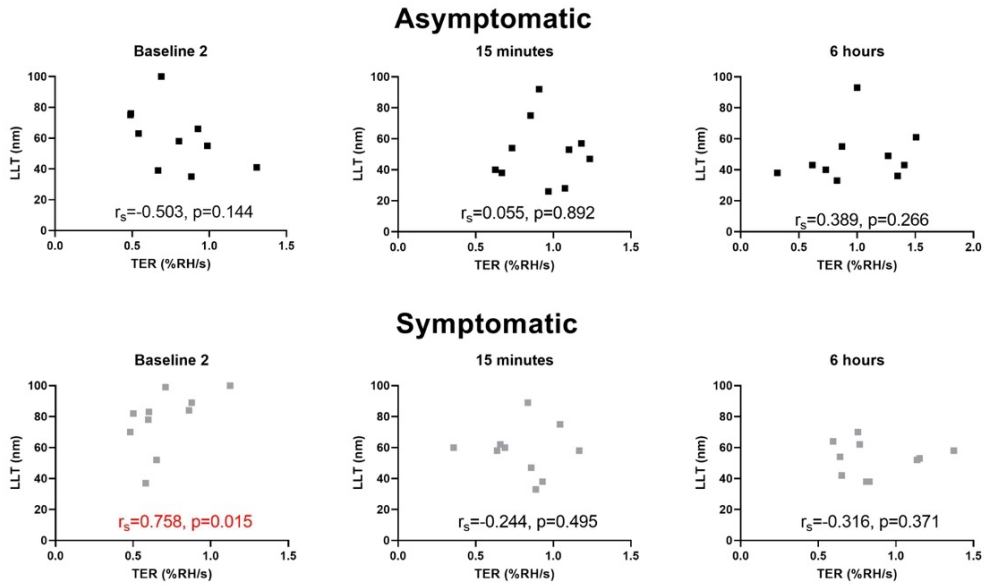

Figure 8-30: Correlations between the tear evaporation rate and minimum objective lipid layer thickness of the left eye. A significant Spearman correlation is shown in red (symptomatic: baseline 2 r_s=0.758, p=0.015, all other r_s<–0.243, all other p>0.370; asymptomatic: all r_s<0.390, all p>0.143).

8.4.6 Correlations Between TER and Ambient Relative Humidity

There was a significant negative correlation between the TER of the left eye and the ambient RH (r_s=–0.595, p<0.0001, Figure 8-31) measured at the second baseline measurement, and after 15 minutes and 6 hours of CL wear.

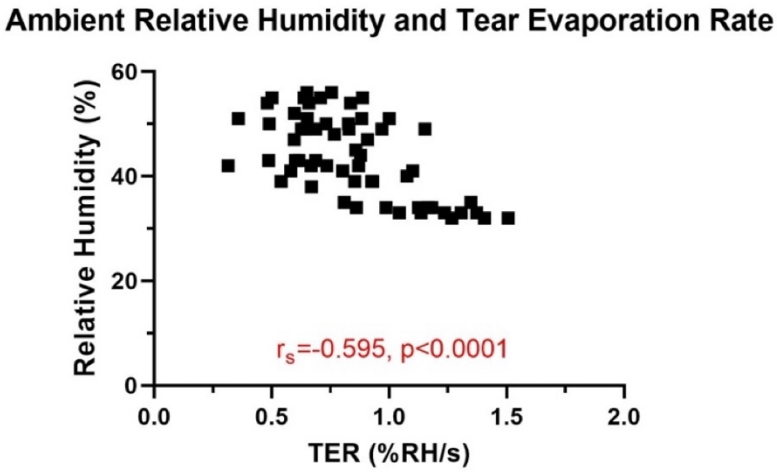

Figure 8-31: Significant Spearman correlation between the tear evaporation rate of the left eye and ambient relative humidity (r_s=–0.595, p<0.0001).

8.5 Discussion

Following *in vitro* testing of the evaporimeter, additional testing was required to investigate whether the instrument had the ability to detect a difference in tear evaporation with a CL *in situ* compared to when a CL was not worn. As previously mentioned in Chapter 7, the new instrument cannot be validated for use in comparison to a "gold standard" [427]. Construct validity [428] was tested by examining the impact of a CL on the TER. The instrument demonstrated the ability to detect a higher TER after 15 minutes and 6 hours of CL wear compared to no lens wear. External validity [429] was tested by comparing the results to other findings in the literature. Although no specific results have been published regarding the TER of delefilcon A or nesofilcon A, higher TERs have been found when CLs are worn [16, 21, 72, 73, 102, 110]. Convergent validity [427, 428, 430] was demonstrated, with strong correlations between TER and LLT (asymptomatic: all r_s>–0.653; symptomatic r_s=0.758).

8.5.1 Changes over Time

The results from the right and left side of the evaporimeter were examined separately to determine whether each side could detect a change in TER. Despite baseline measurements being repeatable in both sides of the symptomatic group and in the right eye of the asymptomatic group, there was a significant decrease in TER between the first and second baseline measurement in the left eye of the asymptomatic group. Since there was a trend towards decreased TERs between the baseline measurements of each eye of the two groups, the tear film may have been destabilized due to the lid eversion performed as part of the safety check at the beginning of the visit or there may have been inadequate adaptation time to the room.

However, both sides of the evaporimeter detected a significant increase in TER over time. The left side of the evaporimeter recorded a higher TER after 15 minutes of CL wear compared to no lens wear, and both sides of the evaporimeter exhibited increased TERs after 6 hours of CL wear compared to baseline. Measurements from the right side of the evaporimeter may not have had a statistically significant change in TER between the second baseline measurement and 15 minutes of CL wear because of a lack of power (observed power=0.40), with a sample size of 61 required for 0.80 power.

Other researchers have measured significantly higher TERs when rigid corneal [73, 102, 175] or soft CLs are worn [16, 21, 73, 102, 110, 175]. A summary appears in Table 8-14. Hamano et al. [73] discovered TER increased when a soft hydroxyethyl methacrylate CL was worn for 30 minutes, but lower TERs were found when a polymethyl methacrylate (PMMA) or a silicone rubber CL were worn for 30 minutes. However, it is unknown whether the decreases in TER were significant, and whether the invasive nature of the evaporimeter may have affected pre-lens TER and could have impacted the participant's ability to blink. Tomlinson and Cedarstaff [102] tested 5 participants with two types of rigid lenses (PMMA, Paragon-18) and three types soft CLs (Cibasoft 38%, Sauflon 70%, Silsoft). The change in TER was monitored for an hour after each CL was inserted into the right eye, while the left eye did not wear a CL. All five types of CLs resulted in a significant increase in TER compared to initial baseline measurements taken prior to CL wear. However, it should be noted that there is a discrepancy in the Sauflon 70% TERs of the first four participants reported in Tables 1 and 2, with the mean TERs in Table 2 also appearing in a paper published the following year [16].

Cedarstaff and Tomlinson [16] also tested 5 participants with three different types of soft CLs (Cibasoft 38%, Cibasoft 55%, Sauflon 70%). Individual variations in response to CL wear were observed, with the majority of participants exhibiting a significant increase in TER when a CL was worn for an hour. Four out

of five participants had a significantly higher TER when wearing Cibasoft 38%, while one participant experienced a significant decrease in TER. Four out of five participants also had a significant increase in TER with Cibasoft 55%, with the remaining participant had a non-significant change in TER. Three participants also had a significant increase in TER with Sauflon 70%, while the other two participants had a non-significant change in TER.

Thai et al. [17] tested habitual CL wearers after wearing five different types of soft CLs (polymacon, omafilcon A, phemfilcon A, balafilcon A, etafilcon A) for 30 minutes. Each type of CL resulted in a higher TER compared to when a CL was not worn. Rohit et al. [21] also reported a significant increase in TER in neophytes were fit with Focus Dailies.

Guillon and Maissa [110] examined differences in TER amongst three separate groups: (i) CL wearers who attended for a visit wearing their habitual CLs, (ii) CL wearers who had not worn CLs on the day of the examination, and (iii) non-CL wearers. Consistent with the findings of Ward et al. [181], they found that all CL wearers had significantly higher TERs than non-CL wearers [110]. CL wearers with lenses *in situ* could also be differentiated from habitual wearers without a CL because the TER was significantly higher for those wearing a CL. In contrast, Dogru et al. [18] were unable to find a significant difference in the TER of neophyte CL wearers before and after two weeks of wearing senofilcon A. However, the TER following two weeks of wear was measured the day after CLs had been worn, with all eyes having removed CLs at least 10 hours prior to evaporimetry. Mathers [175] was also unable to find a significant difference in TER when new rigid or soft CLs were worn for a few minutes compared to when CLs were not worn. However, a significantly higher TER was measured when old CLs were worn.

Table 8-14: Summary of change in tear evaporation rate with and without contact lens wear

	Type of Contact Lens	No Contact Lens Evaporation Rate	ΔEvaporation Rate with Contact Lens	Contact Lens Evaporation Rate
Hamano et al. [73]	Soft HEMA	NR	↑	Δ~30% TER[a]
	PMMA	NR	↓?	Δ–<5% in TER[a,b]
	Silicone rubber	NR	↓?	Δ~–10% in TER[a,b]
Tomlinson and Cedarstaff [102]	PMMA	0.66 ± 0.21[c]	↑	1.42 ± 0.16[c]
	Paragon-18	0.66 ± 0.21[c]	↑	1.42 ± 0.20[c]
	Silsoft	0.66 ± 0.21[c]	↑	1.26 ± 0.22[c]
	Sauflon 70	0.66 ± 0.21[c]	↑	1.16 ± 0.17 [c]/ 1.23 ± NR[c,d]
	Cibasoft 38	0.66 ± 0.21[c]	↑	1.70 ± 0.21[c]
Cedarstaff and Tomlinson [16]	Cibasoft 38	0.74 ± 0.13[c]	↑	Δ0.56 ± 0.26[c] (n = 4),
			↓	Δ–0.19 ± 0.28[c] ↓ (n = 1)
	Cibasoft 55	0.78 ± 0.11[c]	↑	Δ0.35 ± 0.24[c] (n = 4),
			↔	Δ0.51 ± 0.18[c] (n = 1)
	Sauflon 70	0.91 ± 0.18[c]	↑	Δ0.51 ± 0.22[c] (n = 3),
			↔	Δ0.03 ± 0.34[c] (n = 2)
Guillon and Maissa [110]	Soft	15.1 ± 7.3[e,f] ↑ 16.7 ± 7.5[f,g]	↑	23.5 ± 6.8[f]
		11.3 ± 6.8[e,h] ↑ 12.8 ± 7.0[f,h]	↑	18.9 ± 6.2[h]
Dogru et al. [18]	Senofilcon A	4.1[i] ↔ 4.2[i,j]		NM
Mathers [175]	Daily wear for 6 weeks	10.8[k] + 0.9[k]	↑?	New: 14.8[k] + 3.0[k]
			↑?	Old: 23.0[k] + 11.8[k]
	Daily wear for 1 week	25.5[k] + 20.0[k]	↔?	New: 22.4[k] + 12.6[k]
			↑?	Old: 33.4[k] + 12.2[k]
	RGP	17.6[k]	↓?	New: 13.2[k]
			↑?	Old: 28.9[k]
	Single use	17.6[k]	↑?	New: 23.7[k]
			↑?	Old: 37[k]

	Type of Contact Lens	No Contact Lens Evaporation Rate	ΔEvaporation Rate with Contact Lens	Contact Lens Evaporation Rate
Mathers [175]	Combined Lenses	18.0[k] + 13.4[k]	↔	New: 18.5[k] + 8.7[k]
			↑	Old: 28.9[k] + 11.3[k]
Thai et al. [17]	Polymacon		↑	Δ10.67 ± 8.07[l]
	Omafilcon A		↑	Δ8.99 ± 10.89[l]
	Phemfilcon A		↑	Δ16.29 ± 11.20[l]
	Balafilcon A		↑	Δ14.74 ± 12.65[l]
	Etafilcon A		↑	Δ15.75 ± 13.13[l]
	Combined Lenses	39.05 ± 19.03[l]	↑	52.33 ± 18.03[l]
Ward et al. [181]	Etafilcon A, Senofilcon A, Balafilcon A	2.2[i]	↑	3.9[i]
Rohit et al. [21]	Focus Dailies	55.6[l]	↑	90.6[l]
Siddireddy et al. [323]	Etafilcon A, Comfilcon A, Balafilcon A, Lotrafilcon A, Lotrafilcon B	76[i,l] (48)[l]	↑*?*	96[i,l] (28)[l]
	Etafilcon A, Comfilcon A, Balafilcon A, Lotrafilcon A, Lotrafilcon B	88[i,l] (45)[l]	↑*?*	112 [i,l] (54)[l]
This study	Delefilcon A, Nesofilcon A	0.82 ± 0.28[m,n]	↔	15 min: 0.89 ± 0.33[m,n]
			↑	6 hours: 0.94 ± 0.35[m,n]
		0.74 ± 0.23[n,o]	↑	15 min: 0.87 ± 0.23[n,o]
			↑	6 hours: 0.93 ± 0.33[n,o]

Values are reported as mean ±standard deviation and units are x 10^{-7} g/cm^2/s unless specified.

HEMA: hydroxyethyl methacrylate; PMMA: polymethyl methacrylate; TER: tear evaporation rate; NR: specific numerical values not presented in the text; RGP: rigid gas permeable; ↑: significant increase in TER; ↓: significant decrease in TER; ↔: non-significant change in TER; ?: assumed level of significance based on the authors reported values; NM: not measured; n: sample size; min: minutes.

[a]: p-values not reported.
[b]: values estimated from a graph.
[c]: mg/min/cm^2 – values are 100x too large [11].
[d]: values from Table 2 [102].
[e]: non-contact lens wearer.
[f]: 30% humidity.
[g]: contact lens wearer not wearing a contact lens.
[h]: 40% humidity.
[i]: median.
[j]: after 2 weeks of contact lens wear.
[k]: type of descriptive statistic was not specified.
[l]: g/m^2/h.
[m]: right eye.
[n]: % Relative Humidity/second.
[o]: left eye.

Separate analyses of each eye of the two groups were only able to detect a significant difference in TER between the second baseline measurement and 6 hours of CL wear in the left eye of the symptomatic group. Measurements from the right side of the evaporimeter may not have had a statistically significant change in TER due to a lack of power needed to detect a difference between baseline and 15 minutes after the CLs were inserted (observed power: asymptomatic=0.66; symptomatic=0.24), with larger sample sizes required to achieve 0.80 power (total sample size: asymptomatic=14; symptomatic=63). Similarly, the study was underpowered to achieve a significant change in TER in the right side of the evaporimeter between baseline and 6 hours of CL wear (observed power: asymptomatic=0.53; symptomatic=0.49), with additional participants needed to attain 0.80 power (total sample size: asymptomatic=20; symptomatic=22). Although the left eye of the asymptomatic group was adequately powered (observed power=0.92) to detect a difference between baseline and 15 minutes after CL insertion, a significant change may not have been detected due to multiple time points being tested. A significant change in TER for the left side of the evaporimeter was likely not found due to the lack of power between baseline and 6 hours in the asymptomatic group (observed power=0.62), and baseline and 15 minutes in the symptomatic group (observed power=0.49), with more participants needed to reach 0.80 power (total sample size: asymptomatic=16; symptomatic=22). An interim data analysis was conducted after the first 20 participants had completed the study, with approval to include a maximum of 60 people. However, it was not possible to include additional participants because research was ceased due to the COVID-19 pandemic.

Insertion of a CL into the eye causes the pre-ocular tear film to split in two to form a pre-lens and post-lens tear film [288]. In addition to changes in TER over time, a significant decrease in NITBUT occurred between baseline and 6 hours of CL wear in the symptomatic group. Glasson et al. [450] found a significant reduction in NITBUT in tolerant CL wearers after 6 hours of wearing time, although there was no significant change in intolerant CL wearers. Delefilcon A and nesofilcon A can significantly reduce the NITBUT after 20 minutes and 8 hours of wear [294], with shorter NITBUTs encountered as wearing time increases [244].

Changes in subjective and objective LLT were also observed over time in both the symptomatic and asymptomatic groups, with the majority of significant decreases in LLT occurring between baseline and 6 hours of CL wear. The pre-corneal tear film is significantly thicker than the pre-lens tear film [17, 165], and the lipid layer continues to thin over the duration of CL wearing time [314].

8.5.2 Baseline Differences Between Eyes

Differences between the eyes were analyzed at the screening visit to examine whether any characteristics may have affected the ability of the evaporimeter to detect a significant change in TER when CLs were worn. The statistically significant difference in habitual CL power between the eyes of the symptomatic group was not clinically significant because the mean difference in power was 0.25 DS. However, the poorer meibomian quality of expression in the left eye of both groups could be a reason why increased TERs were detected at each time point after CLs were worn when the two groups were combined.

The ocular surface area of the asymptomatic group was also larger in the left eye than the right eye. As discussed in Sections 7.5.2 and 7.6.2, this may be due to normal anatomical variation or because the eyes were not simultaneously photographed. The size of the ocular surface of all the eyes was within the range of previously reported values [97, 434].

8.5.3 Differences Between Groups

Symptomatic participants had significantly higher CLDEQ-8 scores than the asymptomatic group at the screening visit, as required by the inclusion criteria. A CLDEQ-8 score of ≥12 identifies CL wearers that could benefit from some type of invention to improve their symptoms [448]. Symptomatic participants also had poorer quality of meibomian gland expression in both eyes, which could impact the quality of the tear film. Worsened meibomian gland quality and fewer expressible glands have been found in other symptomatic CL wearers [323].

None of the subjective comfort ratings or clinical tests conducted at the dispensing or follow-up visit revealed a significant difference between the symptomatic and asymptomatic group. Insua Pereira and Lira [359] reported no significant difference in subjective comfort or dryness between delefilcon A and nesofilcon A after 1 hour of wear. However, after 7 hours of wear delefilcon A felt significantly more comfortable and less dry than nesofilcon A.

Although a significant difference in TER was not detected between the two groups, there was a trend towards a lower TER in symptomatic CL wearers. This may be related to the mean dispensed power of nesofilcon A being higher in the asymptomatic group than the symptomatic group (–3.55 DS vs –2.40 DS). Despite attempts to ensure all participants enrolled in the study had similar prescriptions in both eyes, the thickness of the CLs may have affected the results, because nesofilcon A is significantly

thinner than delefilcon A after 20 minutes and 8 hours of wear [253]. Because the primary outcome in this study was to test whether the evaporimeter could detect a change in TER over time, contralateral testing, rather than a crossover design, was chosen to ensure both CLs would be tested under the same ambient temperature and RH conditions. Although all participants could theoretically be tested using the same power of CL, this would not be feasible for participants who had significantly different prescriptions from the selected test power. In the future, it may be worthwhile narrowing the inclusion criteria regarding the habitual CL power even further to avoid a potential difference in CL thickness from affecting the results.

Siddireddy et al. [323] tested the TER of a group of CL wearers, the majority of whom habitually wore a frequent replacement CL (60% asymptomatic, 80% symptomatic). Symptomatic lens wearers had higher TERs, regardless of whether or not CLs were worn. A difference in TER may have been detected between the groups because the median (IQR) age of the participants' habitual CLs were 10 (12) days and 5 (11) days in the symptomatic and asymptomatic group, respectively. A formula was subsequently developed based on different clinical findings, including TER without CL wear, to differentiate CL wearers based on symptomology [161].

The TER in this study increased by 17% in the asymptomatic group and 16% in the symptomatic group, 15 minutes after the CLs were inserted. The increase in TER after 6 hours of wear compared to baseline was 27 and 24% in the asymptomatic and symptomatic group, respectively. Siddireddy et al. [323] reported a 26% increase in asymptomatic CLs wearers and 27% in symptomatic CL wearers after their habitual hydrogel or silicone hydrogel CLs were worn for a minimum of 30 minutes. A smaller increase in TER may have observed in this study due to the use of daily disposable lenses and different CL materials.

No significant difference in subjective [451] or objective LLT [323] has been reported in asymptomatic and symptomatic CL wearers when CLs are not worn. Rohit et al. [452] were also unable to find a significant difference in LLT between symptomatic and asymptomatic CL wearers after nelfilcon A lenses were worn for 6 to 8 hours. However, significantly shorter break-up times have been measured in symptomatic CL wearers both with [445, 452] and without CLs [323, 451].

8.5.4 Difference in TER Between Contact Lenses

There was no significant difference in TER between delefilcon A and nesofilcon A after 15 minutes or 6 hours of wear. To achieve 0.80 power, a total of 234 participants would have been required to

detect a difference in TER between the two types of CLs after 6 hours of wearing time (observed power=0.12). Although a direct comparison between this study and previous evaporimetry studies was not possible because different types of CL have been investigated in the past, the increase in TER that occurs with CL wear has not been found to be related to the initial water content [16, 17] or material of the lens [17, 102].

Thai et al. [17] could not find a significant difference in TER between 5 different types of soft CLs (balafilcon A, etafilcon A, omafilcon A, polymacon, phemfilcon A) that were worn for 30 minutes. However, Tomlinson and Cedarstaff [16, 102] reported significant differences in TER between a 38% hydrogel and 70% hydrogel. Despite the fact that the same five participants were presumed to have been tested because the initials of the participants were identical in the two papers, the findings were inconclusive. In one report, four out of five participants had a higher TER with the low water content CL, while the remaining participant had an increased TER with the high water content CL [102]. The following year, the authors found only two participants had a higher TER with the 38% hydrogel, whereas the remaining three participants had an increased TER with the 78% hydrogel [16]. Gravimetric water loss was proportionally similar regardless of the initial water content, and the amount of water loss was not a significant cause of the increased TER observed with CL wear [16].

Kojima et al. [155] investigated the effect of exposing participants to a controlled adverse chamber environment set to 18% RH for 20 minutes. Participants wearing etafilcon A had a significant increase in TER following exposure to the environmental chamber, while the TER of participants with narafilcon A did not significantly change. A separate study measured the TER of omafilcon A and stenfilcon A in 10 minute intervals with a ServoMed Evaporimeter and Delfin Eye-VapoMeter [368]. The ServoMed was unable to detect a difference between the TER of two types of CLs over 30 minutes of wear. However, the Eye-VapoMeter was able to detect a significant increase in TER with omafilcon A, whilst the TER of stenfilcon A did not significantly change over time.

Previous research has found that different types of soft CLs cause the TER to change by –12 [175] to 158% [102] compared to no lens wear. The TER in this study increased by 22 and 28% after 6 hours of nesofilcon A and delefilcon A wear, respectively. Although the percentage increase in TER for nesofilcon A was towards the lower end of previously reported values, this may be due to the advent of newer materials that incorporate water gradient technology [246] or HyperGel material [453]. One study reported that delefilcon A rapidly lost its surface water within the first 15 minutes of wear,

whereas the water content of nesofilcon A underwent minimal changes to the surface water over the same period [245]. A separate study found the water content of nesofilcon A decreased by an average of 1.7% over the course of its wearing time, while the water content of delefilcon A increased by 4.1% [359].

8.5.5 Correlations Between TER and Other Clinical Measurements

No significant correlations were found between the TER and the CLDEQ-8 or the subjective comfort ratings. Siddireddy et al. [323] were also unable to find a correlation between the CLDEQ-8 score and the TER measured with and without CLs in CL wearers, or in a sub-group of asymptomatic participants. However, symptomatic CL wearers had positive correlations between the TER tested with and without CLs and the CLDEQ-8 score. As discussed in Section 8.5.3, this may be due to examining participants wearing their older, habitual CLs compared to the new daily disposable CLs tested in this study. Additional analysis using a point-biserial correlation matrix found a significant correlation between the TER without CL wear and the CLDEQ-8, although no significant correlation was reported between the TER with CL wear [161]. Since there was asymmetry between the horizontal and vertical headings of the correlation matrix of their Table 3, the values in the upper right-hand side of the matrix were used because only this section was completed in the first author's dissertation [330]. Significantly higher TERs have been reported following exposure to a dry environmental chamber with etafilcon A, which were accompanied by increased foreign body sensation and worsened dryness scores [155]. However, no significant changes in comfort were observed in participants wearing narafilcon A.

No significant correlation was found between the TER and the NITBUT, although Abusharha et al. [115] reported a weak positive correlation between them. As discussed in Section 7.5.4 a significant correlation may not have been found because the two tests measure different components of the tear film, and because evaporimetry was tested in this study using a frequent blink rate.

Significant negative correlations were found between the TER and the subjective LLT (r_s=–0.706, p=0.029) and maximum LLT (r_s=–0.653, p=0.048) of the asymptomatic group at the second baseline measurement. Others have previously reported negative correlations between TER and LLT [49, 182], with negative correlations also found between the TER assessed with and without CLs and the objective LLT in a group of CL wearers [323].

Interestingly, a significant positive correlation was found between the TER and the minimum LLT (r_s=0.758, p=0.015) in the symptomatic group at the second baseline measurement. Abusharha et al.

[115] reported a weak positive correlation between TER and LLT. However, it should be noted that there is a discrepancy between the main text that describes a negative correlation, but the rho value (r_s=0.43) and figure 2C in their paper both indicate a positive correlation. The correct correlation is believed to be the positive value, as this reflects the trendline on the figure. However, despite a positive correlation occurring between TER and LLT, the true correlation is likely to be negative as reported by other researchers [49, 182, 323], Chapter 7, and the other significant LLT correlations described in this chapter.

The failure to find any significant correlations between TER and the subjective comfort ratings or other clinical tests, provides further evidence that signs and symptoms do not always match. An investigation found that 23% soft CL wearers who reported significant symptoms of dryness did not show any clinical signs, while signs of dryness were observed in 15% of asymptomatic CL wearers [444].

No significant correlations were found between TER and the ocular surface area or air volume within the evaporimeter. As found was also found in Section 7.4.4.1, volume did not have a significant relationship with TER. Although *in vitro* measurements showed that TER was affected by the ocular surface area (Section 5.3.2.4) and strong positive correlations were observed in the non-dry eye group between TER and ocular surface area 10 and 60 minutes after instillation of Systane® Complete (Section 7.5.4), no significant correlation was found between TER and ocular surface area in this study.

8.6 Future Work

8.6.1 Possible Sources of Error

Although the study was a small, contralateral eye investigation, each side of the evaporimeter was able to detect an increase in TER following CL wear. As reported by other researchers [16, 17, 73, 102], there was an individual response to TER following CL wear. The individual variation in TER may have affected the evaporimeter's ability to find a change in TER after 15 minutes of CL wear in the right eye, or differences over time in each eye of the two groups. An attempt to re-analyze the change in TER from the second baseline measurement to 6 hours of CL wear, by excluding participant 18 from the asymptomatic group, still resulted in non-significant changes in TER over time (right: p=0.059, left eye: p=0.071). Therefore, the reported results include the analysis of all the participants that completed the study.

The study was underpowered. The investigation would have ideally resumed following the interim data analysis to achieve adequate power to detect a change in TER in each eye of the two groups.

The participants were predominantly female and the majority of CL fits performed in Canada and worldwide throughout 2020 were on females [265]. However, a more representative sample of the overall CL population would have been preferable. Females ≥45 years old have been reported to have significantly higher TERs [111]. Although the two groups were well balanced for older females (asymptomatic: 1 participant; symptomatic: 2 participants), age and sex-matching or excluding certain age groups in the future could help minimize the individual TER response observed between participants.

A significant difference in TER may not have been detected between the two groups because classification was based on the CLDEQ-8 score, which assesses the presence of CL discomfort [266, 447], but not dry eye. Two participants in the asymptomatic group reported habitually using ocular lubricants and additional criteria would have been helpful to minimize the inclusion of possible dry eye participants from the asymptomatic group. The number of comfortable hours of wear should have been recorded [443] and compared to the total wearing time. Anyone that used eye drops or had been told/felt they had dry eye should also have been excluded from the asymptomatic group. As both groups had a median NITBUT of <10 seconds, a minimum NITBUT requirement for the asymptomatic group could be included in the future. In addition, a significant difference in TER between the groups may not have been detected because habitual CLs were not tested in the study. As 80% of symptomatic wearers habitually wore frequent replacement CLs, the TER may have been similar between the groups due to the use of daily disposable CLs and because the CLs were only tested after 6 hours of wear.

The left eye of the asymptomatic group had a significant decrease in TER between the two baseline measurements. As there was a trend towards decreased TER between the baseline measurements of each eye of the two groups, this supports the theory that the tear film could have been destabilized due to the lid eversion performed beginning of the visit (Section 7.6.1).

Due to the lack of an environmental chamber to control the ambient temperature and RH (Section7.6.1), the temperature varied between 70 to 79°F (21.1 to 26.1°C) and RH fluctuated between 32 and 56%. Although Abusharha et al. [115] did not find a significant difference in TER between 15 to 25ºC at 40% RH, a dehumidifier was not used because it would have caused an uncomfortable increase in temperature inside the room.

As discussed in Section 7.6.1, the LipiView II LLT was limited by the range of values that the instrument produces. Although the manufacturers advise CLs should be removed at least 4 hours prior to use with the instrument, the LipiView II was used as an additional method of measuring LLT to complement the subjective assessment with the Tearscope Plus. The results from this study indicate the LipiView II was able to detect changes in LLT in response to CL wear.

In addition, a lack of a correlation between the baseline subjective ratings and TER could have been affected by only completing a subjective comfort rating at the first baseline measurement. Subjective ratings after 15 minutes were assumed to not be required because all of the tests performed were non-invasive and performed within the same room. However, in future, subjective ratings should be performed at each time point to ensure an accurate representation of the eyes.

8.6.2 Refining the Evaporimetry Technique

In addition to the suggestions presented in Section 7.6.2, this study highlights the need for a correction factor to modify the TER based on the ambient RH. Rohit et al. [21] also encountered a wide range of RH (32 to 80%) and described a negative relationship between TER and RH measured with and without CLs. Results from an evaporimeter that measures the TER over two different RH ranges found the TER was 28.33 to 59.42% higher at 25–35% RH compared 35–45% RH [107]. Other work by the same group found that the average increase in TER when the RH changed from 40–45% to 20–25% was 85.59 to 117.09% [108]. Although some have chosen to correct the TER to an arbitrarily chosen temperature and RH [49] or to normalize the values to 40% RH [2], a correction factor could also be generated by testing the novel evaporimeter with model eyes inside an environmental chamber, as this will eliminate any individual variability from human variations and allow for a wide range of RHs to be tested.

Future work should also be conducted to determine the adequate amount of adaptation time required for participants since not all the baseline measurements were repeatable. Repeated measurements should be taken over a period of time, without performing any tests prior to evaporimetry that could affect the tear film. Additional testing should also determine whether a difference in TER can be detected between symptomatic and asymptomatic wearers. Measurements of TER when CLs are worn for longer periods of time and/or with habitual CLs that are towards the end of their lifespan may be the best way to determine a difference between the groups.

After a larger sample size has been tested, the decision whether to correct for the TER individual variations in ocular surface area and volume inside evaporimeter can be made. Contrary to results from Chapter 7, no correlation was found between the TER and the area of ocular surface. This finding suggests that if fixation is controlled, the size of the ocular surface does not significantly affect the *in vivo* TER. Since a significant correlation was also not found between the TER and evaporimeter volume in Chapters 7 or 8, the significant effect of ambient RH on TER [21] appears to outweigh the impact of the ocular surface area or air volume inside the evaporimeter.

8.6.3 Future Contact Lens Work

This study demonstrated that the evaporimeter can detect a difference in TER in the left side of the evaporimeter after 15 minutes of CL wear and in both sides of the evaporimeter after 6 hours of CL wear. Additional testing with a larger sample size is required to determine whether a difference can be detected between symptomatic and asymptomatic CL wearers or between different types of CLs. It is worthwhile to begin with testing participants using their habitual frequent replacement CLs. A comparison could be made on the first day of wear following insertion of a new pair of CLs and compared to a minimum of 8 hours of wear just prior to when the CLs are due to be replaced, to see if a difference between symptomatic and asymptomatic wearers can be detected. Differences between CLs could also be examined using conventional low water and high water content CLs. If further work demonstrates the evaporimeter is capable of detecting differences between various types of CLs, additional testing can be done to compare hydrogel to silicone hydrogel CLs of similar water contents, or the newest CLs on the market versus their predecessors.

8.7 Conclusions

Pilot testing of the novel binocular evaporimeter validated its use for measuring the TER of CL wearers. The evaporimeter detected changes in TER in each eye when a CL was worn, with higher rates of evaporation after 15 minutes of CL wear in the left eye and increased TERs after 6 hours of wear compared to baseline in both eyes. The evaporimeter also measured a significantly higher TER after 6 hours of wear in the left eye of the symptomatic group. The only clinical tests with significant changes over time in both the asymptomatic and symptomatic groups were subjective LLT and the mean objective LLT.

None of the clinical tests were able to detect a difference between the two groups, further demonstrating the importance of ensuring CL wearers undergo a symptomology assessment. In addition, no significant difference in TER was measured between delefilcon A or nesofilcon A after 15 minutes or 6 hours of wear.

Future work is warranted using the evaporimeter to test larger sample sizes. The ability to detect differences among the two groups can be improved by exerting caution to avoid the inclusion of dry eye participants from the asymptomatic group. Better correlations between signs and symptoms over time and between groups may be found in the future by testing participants using their habitual CLs towards the end of their comfortable wearing time.

Chapter 9
Conclusions and Future Work

9.1 Novel Evaporimeter

This thesis describes the successful development and testing of a novel binocular evaporimeter. For the first time, a sensor has been incorporated directly into the front lens of a swimming goggle, which required minimal modification to the existing design of the goggle and reduced the amount of extraneous air space within the closed-chamber system. Testing of the non-invasive instrument demonstrated its ability to quickly and simultaneously measure the rate of tear evaporation from both eyes.

Use of the evaporimeter with a range of participants showed that it can detect higher rates of tear evaporation in people with signs and symptoms of dry eye disease, and in response to contact lens (CL) wear. In addition, the evaporimeter also detected significant decreases in tear evaporation rate (TER) following the single instillation of an artificial lubricant to treat dry eye.

The minimal equipment that comprises the evaporimeter (Chapter 4) ensures that the instrument is clinician-friendly. Advantages of the instrument and reasons to advocate for its incorporation into everyday clinical practice include the ease of use, short measurement time, small size, and the fact that it does not require specialist laboratory equipment for ventilation, such as anhydrous calcium sulfate [3, 99, 102], silica gel [101], or compressed air [1].

In vitro testing demonstrated the ability of both sensors to measure a change in relative humidity (RH) when placed over a source of evaporation (Chapter 5). *In vivo* work showed the RH measurement in each goggle was similar, with no statistically significant difference detected between the two sensors when the eyes were open or closed (Chapter 6).

The new instrument provided much faster TER measurements compared to previous binocular evaporimeters that recorded the change in RH over 110 seconds [2] or required 30 minutes of calibration measurements [102]. The short measurement did not appear to cause any condensation inside the closed system, which was a problem reported with prolonged measurements taken with an open-chamber ServoMed Evaporimeter [49]. The comfort of the person being examined was taken into consideration when optimizing the methodology for performing evaporimetry with the new instrument (Chapter 6). By allowing the person to rest their elbows on a table during the measurement, this position

is not expected to result in fatigue or discomfort, especially in comparison to the Mathers evaporimeter, which required a 2-minute measurement with the eyes closed, a 2-minute measurement with the eyes open, in addition to time required for the RH in the system to be lowered to 5% [3]. The fast measurement time is also short enough to ensure fixation can be maintained for the duration of the measurement without suffering from a loss of concentration.

The closer proximity of the sensor to the eye should also reduce the chance of poor mixing of the air within the goggle and reduce temperature variations within the goggle [99], without interfering with the eyelids or eyelashes. The small size of the sensors and the placement reduced the total air volume within the evaporimeter, especially when compared to the 44 ml cylinder used in the Tsubota-Yamada binocular evaporimeter [2]. The results of *in vitro* testing showed that the evaporation rate of the smallest volume tested was at times significantly lower than larger volumes inside the evaporimeter (Chapter 5). These findings are supported by Mathers et al. [105], who reported lower TERs after modifying their evaporimeter to have a smaller volume over the eye.

In vitro testing also demonstrated that the evaporimeter was able to measure higher rates of evaporation as the surface area of the elliptical model eyes increased. This was an expected outcome due to the relationship between surface area and the rate of evaporation [53]. Pilot testing confirmed that the evaporimeter was also able to detect *in vivo* changes, with a smaller RH change occurring when looking down, due to the smaller ocular surface area (Chapter 6). This is consistent with results reported by Tsubota and Nakamori [151].

One important feature of the evaporimeter is that it allows the change in RH to be monitored multiple times per second over the entire duration of the measurement period. This overcomes one criticism of the VapoMeter in that the instrument only provides a single value of the evaporation rate and does not show how water loss occurs over time [60]. Additionally, being able to visualize the change in RH deals with another disadvantage of the VapoMeter and allows the operator to ensure the RH has returned to ambient levels prior to beginning the next measurement. This is of particular importance following a high rate of evaporation. Being able to monitor the RH in the device ensures that any water vapor that has built up in the goggle has dissipated before starting another measurement, rather than automatically resetting after a two-minute interval.

After proving that the instrument was able to measure *in vitro* changes in evaporation, further work was required to investigate potential clinical applications of the new device. Since dry eye disease is one of

the most common problems encountered in practice [121, 334], it was important to examine the use of the evaporimeter in the field of dry eye. Due to the multifactorial nature of dry eye [119], multiple tests can be used to diagnose dry eye [143]. *In vivo* testing of the instrument in Chapter 7 showed that the new evaporimeter measured higher TERs in people with dry eye disease compared to those without dry eye, which was consistent with the findings of most other researchers [3, 4, 8, 9, 74, 78, 88, 93, 101, 105, 109, 113, 118, 152, 153, 156, 158, 177]. Therefore, one potential clinical application of the novel evaporimeter is as a quick screening tool or way of diagnosing dry eye.

Investigation of the new evaporimeter also showed that it can be used to evaluate the effectiveness of an over-the-counter ocular lubricant to treat for dry eye. A significant decrease in TER occurred in the dry eye group between baseline and 30 minutes after the single instillation of a small volume of a lipid nano-emulsion. Uchiyama et al. [12] reported a similar change after a single drop of an artificial lubricant containing hydroxypropyl-guar was instilled in a group of participants with aqueous deficient dry eye. However, most research was unable to measure a significant change in TER between 10 to 60 minutes after the use of a dry eye treatment [12, 13, 82, 95, 98, 193]. A significant reduction in TER was also observed in the non-dry eye group between 10 and 30 minutes after instillation of the ocular lubricant. The higher TER measured at 10 minutes could be due to the destabilization of the lipid layer or may have occurred due to the additional volume of fluid in the eye following the addition of the eye drop.

Due to the high prevalence of CL discomfort [277, 278] and high levels of discontinuation [279-282], it was also imperative to test the evaporimeter with habitual CL wearers. Chapter 8 showed that the novel evaporimeter could measure higher TERs after 15 minutes and 6 hours of daily disposable CL wear, with TER increasing by 22 to 28% after 6 hours of wear. The higher TER was consistent with the findings of most previous researchers [16, 17, 21, 73, 102, 110, 323]. This may be related to the CL dividing the tear film into two and leading to an unstable tear film [287]. Although no significant difference in TER was detected between the two types of CLs, this may be due to the design of nesofilcon A to replicate the lipid layer of the tear film [454] and the water gradient technology of delefilcon A [236]. Similar results were reported by Thai et al. [17], who were also unable to find a difference between five types of hydrogel and silicone hydrogel CLs.

In summary, a new instrument was developed in response to the TFOS DEWS II suggestion that an evaporimeter was needed for clinical use that could be used in different temperatures and RHs [10].

The novel evaporimeter can quickly and simultaneously measure TER from both eyes. The work conducted in this thesis demonstrates that the new evaporimeter can be used to detect dry eye, to evaluate the efficacy of dry eye treatments, and is sensitive enough to detect changes in evaporation rate following CL wear. The initial evaluation of the evaporimeter in a small group of participants revealed findings consistent with the majority of previous evaporimetry work, while overcoming a couple of disadvantages associated with the only commercially available evaporimeter. Suggestions for future work and improvements to the instrument are discussed in Section 9.4.1.

9.2 Contact Lens Dehydration

One objective of this thesis was to investigate the ability of the Eye-VapoMeter to measure the *in vitro* evaporation rate from CLs. To the best of our knowledge, Chapter 2 includes the first description of an evaporimeter being used to perform this task. The Eye-VapoMeter made an excellent choice to investigate the new technique because the instrument is small, wireless, and able to automatically log the evaporation rate in a proprietary software program.

Testing of the new method showed that the instrument was able to successfully distinguish different rates of evaporation between a range of hydrogel and silicone hydrogel CLs. Significant correlations between the equilibrium water content and the rate at which water evaporated from a CL were observed. Identification of CLs that underwent a rapid loss of water from the surface of various lens materials that resulted in high rates of evaporative loss per minute could be helpful in identifying CLs that cause CL discomfort. Use of the Eye-VapoMeter to successfully measure *in vitro* evaporation rate of CLs expands its function beyond its marketed clinical application and also makes the instrument helpful for conducting laboratory investigations. Advantages of *in vitro* investigations include the ability to control the ambient environment within an environmental chamber, less cost in comparison to clinical trials, and the removal of intra- and inter-subject variability from the measurements.

9.3 Eye-VapoMeter

Another objective of this thesis was to repeat the *in vitro* validation tests originally conducted with the Eye-VapoMeter [21]. The resulting graphs of the correction factors and air volume within the evaporimeter were best fit with second order non-linear regressions rather than the strong negative linear relationship described by Rohit et al. [21].

Additional testing with the novel evaporimeter, which used the same swimming goggle as the one found on the marketed version of the Eye-VapoMeter, measured a range of participant air volumes inside the evaporimeter from 13 to 22 cm^3 (Chapters 7 and 8), which is more than double the volumes of 6 to 11 cm^3 [21] reported during the validation process. Calibration measurements made with the instrument used during the validation process may not be applicable to the currently available Eye-VapoMeter because of the differently sized swimming goggle. Over the years there appears to have been at least two other versions of the ocular VapoMeter [21, 330], which are different in appearance to the one marketed by Delfin Technologies Ltd. This suggests that the use of representative evaporimeter volumes for different races [161] based on the results of Rohit et al. [21] may not have accurately described the air space within the evaporimeter, since both the instrument and swimming goggle used may have changed between studies conducted within the same group.

However, despite the *in vitro* discrepancies reported between this thesis and the validation of the instrument [21], the smaller correction factors measured as the evaporimeter volume decreased do not represent typical air volumes encountered with the Eye-VapoMeter. Since significant correlations were also not obtained between the air volume and TER in Chapters 7 and 8 with the new evaporimeter, the effect of the air volume enclosed within the evaporimeter does not seem to be as significant as previously reported [21].

9.4 Future Work

9.4.1 Novel Evaporimeter

9.4.1.1 Methodology

In order to overcome some of the limitations of this thesis, additional work needs to be conducted to further refine the methodology. Although the majority of baseline measurements in Chapters 6, 7, and 8 were repeatable and the adaptation period was consistent between testing, the TER in the left eye of the asymptomatic CL group significantly decreased between the two baseline measurements. This was most likely due to lid eversion being included in the safety slit-lamp biomicroscopy check performed at the beginning of the study visit in Chapters 7 and 8. Further testing should avoid touching the eye prior to evaporimetry measurements or allow a longer adaptation period to ensure the tear film has stabilized before measuring the baseline TER.

The three second blink rate tested in Chapters 7 and 8 is similar to the blink interval of people without dry eye when engaged in conversation [455]. The blink rate increases to every 1.4 and 1.8 seconds in aqueous deficient dry eye and meibomian gland dysfunction, respectively [455]. Altering the methodology to test the evaporation rate with the eyes held open for as long as it feels comfortable might be able to improve the ability to distinguish differences between dry eye and non-dry eye, or between symptomatic and asymptomatic CL wearers.

In addition, further work should be done to determine whether three repeated open and closed eye measurements are necessary, or whether only one or two measurements would suffice. Visual inspection of the graphs obtained over the entire measurement period show similarities between the three sets of measurements, therefore the length of the test could be further reduced if fewer rounds of testing are needed.

9.4.1.2 Instrumentation and Software

Repeated testing of the device has found that there are fragile electrical connections located at the temporal edge of the left goggle that occasionally prevented the system from logging the temperature and RH. These weak connections are likely due to compression of the wires as they changed course over the top of the goggle and curved down towards the microcontroller. The connections needed to be reinforced in order to restore the instrument back to working order. Future iterations of the design could look at making the device wireless to ensure these problems are not encountered in the future.

An additional step that needs to be completed is conversion of the TER from units of %RH/s into units of x 10^{-7} g/cm^2/s. This task was not completed because the COVID-19 lockdown prevented access to the evaporimeter and spare pairs of swimming goggles.

Testing of the evaporimeter during the *in vivo* CL measurements provided further proof that ambient RH affects TER. In order to ensure that the instrument can be used in a range of temperatures and RHs, measurements should either be converted to an arbitrary temperature and RH [49], or correction factors should be created. Correction factors for different RHs could be obtained from testing in the environmental chamber, although lowering the temperature of the chamber below ambient conditions will be difficult since the chamber only has a heating capability.

The proof of concept of the novel evaporimeter has been demonstrated in this thesis. If it is determined that a table of correction factors should be used, consideration should be given to as to whether a new

evaporimeter should be created. One advantage of creating a new evaporimeter would be the assumption that the anti-fog coating would be intact, which would slow the rate of change of RH and probably produce more similar results to the commercially available Eye-VapoMeter. However, perhaps the anti-fog coating should automatically be removed from any future designs because it can wear off over time [406] and to maintain consistency with the work conducted in this thesis.

The software system used with the instrument should also be upgraded to make it more user-friendly. An extra timer should be added so that when an open or closed eye measurement begins, the researcher can easily determine that a set amount of time has elapsed and the evaporimeter can be removed from the eye. Ideally the system will also be able to automatically determine the TER between a predetermined interval and will also be able to calculate the TER as a value in units of x 10^{-7} g/cm^2/s. It would also be helpful to be able to have an automatic log of all of the measurements to refer to or be able to access previous measurements inside the software.

9.4.1.3 Future Studies

The binocular design of the instrument makes it an ideal choice for performing contralateral testing, either by using one eye as a control, or by testing different products in each eye to ensure the same conditions are encountered during the testing period. Larger sample sizes should be investigated because the studies conducted in Chapters 7 and 8 were underpowered.

Further dry eye testing could involve investigating the TER of the different subtypes, or comparisons of the different ocular lubricants or adjunct treatments.

Although significant differences were not detected between types of CLs, more work with lenses would be beneficial. Since the relatively short wearing time of 6 hours at the follow-up visit in Chapter 8 was substantially shorter than the participants regular wearing time of >11 hours, testing with longer wearing times or with habitual CLs could improve the ability to detect differences between symptomatic and asymptomatic wearers. Individual differences in TER were also observed with CL wear. Identification of CLs that result in high TERs may be a way for practitioners to quickly assess whether a lens might result in discomfort for an individual. Gravimetric and refractometry methods could also be used to investigate water loss during the course of CL wear, and these results could be compared to the TER.

In addition, since open-chamber devices rely on Fick's law [66] and the sensors must be placed parallel to the measurement surface, the ability of the new evaporimeter to measure the TER evaporation rate in different positions of gaze could be investigated. Since the instrument is not set in a fixed position, the >3 meters of cable attached to the evaporimeter allows for freedom of movement with the device. Varying the position of gaze during a measurement would give a more accurate representation of the TER when performing certain tasks, such as when using digital devices.

9.4.2 Contact Lens Dehydration

Further *in vivo* work should be done to build upon the confirmation that evaporimeters can detect *in vitro* differences in the evaporation rate of contemporary CLs. The technique was sensitive enough to detect differences between CLs made of the same material, therefore additional work should be done to investigate the effect of different wetting agents and surface treatments on the rate of evaporation.

9.4.3 Eye-VapoMeter

Since the Eye-VapoMeter is still available to purchase, additional work into the effect of volume on the resultant evaporation rate is warranted. Although the modelling clay was placed against the back surface of the lens of the swimming goggle to decrease the volume during the calibration measurements, this does not represent how the eye would naturally protrude into the goggle. In order to improve the technique, it would be worthwhile 3D printing plastic inserts that could reduce the air volume inside the goggle and be placed against the model eye. This would be a better representation of the normal position of the eye and would also give the reassurance that the plastic will not absorb any water vapor during the measurement. These inserts could be tested with both the Eye-VapoMeter and the novel evaporimeter.

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Appendix A

In Vitro Measurement of Contact Lens Evaporation Rate Using the Eye-VapoMeter

Table A1: Post-hoc Dunn's multiple comparisons test changes in evaporation rate per minute

	0 to 10 Minutes Adjusted p-value	**10 to 20 Minutes** Adjusted p-value	**20 to 30 Minutes** Adjusted p-value	**30 to 40 Minutes** Adjusted p-value
etafilcon A (2 week) vs. delefilcon A	0.3531	>0.9999	**0.0495**	>0.9999
etafilcon A (2 week) vs. narafilcon A	>0.9999	>0.9999	**0.0071**	>0.9999
lotrafilcon B vs. nesofilcon A	**0.0264**	0.2954	>0.9999	0.3302
lotrafilcon B vs. nelfilcon A	>0.9999	**0.0471**	0.522	0.7168
lotrafilcon B vs. etafilcon A (daily)	0.4495	**0.0382**	>0.9999	0.4029
lotrafilcon B vs. omafilcon B	0.4901	**0.0191**	**0.0166**	>0.9999
lotrafilcon B vs. hilafilcon B	>0.9999	**0.0037**	>0.9999	>0.9999
comfilcon A vs. nelfilcon A	>0.9999	**0.0202**	>0.9999	>0.9999
comfilcon A vs. etafilcon A (daily)	>0.9999	**0.0162**	>0.9999	>0.9999
comfilcon A vs. omafilcon A	>0.9999	**0.0403**	>0.9999	>0.9999
comfilcon A vs. omafilcon B	>0.9999	**0.0078**	0.6319	>0.9999
comfilcon A vs. hilafilcon B	>0.9999	**0.0014**	>0.9999	>0.9999
ocufilcon D vs. hilafilcon B	>0.9999	**0.011**	>0.9999	>0.9999
nesofilcon A vs. delefilcon A	**0.0020**	>0.9999	>0.9999	>0.9999
nesofilcon A vs. senofilcon A (1 day)	**0.0294**	>0.9999	>0.9999	**0.0092**
nesofilcon A vs. senofilcon A (2 week)	**0.0294**	>0.9999	>0.9999	**0.0301**
nesofilcon A vs. omafilcon B	>0.9999	>0.9999	**0.0224**	>0.9999
nesofilcon A vs. polymacon	**0.0459**	>0.9999	>0.9999	**0.0286**
nesofilcon A vs. narafilcon A	**0.0171**	>0.9999	>0.9999	**0.0055**
nelfilcon A vs. senofilcon A (1 day)	>0.9999	>0.9999	0.1472	**0.025**
nelfilcon A vs. narafilcon A	>0.9999	>0.9999	**0.0141**	**0.0153**
delefilcon A vs. omafilcon B	0.0593	0.3858	**0.0018**	>0.9999
etafilcon A (daily) vs. senofilcon A (1 day)	0.4901	>0.9999	>0.9999	**0.0119**
etafilcon A (daily) vs. senofilcon A (2 week)	0.4901	>0.9999	>0.9999	**0.0382**
etafilcon A (daily) vs. polymacon	0.7023	>0.9999	>0.9999	**0.0363**
etafilcon A (daily) vs. narafilcon A	0.3159	>0.9999	0.5001	0.0071
senofilcon A (1 day) vs. omafilcon B	0.534	>0.9999	**0.0033**	0.3772
senofilcon A (1 day) vs. hilafilcon B	>0.9999	0.594	0.4688	**0.0447**
senofilcon A (2 week) vs. omafilcon B	0.534	>0.9999	**0.004**	0.9355

	0 to 10 Minutes Adjusted p-value	**10 to 20 Minutes** Adjusted p-value	**20 to 30 Minutes** Adjusted p-value	**30 to 40 Minutes** Adjusted p-value
omafilcon B vs. narafilcon A	0.3454	>0.9999	**0.0002**	0.2519
hilafilcon B vs. narafilcon A	>0.9999	>0.9999	0.0548	**0.0278**

Bold indicates significant differences.

Table A2: Post-hoc Dunn's multiple comparisons test for duration of evaporation

	Adjusted p-value
ocufilcon D vs. senofilcon A (1 day)	**0.0082**
ocufilcon D vs. hilafilcon B	**0.0185**
ocufilcon D vs. narafilcon A	**0.0014**
polymacon vs. senofilcon A (1 day)	**0.0019**
polymacon vs. hilafilcon B	**0.0046**
polymacon vs. narafilcon A	**0.0003**
senofilcon A (1 day) vs. comfilcon A	**0.0381**
comfilcon A vs. narafilcon A	**0.0075**
delefilcon A vs. narafilcon A	**0.0129**
narafilcon A vs. omafilcon B	**0.0122**
ocufilcon D vs. senofilcon A (1 day)	**0.0082**

Bold indicates significant differences.

Appendix B

Grading Scales from Comparison of Tear Evaporation Rate with Systane® Complete in Dry Eye and Non-Dry Eye

Table B1: Summary of clinical tests and grading scales

Clinical tests	Grading scale
Meibomian quality of expression	0: clear fluid 1: cloudy fluid 2: cloudy particulate fluid 3: inspissated, like toothpaste 4: waxy, inexpressible
Meibomian glands yielding liquid secretion	0: >75% (almost all) 1: 50-75% (more than half) 2: 25-50% (less than half) 3: <25% (only a few) 4: ~0% (close to none)
Meibomian gland dropout (meiboscore)	0: no meibomian gland loss 1: area of loss <1/3 of the total meibomian gland area 2: area of loss between 1/3 and 2/3 of the total meibomian gland area 3: area of loss >2/3 of the total meibomian gland area
Vascularity of eyelid margin	0: none 1: minimal 2: mild 3: moderate 4: severe
Lash loss	0: none 1: minimal 2: mild 3: moderate 4: severe
Edema of lid margin	0: absent 1: present
Telangiectasia	0: none 1: single telangiectasia 2: 2-5 telangiectasia 3: >5 telangiectasia 4: severe – entire lid involvement
Tear film debris	0: absent 1: present

Clinical tests	Grading scale
Infiltrates	Diameter of largest infiltrate: 0: none 1: <0.5 mm 2: 0.5 – 1 mm 3: 1 – 1.5 mm 4: >1.5 mm Depth of largest infiltrate: 0: none 1: epithelial 2: sub-epithelial 3: mid-stromal 4: deep stromal
Hyperemia (bulbar and limbal)	0: normal 1: trace 2: mild 3: moderate 4: severe
Conjunctival staining	0: none 1: minimal diffuse punctate 2: coalescent punctate 3: confluent 4: deep confluent Total conjunctival staining score = Sum of the staining in all four quadrants
Corneal staining	Type: 0: no staining 1: trace, minimal superficial diffuse staining or stippling, or trace abrasion or foreign body tracks 2: mild, regional or diffuse punctate staining, or mild abrasion or foreign body tracks 3: moderate, significant dense coalesced staining, corneal abrasion or foreign body tracks 4: severe abrasions greater than 2mm diameter, ulcerations, epithelial loss, or full thickness abrasion Extent: 0: no staining 1: 1-15% of area 2: 16-30% of area 3: 31-45% of area 4: >45% of area Depth: 0: no staining 1: superficial epithelium 2: deep epithelium, delayed stromal glow 3: immediate localized stromal glow 4: immediate diffuse stromal glow, or full thickness abrasion Zone score = Type*Extent*Depth Total corneal staining score = Sum of the zone scores for each zone

Clinical tests	Grading scale
Palpebral hyperemia	0: none 1: trace, slight injection of conjunctival vessels 2: mild injection 3: moderate injection 4: severe injection
Palpebral roughness	0: uniform satin appearance of conjunctiva 1: trace, slight loss of smoothness 2: mild, or scattered papillae/follicles <1mm in diameter 3: moderate, significant papillae/follicles <1mm in diameter 4: severe, localized or generalized papillae/follicles 1mm or more in diameter

Appendix C
Calculation of the Time Interval to Measure the Tear Evaporation Rate

Statistical analyses were conducted using IBM® SPSS® Statistics v.26 (IBM Corporation, Armonk, New York, USA). Data was analyzed in 0.5 second increments from 0 to 20 seconds and tested for normality using a Shapiro-Wilk test.

OPEN EYE measurements:

Participants were analyzed individually. For each study time point, the 3 separate OPEN EYE RH values of the right eye were combined to determine the range of RH between 0 to 20 seconds after the evaporimeter was placed over the eyes. A Friedman test was used to investigate change in RH over time (Systane Complete: OD all $p < 0.001$, OS all $p < 0.001$; delefilcon A and nesofilcon A: OD all $p < 0.001$, OS: all $p < 0.385$).

Data underwent post-hoc testing using a Tukey Honestly Significant Difference test to identify homogenous subsets at the beginning of the measurement (initial subset) and end of the measurement (end subset) over which there was no significant change in RH. The final value of the initial subset (INITIAL VALUE) and the first value of the end subset (END VALUE) were recorded (Appendix D).

The same procedure was repeated for the left eye.

All of the INITIAL VALUES for each eye were combined to find the frequency of the 95^{th} percentile. The same procedure was followed using the END VALUES.

CLOSED EYE measurements:

The same procedure as described in the OPEN EYE measurements section was repeated using values taken while the eyes were closed. A Friedman test was used to investigate change in RH over time (Systane Complete: OD all $p < 0.001$, OS all $p < 0.001$; delefilcon A and nesofilcon A: OD all $p < 0.001$, OS: all $p < 0.001$).

The INITIAL VALUES and END VALUES for the two *in vivo* TER studies are shown in Table C1. Because the results of the two studies were similar, the data was combined and used to calculate the TER.

Table C1: 95th percentile initial values and end values

		OPEN EYE		CLOSED EYE	
Study	**n**	**Initial Value (s)**	**End Value (s)**	**Initial Value (s)**	**End Value (s)**
1	200	6.98	17.50	9.50	17.50
2	160	7.00	17.50	10.50	17.48
1 & 2	360	7.00	17.50	10.00	17.50

Study 1: Systane Complete; Study 2: delefilcon A and nesofilcon A

Appendix D

Initial Values and End Values

Table D1: Initial values and end values based on Tukey homogeneous subsets for the comparison of tear evaporation rate with Systane® Complete in dry eye and non-dry eye

			OPEN EYE		CLOSED EYE	
Participant	**Goggle**	**Time**	**Initial Value (s)**	**End Value (s)**	**Initial Value (s)**	**End Value (s)**
2	Right	0	4.50	17.50	9.50	10.00
2	Left	0	4.50	16.00	9.00	9.50
2	Right	0R	6.00	14.00	4.00	16.50
2	Left	0R	5.00	16.00	4.00	16.00
2	Right	10	3.00	17.50	5.00	15.50
2	Left	10	3.50	17.50	4.50	15.00
2	Right	30	3.50	15.50	5.50	16.00
2	Left	30	5.00	15.50	5.50	14.00
2	Right	60	3.50	17.00	5.50	14.50
2	Left	60	2.50	18.50	2.50	17.00
3	Right	0	5.50	16.00	5.00	13.00
3	Left	0	9.50	11.00	7.50	12.50
3	Right	0R	4.50	14.50	3.50	16.50
3	Left	0R	6.00	13.50	5.50	14.50
3	Right	10	3.50	16.00	9.00	10.00
3	Left	10	3.00	17.50	7.50	13.00
3	Right	30	4.00	15.50	4.00	15.50
3	Left	30	6.00	12.00	5.50	13.50
3	Right	60	4.00	15.00	11.50	5.00
3	Left	60	6.00	12.00	11.50	5.00
5	Right	0	4.50	15.50	4.50	16.00
5	Left	0	3.00	17.50	7.00	15.00
5	Right	0R	3.50	15.50	5.00	13.00
5	Left	0R	6.00	15.00	3.50	18.00
5	Right	10	5.50	13.00	4.00	16.00
5	Left	10	6.00	13.00	4.50	16.00
5	Right	30	7.50	11.00	8.00	9.00
5	Left	30	3.50	16.00	8.50	11.00

			OPEN EYE		CLOSED EYE	
Participant	**Goggle**	**Time**	**Initial Value (s)**	**End Value (s)**	**Initial Value (s)**	**End Value (s)**
5	Right	60	6.00	14.50	5.50	14.50
5	Left	60	4.50	17.00	5.50	13.50
8	Right	0	4.50	11.50	4.00	13.00
8	Left	0	6.00	11.00	3.50	15.00
8	Right	0R	6.00	9.50	4.50	13.00
8	Left	0R	6.50	11.00	4.50	11.00
8	Right	10	2.50	15.50	6.00	12.00
8	Left	10	3.00	14.50	9.00	10.50
8	Right	30	3.00	14.00	7.00	7.50
8	Left	30	6.50	12.50	7.00	11.50
8	Right	60	4.50	15.00	5.50	11.50
8	Left	60	3.00	16.00	4.50	12.50
9	Right	0	4.50	14.50	3.50	15.50
9	Left	0	3.50	16.00	5.00	14.50
9	Right	0R	6.00	12.50	4.50	12.50
9	Left	0R	3.00	17.00	3.00	17.00
9	Right	10	8.50	9.50	7.00	11.50
9	Left	10	7.00	11.50	6.50	12.50
9	Right	30	5.00	10.00	3.50	16.50
9	Left	30	4.00	11.00	4.50	14.00
9	Right	60	2.50	16.00	5.00	12.50
9	Left	60	5.50	12.00	2.00	18.00
11	Right	0	3.00	15.00	5.50	10.50
11	Left	0	3.50	17.00	3.50	15.00
11	Right	0R	3.00	15.50	10.50	7.50
11	Left	0R	3.50	15.00	4.50	11.00
11	Right	10	2.50	17.00	8.50	11.00
11	Left	10	4.50	13.50	9.00	10.50
11	Right	30	5.50	11.50	2.50	16.00
11	Left	30	5.00	11.50	7.00	11.50
11	Right	60	2.00	15.50	4.00	16.00
11	Left	60	2.50	16.50	4.50	12.50
12	Right	0	3.50	14.50	3.00	15.00
12	Left	0	4.50	14.50	7.00	9.50

			OPEN EYE		CLOSED EYE	
Participant	**Goggle**	**Time**	**Initial Value (s)**	**End Value (s)**	**Initial Value (s)**	**End Value (s)**
12	Right	0R	2.00	16.50	2.00	16.00
12	Left	0R	3.00	16.00	2.50	16.50
12	Right	10	2.50	15.00	5.00	10.50
12	Left	10	2.50	16.50	4.50	12.50
12	Right	30	3.00	14.50	1.50	16.50
12	Left	30	4.00	14.00	4.50	12.50
12	Right	60	1.50	17.00	2.00	15.50
12	Left	60	1.00	18.50	2.00	17.00
18	Right	0	4.00	13.50	11.00	8.00
18	Left	0	4.00	15.00	7.50	12.00
18	Right	0R	3.00	14.50	8.50	11.00
18	Left	0R	4.00	13.00	6.00	12.50
18	Right	10	2.50	15.50	6.00	10.50
18	Left	10	3.50	15.00	5.00	11.50
18	Right	30	2.50	13.50	3.00	15.50
18	Left	30	3.00	15.00	2.50	17.50
18	Right	60	4.50	13.00	4.00	17.00
18	Left	60	2.50	16.00	3.00	15.50
19	Right	0	3.50	15.50	4.50	12.00
19	Left	0	2.50	17.00	3.50	16.00
19	Right	0R	6.00	12.50	5.50	12.50
19	Left	0R	6.50	12.00	3.50	15.50
19	Right	10	4.00	15.00	4.00	12.50
19	Left	10	4.00	15.00	3.00	13.50
19	Right	30	1.50	18.50	3.50	15.50
19	Left	30	4.00	14.50	2.00	17.00
19	Right	60	2.00	17.50	3.00	15.00
19	Left	60	3.00	16.00	5.00	11.50
20	Right	0	2.50	15.00	1.50	18.50
20	Left	0	4.00	13.00	3.00	13.50
20	Right	0R	3.50	13.50	2.50	14.00
20	Left	0R	3.00	14.00	1.50	17.50
20	Right	10	2.50	16.00	5.00	9.50
20	Left	10	3.00	15.50	4.00	14.00

			OPEN EYE		CLOSED EYE	
Participant	**Goggle**	**Time**	**Initial Value (s)**	**End Value (s)**	**Initial Value (s)**	**End Value (s)**
20	Right	30	3.00	15.00	4.00	12.00
20	Left	30	2.00	17.50	4.00	12.00
20	Right	60	2.00	17.00	2.50	13.50
20	Left	60	1.50	18.00	2.50	13.50
23	Right	0	5.50	13.50	2.00	15.50
23	Left	0	4.00	16.00	3.00	13.50
23	Right	0R	2.00	17.50	2.50	15.00
23	Left	0R	3.00	16.50	3.00	15.00
23	Right	10	2.50	15.00	5.00	11.50
23	Left	10	4.00	13.00	3.00	15.00
23	Right	30	4.50	12.50	4.50	11.50
23	Left	30	5.00	13.00	4.50	10.00
23	Right	60	5.50	11.00	3.50	11.00
23	Left	60	5.00	12.50	3.50	11.00
24	Right	0	4.00	14.00	6.50	10.00
24	Left	0	5.00	13.00	7.50	11.00
24	Right	0R	4.00	15.50	3.50	18.00
24	Left	0R	4.50	14.00	5.00	17.00
24	Right	10	2.50	15.50	4.50	15.00
24	Left	10	3.00	16.50	5.50	13.00
24	Right	30	3.00	15.50	4.00	16.50
24	Left	30	2.00	18.00	3.50	16.50
24	Right	60	4.00	10.50	3.50	17.00
24	Left	60	3.00	15.00	5.00	15.50
25	Right	0	6.00	12.00	15.00	3.50
25	Left	0	5.00	15.00	13.00	4.50
25	Right	0R	7.50	9.00	5.50	11.50
25	Left	0R	5.50	12.50	3.00	16.50
25	Right	10	2.50	15.50	3.50	14.00
25	Left	10	3.50	15.00	3.50	16.00
25	Right	30	4.50	14.50	2.50	16.50
25	Left	30	5.50	13.00	7.00	11.50
25	Right	60	3.00	15.50	2.50	16.50
25	Left	60	4.00	14.50	3.50	15.50

			OPEN EYE		CLOSED EYE	
Participant	**Goggle**	**Time**	**Initial Value (s)**	**End Value (s)**	**Initial Value (s)**	**End Value (s)**
26	Right	0	6.50	14.00	7.50	12.00
26	Left	0	7.00	13.00	3.50	17.00
26	Right	0R	11.00	6.00	5.50	13.50
26	Left	0R	8.00	10.50	5.50	14.50
26	Right	10	9.50	8.50	8.50	8.00
26	Left	10	7.00	13.00	6.00	11.00
26	Right	30	4.00	13.00	11.50	8.00
26	Left	30	5.00	14.50	8.50	13.50
26	Right	60	3.50	17.00	4.00	16.50
26	Left	60	5.00	13.00	10.50	8.50
28	Right	0	4.50	13.00	5.50	11.00
28	Left	0	3.00	15.50	4.00	13.00
28	Right	0R	3.50	16.00	4.00	14.00
28	Left	0R	2.50	18.00	3.00	15.50
28	Right	10	4.50	13.00	7.00	10.50
28	Left	10	3.50	14.50	5.50	11.00
28	Right	30	2.50	14.50	2.00	16.50
28	Left	30	3.50	13.00	2.00	16.50
28	Right	60	2.00	13.00	.50	18.50
28	Left	60	3.00	13.50	2.50	16.50
30	Right	0	5.50	15.00	3.50	14.00
30	Left	0	4.00	16.00	4.50	13.00
30	Right	0R	2.50	18.00	2.50	15.00
30	Left	0R	2.50	17.00	5.00	12.00
30	Right	10	3.00	15.50	3.50	14.50
30	Left	10	4.00	14.50	3.00	15.00
30	Right	30	5.00	13.00	4.50	13.50
30	Left	30	5.50	12.00	2.00	17.50
30	Right	60	6.50	10.50	6.00	12.00
30	Left	60	4.00	12.00	4.50	13.00
31	Right	0	4.50	11.50	7.00	8.00
31	Left	0	3.50	13.50	3.00	13.00
31	Right	0R	2.50	14.00	2.00	18.50
31	Left	0R	3.50	14.50	3.00	16.50

			OPEN EYE		CLOSED EYE	
Participant	**Goggle**	**Time**	**Initial Value (s)**	**End Value (s)**	**Initial Value (s)**	**End Value (s)**
31	Right	10	2.00	12.50	5.00	11.00
31	Left	10	3.50	11.50	5.00	8.00
31	Right	30	2.00	12.00	4.00	14.50
31	Left	30	2.50	12.00	4.00	12.50
31	Right	60	2.50	12.00	4.50	13.00
31	Left	60	3.00	12.00	3.50	13.50
34	Right	0	2.50	15.50	4.00	14.00
34	Left	0	3.50	15.00	5.50	12.00
34	Right	0R	2.00	17.50	3.50	15.50
34	Left	0R	4.00	16.00	3.00	17.00
34	Right	10	2.50	14.00	2.00	16.00
34	Left	10	3.50	13.50	2.50	15.50
34	Right	30	2.50	13.00	2.00	15.50
34	Left	30	2.00	15.00	3.50	13.50
34	Right	60	3.00	13.50	3.00	16.50
34	Left	60	3.00	15.00	3.00	17.00
35	Right	0	5.50	11.50	2.50	16.00
35	Left	0	2.50	14.50	1.50	17.00
35	Right	0R	2.50	17.00	2.00	17.00
35	Left	0R	2.50	15.00	4.00	13.00
35	Right	10	2.00	16.50	7.50	11.00
35	Left	10	3.50	14.00	6.00	11.00
35	Right	30	1.50	16.50	9.50	5.00
35	Left	30	2.50	15.50	5.50	7.00
35	Right	60	1.50	16.50	3.50	11.50
35	Left	60	1.50	17.00	4.00	12.00
36	Right	0	4.50	17.50	2.50	19.00
36	Left	0	4.50	16.00	3.50	16.00
36	Right	0R	6.00	14.00	9.50	9.00
36	Left	0R	5.00	16.00	6.00	12.00
36	Right	10	3.00	17.50	3.00	18.00
36	Left	10	3.50	17.50	3.00	17.50

			OPEN EYE		CLOSED EYE	
Participant	**Goggle**	**Time**	**Initial Value (s)**	**End Value (s)**	**Initial Value (s)**	**End Value (s)**
36	Right	30	3.50	15.50	5.00	15.00
36	Left	30	5.00	15.50	4.00	15.50
36	Right	60	3.50	17.00	4.00	16.50
36	Left	60	2.50	18.50	2.50	18.00

Time 0: Baseline 1; Time 0R: Baseline 2; Time 10: 10 minutes post-drop; Time 30: 30 minutes post-drop; Time 60: 60 minutes post-drop

Table D2: Initial values and end values based on Tukey homogeneous subsets for the comparison of tear evaporation rate with delefilcon A and nesofilcon A

			OPEN EYE		CLOSED EYE	
Participant	**Goggle**	**Time**	**Initial Value (s)**	**End Value (s)**	**Initial Value (s)**	**End Value (s)**
1	Right	0	7.00	12.00	7.00	13.00
1	Left	0	6.00	12.50	7.00	11.50
1	Right	0R	2.50	16.50	7.50	13.50
1	Left	0R	4.00	12.00	11.00	12.00
1	Right	15	3.00	14.50	6.00	13.50
1	Left	15	3.00	14.50	7.00	11.50
1	Right	360	3.50	15.00	7.00	13.50
1	Left	360	4.50	13.50	7.00	13.00
2	Right	0	4.00	16.50	6.50	15.00
2	Left	0	3.00	18.00	4.50	17.00
2	Right	0R	4.50	16.50	7.50	12.50
2	Left	0R	3.50	17.50	7.00	12.00
2	Right	15	4.50	15.50	7.00	14.00
2	Left	15	5.50	14.00	7.50	13.00
2	Right	360	7.00	12.50	8.00	11.00
2	Left	360	4.00	15.50	10.50	9.00
3	Right	0	6.00	12.50	4.50	13.50
3	Left	0	5.00	13.50	4.50	14.50
3	Right	0R	5.00	15.00	2.50	15.00
3	Left	0R	3.00	17.00	4.00	14.00

			OPEN EYE		CLOSED EYE	
Participant	**Goggle**	**Time**	**Initial Value (s)**	**End Value (s)**	**Initial Value (s)**	**End Value (s)**
3	Right	15	3.50	15.50	6.00	14.00
3	Left	15	3.00	17.00	2.50	18.00
3	Right	360	3.00	15.00	4.50	15.00
3	Left	360	2.50	16.00	5.00	14.50
4	Right	0	5.50	13.00	8.00	11.00
4	Left	0	7.00	12.00	9.00	8.00
4	Right	0R	5.50	14.00	6.00	12.00
4	Left	0R	5.00	14.50	5.00	13.50
4	Right	15	2.50	16.00	3.50	14.50
4	Left	15	4.00	15.50	5.00	12.50
4	Right	360	5.00	13.50	2.50	16.50
4	Left	360	4.50	13.50	6.50	11.00
5	Right	0	2.50	17.50	9.00	9.00
5	Left	0	4.00	16.00	10.00	6.50
5	Right	0R	4.50	14.00	4.50	16.00
5	Left	0R	2.50	16.50	5.00	16.00
5	Right	15	4.00	16.00	5.50	15.00
5	Left	15	4.50	15.50	5.50	15.50
5	Right	360	4.50	14.00	3.50	15.00
5	Left	360	4.50	15.00	3.50	16.00
6	Right	0	6.50	12.50	8.50	9.50
6	Left	0	6.00	13.50	5.00	15.50
6	Right	0R	8.00	12.00	5.50	13.00
6	Left	0R	2.50	17.50	3.50	14.50
6	Right	15	4.50	15.00	7.00	12.00
6	Left	15	3.50	16.00	5.00	12.50
6	Right	360	3.50	15.50	5.00	15.00
6	Left	360	5.50	13.00	3.50	16.00
7	Right	0	4.50	16.00	10.00	8.00
7	Left	0	5.00	15.50	6.50	12.50
7	Right	0R	8.00	12.00	14.50	4.00
7	Left	0R	8.50	12.00	15.50	3.50
7	Right	15	3.50	17.50	3.50	14.50
7	Left	15	3.50	17.50	4.00	16.00

			OPEN EYE		CLOSED EYE	
Participant	**Goggle**	**Time**	**Initial Value (s)**	**End Value (s)**	**Initial Value (s)**	**End Value (s)**
7	Right	360	2.50	17.50	6.50	12.00
7	Left	360	3.00	17.50	3.50	16.50
8	Right	0	3.00	11.50	5.50	11.50
8	Left	0	3.00	10.50	4.50	13.00
8	Right	0R	2.50	11.50	5.50	12.00
8	Left	0R	2.00	13.00	4.50	14.50
8	Right	15	1.50	12.50	4.00	14.50
8	Left	15	1.50	13.50	3.50	15.50
8	Right	360	2.50	10.50	5.50	9.00
8	Left	360	2.00	9.50	3.50	13.50
9	Right	0	4.50	14.00	7.50	9.50
9	Left	0	7.50	10.50	9.50	9.00
9	Right	0R	4.50	15.00	3.50	13.50
9	Left	0R	5.00	14.00	7.00	9.50
9	Right	15	3.00	16.50	1.50	16.50
9	Left	15	4.00	15.00	6.50	8.50
9	Right	360	4.00	15.00	12.50	6.00
9	Left	360	3.50	15.50	9.00	8.50
10	Right	0	4.50	12.00	7.00	8.00
10	Left	0	5.00	11.50	5.50	10.50
10	Right	0R	4.00	10.00	3.00	16.00
10	Left	0R	2.50	14.50	3.50	15.50
10	Right	15	2.50	12.50	2.50	13.00
10	Left	15	4.00	14.00	2.50	15.50
10	Right	360	2.50	11.50	3.00	11.00
10	Left	360	2.50	16.50	3.50	12.50
11	Right	0	6.50	13.00	19.50	1.00
11	Left	0	6.50	13.50	8.50	12.00
11	Right	0R	6.00	16.00	12.00	8.50
11	Left	0R	7.00	13.00	8.00	12.50
11	Right	15	7.00	13.50	10.50	9.00
11	Left	15	6.50	12.50	11.50	7.50
11	Right	360	8.50	12.50	5.00	16.00
11	Left	360	6.50	15.00	4.50	16.00

			OPEN EYE		CLOSED EYE	
Participant	**Goggle**	**Time**	**Initial Value (s)**	**End Value (s)**	**Initial Value (s)**	**End Value (s)**
12	Right	0	2.00	16.00	5.50	10.50
12	Left	0	3.00	15.50	6.50	10.50
12	Right	0R	1.00	19.00	3.00	15.50
12	Left	0R	3.00	14.50	5.50	12.00
12	Right	15	2.00	16.50	3.00	14.50
12	Left	15	2.00	17.50	1.50	18.50
12	Right	360	4.50	12.50	2.50	15.50
12	Left	360	3.00	14.00	3.50	14.00
13	Right	0	3.00	13.50	3.00	14.00
13	Left	0	3.00	15.00	2.00	17.00
13	Right	0R	2.00	15.00	4.00	13.00
13	Left	0R	2.00	15.00	3.00	16.00
13	Right	15	2.50	15.00	3.50	14.50
13	Left	15	2.50	16.00	3.00	17.00
13	Right	360	2.00	15.00	5.00	13.00
13	Left	360	2.50	15.00	4.00	13.50
14	Right	0	6.00	13.00	9.00	11.50
14	Left	0	6.50	13.50	7.00	13.50
14	Right	0R	6.00	14.00	9.00	13.00
14	Left	0R	5.50	15.00	6.00	15.00
14	Right	15	3.50	17.00	8.00	9.50
14	Left	15	4.00	17.00	5.00	15.50
14	Right	360	4.50	14.50	6.50	10.50
14	Left	360	3.00	16.50	5.50	13.50
15	Right	0	5.50	15.50	5.50	15.00
15	Left	0	4.50	17.50	4.50	16.00
15	Right	0R	5.00	15.50	4.50	16.50
15	Left	0R	5.00	15.00	2.00	18.00
15	Right	15	3.50	14.50	4.50	15.50
15	Left	15	4.00	13.50	4.00	15.50
15	Right	360	3.00	17.50	6.00	14.00
15	Left	360	3.00	17.00	2.50	17.50

			OPEN EYE		CLOSED EYE	
Participant	**Goggle**	**Time**	**Initial Value (s)**	**End Value (s)**	**Initial Value (s)**	**End Value (s)**
16	Right	0	3.00	12.00	5.50	12.00
16	Left	0	3.00	14.00	4.00	14.00
16	Right	0R	5.00	10.50	2.50	16.50
16	Left	0R	3.50	13.50	4.00	15.00
16	Right	15	2.50	11.00	2.50	16.00
16	Left	15	4.00	11.00	4.00	14.50
16	Right	360	1.00	16.00	4.00	12.50
16	Left	360	1.50	17.00	4.50	12.00
17	Right	0	5.00	11.00	6.00	12.00
17	Left	0	3.00	13.50	3.00	17.50
17	Right	0R	3.00	15.00	2.50	18.00
17	Left	0R	2.00	17.50	3.50	16.00
17	Right	15	3.00	11.50	4.00	16.00
17	Left	15	3.00	13.00	5.00	14.00
17	Right	360	2.00	17.00	3.50	15.00
17	Left	360	3.00	14.00	2.50	16.00
18	Right	0	3.50	15.50	6.50	14.00
18	Left	0	2.50	18.00	4.50	16.00
18	Right	0R	4.50	15.50	3.00	16.00
18	Left	0R	3.00	17.50	3.00	17.00
18	Right	15	3.00	15.50	5.50	11.50
18	Left	15	3.50	16.00	3.00	15.00
18	Right	360	4.00	13.50	8.50	9.00
18	Left	360	6.00	12.00	8.00	9.50
19	Right	0	9.50	9.00	7.00	10.00
19	Left	0	6.00	12.50	2.00	15.00
19	Right	0R	6.50	13.50	3.00	16.00
19	Left	0R	3.00	17.00	2.00	16.50
19	Right	15	6.50	12.00	3.00	15.00
19	Left	15	5.50	13.50	5.00	13.50
19	Right	360	4.50	15.00	5.00	13.00
19	Left	360	4.00	14.50	5.00	11.00
20	Right	0	3.00	16.00	3.50	16.50
20	Left	0	2.50	17.50	6.00	13.50

			OPEN EYE		CLOSED EYE	
Participant	**Goggle**	**Time**	**Initial Value (s)**	**End Value (s)**	**Initial Value (s)**	**End Value (s)**
20	Right	0R	3.00	14.00	2.00	18.50
20	Left	0R	2.50	16.50	2.50	18.00
20	Right	15	2.00	16.00	2.00	17.00
20	Left	15	2.00	17.50	2.50	17.00
20	Right	360	3.50	14.50	8.00	8.50
20	Left	360	4.50	15.00	6.00	12.50

Time 0: Baseline 1; Time 0R: Baseline 2; Time 15: 15 minutes of contact lens wear; Time 360: 360 minutes of contact lens wear

Appendix E

Analysis of Comparison of Tear Evaporation Rate with Systane® Complete in Dry Eye and Non-Dry Eye (All Time Points)

There was no statistically significant three-way interaction between time, eye, or group (p=0.682) or two-way interaction between time and group (p=0.066, Figure E1). However, there was statistically a significant two-way interaction between time and eye (p=0.003). Although the simple two-way interaction between time and eye for non-dry eye participants was not statistically significant (p=0.106), the interaction was statistically significant for dry eye participants (p=0.015). There were no statistically significant simple main effects of time on the right eye (p=0.083) or the left eye (p=0.418) of dry eye participants.

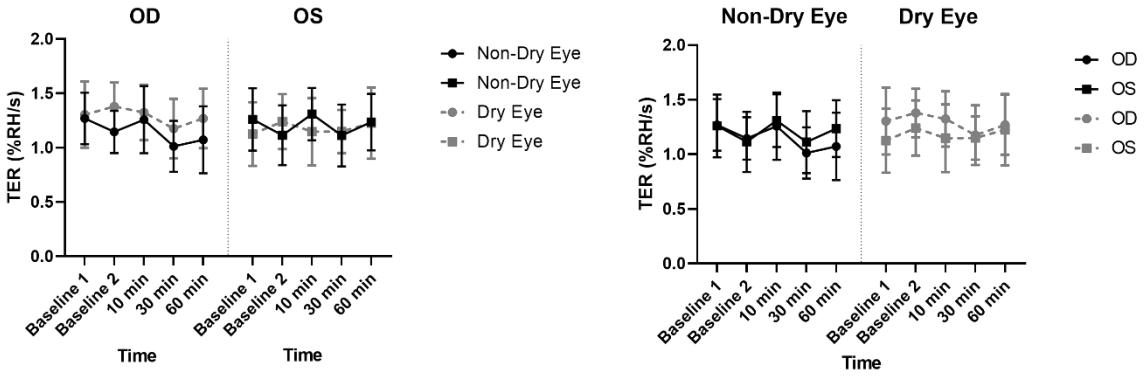

Figure E1: Change in tear evaporation rate for the right and left eye of each group. Error bars indicate standard deviation. There was no significant interaction between time and group (p=0.066). However, there was a significant interaction between time and eye (p=0.003) for the dry eye group (p=0.015), but not the non-dry eye group (p=0.106).

Table E1: Summary of two-way and three-way interactions between time, eye, and group

Interactions		p-value
time*group*eye		0.682
time*group		0.066
time*eye		**0.003**
	non-dry eye	0.106
	dry eye	**0.015**

Bold indicates significance.

Table E2: Summary of tear evaporation rate over time

	Tear Evaporation Rate (%RH/s)			
	Non-Dry Eye		Dry Eye	
	Right Eye (n = 10)	**Left Eye (n = 0)**	**Right Eye (n = 10)**	**Left Eye (n = 10)**
Baseline 1	1.27 ± 0.24	1.26 ± 0.29	1.30 ± 0.30	1.13 ± 0.29
Baseline 2	1.15 ± 0.19	1.11 ± 0.27	1.38 ± 0.22	1.24 ± 0.25
10 minutes post-drop	1.26 ± 0.31	1.31 ± 0.24	1.32 ± 0.25	1.15 ± 0.31
30 minutes post-drop	1.01 ± 0.24	1.11 ± 0.28	1.18 ± 0.27	1.15 ± 0.20
60 minutes post-drop	1.07 ± 0.31	1.24 ± 0.26	1.27 ± 0.27	1.23 ± 0.33
p-value	**0.005**	0.053	0.083	0.418

Bold indicates significant differences.

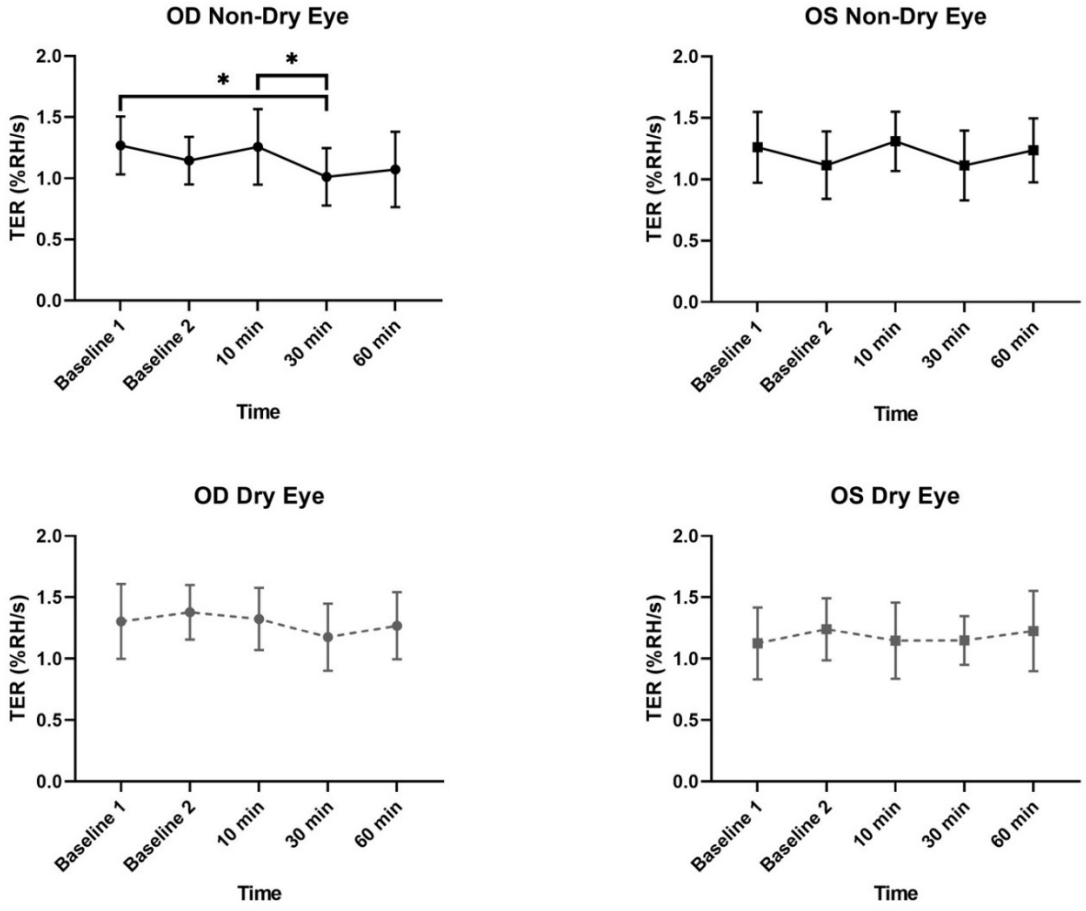

Figure E2: Average change in tear evaporation rate over time for each eye of the non-dry eye and dry eye group. Error bars indicate standard deviation (SD). There was a significant change in the tear evaporation rate over time for the right eye of the non-dry eye group (p=0.005). Post-hoc Tukey's multiple comparisons test found a lower tear evaporation rate at 30 minutes compared to the first baseline measurement (p=0.013) and 10 minutes post-drop (p=0.020). No other significant changes over time were observed (left eye non-dry eye: p=0.053; right eye dry eye: p=0.083; left eye dry eye: p=0.418).

Table E3: Summary of subjective comfort and clinical measurements for the right eye

	Non-Dry Eye Group (n = 10 eyes)	Dry Eye Group (n = 10 eyes)	p-value
Comfort Rating			
Baseline 1	100.00 (11)	72.50 (35)	**0.001**
Baseline 2	100.00 (6)	70.00 (28)	**<0.001**
10 minutes post-drop	100.00 (6)	72.50 (23)	**<0.001**
30 minutes post-drop	100.00 (11)	67.50 (29)	**0.001**
60 minutes post-drop	100.00 (11)	73.00 (13)	**<0.001**
p-value	0.686	0.638	
Dryness Rating			
Baseline 1	100.00 (4)	67.50 (33)	**<0.001**
Baseline 2	100.00 (3)	57.50 (24)	**<0.001**
10 minutes post-drop	100.00 (6)	70.00 (26)	**0.001**
30 minutes post-drop	100.00 (5)	63.00 (30)	**0.001**
60 minutes post-drop	100.00 (5)	60.00 (24)	**<0.001**
p-value	0.372	0.493	
Stinging/Burning Rating			
Baseline 1	100.00 (0)	90.00 (83)	**0.036**
Baseline 2	100.00 (1)	90.00 (56)	0.076
10 minutes post-drop	100.00 (0)	94.50 (83)	**0.019**
30 minutes post-drop	100.00 (0)	97.50 (90)	0.056
60 minutes post-drop	100.00 (0)	100.00 (81)	0.091
p-value	0.558	0.865	
Tear Meniscus Height			
Baseline 1	0.27 (0.08)	0.29 (0.15)	0.344
Baseline 2	0.29 (0.12)	0.28 (0.12)	0.791
10 minutes post-drop	0.26 (0.08)	0.28 (0.13)	0.545
30 minutes post-drop	0.33 (0.12)	0.25 (0.11)	0.130
60 minutes post-drop	0.28 (0.12)	0.22 (0.19)	0.405
p-value	**0.005**	0.115	
Tear Evaporation Rate			
Baseline 1	1.27 ± 0.24	1.30 ± 0.31	0.783
Baseline 2	1.14 ± 0.19	1.38 ± 0.22	**0.022**
10 minutes post-drop	1.26 ± 0.31	1.32 ± 0.25	0.595
30 minutes post-drop	1.01 ± 0.24	1.18 ± 0.27	0.171
60 minutes post-drop	1.07 ± 0.31	1.27 ± 0.27	0.150
p-value	**0.005**	0.088	
Non-Invasive Keratograph Break-up Time			
Baseline 1	12.17 (17.36)	4.31 (2.15)	**0.001**
Baseline 2	12.04 (16.37)	4.05 (3.76)	**0.003**
10 minutes post-drop	10.29 (10.60)	3.63 (3.16)	**0.005**

	Non-Dry Eye Group (n = 10 eyes)	Dry Eye Group (n = 10 eyes)	p-value
Non-Invasive Keratograph Break-up Time			
30 minutes post-drop	14.21 (13.81)	3.63 (1.81)	**0.001**
60 minutes post-drop	7.91 (19.85)	3.92 (3.92)	**0.012**
p-value	0.400	0.855	
Objective Lipid Layer Thickness (mean)			
Baseline 1	65.70 ± 16.17	69.40 ± 21.61	0.670
Baseline 2	61.10 ± 13.56	69.40 ± 18.08	0.261
10 minutes post-drop	68.70 ± 20.21	75.00 ± 18.32	0.475
30 minutes post-drop	63.30 ± 17.84	73.00 ± 20.17	0.270
60 minutes post-drop	67.00 ± 17.83	70.20 ± 16.52	0.682
p-value	0.619	0.389	
Objective Lipid Layer Thickness (maximum)			
Baseline 1	97.50 (33)	84.00 (30)	0.938
Baseline 2	81.50 (36)	80.00 (27)	0.757
10 minutes post-drop	84.50 (39)	93.50 (21)	0.506
30 minutes post-drop	89.50 (33)	85.00 (28)	0.646
60 minutes post-drop	87.50 (30)	81.50 (27)	0.820
p-value	0.663	0.052	
Objective Lipid Layer Thickness (minimum)			
Baseline	61.50 (24)	59.00 (27)	0.910
Baseline 2	53.50 (15)	56.50 (14)	0.383
10 minutes post-drop	57.00 (34)	59.50 (13)	0.405
30 minutes post-drop	52.50 (32)	57.50 (14)	0.344
60 minutes post-drop	55.50 (29)	60.50 (14)	0.520
p-value	0.275	0.911	

Bold indicates significant differences.

Post-hoc testing with a Dunn's multiple comparisons test found the non-dry eye group had a higher tear meniscus height 30 minutes after the drop was instilled compared to the first baseline measurement (p=0.003).

Appendix F

Grading Scales from Comparison of Tear Evaporation Rate with Delefilcon A and Nesofilcon A

Table F1: Summary of clinical tests and grading scales

Clinical tests	Grading scale
Meibomian quality	0: no secretion (including capped orifices) 1: inspissated (semi-solid, toothpaste like) 2: colored/cloudy liquid 3: clear, liquid oil
Vascularity of eyelid margin	0: none 1: minimal 2: mild 3: moderate 4: severe
Lash loss	0: none 1: minimal 2: mild 3: moderate 4: severe
Edema of lid margin	0: absent 1: present
Telangiectasia	0: none 1: single telangiectasia 2: 2-5 telangiectasia 3: >5 telangiectasia 4: severe – entire lid involvement
Tear film debris	0: absent 1: present
Infiltrates	Diameter of largest infiltrate: 0: none 1: <0.5 mm 2: 0.5 – 1 mm 3: 1 – 1.5 mm 4: >1.5 mm Depth of largest infiltrate: 0: none 1: epithelial 2: sub-epithelial 3: mid-stromal 4: deep stromal
Hyperemia (bulbar and limbal)	0: normal 1: trace 2: mild 3: moderate 4: severe

Clinical tests	Grading scale
Conjunctival staining	0: none 1: minimal diffuse punctate 2: coalescent punctate 3: confluent 4: deep confluent
Conjunctival indentation	0: none 1: very slight 2: slight 3: moderate 4: severe
Corneal staining	Type: 0: no staining 1: trace, minimal superficial diffuse staining or stippling, or trace abrasion or foreign body tracks 2: mild, regional or diffuse punctate staining, or mild abrasion or foreign body tracks 3: moderate, significant dense coalesced staining, corneal abrasion or foreign body tracks 4: severe abrasions greater than 2mm diameter, ulcerations, epithelial loss, or full thickness abrasion Extent: 0: no staining 1: 1-15% of area 2: 16-30% of area 3: 31-45% of area 4: >45% of area Depth: 0: no staining 1: superficial epithelium 2: deep epithelium, delayed stromal glow 3: immediate localized stromal glow 4: immediate diffuse stromal glow, or full thickness abrasion
Palpebral hyperemia	0: none 1: trace, slight injection of conjunctival vessels 2: mild injection 3: moderate injection 4: severe injection
Palpebral roughness	0: uniform satin appearance of conjunctiva 1: trace, slight loss of smoothness 2: mild, or scattered papillae/follicles <1mm in diameter 3: moderate, significant papillae/follicles <1mm in diameter 4: severe, localized or generalized papillae/follicles 1mm or more in diameter

Appendix G

Analysis of Comparison of Tear Evaporation Rate with Delefilcon A and Nesofilcon A (All Time Points)

The change in TER over time was not statistically significantly for the right side of the evaporimeter (p=0.090). The left side of the evaporimeter had a significant change in TER over time (p=0.002, Figure G1). Post-hoc testing with a Tukey's multiple comparisons test found a significant change in TER between first baseline measurement and the second baseline measurement (p=0.019), between the second baseline measurement and 15 minutes after contact lenses were inserted (p=0.005), and between the second baseline measurement and after 6 hours of contact lens wear (p=0.008).

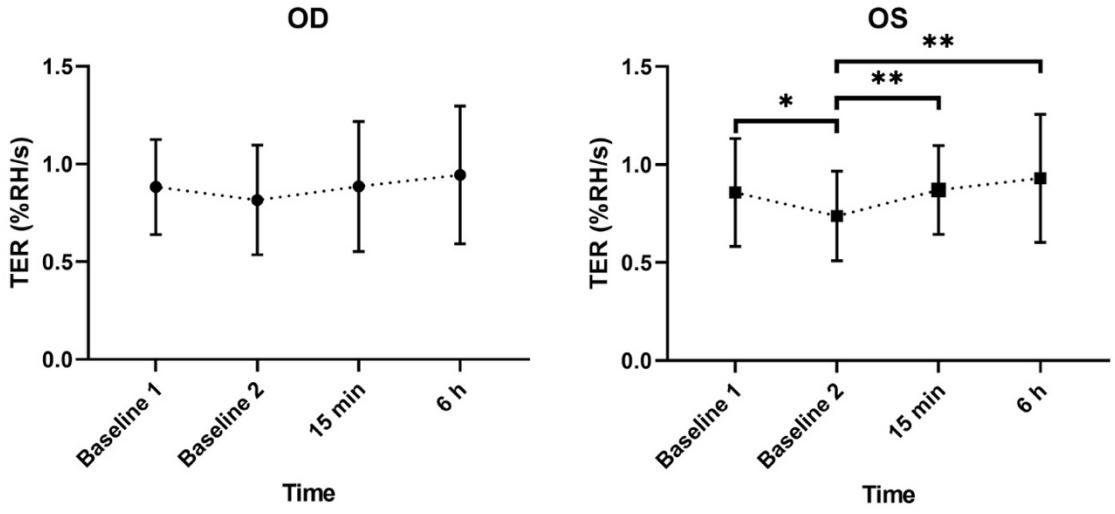

Figure G1: Average change in tear evaporation rate over time for each eye of all participants. Error bars indicate standard deviation. The change in tear evaporation rate over time was significant for the left eye (p=0.002), but not the right eye (p=0.090).

The change in TER over time was not statistically significant for either eye of the asymptomatic group (right eye: p=0.339; left eye: p=0.118). The change in TER over time was significantly different for both eyes of the symptomatic group (right eye: p=0.026; left eye: p=0.028, Figure G2). Post-hoc testing with an uncorrected Dunn's test and Bonferroni correction found the TER in the right eye of the symptomatic group was significantly lower at 15 minutes compared to 6 hours of wearing time

(p=0.009). Post-hoc testing with a Dunn's multiple comparisons test detected that the TER in the left eye of the symptomatic group was significantly higher 6 hours after the contact lenses were instilled compared to the second baseline measurement (p=0.034).

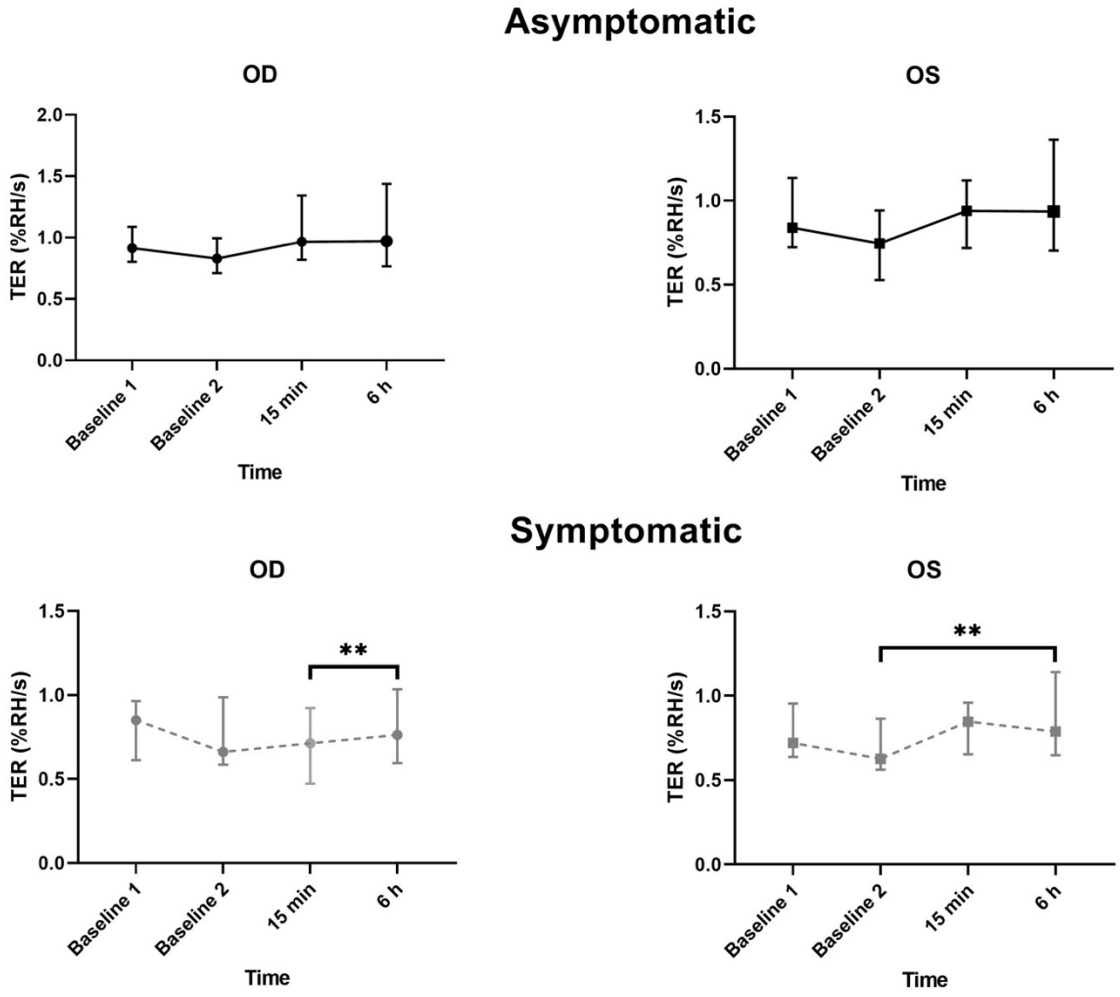

Figure G2: Median change in tear evaporation rate over time for each eye of the asymptomatic and symptomatic group. Error bars indicate IQR. No significant change in tear evaporation rate was observed for either eye of the asymptomatic group (right eye: p=0.339; left eye: p=0.118). The change in tear evaporation rate was significant for both eyes of the symptomatic group (right eye: p=0.026; left eye: p=0.028).

Table G1: Summary of clinical measurements for the left eye

	Asymptomatic Group (n = 10 eyes)	Symptomatic Group (n = 10 eyes)	p-value
Tear Evaporation Rate			
Baseline 1	0.84 (0.41)	0.72 (0.32)	0.166
Baseline 2	0.75 (0.41)	0.63 (0.30)	0.481
15 minutes post-lens	0.94 (0.40)	0.85 (0.31)	0.248
6 hours post-lens	0.94 (0.66)	0.79 (0.49)	0.393
p-value	0.118	**0.028**	
Non-invasive Break-up Time			
Baseline 1	7.90 (5.98)	8.27 (5.18)	0.971
Baseline 2	6.47 (8.39)	8.82 (3.94)	0.314
10 minutes post-lenses	6.99 (6.78)	7.62 (10.16)	0.684
360 minutes post-lenses	6.10 (3.15)	5.19 (5.03)	0.529
p-value	**0.020**	0.050	
Subjective Lipid Layer Thickness			
Baseline 1	3.50 (2.50)	4.50 (2.00)	0.533
Baseline 2	5.00 (2.00)	5.00 (1.25)	0.880
10 minutes post-lenses	3.00 (2.25)	3.00 (2.50)	0.903
360 minutes post-lenses	1.00 (2.00)	2.00 (2.00)	0.650
p-value	**0.0003**	**0.0003**	
Objective Lipid Layer Thickness (mean)			
Baseline 1	74.00 (44.75)	85.00 (30.75)	0.418
Baseline 2	76.50 (42.50)	93.50 (25.50)	0.391
10 minutes post-lenses	59.50 (31.25)	70.50 (22.00)	0.382
360 minutes post-lenses	58.50 (15.75)	63.50 (12.75)	0.240
p-value	0.073	0.004	
Objective Lipid Layer Thickness (maximum)			
Baseline 1	94.00 (29.00)	100.00 (1.25)	0.225
Baseline 2	94.50 (27.50)	100.00 (4.00)	0.183
10 minutes post-lenses	71.50 (39.50)	86.00 (17.50)	0.191
360 minutes post-lenses	74.00 (32.00)	82.50 (17.75)	0.211
p-value	0.069	**0.016**	
Objective Lipid Layer Thickness (minimum)			
Baseline 1	56.00 (28.25)	77.50 (33.25)	0.127
Baseline 2	60.50 (34.75)	82.50 (26.00)	0.065
10 minutes post-drop	50.00 (26.00)	59.00 (20.50)	0.224
30 minutes post-drop	43.00 (19.00)	53.50 (21.50)	0.270
p-value	0.175	**0.001**	

Data are presented as mean ± SD or mean (IQR). **Bold** indicates significant differences.

Table G2: Correlations between tear evaporation rate of the left eye and other clinical measurements

		Asymptomatic Group (n = 10 eyes)	p-value	Symptomatic Group (n = 10 eyes)	p-value
Ocular and Evaporimeter Characteristics					
Ocular surface area					
	Baseline 1	-0.171	0.637	-0.264	0.462
	Baseline 2	-0.112	0.758	-0.367	0.298
	15 minutes	0.188	0.604	-0.067	0.855
	6 hours	0.340	0.337	-0.368	0.296
Evaporimeter volume					
	Baseline 1	0.153[a]	0.673	0.384[a]	0.271
	Baseline 2	0.601[a]	0.071	0.456[a]	0.185
	15 minutes	0.301[a]	0.397	0.345[a]	0.326
	6 hours	0.350[a]	0.321	0.072[a]	0.849
Symptomology					
CLDEQ-8					
	Baseline 1	-0.192	0.595	0.050	0.890
	Baseline 2	-0.367	0.296	-0.159	0.662
	15 minutes	-0.394	0.260	-0.048	0.896
	6 hours	-0.393	0.262	-0.122	0.737
Comfort rating					
	Baseline 1	-0.188[a]	0.598	0.123[a]	0.736
	Baseline 2*	-0.104[a]	0.778	0.314[a]	0.372
	15 minutes	0.121[a]	0.739	0.351[a]	0.321
	6 hours	0.130[a]	0.720	0.232[a]	0.515
Dryness rating					
	Baseline 1	-0.126[a]	0.729	0.350[a]	0.323
	Baseline 2*	0.151[a]	0.675	0.546[a]	0.109
	15 minutes	-0.348[a]	0.328	0.522[a]	0.127
	6 hours	0.162[a]	0.654	0.025[a]	0.952
Stinging/burning rating					
	Baseline 1	0.009[a]	0.999	0.130[a]	0.733
	Baseline 2*	0.311[a]	0.400	0.510[a]	0.156
	15 minutes	0.623[a]	0.067	-0.290[a]	0.600
	6 hours	0.415[a]	0.244	-	--
Clinical Tests					
Non-invasive tear film break-up time					
	Baseline 1	0.394[a]	0.263	**-0.661[a]**	**0.044**
	Baseline 2	0.212[a]	0.560	-0.460[a]	0.184
	15 minutes	0.285[a]	0.427	-0.442[a]	0.204
	6 hours	-0.079[a]	0.838	0.127[a]	0.733

	Asymptomatic Group (n = 10 eyes)	p-value	Symptomatic Group (n = 10 eyes)	p-value
Lipid layer thickness (subjective)				
Baseline 1	-0.553[a]	0.102	0.479[a]	0.164
Baseline 2	**-0.706[a]**	**0.029**	0.254[a]	0.479
15 minutes	-0.505[a]	0.144	-0.415[a]	0.236
6 hours	0.315[a]	0.394	-0.035[a]	0.999
Lipid layer thickness (mean)				
Baseline 1	-0.620[a]	0.063	0.423[a]	0.226
Baseline 2	-0.591[a]	0.078	0.532[a]	0.117
15 minutes	-0.200[a]	0.584	-0.219[a]	0.542
6 hours	-0.244[a]	0.495	-0.127[a]	0.733
Lipid layer thickness (maximum)				
Baseline 1	-0.588[a]	0.080	0.500[a]	0.156
Baseline 2	**-0.653[a]**	**0.048**	0.147[a]	0.689
15 minutes	-0.295[a]	0.404	0.259[a]	0.468
6 hours	-0.171[a]	0.635	0.061[a]	0.871
Lipid layer thickness (minimum)				
Baseline 1	-0.230[a]	0.396	0.304[a]	0.390
Baseline 2	-0.503[a]	0.144	**0.758**[a]	**0.015**
15 minutes	0.055[a]	0.892	-0.244[a]	0.495
6 hours	0.389[a]	0.266	-0.316[a]	0.371

[*]: Baseline 1 subjective comfort ratings versus Baseline 2 TER; [a]: Spearman rank correlation; -: horizontal line; --: no p-value calculated.

Bold indicates significant correlations.

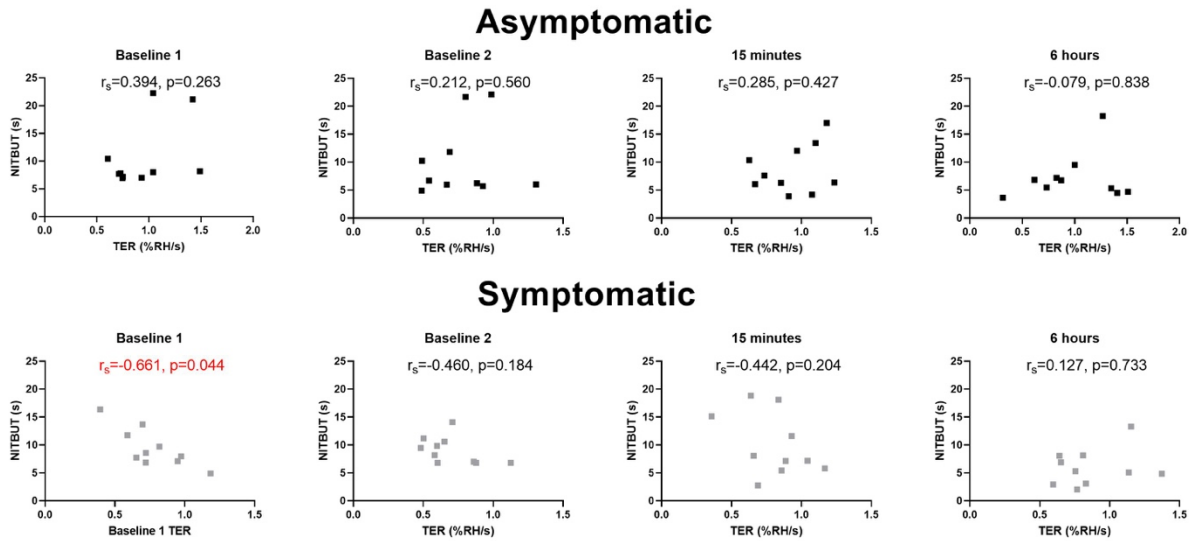

Figure G3: Correlations between the tear evaporation rate and the NITBUT of the left eye. A significant Spearman correlation is shown in red (symptomatic: baseline 1 r_s=-0.661, p=0.044, other r_s<0.128, other p>0.183; asymptomatic: all r_s<0.395, all p>0.262).

Appendix H

VapoMeter Calibration Certificates

Delfin

Certificate of Calibration

Certificate number: 19122014-SWL4644

Instrument type: VapoMeter SWL4

Serial number: SWL4644

Date of calibration: 15th December 2014
Due: 11 / 2016

Customer: University of Waterloo

Instrument condition on return

Meets all specifications

Calibration procedure

Standard calibration according to Working Instructions Version 4
(Externally Audited Quality Handbook, ISO 13485:2003)

We certify that the above equipment meets or exceeds published specifications and has been duly inspected and calibrated using standards and instruments whose accuracies are traceable to International Standards, standard measuring equipment and methods for the realization of physical units of measuring according to the International Systems of Units (SI).

Calibration performed by

Juha Miettinen

Certification approved by

Heikki Meriranta

The calibration has been performed at Delfin's manufacturing plant:
Delfin Technologies Ltd, PO. Box 1199, 70211 Kuopio, Finland

Delfin Technologies Oy
Microkatu 1
P.O.Box 1199
70211 Kuopio
Finland

Calibration results VapoMeter S/N SWL4644

Calibration level	**Evaporation source ($g/m^2/h$)**	**Difference (%)** (Tolerance ± 10 %)	**Coefficient of variation (%)** (Tolerance ± 10 %)
Standard mode results			
Low level accuracy	8,5	-1,4	5
Mid level accuracy	59	+0,8	2
High level accuracy	181	-0,3	1
Nail mode results			
Low level accuracy	8,5	+8,7	3
Mid level accuracy	59	+1,7	2
High level accuracy	181	-6,2	2

Surrounding calibration room conditions:

Relative humidity: (41,7 ± 1,2) % RH
Temperature: (23,2 ± 0,8) °C

Equipment used in calibration:

Laboratory scale

Type	Setra EL-410S
Serial number	C2416
Readability	0,001 g
Reproducibility	± 0,001 g
Linearity	± 0,002 g
Calibration	Kern & Sohn GmbH G012247 - F1 - 200 g weight

Air temperature and relative humidity meter

Measurement Indicator

Type	Vaisala MI70
Serial number	G4740009

Humidity and Temperature Probe

Type	HMP75
Serial number	G5040001
Accuracy	± 0.4 %RH (0,3 to 90,3 %RH) / ±0.0 °C
Calibration	Vaisala certificate Nr. H17-14070048
Calibration date	2014-February-13

Delfin Technologies Oy
Microkatu 1
P.O.Box 1199
70211 Kuopio
Finland

Certificate of Calibration

Certificate number: 13122016-SWL4644

Instrument type: VapoMeter SWL4

Serial number: SWL4644

Date of calibration: 12th December 2016
Due: 1 / 2019

Customer: University of Waterloo

Instrument condition on return

Meets all specifications

Calibration procedure

Standard calibration according to Working Instructions Version 4
(Externally Audited Quality Handbook, ISO 13485:2003)

We certify that the above equipment meets or exceeds published specifications and has been duly inspected and calibrated using standards and instruments whose accuracies are traceable to International Standards, standard measuring equipment and methods for the realization of physical units of measuring according to the International Systems of Units (SI).

Calibration performed by

Juha Miettinen

Certification approved by

Heikki Meriranta

The calibration has been performed at Delfin's manufacturing plant:
Delfin Technologies Ltd, PO. Box 1199, 70211 Kuopio, Finland

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Microkatu 1
P.O.Box 1199
70211 Kuopio
Finland

Calibration results VapoMeter S/N SWL4644

Calibration level	Evaporation source ($g/m^2/h$)	Difference (%) (Tolerance ± 10 %)	Coefficient of variation (%) (Tolerance ± 10 %)
Small adapter mode results			
Low level accuracy	7,6	-5,1	4
Mid level accuracy	57,0	-7,3	2
High level accuracy	169,0	-8,3	1

Surrounding calibration room conditions:

Relative humidity: (52,0 ± 1,4) % RH
Temperature: (22,4 ± 0,1) °C

Equipment used in calibration:

Laboratory scale

Type Precisa ES 220A
Serial number 4601260
Adjusting Kern & Sohn GmbH G012247 - F1 - 200 g weight

Air temperature and relative humidity meter

Measurement Indicator

Type Vaisala MI70
Serial number G4740009

Humidity and Temperature Probe

Type HMP75
Serial number G5040001
Accuracy ± 0.4 %RH (11,7 to 90,6 %RH) / ±0.0 °C
Calibration Vaisala certificate Nr. H52-16100074
Calibration due date 2017-March-11

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