# The Investigation of Tear Film Osmolality as a Clinical Instrument Used in Assessments of the Tear Film and Dry Eye Disease

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

Tear film osmolality is a product of the varying concentrations of dissolved solutes (proteins, lipids and mucins) in the tear fluid. Research suggests that a hyperosmotic tear film is a trait common to all forms of dry eye, and it may be the driving force causing the discomfort, ocular surface damage and inflammation found in both evaporative and tear deficient forms of dry eye disease. Tear film osmolality has been proposed to be the "gold standard" diagnostic test for the evaluation of dry eye disease, as a distinct separation between tear film osmolalities in normal and dry-eyed (aqueous deficient or evaporative) populations has become evident.

Historically, tear film osmolality could only be measured in a laboratory setting and required a highly skilled technician to use the instrumentation. The recent development of easy-to-use, small volume osmometers has made it possible for tear film osmolality to be measured clinically. As these instruments are quite new, there has been very little research completed with them. Therefore, a series of studies was conducted to investigate the utility of one of these new osmometers – the Advanced Instruments Model 3100 Nanolitre Osmometer.

The specific aims of each chapter were:

- Chapter 3: To determine if the Advanced Instruments Model 3100 Nanolitre Osmometer was capable of quantitatively measuring tear film osmolality in a normal population, using 0.5μL tear samples.
- Chapter 4: Previous studies have shown the Advanced Instruments Model 3100 Nanolitre Osmometer not significantly different from another commercially available osmometer (Wescor Vapor Pressure Osmometer) for the measurement of human tears. This chapter examined the repeatability of the new instrument over multiple measurements on the same sample and over multiple days.
- Chapter 5: To determine if tear film osmolality values varied significantly over the course of a normal working day in a population that was primarily free from symptoms of dry eye.
- Chapter 6: To investigate the relationships between tear film osmolality and other commonly used clinical tests for dry eye disease. The clinical tests examined included various questionnaires designed to assess patient symptoms (Single Item Dry Eye Questionnaire

(SIDEQ), the Ocular Surface Disease Index (OSDI), and the McMonnies Dry Eye Questionnaire (MMDEQ) and a linear analogue comfort scale (LACS)), a non-invasive tear break-up time test (NIBUT), and examination of ocular surface redness and tear ferning (TF). Secondarily to determine if the other clinical tests demonstrated significant diurnal variations over the course of a normal working day.

- Chapter 7: To measure tear film osmolality in a population with mild to moderate symptoms of dry eye disease, and to compare this value with the osmolality of a population of age-matched controls without the disease. Secondarily, to investigate the relationship between tear film osmolality and patient comfort in a population with mild to moderate symptoms of dry eye disease.

## Methods

- Chapter 3: Tears were collected from 40 volunteer participants with a capillary tube. Some participants were non-contact lens wearers (Non-CL), while others wore either soft or rigid contact lenses (CL). Tear film osmolality was measured with the Advanced Instruments Model 3100 Nanolitre Osmometer.
- Chapter 4: Tears were collected from 10 volunteer participants using two different collection techniques. Collections were repeated on three separate days (6 study visits total); three osmolality measurements per collection were taken using the Advanced Instruments Model 3100 Nanolitre osmometer.
- Chapter 5: Tears were collected from 40 volunteer participants in two separate studies (n=80 in total). Tears were collected with a capillary tube three times a day (morning, mid-day and afternoon), on two separate days (6 study visits total). Tear film osmolality was measured with the Advanced Instruments Model 3100 Nanolitre Osmometer.
- Chapter 6: Clinical tests were administered and tear samples were collected using a capillary tube from 40 volunteer participants. Measurements were taken three times a day (morning, mid-day and afternoon), on two separate days (6 study visits total). Tear film osmolality was measured with the Advanced Instruments Model 3100 Nanolitre Osmometer.
- Chapter 7: Participants were classified as either having dry eye disease (DE) or not having dry eye disease (NDE) based on a clinical examination that included a case history, phenol

red thread test and biomicroscopy (white light and sodium fluorescein assessment). Tear samples were then collected from all participants using a capillary tube and tear film osmolality was measured with the Advanced Instruments Model 3100 Nanolitre Osmometer. Participants also completed the SIDEQ, the OSDI, and the MMDEQ.

## Results

- Chapter 3: The mean tear film osmolality of the population was 298.7±11.4mOsm/Kg. CL wear (soft or rigid) did not appear to have a significant effect on tear film osmolality (CL: 298.5±11.2mOsm/Kg vs. Non-CL: 298.9±11.5mOsm/Kg), although this study was not designed to specifically look at the effects of contact lens wear on tear film osmolality.
- Chapter 4: There was reasonably good concordance between measurements of tear film osmolality taken with the Advanced Instruments Model 3100 Nanolitre Osmometer (intraclass correlations range from 0.6497 ($F= 0.0582$) to 0.9550 ($F = 0.5893$)). Repeatability appeared to be affected by significant changes in ambient humidity (>10% per day). Concordance was similar with both sampling techniques.
- Chapter 5: In the first study, no significant diurnal change in tear film osmolality was found ($p>0.05$), although a significant difference in measurements taken on Day 1 compared to Day 2 was found ($p=0.040$). When the first and last 10 participants enrolled were compared, the difference between days was present in the first 10 participants, but not in the last 10; it is likely that the investigator underwent a learning process during the period of the study, and that reflex tearing occurred more often in the early portion of the study compared with the latter portion. In the second study, no significant diurnal change in tear film osmolality was found ($p>0.05$) and no significant difference in measurements taken on Day 1 compared to Day 2 was found ($p>0.05$). When tear film osmolality was compared with the number of hours participants were awake, no significant correlation was found ($r = 0.07044$).
- Chapter 6: Significant correlations were not found between tear film osmolality and SIDEQ ($r = 0.1347$), OSDI ($r = 0.0331$), MMDEQ ($r = 0.2727$), LACS ($r = -0.1622$), NIBUT ($r = -0.2280$), subjectively graded redness ($r=-0.2280$), or objectively measured redness ($r = 0.1233$). A weakly significant correlation was found between TF and tear film osmolality ($r = 0.3978$). None of the clinical measures (LACS, NIBUT, subjective or objective redness or TF) varied significantly over the course of the day.

- Chapter 7: Tear film osmolality was higher in both the right (DE = 311.1±12.4mOsm/Kg, NDE = 306.2±11.2mOsm/Kg) and left eyes (DE = 313.2±11.9mOsm/Kg, NDE = 304.0±7.5mOsm/Kg) of participants, but the difference was only statistically significant in the left eye. Tear film osmolality did not correlate significantly with DE patient symptoms using any of the questionnaires (SIDEQ, OSDI, MMDEQ).

## Conclusions

- Chapter 3: The Advanced Instruments Model 3100 Nanolitre Osmometer appeared to be capable of measuring tear film osmolality in a normal population. Our population mean was slightly lower than what is reported to be normal (305mOsm/Kg), but it still fell within the range of values reported as normal (297 – 318mOsm/Kg).
- Chapter 4: The Advanced Instruments Model 3100 Nanolitre Osmometer demonstrated reasonably good repeatability for the measurement of human tear samples. Unfortunately, the instrumentation appeared to be affected by dramatic weather changes. Maintaining the instrument in a humidity controlled environment may resolve this problem.
- Chapter 5: Tear film osmolality did not appear to vary significantly over a normal working day. Inducing reflex tearing, perhaps with an unskilled investigator collecting the tears, can be a significant source of error (as demonstrated in the first study).
- Chapter 6: Tear film osmolality did not correlate well with other clinical instruments designed to assess either patient symptoms or signs of dry eye disease in a normal population. Tear film osmolality and tear ferning did demonstrate a weakly significant positive correlation. None of the clinical measures assessed demonstrated a significant diurnal variation over the course of a normal working day.
- Chapter 7: Tear film osmolality appeared to be higher in participants with mild to moderate symptoms of dry eye when compared with age matched, asymptomatic controls. Tear film osmolality did not correlate well with patient symptoms in a population of mild to moderate severe dry eyed individuals.

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# Dedication

*"Life isn't about finding yourself. Life is about creating yourself" – Author Unknown*

*To all of those who have helped me create – Doug, Sandie, Johanna, Bryan, Rock & Ida, Harold and Olga, Kathryn, Nikki, Viena, Bonnie and Jeevan.*

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# Chapter 1
# Literature Review on Tear Film Osmolality

## 1.1 Dry Eye Disease

### 1.1.1 Definition of Dry Eye

In 1995, the National Eye Institute/Industry workshop defined dry eye as "a disorder of the tear film due to deficiency or excessive tear evaporation which causes damage to the interpalpebral ocular surface and is associated with symptoms of ocular discomfort".[1] This definition was recently updated in 2007, at the International Dry Eye Workshop, where dry eye was defined as a "multifactorial ocular surface disease diagnosed by symptoms of discomfort and signs of visual disturbance, tear film instability and ocular surface damage, accompanied by increased osmolarity of the tear film and ocular surface inflammation."[2]

Dry eye disease is an umbrella term which refers to a breakdown of the ocular surface functional unit as a whole. The functional unit is composed of the ocular surface (cornea, conjunctiva and meibomian glands), the lacrimal glands, the eyelids, and the sensory and motor nerves that connect them.[3] Under this umbrella, there are many different types of dry eye disease, which have been classified based on their etiopathogenic origins (Figure 1.1). Examples of the various types of dry eye disease include aqueous-deficient dry eye, evaporative dry eye, Sjögren's syndrome, and contact lens induced dry eye.[2]

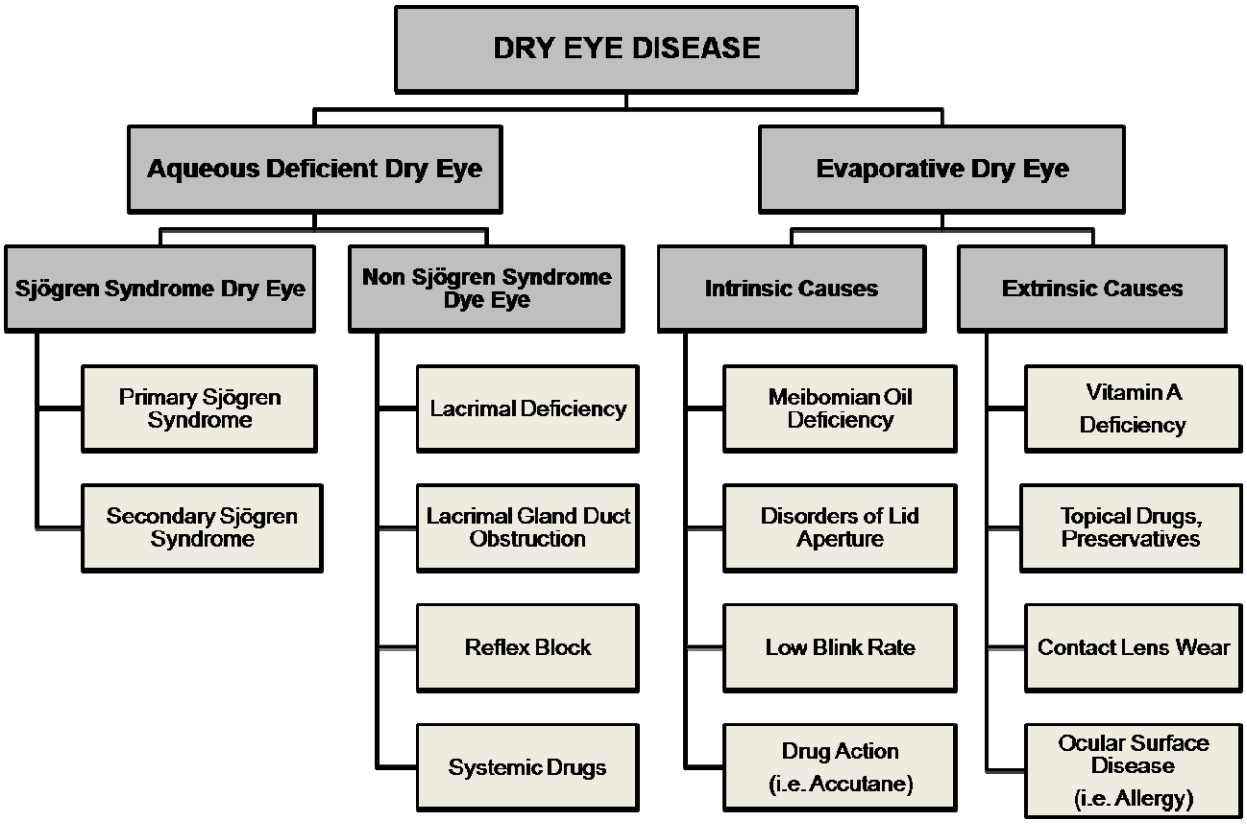



**Figure 1-1: Etiopathogenic classification of dry eye disease. (Adapted from: The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye Workshop (2007) Ocul Surf 2007; 5:75-92).**

### 1.1.2 Prevalence of Dry Eye Disease

Dry eye disease is one the most frequently diagnosed ocular problems in optometry clinics throughout North America and the world.[4] It has been estimated that approximately one-third of the general population have occasional symptoms of dry eye,[5] while one in every four patient visits to an ophthalmologist are related to complaints of dry eye,[6] and 17% of visits to eye care centers are due to dry eye issues.[7] Over the past number of years, a multitude of epidemiological studies have been undertaken in an attempt to determine the prevalence of dry eye disease in the general population, and they have determined that the dry eye disease has a prevalence somewhere in the range of approximately 3.5% to 58%.[5, 7-19]

A major issue which arose out these studies was that no two studies used the same criteria to define dry eye. Many of the studies have used symptoms as their only criteria, while others have used various combinations of symptoms and clinical signs. Add to that the fact that they have studied different age groups in different geological locations, using varying sampling and measurement techniques and different cut-off values, and we start to understand why there is such a vast range of numbers quoted as the prevalence rate of dry eye.[20]

In terms of cost, a decade ago Americans were estimated to be spending $100 million annually on artificial tear products (prescribed or self-medicated).[21] That $100 million did not include the costs of visits to eye care professionals, other treatment costs, or the impact dry eye has on the health and productivity of patients or the number of work hours lost as a result of the disease.[22] In the last ten years, these costs have only gone up. In gaining a greater understanding of the pathology underlying dry eye disease we have become more aware of the impact it has on the everyday life of patients.

## 1.2 Clinical Evaluation of Dry Eye Disease

As mentioned previously, one of the greatest challenges with the study of dry eye disease is the lack of a set of simple, concise, globally accepted diagnostic criteria[20] defining not only the disease itself, but also the accepted levels of disease severity. Currently, symptoms of discomfort, tear film instability, tear film hyperosmolality, ocular surface inflammation and ocular surface damage are thought to be characteristics common to most forms of dry eye disease.[1, 2] Therefore, one would expect that an accepted diagnostic criteria would in some way test for many, or all, of these problems.

At this point, it needs to be clarified that the emphasis of this review is clinical; diagnostic procedures that can only be performed in laboratory or specialized research settings have not been included, as they are not readily available to the average clinician, in a typical clinical setting. A globally accepted set of diagnostic criteria would be useless if it could not be applied in the majority of optometric clinics around the world. A summary of the diagnostic tests readily available to clinicians for the evaluation of dry eye disease is listed in Figure 1.2.

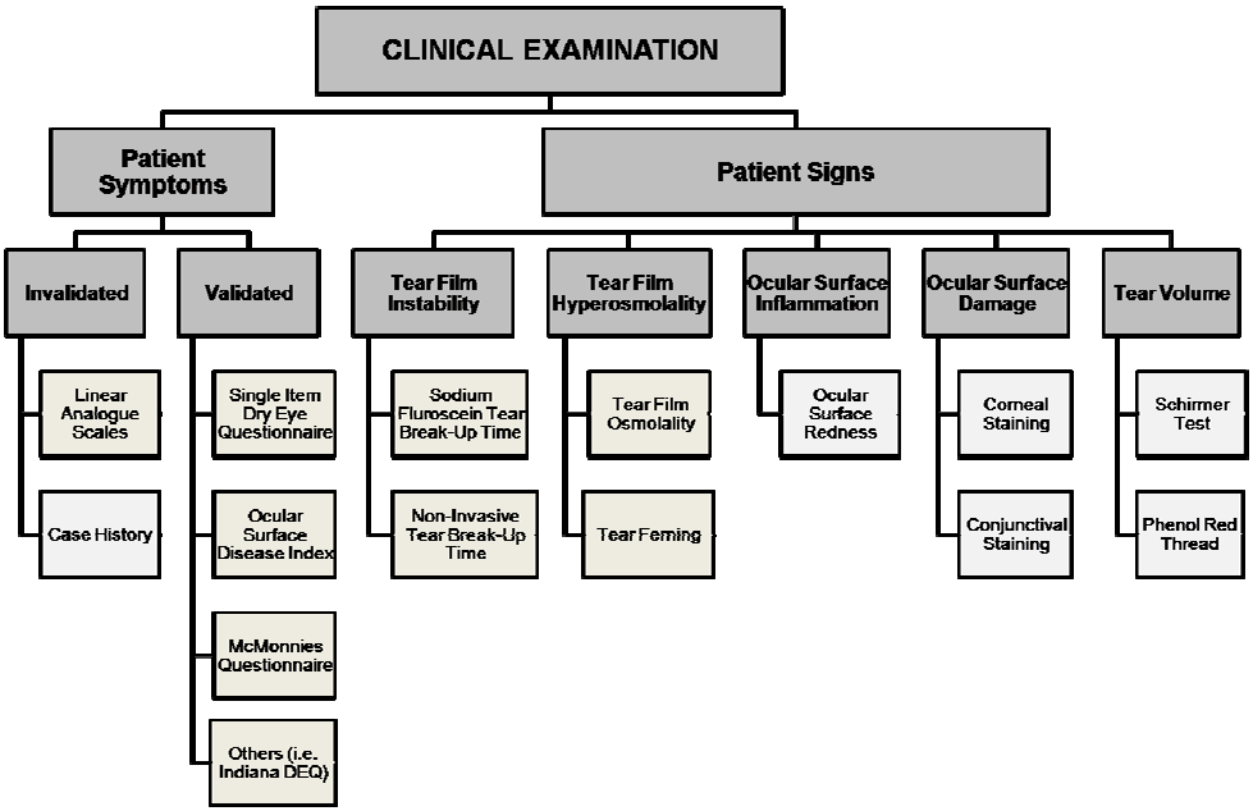



**Figure 1-2: A summary of commonly used clinical tests in the evaluation of dry eye disease. (Adapted from: The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye Workshop (2007) Ocul Surf 2007; 5:75-92).**

### 1.2.1 Evaluation of Patient Symptoms

#### 1.2.1.1 Patient Symptoms – Validated Techniques

Questionnaires, particularly those which have been validated and have accepted scoring criteria, are one of the most simple and effective ways to asses patient symptoms. They can be administered by clinical staff and completed by patients before they even enter the exam room. Currently there are a number of validated questionnaires that can be used, but the question of "which works best?" is still unanswered at this time. The difficulty in answering this question lies in the fact that all of the

available questionnaires are designed differently and they assess slightly different things. They also vary greatly in their length and level of detail. Three commonly used questionnaires are the Single Item Dry Eye Questionnaire (SIDEQ),[23] the Ocular Surface Disease Index (OSDI)[24, 25] and the McMonnies Questionnaire.[26, 27] These three questionnaires are all a single page long and have pre-defined scoring systems capable of classifying dry eyed patients based upon their disease severity. Other questionnaires, such as the Indiana Dry Eye Questionnaire,[28, 29] are being developed, but they do not yet have an accepted scoring system or they have not been validated.

The SIDEQ is a single item, self assessment questionnaire that asks patients to rate their ocular surface comfort on a 0 to 4 scale.[23] A score of "0" corresponds to no discomfort or no dry eye disease, while a score of "4" corresponds to severe symptoms of ocular surface discomfort, often associated with advanced dry eye disease.[23]

The OSDI is a twelve item quality of life questionnaire designed to measure the severity of ocular surface disease and its impact on vision related functions.[24, 25] Participants are asked to evaluate each of the twelve items on a 5-point Likert scale (all of the time, most of the time, half of the time, some of the time, none of the time) over a recall period of the last week. The items are divided into three subgroups - "Ocular Symptoms", "Vision-Related Functioning", and "Environmental Triggers". A total OSDI (between 0-100) score is obtained by adding the scores for each of the subgroups together. The higher a participant's score, the greater the disability they experience as a result of their disease.[24, 25] Scores of 0-12 are considered to be normal, 13-22 indicative of mild dry eye, 23-32 of moderate dry eye, and 33-100 of severe dry eye disease.

The McMonnies questionnaire is made up of fifteen questions, fourteen of which focus on clinical "risk factors" for dry eye disease that have been derived from the literature. These "risk factors" include patients' age, gender, contact lens history, dry eye symptoms, previous dry eye treatments, secondary symptoms (associated with environmental stimuli), medical conditions associated with dry eye syndrome (arthritis, Sjögren syndrome, thyroid disease), dryness of mucous membranes (mouth, throat, chest, or vagina), and medication use.[30] The McMonnies questionnaire uses a weighted-scale scoring algorithm to obtain an overall "Index" score. The Index score can fall between 0 and 45; higher scores being again indicative of greater dry eye disease.[27] The instrument has a recommended cut-point of 14.5, with scores higher than this thought to be associated with dry eye disease.[26, 27] Patients' Index scores can also be used to categorize them based upon their severity of dry eye disease. When used in this way, a score of 0-10 is considered to be normal, a score of 11-19

suggestive of borderline dry eye, and a score of 20 or greater indicative of moderate to severe dry eye disease.[26, 27]

### 1.2.2 Patient Symptoms – Other Techniques

Although not validated, another way to assess participant ocular surface comfort is through the use of linear analogue scales.[31] These scales are simple linear scales from 0 to 100, and participants are asked to record on the scale the comfort of their eyes at that particular moment. 0 is equivalent to complete ocular surface discomfort, while 100 is representative of complete ocular surface comfort. Scores can be expressed as a percentage of ocular surface comfort.

Finally, the importance of a detailed clinical history taken by an experienced clinician cannot be overlooked. Although case histories cannot be validated and given a score, there are important questions that should be asked of patients who have been previously diagnosed with, or who are suspected of having, dry eye disease. Some of these questions include information about the patient's age, general health, systemic medication use, smoking habits, contact lens practices, ocular surface comfort, allergies (systemic and ocular), and artificial lubricant use.[30]

### 1.2.3 Evaluation of Clinical Signs of Ocular Surface Damage

#### 1.2.3.1 Tear Film Instability

A stable tear film is essential for maintaining crisp, clear vision. It is also vital to the health of the epithelial cells on the ocular surface as the tears are responsible for sustaining cell hydration, providing nutrition and antibacterial protection, and removing waste.[32, 33]

Tear film stability is thought to be a product of many factors, including tear film viscosity and surface tension, tear meniscus radius, tear film thickness, and tear film composition.[34] Tear film instability, on the other hand is thought to be the result of a breakdown of one or many of these factors, and many mechanisms of tear film break up have been presented. Holly[35] proposed that tear film break up was a result of contamination of the mucin layer of the tear film by the inward movement of the superficial lipids. Liotet *et al*.,[36] suggested that perhaps it was the inability of the corneal epithelial cells to manufacture the glycocalyx, which in turn would prevent the tear film mucins from attaching properly to the corneal surface. Sharma and Ruckenstein[37] felt that Van der Waals dispersion forces (attractive or repulsive forces that exit between molecules), may contribute to the disruption of the tear film. Other theories have suggested that tear break-up may be initiated at

points where a surface epithelial cell has been recently sloughed leaving a newly exposed cell with an immature glycocalyx and a slightly lower wettability,[38] that gravity and tear drainage may have an effect on tear film instability,[34] and that a rising tear film meniscus height, which reaches the effective range of the dewetting intermolecular forces, could be contributing as well.[39]

Regardless of the underlying mechanism leading to tear film instability, it has been found to be associated with dry eye disease. Tear film break up is thought to increase tear film osmolality and local drying of the exposed ocular surface, which in turn excites inflammatory cell markers, triggering epithelial cell damage and apoptotic cell death.[2]

Clinically, tear film instability is commonly assessed by determining the "tear film break-up time". This can be measured either by instilling sodium fluorescein (NaFl) dye into the tears and watching the tear film under cobalt blue light at a biomicroscope until "black spots", or dry areas, appear, or by reflecting a pattern of rings off the tears and watching for the first sign of distortion of the ring pattern. The first technique is an invasive technique in that the tear film is physically altered (and its volume significantly increased) by the instillation of the dye.[40, 41] The second technique does not invade upon or alter the tear film significantly in any way, and is often referred to by clinicians as a "Non-Invasive Tear Break-Up Time" for exactly that reason.[42, 43] Using either technique, clinicians are able determine the length of time after a blink (in seconds) that the tear film remains intact, or stable, on the ocular surface. Shorter tear break-up times have been found to be associated with dry eye disease.[1, 42, 44]

#### 1.2.3.2 Tear Film Hyperosmolality

Tear film hyperosmolality is thought to be one of the core mechanisms responsible for driving the inflammation associated with dry eye disease, which eventually leads to ocular surface damage and patients' symptoms of discomfort.[2, 45] Faster tear thinning rates have been found in normal individuals with higher tear film osmolarities,[46] and it has been suggested that a higher rate of tear thinning is a risk factor for tear film hyperosmolality.[2]

It has been suggested that tear film hyperosmolality stimulates inflammatory events involving the mitogen-activated protein kinase (MAP kinase) and nuclear factor – kB (NFkB) signalling pathways.[47] It has also been thought to contribute to the generation of inflammatory cytokines, primarily interleukin 1α (IL 1α), interleukin 1β (IL 1β), tumor necrosis factor α (TNF α) and matrix

metalloproteinases (MMPs), all of which lead to the stimulation of inflammatory cells at the ocular surface, and eventually cause the ocular surface damage associated with dry eye disease.[48]

Some researchers suggest that the measurement of tear film osmolality could become the "gold standard" diagnostic test for the evaluation of dry eye disease, as a distinct separation between tear film osmolalities of normal and dry-eyed populations has been proposed.[1, 45, 49, 50] A hyperosmotic tear film appears to be a trait common to both aqueous deficient and evaporative forms of dry eye,[45] and this makes it a good candidate as the single, clinical diagnostic test of dry eye disease. The current literature and our knowledge of tear film osmolality will be discussed in more detail shortly.

Tear ferning is another simple clinical test which, although it does not provide information regarding the osmolality of tears, is capable of providing information regarding the quality of patient's tears. This technique is based upon the evaluation of the crystallization patterns that form when tears are left to air dry at room temperature on a clean, clear microscope slide.[51, 52] Initially, tear ferning patterns were thought to be caused by mucus,[51, 53] but researchers have suggested that the electrolyte concentration, particularly the ratios of sodium chloride to other ions (potassium, calcium and magnesium)[54] and to macromolecules (mucins, lipids, proteins)[55] are responsible. Increased amounts of lipid-contaminated mucus, altered tear rheology and reduced protein and mucin levels, in combination with raised tear film osmolality have also been implicated in the development of tear ferning patterns.[55]

Regardless of the exact mechanism driving their formation, tear ferning patterns, much like tear film osmolality, are dependent upon the concentrations of dissolved solutes (including mucins, lipids, proteins and salt ions) within the tear film itself. Tear ferning then, while not a direct measure of tear film osmolality, can provide a general idea of the composition of the tear film, which ultimately is responsible for its osmolality. Differences in tear ferning patterns have been found between individuals with and without dry eye disease.[52, 55]

This test is relatively simple and quick to perform and it does not require a large amount of specialized equipment. The clinician simply collects a small sample (approximately 0.3µL) of basal tears with a capillary tube, transfers them to a clean, clear microscope slide, leaves them to dry for about 10 minutes, and then examines the sample under a microscope. Ferning patterns can then be graded on a simple 1 to 4 scale, which has been shown to be repeatable[56] and will be discussed in more detail in Chapter 2 (General Methods).

#### 1.2.3.3 Ocular Surface Inflammation

"*Rubor* (redness), *calor* (increased heat), *tumor* (swelling), and *dolor* (pain)." These are the four classical signs of inflammation which were originally described by Celsus (ca 30 BC–38 AD), while *functio laesa* (loss of function) was added later.[57-60] These five signs are commonly found with acute inflammation on the body's surface, but in cases of chronic inflammation, which is typical in dry eye disease, they may not all be present.

Bulbar conjunctival hyperaemia, or the classic "red eye", is associated with many types of ocular surface pathology, including acute microbial or viral infections, abrasions, allergies, and dry eye disease, and it is a common complaint of individuals who suffer from irritated and uncomfortable eyes.[61, 62] For this reason, ocular surface redness (typically measured from the bulbar conjunctiva), appears to be a promising variable through which to measure and study the inflammatory process taking place in dry eye disease.

Currently there are two general methods for measuring redness – a subjective method based upon grading scales, and an objective method using a spectrophotometer. Currently, the first method is the most commonly used clinically, as spectrophotometers tend to be used primarily for research purposes. Spectrophotometers tend to be mounted on a biomicroscope, therefore it would not be unreasonable to eventually adapt them for clinical use, particularly if research demonstrates that ocular surface redness has significant potential to be used as a diagnostic test in dry eye disease.

Subjectively, there are many different clinical grading scales available for clinician use. Some scales run from 0-3 or 1-4, others are divided into increments of 10,[63] and still others, such as the one used at the Center for Contact Lens Research (CCLR), University of Waterloo, are based on a 0-100 scale. The 0-100 scale used at the CCLR, has been adapted from a CCLRU 1-4 scale, and grades redness in terms of "percentage", where a grade of 0 is considered to be negligible redness, and a grade of 100 is considered to be severe redness.[64-66]

Objectively, bulbar conjunctival redness can be measured using a SprectraScan PR650© Spectrophotometer (Photo Research Inc., Chatsworth, CA, USA). This instrument is a table top device which measures luminance and chromaticity values through the measurement of absolute intensity at each wavelength of light, and then calculates of the equivalent CIE (Commision Internationale d'Eclairage) u' value.[66, 67] A higher u' value has been shown to be equivalent to greater bulbar conjunctival redness,[66, 67] although work qualifying the relationship between photometric measurements and redness grading scales is still underway.[68]

#### 1.2.3.4 Ocular Surface Damage

Some, albeit not all, ocular surface damage to the corneal and conjunctival epithelial cells is thought to be caused by a combination of tear film instability, tear film hyperosmolality and the stimulation of inflammatory cascades at the ocular surface.[2, 48] Clinically, ocular surface damage has typically been measured through the assessment and grading of corneal and conjunctival staining. Vital dyes, such as sodium fluorescein, lissamine green and rose bengal, are thought to stain dead or damaged epithelial cells on the ocular surface, thereby making them visible to clinicians.[1, 2, 62, 69, 70] Like ocular surface redness, there are many different grading scales available for quantifying corneal and conjunctival staining in dry eye research, including the Oxford grading system[69], the van Bijsterveld scale,[70] and the CLEK system.[1, 71]

#### 1.2.3.5 Tear Volume

Although not specifically listed in the most recent definition of dry eye disease, tear volume is often measured clinically, because a decreased tear volume has been shown to be commonly found in patients with dry eye disease.[1, 2, 20, 62, 72, 73] The Schirmer test and the Phenol Red Thread test are two tests available for clinical assessment of tear volume. Of the two tests, the Phenol Red Thread test is easier and more comfortable to perform, but questions exist regarding the validity of this test.[62, 74-76]

The Schirmer test remains the standard test for evaluating tear film volume in dry eye disease. If the ocular surface is anaesthetized prior to the test, reflex tear flow is not stimulated and it is believed that this test can measure the volume of tear fluid present on the ocular surface, but if the ocular surface is not anaesthetized then it may be that the test is actually determining the ability of the lacrimal gland to respond to stimulation.[38] Despite the increased discomfort for the patient, research suggests that the test is more repeatable and reliable when anaesthesia is not used, and that it should be performed without anaesthesia when used as a diagnostic test for dry eye disease.[2, 70, 77]

## 1.3 Tear Film Properties

The tear film, which has been reported to have a thickness somewhere in the range of 1.5µm to 45µm in normal individuals, [78-80] is an essential element of the ocular surface. Without it, the ocular surface would not be able to function and our vision would not be of the same quality.[81-84]

### 1.3.1 Structure of the Tear Film:

The human tear film has historically been defined as a three layered structure composed of an outer lipid layer, a middle aqueous layer and an inner mucin layer which lies directly adjacent to the cornea.[83, 85-87] The lipid layer acts a barrier to tear film evaporation, while the aqueous layer is largely responsible for the nutritional needs of the cornea, and contains glucose, lysozyme and other proteins, various dissolved salts, and urea. The mucin layer coats the corneal surface rendering it hydrophilic, and anchors the tear film to the corneal surface.[85, 86]

Recently, models of the tear film have been revised to reflect a more complex system. Instead of having three separate layers, the tear film has been proposed to exist as a dynamic gradient, with the lipid, aqueous and mucin layers mixing and interacting throughout.[88-92] Most recently, the tear film has been defined as a bi-layered structure composed of a superficial lipid layer overlying an aqueous/mucinous layer.[93] In spite of recent work, the concept of a three-layered tear film is still a valuable model in the study of the ocular surface.[94]

The lipid layer is still believed to float on the outer surface of the tear film, but the aqueous and mucin layers are no longer mutually exclusive. The aqueous layer is thought to contain free floating gel-like mucins, while the mucin layer contains a layer of mucin molecules bound to the corneal surface. The free floating mucins are thought to perform different functions than those attached to the corneal surface. In fact, research has found that the tear film contains many unique, chemically and functionally different mucin compounds which work together to create a stable, healthy environment.[88, 89, 95] An abnormality of one or more of the tear film structures can have devastating effects on the ocular surface, and can lead to ocular surface dryness, inflammation, and damage.

### 1.3.2 Tear Film Components

The major components of the tear film are water, proteins, lipids, mucins, electrolytes and other small molecules (i.e. defensins and collectins).[38] Many other compounds, such as inflammatory mediators, cytokines, growth factors, white blood cells, antigens, signalling molecules, complement components and remodelling enzymes have also been found in the tear film. These components are considered to be minor components, some of which have been shown to change in the presence of various pathologies.[38] For the purpose of this review, the emphasis of the following discussion will be on the major components of the tear film (proteins, lipids and mucins), although some time will be spent

looking at electrolytes as well, as they have been found to play a role in dry eye disease and in controlling osmolality.

#### 1.3.2.1 Tear Film Proteins

To date, over 60 proteins have been identified in the human tear film,[83, 96, 97] although some estimates suggest there may be upwards of 80-100 proteins present.[38] Tear proteins are present in the aqueous layer of the tear film, with lysozyme, lactoferrin and lipocalin being prominent components.

Lysozyme comprises 20-40% of the total tear protein,[98] and its concentration is higher in the tears than in any other bodily fluid.[83] Lysozyme plays a key role in the antibacterial defence properties of tears.[99, 100]

Lactoferrin possesses antibacterial properties[101, 102] and acts as a scavenger for free radicals.[103] Recent work by Ohashi *et al*. using enzyme-linked immunosorbent assays (ELISA), has determined the concentration of lactoferrin in tears to be upwards of 1.6ng/mL.[104]

Lipocalin can bind fatty acids, and when complexed with other tear components, may contribute to the high, non-Newtonian (shear thinning) viscosity and the low surface tension of the tear film.[105] Therefore it is thought to play an essential role in the maintenance of tear film stability.[105, 106]

Other important tear proteins include serum albumin, transferrin, the immunoglobulins (IgE, IgG, IgM), ceruloplasmin and aquaporin 5.[83, 107] Numerous peptide growth factors including EGF, HGF, TGF β are also found in the aqueous layer of the tear film.[97] EGF (epidermal growth factor) is thought to play a potential regulatory role for the lacrimal gland in maintaining the ocular surface, control of corneal would healing, and in diseases of the ocular surface.[108, 109]

#### 1.3.2.2 Tear Film Lipids

Tear film lipids are formed in the meibomian glands of the upper and lower eyelids,[110] although the Glands of Moll and lash follicle Glands of Zeis also produce some lipid.[111] Tear film lipids form a thin smooth film on the outermost layer of the tears, composed of an outer non-polar lipid layer and an inner polar lipid layer.[112] The non-polar layer is a relatively thick layer and forms the bulk of the entire lipid layer, and contains many elements, including wax esters, sterol esters, hydrocarbons, triglycerides and free fatty acids.[113] It is separated from the main body of the tear film by the thinner, inner polar layer of lipids, which are composed primarily of phospholipids.[113] Polar lipids have

surfactant properties, which facilitate their interaction with both the aqueous tear layer and the non-polar lipid layer..[112, 114]

Wax monoesters and sterol esters make up approximately 77% of the meibomian gland fluid, and are the major class of lipids present in the tear film.[112, 115, 116] Di- and trigylcerides account for 7% of tear film lipids, hydrocarbons for 2%, and diesters that form ester linkages with fatty acids, fatty alcohols, or sterols account for about 8%. Trace amounts of cerebrosides and ceramides are also present.[83, 117]

#### 1.3.2.3 Tear Film Mucins

Tear film mucins are found primarily in the mucus layer of the tear film, along with various other molecules including immunoglobulins, urea, salts, glucose, leukocytes, cellular debris and enzymes.[71] To date, 20 different epithelial mucins have been identified, 16 of which (MUC1, MUC4, MUC 11, MUC13, MUC15, MUC16, MUC17, and MUC20, and the secretory mucins MUC2, MUC5AC, MUC5B and MUC7)[73, 83, 90, 118, 119] have been found to be produced by the corneal and conjunctival epithelial cells or the lacrimal gland.

Membrane bound mucins are a major component of the glycocalyx covering the corneal and conjunctival epithelial cells.[120-122] Of the membrane bound mucins, MUC1, MUC4 and MUC16, have received the most attention in tear film research.

MUC1 mRNA is expressed in all corneal and conjunctival epithelial cells, but the protein is present only in the apical surface cells of the cornea and in the apical and sub-apical cells of the conjunctiva.[123-130] MUC1 is thought to be involved in signal transduction pathways[131] and to be associated with the actin cytoskeleton of epithelial cells.[132]

MUC4 is generally considered to be a membrane bound mucin that is predominately found in the conjunctival epithelial cells, although it has been found in small quantities in corneal epithelial cells.[93, 124, 125, 127, 130, 133-135] It is suggested that MUC4 may have a signalling function, and may be involved in growth regulation.[136, 137] A soluble form of MUC4 has been identified in the lacrimal gland and tears, although the exact function of this form is unknown.[124, 138]

MUC16 has been found in the apical cells of the corneal epithelium, as well as the apical and sub-apical cells of the conjunctival epithelium.[73, 90, 130, 139] MUC16 appears to interact with the actin cytoskeleton of epithelial cells, much like MUC1, and it appears form a protective barrier at the

epithelial surface, which helps to prevent the adhesion of pathogens.[140] MUC16 has been found to be free floating in the tear film as well, and the stimuli triggering its release are still being studied.[141, 142]

The small, soluble mucin MUC7, [124, 129, 135, 143] and the large, gel-forming mucin MUC5AC, [126, 127, 129, 134, 144] are the primary free floating soluble mucins found in the human tear film.

MUC7 has been found to be released by both the conjunctival epithelium and the lacrimal gland.[124, 129, 135, 143] It is a small, monomeric molecule, and is believed to have antifungal or anticandidacidal activity.[145, 146]

MUC5AC is expressed by the conjunctival goblet cells.[125, 127, 130, 134, 135, 144, 147, 148] It is a large gel-forming mucin, whose structure appears to permit the formation of multiple disulfide bonds between its molecules, thereby creating a mucin network within the aqueous tear layer.[90]

#### 1.3.2.4 Tear Film Electrolytes

Electrolytes such as sodium, potassium, magnesium, calcium, chloride, bicarbonate, and phosphate ions are found within the tear film aqueous layer. They are thought to be responsible for controlling tear film osmolality,[149] act as buffers to preserve tear film pH,[150] and maintain the integrity of the ocular surface epithelium.[151]

### 1.3.3 Functions of the Tear Film:

#### 1.3.3.1 The Lipid Layer

The lipid layer reduces the rate of tear film evaporation and lubricates the eyelids as they pass over the ocular surface. It also alters the tear film surface tension to prevent it from overflowing the lower lid margin, and thickens and stabilizes the tear film via interactions with the aqueous layer.[81, 82, 84, 152, 153]

#### 1.3.3.2 The Aqueous Layer

The aqueous layer provides atmospheric oxygen and removes metabolic waste products from corneal epithelial cells. It contains substances which act as antibacterial agents and is capable of washing large particles of debris away from both the cornea and the conjunctiva. It helps maintain the tonicity and pH of the tear film and provides a smooth refractive surface, as it masks many corneal irregularities.[79-82, 84, 153]

#### 1.3.3.3 The Mucin Layer

The mucin layer coats the hydrophobic epithelial cells of the cornea and conjunctiva surfaces rendering them hydrophilic, thus stabilizing the tear film. Mucins are critical in sustaining proper ocular surface hydration and ease the spread of the aqueous layer over the corneal surface, creating a smooth refractive interface. Mucins coat tear film debris and help prevent corneal trauma. They also alter the surface tension of the tear film, minimizing the force exerted on the cornea and conjunctiva by the eyelids during a blink.[78, 80-82, 84, 154, 155]

### 1.3.4 Physical Properties of Tear Film

#### 1.3.4.1 pH

Human tears have been reported to have a pH which falls in the 6.6 to 7.8 range[150] and are relatively neutral, while the eye has been reported to be capable of tolerating a much wider range of pH, from 6.2 to 9.0 at 0.2M strength.[156] Khurana *et al*. compared the pH of the tear film in both normal and dry eyed populations, but did not find a significant difference between the two groups.[44]

#### 1.3.4.2 Viscosity

Tears have been shown to have what is termed a "non-Newtonian" viscosity, in that their viscosity changes as the shear rate they are exposed to changes. At 25.0°C, the viscosity of human tears has been demonstrated to decrease from approximately 5.0 to 1.5cP with increasing shear rate.[157, 158] It has been suggested that tear film viscosity is a product of the composition of its proteins, mucins and lipids[157] although the exact components responsible have not been determined.

Viscosity is an important property of the tear film, as tears are exposed to a wide range of shear stresses with every blink. The viscosity of tears is highest at low shear rates (when the eye is open) which helps to maintain tear coverage of the ocular surface, and lowest at high shear rates (i.e. blinking) as this helps to minimize the frictional and mechanical forces exerted on the cornea during lid opening and closure. Presently, tear viscosity has been thought to depend, at least in part, upon the binding of lacrimal lipids to tear-specific lipocalin, and in part on associations between major tear proteins, lysozyme in particular.[38] The non-Newtonian viscosity of tears may be due to the presence of a loose aggregation of the lipocalin-lipid complex and other proteins at low shear rates, which is torn apart with higher shearing forces. This is thought to be a progressive, but reversible process.[38]

It is possible that alterations in the normal concentrations of tear film proteins, mucins and lipids, as seen in dry eye disease, may have a detrimental effect on tear film viscosity, although research in this area has been limited by the large sample volumes needed to obtain measurements. Tiffany (1991) has compared normal and mild dry eye individuals, and found no differences in their tear film viscosities.[158]

#### 1.3.4.3 Surface Tension

Pure water has a surface tension of approximately 72mN/m, whereas human tears have a surface tension which falls in the range of 40 to 46mN/m.[154] Although early theories proposed that tear film mucins were primarily responsible for the surface tension of tears, recent work has demonstrated that tear film surface tension is dependent upon the binding of lacrimal lipids to tear lipocalin, much like tear film viscosity.[159]

Current research regarding the effect of dry eye disease on tear film surface tension has yet to demonstrate any marked differences between individuals with and without the disease. Tiffany *et al*. (1989) found a broad spread of surface tensions in groups of normal and dry eyed individuals, with considerable overlap in results between the groups. Normal tears were generally found to be more surface-active (i.e. had lower surface tension values).[160] Reduced surface activity of tears (i.e. higher surface tension values), would effectively reduce the ability of tears to spread out and form a stable tear film following a blink, and may be a result of either a decrease in the lipocalin or lipid components of the tear film, or competitive binding by other types of lipids.[38] Holly *et al*. (1977) only found a slight decrease in surface activity (increase in surface tension) in patients with keratoconjunctivitis sicca and ocular pemphgoid,[161] while Showenwald *et al*. (1998) demonstrated that the elevated surface tension in dry eye was at least partially corrected by an increased output of other tear proteins.[106] Although the protein was not specifically named, it was shown that improvement in surface activity was associated with increased levels of lipocalin.[38, 106]

#### 1.3.4.4 Osmolality

On average, human tears have an osmolality of 305mOsm/Kg in patients without dry eye disease.[49, 162-165] Tear film osmolality is higher in patients who wear contact lenses or have dry eye disease.[2, 49, 98, 166-168] Tear film osmolality will be discussed in detail in the following section, as it is the emphasis of this review.

## 1.4 Osmolality

### 1.4.1 Definition of Osmometry:

Osmometry is a measure of solute concentration. It differs from the measurement of molarity, in that molarity is the measure of the number of moles of solute in a solution, while osmometry is a measure of the number of solute particles in a solution. Osmometry takes into account the disassociation of solutes in solution, irrespective of their size, density, molecular weight or electric charge.[169] Osmometry measurements are expressed as "Osm", which is pronounced "osmolar".

### 1.4.2 Definitions of Osmolarity and Osmolality

The terms "osmolarity" and "osmolality" are often used interchangeably, as they are both measures of osmometry, but in actual fact they are not interchangeable.

Osmolarity is a measure of the number of osmoles of solute per litre of solution (Osm/L or mOsm/L), [10] The difficulty with this term is that the volume of a solution can change as its temperature changes. Therefore osmolarity is rarely used when osmometry measurements are taken, as they are temperature dependent.

Osmolality on the other hand, is a temperature independent measure, as it is a measure the number of osmoles of solute per kilogram of solution (Osm/Kg or mOsm/Kg).[170, 171] This is typically the more commonly used measurement in osmometry, and is the correct unit of measurement for techniques such as freezing point depression osmometry, where the sample temperature is purposely altered during the measurement process.

Unfortunately, in the much of the literature available on tear film osmolality, both osmolarity and osmolality terms are used. This can cause confusion when comparing values between studies. Fortunately, when the concentration of solutes in a solution is very low, as it is in human tears, osmolarity and osmolality are considered to be equivalent.

Throughout the remainder of this work, the author will use the term osmolality to refer to all tear film osmometry measurements, except when citing work which has previously been published using the osmolarity term.

## 1.5 Measuring Tear Film Osmolality

When a solute is dissolved in a pure solvent, it depresses the freezing point of the solvent, raises its boiling point, increases its osmotic pressure and lowers its vapor pressure. Freezing points, boiling points, osmotic and vapor pressures are known as "colligative" or concentrative properties of solvents – when a solute is added to a solution, its colligative properties change, within reasonable limits, in direct proportion to the solute concentration. As instruments, osmometers are designed to detect and measure changes in one of these colligative properties, thereby enabling them to determine the concentration of dissolved particles of solute, or the osmolality, of a particular solution.[172] There are limitations to using each of the colligative properties for the measurement of osmolality, and different instruments have been developed to try and deal with these issues.

### 1.5.1 Freezing Point Depression Osmometry

The freezing point depression technique is based on the fact that the freezing point of pure water ($H_2O$) is precisely +0.010°C, and that one mole of a non-dissociating solute (a solute that remains intact and does not dissociate into ionic species) such as glucose, when dissolved in one kilogram of pure water will depress water's freezing point by 1.858°C. This value is known as the freezing point depression constant for water. If solutions are ionic, and they dissociate into their separate ionic species upon being dissolved in water, than the freezing point of water is depressed by 1.858°C for each ionic species. For example, a solution containing one mole sodium chloride dissolved in one kilogram of water would have a freezing point that was depressed by 3.716°C. This simple example assumes that complete dissociation of the sodium chloride into its constituent ions ($Na^+$ and $Cl^-$) occurred. In reality, dissociation is never complete and calculations must be adjusted by an osmotic coefficient factor.[172]

For simple solutions such as glucose or sodium chloride, reference tables can be used to determine the relative concentrations of each species, but in more complex solutions containing both ionized and non-dissociated species, it is not possible to easily determine the concentration of each specific solute. This problem is common to all of the colligative properties, but it can be addressed by using different calculations and units of measurement, specific to each colligative property. Unfortunately, values in different units are hard to compare, thus osmolality is used as a common unit for concentration measurements instead.[172]

#### 1.5.1.1 Clifton Nanolitre Osmometer

The Clifton Nanolitre Osmometer is designed to determine the antifreeze activity, or the thermal hysteresis, of a solution, which is the difference between the melting point and freezing points of that solution. It only requires a 200nL sample, but is an extremely complex instrument to use.

This instrument consists of a controller box, a cooling stage and a sample holder, but it cannot be used without additional micrometer syringes, immersion oils and microscopes, and an experienced technician. This instrument is no longer in production and has rarely been used in tear film studies which have taken place since the early 1990's.

#### 1.5.1.2 Advanced Instruments Model 3100 Tear Osmometer

The Advanced Instruments Model 3100 Nanolitre Osmometer is a freezing point depression osmometer that uses high-precision thermistors to sense the sample's temperature and to control the freezing process. When working with nanolitre samples, warming rates can be controlled with more precision than cooling rates, therefore the operating software of the Model 3100 Osmometer is designed to detect the sample melting point, rather than its freezing point. Through image analysis, the software is capable of detecting the exact point at which the sample changes from a solid to a liquid (i.e. the exact point when the last ice crystal in the sample has melted).[172] It requires a sample volume of 500nL, which makes it useful for working with tear samples.

### 1.5.2 Vapor Pressure Osmometry

Vapor pressure osmometry is based upon Raoult's law, which states that the vapor pressure of an ideal solution is dependent upon the vapor pressure of each chemical component and the mole fraction of each component present in the solution. The vapor pressure of each component, adjusted for the mole fraction of the component present, can be called its partial pressure. The sum of all of the partial pressures of the components of a solution is equal to the solution's vapor pressure. Therefore, as the number of components present in a solution increases, the individual partial pressures of each component decreases.[173]

Vapor pressure osmometers typically contain two chambers – one for pure solvent and the other for the test solution. Thermistors in each chamber provide an electrical signal, which is the actual measurement of the differential amount of heating required in each chamber to achieve vapor pressure equilibrium between the chambers.

#### 1.5.2.1 Wescor Vapor Pressure Osmometer

The Wescor Vapor Pressure Osmometer specifically, is based upon the concept of dew point temperature depression. Dew point temperature depression is a function of a solution's vapor pressure, thus it is in turn an indirect measurement of osmolality. The sensing element of the osmometer is a fine-wire thermocouple hygrometer. The Wescor Vapor Pressure Osmometer can be adapted for use with samples of various sizes, although the smallest sample size that can be used is 1000nL or 1μL. Samples take approximately 80s to run, and the osmolality reading is displayed in mOsm/Kg. [174]

### 1.5.3 Electrical Conductance

Electrical conductance is a measure of how easily electricity flows along a certain path through an electrical element. Electrical conductance is not a coligative property of solutions. Instruments developed based upon this principle are capable of measuring the resistance to flow of electricity in solutions and use the resistance values to calculate solution osmolality. The theory behind this principle is that increased concentrations of solutes dissolved in a solution would increase the amount of resistance to electrical flow in a particular solution. As of yet, very little information is available about how this technique works, as it is still very new, and instruments developed using this technique are only now starting to be tested.

#### 1.5.3.1 OcuSense

The OcuSense is a tear film osmometer which has been recently developed to measure tear film osmolality based upon the concept of electrical conductance. This instrument has the smallest required sample volume of any of the instruments discussed, as it only requires a sample of 50nL. This instrument is quite new and is still undergoing evaluation so there is very little information available regarding how it works.

## 1.6 Tear Film Osmolality

### 1.6.1 Tear Film Components Influencing Osmolality

Tear film osmolality is a product of the varying concentrations of dissolved solutes (proteins, lipids and mucins) in the tear fluid.[175] Tear film electrolytes such as sodium, potassium, magnesium, calcium, chloride, bicarbonate, and phosphate ions are also thought to be involved in the regulation of tear film osmolality.[149]

### 1.6.2 "Gold Standard" Theory

Tear film osmolality has been reported to be the "gold standard" diagnostic test for the evaluation of dry eye disease, as a distinct separation between tear film osmolalities in normal and dry-eyed populations has become evident.[1, 45, 49, 50] Research suggests that a hyperosmotic tear film is a trait common to all forms of dry eye, and it may be the driving force causing the discomfort, ocular surface damage and inflammation found in both evaporative and tear deficient forms of dry eye diseaes.[45] In light of new instrumentation becoming available, tear film osmolality is becoming a test that can be easily measured in a clinical setting on the majority of patients, and work with this tear film property has once again attracted significant amounts of research attention in recent years.

### 1.6.3 Clinical use of Tear Film Osmolality as a Diagnostic Test

Normal tear film dynamics, including the distribution, turnover and drainage, evaporation, and absorption of tears, require adequate tear production, retention on the ocular surface and balanced elimination.[176] Tear film osmolality measurements are thought to represent the end product of changes in tear film dynamics,[177] and this is one of the reasons that tear film osmolality is thought of as being an attractive index for dry eye diagnosis.[162] With the recent advent of relatively simple, easy to use clinical instrumentation (Advanced Instruments Model 3100 Nanolitre Tear Film Osmometer, OcuSense) for the measurement of tear film osmolality, the likelihood of this becoming a commonly used clinical test has increased significantly.

One of the challenges with any diagnostic test is to determine what is considered to be a normal value, and then what is a sensitive and specific cut-off value for classifying individuals as abnormal. Table 1.1 summarizes the results of the last thirty years of tear film osmolality research, and lists findings for both normal and dry eyed individuals, as well as individuals with other pathologies or contact lens wear. The average, normal tear film osmolality, calculated from all of these studies is approximately 306.7mOsm/Kg, although tear film osmolality has been shown to range from as low as 297mOsm/Kg[46] to as high as 318mOsm/Kg[165] for normal individuals. Some of the differences between the various reported values may be a result of the different instrumentation and collection techniques used.

Individuals with dry eye disease have been reported to have a tear film osmolality, on average, of 333mOsm/Kg, with a range of 313mOsm/Kg[178] to 365mOsm/Kg.[166] The struggle in setting a referent value for dry eye diagnosis comes from the significant overlap in tear film osmolality values that

occurs between 300-320mOsm/Kg for both normal and dry eyed individuals.[162] Originally, the cut-off value for dry eye disease was set at 312mOsm/L by Farris and Gilbard in order to provide the maximum sensitivity in diagnosis.[179] It was felt that some possible over-diagnosis was preferred to under-diagnosis of dry eye disease. Since then Craig has suggested using values over 320mOsm/L,[180] and Mathers and Choi suggested a referent of 318mOsm/L.[181] Mathers and Choi determined their referent value by the criterion of one standard deviation from the mean of a cluster analysis of patients with the condition.[178] Tomlinson has suggested using cut-off values in the range of 312-322mOsm/L,[182] and Sullivan has suggested anything over 318mOsm /L be considered as diagnostic of dry eye disease.[183] The most recent recommendation for a referent for dry eye disease was the 2008 report from Khanal *et al*, who proposed a cut-off value of 317mOsm/L.[184] In this most recent publication, tear osmolarity was determined to be the best single test for the diagnosis of dry eye, although a series of tests using a weighted comparison of tear turnover rate, evaporation and osmolarity was more effective.[184] Recent work with tear film osmolality as a diagnostic measure for dry eye is a direct result of the improved availability of instrumentation, which has made it feasible as a clinical measure. Currently, it appears that a cut-off value of approximately 316-317mOsm/Kg provides good accuracy in the diagnosis of dry eye disease. Research suggests that despite the good overall predictive power of tear film osmolality in separating dry eyed individuals from normal individuals, tear film osmolality will not be a good test for differentiating different types or severities of dry eye. Tear film osmolality, as an end stage measurement, will not be able to pick up the subtle changes in tear film dynamics that differentiate the various forms of dry eye disease.[162]

**Table 1-1: Summary of published literature investigating tear film osmolality in normal and diseased individuals (DE = dry eye, MGD = meibomian gland dysfunction, CL = contact lens). Reported units are those originally published. *1981 Farris, Stuchell & Mandell study reported in mOsm/L.**

| Publication | Instrument | Sample Size | Normal | | KCS/Dry Eye | | Other | |
|---|---|---|---|---|---|---|---|---|
| **Gilbard, Farris & Santamaria (1978)[169]** | Clifton | n = 61 (normal=31, DE=30) | 302 ± 6.3mOsm/L | | 343 ± 32.3mOsm/L | | Conjunctivitis 298mOsm/L | |
| **Terry & Hill (1978)[164]** | Thermocouple hygrometer | n = 6 | 310 ± 5.7mOsm/Kg | | | | Prolonged lid closure 285±2.4mOsm/Kg | |
| **Gilbard & Farris (1979)[166]** | Clifton | n = 20 eyes | | | 365 ± 77mOsm/L | | With treatment 329± 47mOsm/L | |
| ***Farris, Stuchell & Mandell (1981)[185]** | Clifton | n = 536 eyes (normal=219, DE=123, CL=194) | <41y/o | >40y/o | <41y/o | >40y/o | CL wear | |
| | | | 302±6 | 306±7 | 325±8 | 330±20 | 313±13 | 323±23 |
| | | | 303±8 | 306±7 | 337±16 | 330±11 | 317±30 | 310±15 |
| **Benjamin & Hill (1983)[165]** | Clifton | n = 6 | 318mOsm/Kg | | | | | |
| **Benjamin & Hill (1986)[186]** | Freezing point depression | n = 2 (502 total samples) | 315mOsm/Kg | | 331mOsm/Kg | | | |
| **Farris, Stuchell & Mandell (1986)[163]** | Clifton | n = 134 (normal=67, DE=67) | 305 ± 10mOsm/L (302 ± 5mOsm/L) | | 324 ± 11mOsm/L (324 ± 11mOsm/L) | | | |
| **Craig *et al*. (1995)[187]** | Clifton | n = 40 | 303.7 ± 22.9mOsm/Kg | | | | | |
| **Craig &Tomlinson (1995)[188]** | Clifton | n = 100 | 303.6 ± 13.0mOsm/Kg | | | | | |
| **Mathers, *et al*. (1996)[152]** | Freezing Point Depression | n = 55 (normal=34, DE=21) | 303 ± 10mOsm/L | | 313 ± 9mOsm/L | | MGD 314±10mOsm/L | |
| **Tomlinson and Khanal (2005)[162]** | Freezing Point Depression | n = 14 (DE= 8, MGD=6) | | | 323 ± 17mOsm/L | | MGD 321±12mOsm/L | |
| **Nichols & Sinnot (2006)[46]** | Advanced Instruments (200nL) | n = 360 (normal=161, DE=199) | Contact lenses 297.06± 31.82mOsm/Kg | | | | Contact lenses DE 307.66± 32.39mOsm/Kg | |
| **Khanal *et al*. (2008)[184]** | Freezing Point Depression | n = 73 (normal=32, DE=41) | 308.39 ± 9.29mOsm/L | | 328.71 ± 13.73mOsm/L | | | |

### 1.6.4 Effects of Age and Gender on Tear Film Osmolality

Various studies have looked at the effects of age on the tear film. Tear production has been thought to decrease with age,[189, 190] as does the reflex tear turnover rate,[191] tear film break-up times,[192, 193] and levels of the lacrimal gland proteins (lysozyme and lactoferrin).[194] Yet, tear volume does not appear to change with age when measured with either flurophotometry[195] or inferior tear prism height observations.[196] Tear evaporation rate also remains constant with increasing age.[197, 198]

Farris *et* al. (1981) was one of the first groups to investigate the effects of age on tear film osmolality,[199] although their results were inconclusive at best. No significant difference in tear film osmolality was observed between young (<40 years) or older (>41 years) male patients, nor was a significant difference found between young (<40 years) and older (>41 years) female patients with keratoconjunctivitis sicca (KCS). A difference between young (<40 years) and older (>41 years) normal female patients was observed.[199]

In 1995, Craig and Tomlinson designed a study to investigate the effects of age on tear film osmolality in a large sample (n=100) of gender-matched subjects chosen to cover the major decades of life (17-75 years).[188] In this study, males (307.1±14.4mOsm/Kg) were found to have a significantly higher ($p = 0.006$) tear film osmolality compared to females (300.1±10.4mOsm/kg). A correlation between age and tear film osmolality was not found when all of the subjects were considered together ($r^2=0.014$, $p=0.378$), although there was a significant difference in tear film osmolality between young women (<41years, 297.6±11.2mOsm/Kg) and older women (>41years, 304±6.7mOsm/Kg).[188] This led Craig and Tomlinson to conclude that age did not have a significant effect on tear film osmolality, but that there appeared to be a gender effect, at least in younger females. The difference in tear film osmolality between older (>41years) males and females was not significant.[188]

Work by Mathers *et al*. in 1996, has only confused things further. This group looked at a series of tear film tests including Schirmer test without anaesthetic, steady state tear flow using fluorophotometry, meibomian gland function based on gland drop-out, expressible lipid volume and viscosity, tear loss from evaporation, and tear osmolarity with respect to aging, and found a significant positive correlation (0.59) with tear film osmolarity measurements and aging in normal individuals.[200]

The effect of gender on tear film osmolality has been studied in depth, but unfortunately the collection of published research is as inconclusive as that regarding age. Originally Farris, *et al*.

(1981) observed no statistical difference in tear film osmolality between males and females,[199] although these subjects were not age matched, and data from both eyes were used in the analysis, which was thought to introduce significant statistical errors.[188, 201] In a later study by the same group, 12 males and 39 females were examined, and the tear film osmolality of the male group (306±4mOsm/Kg) was found to be significantly higher than the female group (301±4mOsm/Kg). There are obvious issues in sample sizes here, thus this same study looked at 7 normal males and females in a separate analysis and found there was no significant difference in tear film osmolality between the genders.[163]

Terry and Hill (1978) found that males (312±5.2mOsm/Kg) had higher tear film osmolalities than females (307±6.2mOsm/Kg), but this difference was not significant.[164] Unfortunately, only 3 subjects were enrolled in each group, and they were all young, healthy individuals, as such it is hard to extrapolate this data to a larger population.

Based on a review of the presented literature, there does not appear to be a significant effect of either age or gender on tear film osmolality, as the results are completely inconclusive.

### 1.6.5 Diurnal Variation of Tear Film Osmolality

Some commonly used clinical tests, such as intraocular pressure (IOP) measurements, have been shown to be affected by diurnal variations, and must be taken at multiple times over the day in individuals highly suspect for glaucoma.[202, 203] There has been a small amount of research undertaken in order to determine if tear film osmolality is affected by similar diurnal variations or not. However, most of this research has been focused on measuring diurnal variations in tear film proteins rather than in tear film osmolality itself.

The first study designed to specifically investigate diurnal variations in tear film proteins was published in 1972 by Pietsch and Peralmann. They used Schirmer strips to collect tears over a twenty-four hour period and studied lysozyme concentrations, but found no significant diurnal variation effect.[204] In 1978, Horwitz, *et al*., used capillary tubes to collect tears, and measured both sIgA and lysozyme levels over a 24 hour period. They also found no significant diurnal variation, but they did find that tear protein levels were significantly elevated between 0900 to 1200, significantly reduced between 2400 (midnight) and 0300.[205] Haggerty and Larke were again unable to find a significant diurnal variation in tear film total migrated proteins using gel electrophoresis and densitometry analysis in 1982.[206]

Huth *et al.* were the first to demonstrate a circadian rhythm in total protein concentration (TPC).[175] TPC was found to be at its highest (a twofold increase) after prolonged lid closure, as with sleep. TPC remained fairly stable during the hours of 0800 to 1700 though.[175] Sen and Sarin found significant variations lysozyme concentrations, but these were the lowest at 0600, and the highest at 2200,[207] which is opposite to the results of Horwitz *et al*.

Little tear protein analysis work was published after Sen and Sarin's work in1986, until Ng *et al*. measured TPC, immunoglobulins (sIgA), serum albumin, and regulated proteins (tear-specific prealbumin (TSP), lactoferrin) in human tears with gel electrophoresis and densitometry in 2000. They used two different sampling methods – a yawn-stimulated tear collection with a capillary tube, and an eye flush technique, but did not find any significant diurnal variations in any of the protein levels with either technique.[208] The only exception to this was in serum albumin levels using the eye-flush method, which were found to be significantly elevated between 1300 and 1900 in some participants, although in others they remained stable throughout the day.[208] They concluded that there is not significant diurnal variation in either TPC or any of the major tear protein levels (sIgA, lactoferrin, serum albumin, TSP and lysozyme).[208]

Other studies have looked at levels of tear enzymes (lactate dehydrogenase (LDH) and malate dehydrogenase (MDH)),[209] angiostatin levels,[210] and tear cytokines,[211] but the results of these works have been as ambiguous and those completed on tear film proteins.

The first study looking at diurnal variation of tear film osmolality was published in 1978 by Terry and Hill. In this study, they measured diurnal variations in tear film osmolality with a precision thermocouple hygrometer in six non-contact lens wearing subjects (three males, three females). Measurements were taken every hour between 9am and 10pm over a five day period from each of the subjects, and they were taken immediately upon eye opening (after a period of six to eight hours of sleep) in five of six subjects.[164] A 5µL sample volume was required for each measurement. Certain subjects were found to demonstrate a greater variability in their tear film osmolalities than others, but overall a significant diurnal variation in tear film osmolality was not noticed.[164] No differences between male and female subjects were noted, nor were any correlations with food and fluid intake detected.[164] Interestingly, the measurements taken immediately upon waking were found to be significantly lower than the measurements taken when the eye were open. It was suggested that a reduced rate of evaporation and tear clearance during eye closure was responsible for the a considerable decrease in the osmotic pressure of the tear film.[164]

Benjamin and Hill re-visited the concept of diurnal variation in tear film osmolality in 1982. This time they worked with a freezing point depression osmometer capable of measuring 200nL samples.[165] Six healthy young adults were enrolled in this particular study, and their tear film osmolalities were measured every 10minutes for 8.5hours.[165] An overall trend to increasing tear hypertonicity towards the end of the day was found and tear film osmolality was estimated to increase by approximately 1.43mOsm/Kg•$s^{-1}$. However, two subjects actually demonstrated a mild decrease in tear film osmolality over the day.[165] The smaller sample volume (200nL) was deemed to be advantageous in measuring tear film osmolality as it minimized the risk of reflex tearing and made the rapid collection of reliable samples feasible, thereby making it possible to collect samples at frequent time intervals without depleting the normal tear volume significantly.[165]

### 1.6.6 Effect of Contact Lens Wear on Tear Film Osmolality

Farris, Stuchell and Mandel were one of the first groups to look at tear film osmolality in contact lens wearers. They demonstrated that tear film osmolarity was elevated in patients who did not have dry eye symptoms but who wore either hard or extended wear soft contact lenses. On average, the tear film osmolarity in these particular patients was found to be between 310 – 323mOsm/L.[98, 199] This was significantly higher than in normal controls, while at the same time significantly lower than tear film osmolality values that had been reported in individuals with keratoconjunctivitis sicca.[98, 199] Unfortunately, the presence of a significant increase in tear film osmolality in patients who wore daily-wear soft contact lenses could not be confirmed.[98, 199]

Gilbard *et al*. investigated a potential mechanism for the increase in tear film osmolality in contact lens wearers in 1986.[167] They proposed that tear film osmolarity was elevated in contact lens wearers due the decreased corneal sensitivity associated with both hard and soft contact lens wear.[212-214] To investigate their proposed mechanism, they used a rabbit model and simulated the decreased corneal sensitivity caused by contact lens wear with 0.5% proparicane hydrochloride anaesthetic drops. The rabbits were found to have a significantly higher tear film osmolarity after the instillation of the drops.[167] It was proposed that the increase in tear film osmolarity was caused by a decrease in tear secretory rates as a result of reduced corneal sensitivity.[167] Although the proparicane hydrochloride drops could have been, at least in part, responsible for the increased tear film osmolality, investigators did not feel the effect of the drops was significant in this particular study.

In 2006, Nichols and Sinnott, completed a study looking at factors associated with dry eye disease in contact lens wearers. They found that contact lens wearers with symptoms of dry eye disease did

have a higher tear film osmolality (307.66mOsM) than contact lens wearers without symptoms of dry eye disease (297.06mOsM). Although neither of the tear film osmolality values were as high as those previously reported in the literature, Nichols and Sinnott felt this may have been caused by mild reflex tearing induced when patients removed their contact lenses prior to tears being collected for measurement.

The effect of contact lens wear on tear film osmolality measurements is in need of further investigation. Contact lens wear appears to increase tear film osmolality[46, 98, 199] and this may be due to a decreased tear secretion rate caused by a reduction in corneal sensitivity.[167]

## 1.7 Conclusion

Tear film osmolality is a product of the varying concentrations of the dissolved proteins, lipids, mucins and electrolytes in the tear fluid.[149, 175] It is elevated in patients with dry eye disease, and is believed to be one of the mechanisms driving patients' symptoms of discomfort and the ocular surface damage associated with this disease. [1, 45, 49, 50] Tear film hyperosmolality appears to be a common trait of both aqueous deficient and evaporative forms of dry eye disease,[45] and it is thought to be the end result of the various mechanisms associated with dry eye disease.[162] For these reasons, researchers have suggested that tear film osmolality could become the "gold standard" diagnostic test for the evaluation of dry eye disease.[45]

Historically, the measurement of tear film osmolality in a clinical setting has been limited by the lack of available, easy to use equipment. Most of the tear film osmolality research to date was completed with the Clifton Nanolitre Osmometer, which a complicated instrument to use, and is no longer in production. The recent development of new instruments for measuring tear film osmolality clinically, have driven the recent onslaught of research regarding this tear film property. One of the newest instruments available is the Advanced Instruments Model 3100 Tear Osmometer, which operates on the principle of freezing point depression osmometry. At present, very little information has been published regarding the capability of the Advanced Instruments Model 3100 Tear Osmometer to measure tear film osmolality. For this reason, some of the preliminary work completed in this thesis was developed specifically to evaluate the feasibility of using the Advanced Instruments Model 3100 Tear Osmometer as a clinical diagnostic instrument for the measurement of tear film osmolality.

Intraocular pressure measurements have been shown to be affected by a diurnal variation[202, 203] and there has been speculation as to whether or not tear film osmolality would be affected in a similar manner. Previous work with older instrumentation has shown that tear film osmolality measurements, unlike intraocular pressure measurements, do not appear to be affected by a diurnal variation.[164, 165] Unfortunately, these studies were completed on very small populations, and may not be applicable to larger groups, particularly when newer instrumentation is used. A section of this thesis has been designed specifically to investigate whether or not tear film osmolality displays a diurnal variation in a normal population. This research will be completed on a larger population than previously studied, using the newly available Advanced Instruments Model 3100 Tear Osmometer.

One of the greatest difficulties in dry eye research has been lack of association found between patients' symptoms, and the clinical signs of ocular surface damage observed by clinicians in dry eye disease.[61, 62, 215] The lack of association between tests makes it difficult to develop a universally accepted diagnostic criteria for dry eye disease. In hopes of shedding some light on this challenging situation, one of the studies in this thesis aims to investigate the relationships between tear film osmolality measured with the Advanced Instruments Model 3100 Tear Osmometer, and other commonly used clinical tests of dry eye disease. The commonly used clinical tests of dry eye disease studied include various questionnaires designed to evaluate patient symptoms in conjunction with an assortment of clinical tests developed for the assessment of the tear film and the ocular surface.

Finally, the availability of new clinical instrumentation raises the question of whether or not the increase in tear film osmolality associated with dry eye disease will still be measureable. Therefore the final section of this thesis is devoted to the measurement of tear film osmolality in patients symptomatic of dry eye disease, and comparing these results with the measurements of tear film osmolality in a normal control population. Although research also suggests that there may be an elevation in tear film osmolality associated with contact lens wear, this will not be investigated in this thesis. The effect of contact lens wear on tear film osmolality was felt to be beyond the scope of this particular project, therefore it will be investigated at a later date.

The recent re-definition of dry eye disease in 2007, and the advent of new instrumentation which is makes the clinical measurement of tear film osmolality feasible, makes this a prime time for the study of tear film osmolality in both normal and dry eyed populations. Although many questions remain regarding tear film osmolality in both normal and diseased populations, the author will only be able to attempt to answer a few of them with this thesis. It is this author's dearest hope that the work

conducted in this thesis will act as a starting point to guide future investigators work with the measurement of tear film osmolality.

# Chapter 2
# Materials and Methods

In this chapter, participant involvement, the procedures conducted during the study visits and the instruments used will be described in detail.

## 2.1 Informed Consent

Informed consent was obtained from all participants prior to the commencement of all of the studies completed. All of the studies described in this work received approval from the Office of Research Ethics at the University of Waterloo, Waterloo, Ontario, Canada (ORE#'s 12350, 13990, and 14862).

## 2.2 Dry Eye Questionnaires

Participants were asked to complete an assortment of questionnaires as part of the various studies included in this thesis. The questionnaires used included the Single Item Dry Eye Questionnaire (SIDEQ),[1] the Ocular Surface Disease Index (OSDI),[2, 3] and the McMonnies Questionnaire,[4, 5] which have been previously validated using pre-defined scoring systems. The scoring systems enable participants dry eye symptoms to be classified depending upon their severity (none, mild, moderate or severe). The SIDEQ, OSDI and McMonnies questionnaires were used primarily to assess the level of participants' ocular comfort or discomfort, although the SIDEQ was used in the final study (Chapter 7) to help classify participants into normal and dry eye groups.

### 2.2.1 Single Item Dry Eye Questionnaire (SIDEQ)

The SIDEQ is a single item, self assessment questionnaire that allows participants to rate their ocular surface comfort on a 0 to 4 scale.[1] A score of "0" corresponds to no discomfort or dry eye disease, while a score of "4" corresponds to severe symptoms of ocular surface discomfort, often associated with advanced dry eye disease.[1] (Appendix 1)

### 2.2.2 Ocular Surface Disease Index (OSDI)

The OSDI is a 12-item quality of life questionnaire designed to measure the severity of ocular surface disease, and its impact on vision related functions.[2, 3] Participants are asked to evaluate each of the items on the instrument on a 5-point Likert scale (all of the time = 4, most of the time = 3, half of the time = 2, some of the time = 1, none of the time or not applicable = 0), over a recall period of the last

week. The 12 items are divided into three subgroups - "Ocular Symptoms", "Vision-Related Functioning", and "Environmental Triggers". Individual question scores are summed, and that value is plugged into the following formula, which can be used to calculate an overall score (0-100):

$$\text{OSDI Score} = \frac{\text{(Sum of scores X 100)}}{\text{(Total number of questions X 4)}}$$

The higher a participants score, the greater the disability they experience.[2, 3] Scores of 0-12 are considered to be normal, 13-22 indicative of mild dry eye, 23-32 of moderate dry eye, and 33-100 of severe dry eye disease. (Appendix 2).McMonnies Questionnaire

The McMonnies Questionnaire is made up of 15 questions, 14 of which focus on clinical "risk factors" for dry eye disease, which have been derived from the literature. The "risk factors" include, age, gender, contact lens history, dry eye symptoms, previous dry eye treatments, secondary symptoms (associated with environmental stimuli), medical conditions associated with dry eye syndrome (arthritis, Sjögren syndrome, thyroid diseae), dryness of various mucous membranes (mouth, throat, chest, or vagina), and medication use.[6] It uses a weighted-scale scoring algorithm, where each possible answer has been given a scoring value between 0 and 6; these values are summed together to obtain an overall "Index" score. The Index score can fall between 0 and 45; higher scores are indicative of greater dry eye disease.[5] The instrument has a recommended cut-point of 14.5 for dry eye.[4, 5] Index scores can also be used to categorize participants based on their severity of dry eye disease. When used in this way, a score of 0-10 is considered to be normal, a score of 11-19 suggestive of borderline dry eye, and a score of 20 or greater, indicative of dry eye disease (Appendix 3).[4, 5]

### 2.2.3 Analogue Scales for Comfort Assessment

Although, not validated, another way to assess participant ocular surface comfort is through the use of linear analogue scales.[7] These scales are simple linear scales from 0 to 100, and participants were asked to record on the scale, the comfort of their eyes at that particular moment. 0 was indicative of "complete" ocular surface discomfort, while 100 was representative of "complete" ocular surface comfort. Scores can be expressed as a percentage of ocular surface comfort.

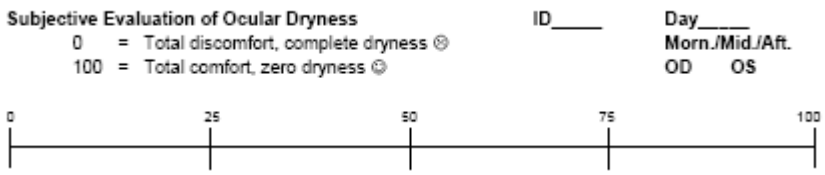



**Figure 2-1: Linear analogue scale for the assessment of patient comfort.**

Some or all of these questionnaires were used throughout the following studies to gain a better understanding of participant's levels of ocular surface comfort. Comfort scores from all of the questionnaires were compared with various clinical signs of dry eye disease, to determine if any correlations existed between participant symptoms and clinical signs (Chapter 6). The SIDEQ questionnaire was also used in one study to classify participants into dry eyed and non-dry eyed groups (Chapter 7).

## 2.3 Tear Film Collection

Tear samples were collected from the inferior-temporal meniscus from either one or both eyes of participants, depending on the specific study design. They were collected with a single use disposable capillary tube (Figure 2.2), without the use of corneal anaesthesia. In order to minimize the stimulation of reflex tearing, care was taken to ensure that the lid margin and corneal surface were not touched. Tear samples were never pooled for analysis; all analysis was done as soon as possible after tear film collection and samples were never stored overnight.

Two capillary tubes were used for tear collection - a disposable, flexible polycarbonate capillary tube (Advanced Instruments Inc, Norwood, MA, USA) was used in the initial pilot studies (Chapter 3, Chapter 5), and a disposable 5µL glass capillary tube (Drummond Scientific Company, Broomall, PA, USA) was used in later studies for reasons explained below.

The Advanced Instruments polycarbonate capillary tube is designed specifically to work with the Advanced Instruments Tear Osmometer Nanodispensing Sampler (Advanced Instruments Inc, Norwood, MA, USA). When tears were collected using this technique, they were transferred directly to the Advanced Instruments Model 3100 Nanolitre Osmometer sample loading tip, for the measurement of tear film osmolality.

The polycarbonate capillary tubes have very narrow bore holes, and did not efficiently draw tears, which can increase the risk of inducing reflex tearing, therefore the glass capillary tube with a larger

diameter (Drummond Scientific Company, Broomall, PA, USA) was used instead during the later studies (Chapter 4-7).

Initially tears were collected with participants seated at a biomicroscope (Chapter 3, 5), but it was felt that this too had the potential to stimulate reflex tearing, so the procedure was modified slightly, with tear collections being taken while participants where reclined in a chair, without the use of a biomicroscope.

Tears collected with the glass capillary tube (Drummond Scientific Company, Broomall, PA, USA) had to be aliquoted into a small 0.2mL polymerase chain reaction (PCR) tube (Axygen Scientific Inc., Union City, CA, USA) prior to being transferred to the Advanced Instruments Model 3100 Nanolitre Osmometer sample loading tip. In order to minimize the effects of evaporation on the small tear samples collected, all of the PCR tubes were chilled prior to use.

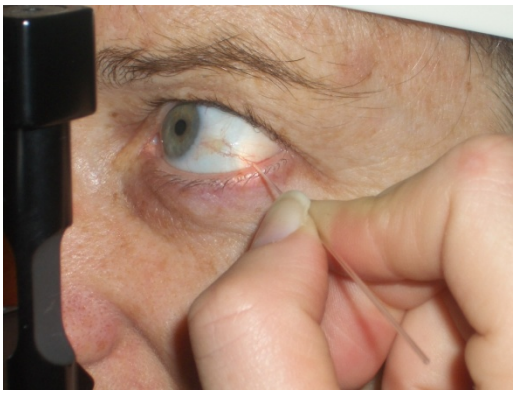


**Figure 2-2: Tear film collection at a slit lamp with a disposable polycarbonate capillary tube.**

### 2.3.1 Tear Film Osmolality

Once collected, tear samples were transferred with the Advanced Instruments Tear Osmometer Nanodispensing Sampler, either directly from the capillary tubes, or from the PCR tubes, to a sample loading tip designed specifically for the Advanced Instruments Model 3100 Nanolitre Osmometer (Figure 2.3).

The Advanced Instruments Model 3100 Nanolitre Osmometer (Figure 2.4) is a freezing point depression osmometer that uses high-precision thermistors to sense the sample's temperature and to control the freezing process. When working with nanolitre samples, warming rates can be controlled with more precision than cooling rates, therefore the operating software of the Model 3100 Osmometer is designed to detect the sample melting point, rather than its freezing point. Through

image analysis, the software is capable of detecting the exact point at which the sample changes from a solid to a liquid (i.e. the exact point when the last ice crystal in the sample has melted).[8]

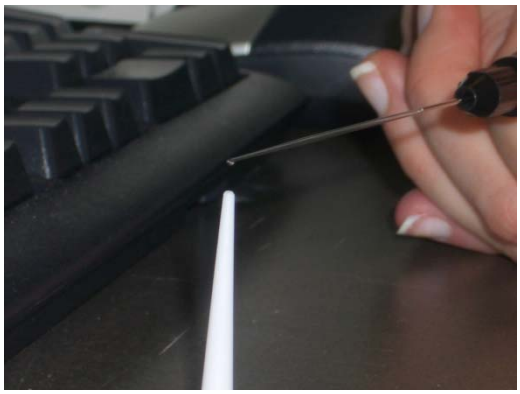


**Figure 2-3:Transfer of tear sample from capillary tube to the Advanced Instruments Model 3100 Nanolitre Osmometer sample loading tip.**

**Figure 2-4: Advanced Instruments Model 3100 Nanolitre Tear Osmometer.**

### 2.3.2 Tear Ferning

Tear Ferning is simple, quick technique that provides practitioners with information regarding the quality of a patients tear film.[9, 10] It is performed by taking a small (0.3µL) droplet of a tear sample, placing it on a clean, grease-free microscope slide, and then leaving it to dry at room temperature for five to seven minutes. It is during this time that the tear components (proteins, lipids, and salts) will trigger the formation of crystallization patterns, or tear ferns. The tear ferning patterns can be microscopically examined and photographed at a magnification of x40 to x100, and their quality graded on a 1-4 scale. A grade of 1 or 2 is considered to be indicative of a normal tear film, and a grade of 3 or 4 is indicative of an abnormal tear film or dry eye disease (Figure 2.5).[9, 10]

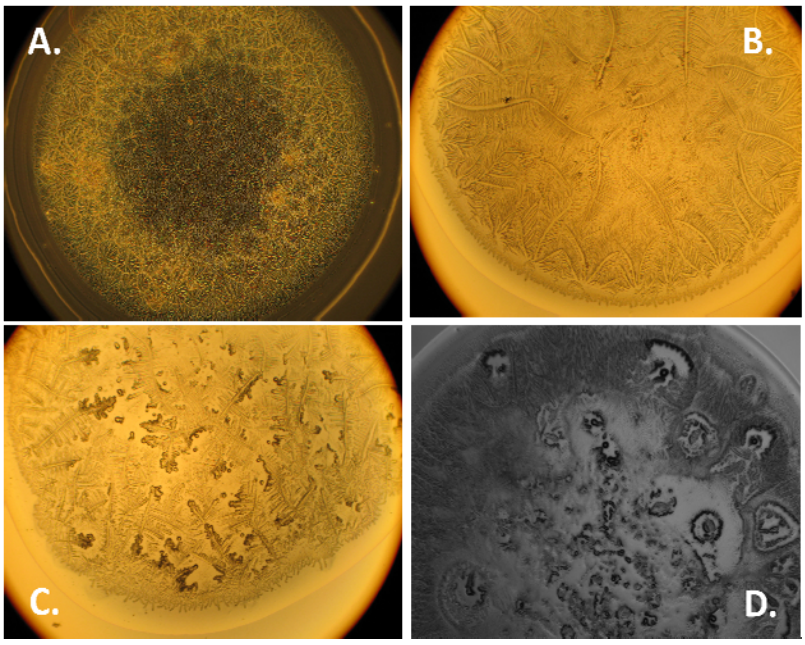



**Figure 2-5: Tear ferning images. Examples of grades 1(A), 2(B), 3(C) and 4(D) are shown below. Grade 1 and 2 ferning patterns are found in individuals who do not have dry eye disease, while grade 3 and 4 patterns are associated with an abnormal tear film or dry eye disease.**

## 2.4 Tear Film Stability

Non-invasive tear break-up time (NITBUT) was measured with the Atlas Topographer, (Carl Zeiss Canada Ltd. Toronto, ON, Canada). The Atlas Topographer is a placido disk topographer, meaning that concentric rings of light are projected onto the cornea, and then their reflection is observed and imaged with a CCD camera. When used to measure NITBUT, the observer watches the reflected concentric rings, looking for the first sign of distortion or disruption in their pattern (Figure 2.6). This is considered to be equivalent to a disruption of the tear film surface, or tear film break-up.[11] The time to the first disruption of the image is measured in seconds, to the nearest 0.1 seconds.

The chin rest and head rest of the instrument were cleaned using alcohol swabs (Isopropyl alcohol 70%, Becton and Dickinson Canada Inc. Oakville, Ontario) prior to each series of measurements. Participants were asked to blink completely three times prior to each measurement, and three measurements were taken per eye. The mean values for the left and right eyes were calculated and reported.[11]

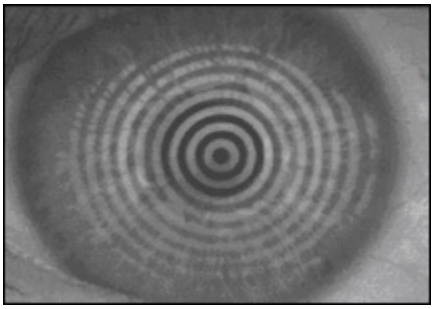


**Figure 2-6: Placido disk image projected onto a corneal surface for the measurement of non-invasive tear break-up time.**

## 2.5 Ocular Surface Redness

Ocular surface redness, or bulbar hyperaemia, was measured both subjectively and objectively.

### 2.5.1 Subjective Grading

Subjective redness measurements were made by one of two experienced clinicians. All measurements were taken from the temporal bulbar conjunctiva of the right and left eyes using a biomicroscope. The chin rest and head rest of the biomicroscope was cleaned prior to use with an alcohol swab (Isopropyl alcohol 70%, Becton and Dickinson Canada Inc. Oakville, Ontario). Participants were initially asked to look straight ahead, and then to direct their gaze to either the left (for right eye measurements) or right (for left eye measurements).

Temporal bulbar redness was graded based upon a modified CCLRU 0-100 scale, where 0 was considered to be negligible redness, and 100 was considered to be severe redness (25 was trace, 50 was mild, and 75 was moderate).[12-14] The participant's gaze was directed appropriately to permit hyperaemia grading of the temporal bulbar conjunctiva of both eyes.

### 2.5.2 Photometry

Objectively, temporal bulbar redness was determined using the SprectraScan PR650© Spectrophotometer (Photo Research Inc., Chatsworth, CA, USA) (Figure 2.7). This instrument is a table top device which measures luminance and chromaticity values through the measurement of absolute intensity at each wavelength of light and then uses these values (luminance and chromaticity) to calculate the equivalent CIE u' (Commision Internationale d'Eclairage) value.[14, 15] u' is one of two chromaticity coordinates (u', v') used to describe the position of a colour in the CIE

colour space diagram (1976) and does not have a specified unit. A higher u' value has been shown to be equivalent to greater bulbar conjunctival redness in previous studies.[14, 15]

Prior to measurements being taken, the instrument chin and head rests were cleaned using an alcohol swab (Isopropyl alcohol 70%, Becton and Dickinson Canada Inc. Oakville, Ontario). Participants sat at the photometer, and their head position was adjusted until it was aligned with the photometer. They were asked to look at fixation lights on either their left or their right, in order to align their temporal bulbar conjunctiva with the instrumentation. Looking through the eye piece, the examiner positioned a black circle (with an area of approximately 19.63mm$^2$) over the area of the temporal conjunctiva that was measured. The area measured in this study was approximately 2mm from the temporal limbus, and centred vertically between the upper and lower lids, on the temporal bulbar conjunctiva (Figure 2.8).

The spectrophotometer was not turned on until just before measurements were taken, and it was turned off immediately after, in order to minimize the amount of time the ocular surface was exposed to the heat given off by the bulb, as the heat increases tear film evaporation. Although previous studies have taken three separate photometry measurements and then averaged them to obtain a value, only one measurement was taken per eye at each visit in our study, as it was felt that multiple measurements had the potential to increase tear film evaporation un-necessarily, there-by interfering with the other tear film measurements being taken at the same time.

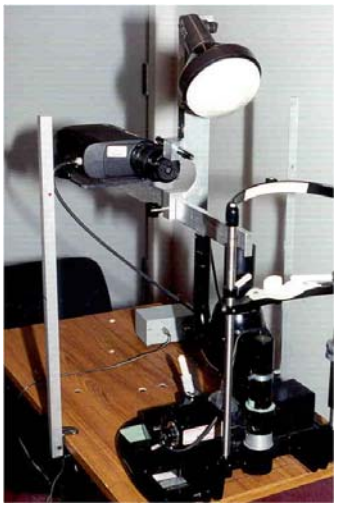


**Figure 2-7: SprectraScan PR650© Spectrophotometer (Photo Research Inc., Chatsworth, CA, USA).**

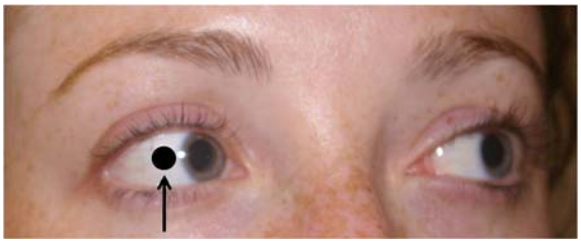


**Figure 2-8: Alignment of measurement area on the temporal bulbar conjunctiva of participants, as seen by the investigator, for the measurement of bulbar conjunctival redness (arrow).**

## 2.6 Phenol Red Thread Test

The Phenol Red Thread (PRT) test (ZONEQUICK, Showa Yakuhin Kako Co., Ltd. Tokyo, Japan), was used in the final study discussed in Chapter 7, as a diagnostic test for dry eye disease. This test was used to measure tear volume in all of the participants. Phenol Red Threads change colour from yellow to red when they are wet by human tears. The length of the thread that changes colour within 15 seconds of exposure to the tear fluid (measured in millimeters) is an indicator of tear volume.

The threads are packaged in pairs, in sterilized packets, and have a bend in them 3mm from one end. Participants' lower lids are gently pulled down, and the bent end of the thread is placed about 1/3 of the distance from the lateral canthus, as shown in Figure 2.9. Participants are asked to look straight ahead and blink normally for 15 seconds, at which time the threads are carefully removed, and the length of the area of colour change is measured. One eye was tested at a time.

As this test was done only for diagnostic purposes, to assist a clinician classify individuals with dry eye disease, the results of this test are not included in this thesis.

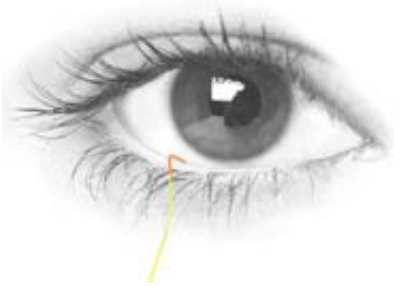


**Figure 2-9: Phenol Red Thread placed 1/3 of the distance from the lateral canthus for measurement of tear film volume.**

## 2.7 Corneal Staining

Corneal staining was a diagnostic test used to differentiate normal and dry eyed participants in the study presented in Chapter 7. Sodium fluorescein (NaFl) ophthalmic strips (Fluorets®, Bausch & Lomb) were used for this procedure in all participants. The strip was wet with a saline (Bausch & Lomb Sensitive Eyes Saline, Bausch & Lomb), and the dye strip was then touched to the lower tarsal conjunctiva, with care being taken to avoid touching the surfaces of both the cornea and bulbar conjunctiva.

Corneal staining was assessed over the entire corneal surface in three separate categories: depth, extent and type. In each of these categories staining was graded on a 0-100 scale, where a grade of zero meant there was no corneal staining observed. Each of the three categories was graded individually, and then the grades were summed as explained in detail in Appendix 4, to give a total corneal staining score.

At the time corneal staining was assessed, the clinician assigned a grade of staining to each of the 5 sectors (nasal, temporal, superior, inferior and central) and then used this plus other criteria to decide if the subject was positive for dry eye or not.

A CCLRU Photographic Scale[16] was used as a reference, and the clinician was asked to grade both the severity and type of staining (superficial punctuate, macropunctate, coalescent patch, etc) present, and to estimate the area of each zone affected. Severity was graded using a scale of 0 (negligible fluorescein staining) to 100 (severe fluorescein staining), while the area of staining in each zone was recorded as a percentage of 1-100%. A score of 100% indicated a zone that was stained over the entire extent of the zone, while a score of "0" indicated that no staining was present in that zone.

A global staining score was calculated from these values (Appendix 4), but at the time the decision regarding the patients' status as being positive for dry eye or not, the clinician did not know the participants' final global staining score. The clinician was asked to make a clinical judgment instead (Figure 2.10). As the clinician had previous experience in the examination of patients with dry eye disease, they were deemed capable of making this decision. Only one clinician performed all of the examinations, eliminating potential inter-observer biases in the assessment of corneal staining.

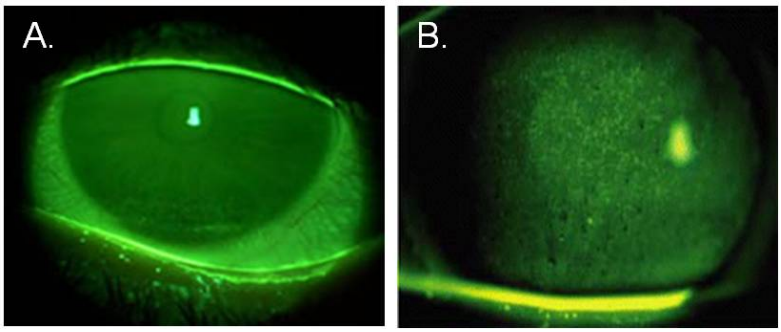



**Figure 2-10: Corneal staining examples of what was considered to be (A) corneal staining present in an individual without dry eye disease, and (B) corneal staining present in an individual with dry eye disease.**

This test was done purely for diagnostic purposes, to assist a clinician differentiate between individuals with and without dry eye disease, therefore this data is not included in this thesis.

# Chapter 3
# Measurement of Tear Film Osmolality in a Normal Population

## 3.1 Introduction

One of the greatest challenges to overcome when using new clinical diagnostic instrumentation is to determine if the instrument measures what it was designed to. The Advanced Instruments Model 3100 Nanolitre Osmometer is a freezing point depression osmometer that was designed to measure human tear film osmolality. It uses a sample volume of only 0.5µL, which is small in comparison with most other commercially available osmometers, which require samples of 5-10µL.[1] This small sample size is of particular importance, as the volume of human tears present on the ocular surface of a normal individual is approximately 7µL.[2] In individuals with dry eye disease, the volume of tears is further reduced.[3]

The larger the sample volume that is required by an osmometer, the more difficult it becomes to collect a tear sample without inducing reflex tearing. Reflex tearing is a source of significant measurement error, as it can lead to artificially low osmolality measurements. However, the smaller sample volumes can be more susceptible to other errors such as evaporation, which can occur during the sample transfer and loading processes. Evaporation of the sample can lead to artificially high osmolality measurements, and needs to be minimized as well.

The purpose of this study was to determine if the Advanced Instruments Model 3100 Nanolitre Osmometer is capable of quantitatively measuring tear film osmolality in a population, using 0.5µL samples.

## 3.2 Methods

The protocol for this study was approved by the Office of Research Ethics at the University of Waterloo (ORE# 12350), prior to the commencement of the study, and informed consent was obtained from all participants. 40 volunteer participants were recruited from the students, staff and faculty, at the School of Optometry, University of Waterloo, and enrolled in this study.

### 3.2.1 Criteria for Participation

The only requirements for participation were that participants were at least 18 years of age, had signed an informed consent, and had not used any artificial lubricants for at least 6 hours prior to any of their study visits.[4]

### 3.2.2 Study Procedures

Tear samples (0.5-1.0μL) were collected at each of six separate visits, from each participant. The tear samples were collected using a single use, disposable, flexible polycarbonate capillary tube (Advanced Instruments, MA), from the inferior temporal meniscus of the left eye. Tear samples were collected without anaesthesia, while participants were seated at a biomicroscope (Figure 2.2). Care was taken to ensure that the lid margin and corneal surfaces were not touched, and participants were asked to look in a superior-nasal direction to further protect the corneal surface.[4]

Tear samples were transferred to the Advanced Instruments Model 3100 Nanolitre Osmometer immediately after collection, in order to minimize evaporation. Tear film osmolality was subsequently measured.

### 3.2.3 Instrument Calibration

The calibration of the Advanced Instruments Model 3100 Nanolitre Tear Osmometer was checked daily. The osmolality of a 304mOsm/Kg standard reference solution (Advanced Instruments Inc, Norwood, MA, USA) was measured a minimum of three times per day, whenever the instrument was used. The calibration of the machine was considered to be acceptable if the mean ± standard deviation of the reference samples were within 304 ± 4mOsm/Kg. If the mean ± standard deviation of the reference samples did not fall within this range, than the instrument was immediately recalibrated before any tear film samples were measured.

### 3.2.4 Statistical Analysis

All data was pooled and the population mean and standard deviation were calculated.[4] All graphing analysis was completed using Graph Pad Prism 5 Software (Graph Pad Software Inc., www.graphpad.com).

## 3.3 Results

As an instrument, the Advanced Instruments Model 3100 Nanolitre Osmometer was relatively simple to use. A significant potential source of error in its operation is in the loading of the tear samples. However, the technique required for this is fairly easy to learn. Once operators are proficient in sample loading, the instrument becomes much easier to use, and the potential for error is markedly decreased.

A sample takes approximately 10-15minutes to run once loaded, depending on it's osmolality. Samples with higher osmolalities melt faster, thus they typically take less time to measure. The Advanced Instruments Model 3100 Nanolitre Osmometer uses a video imaging system to dynamically observe the sample during the measurement process. The instrument software has been designed to detect the initial freezing point of the instrument and its final melting point. Initially when a sample is loaded it appears relatively clear (Figure 3.1A), but as it cools, less light is transmitted through the sample and the image gradually becomes darker (Figure 3.1B). When the sample is completely frozen the image appears to be completely black because it is not possible for light to pass through the frozen sample (Figure 3.1C). The software recognizes the time point when light is no longer being transmitted through the sample as the freezing point of the tears. The gradual warming (and subsequent melting) of the sample is initiated at this time. As the sample melts, ice crystals can be seen to be moving around in the image display (Figure 3.1D,E) – the software system monitors the movement of the ice crystals and calculates the melting point of the sample when movement is no longer detectable (i.e. the sample has completely thawed) (Figure 3.1F).

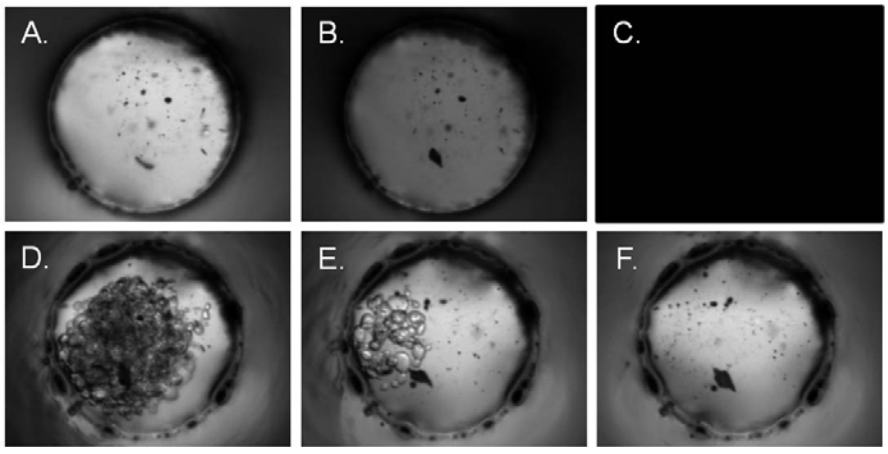



**Figure 3-1: Sample display images used by the Advanced Instruments Model 3100 Nanolitre Tear Osmometer in the calculation of tear film osmolality.**

In the various tear samples analyzed, there were visible differences between the appearances of some samples. Some tear samples were completely transparent (Figure 3.2A), while others had varying levels of debris (Figure 3.2 B,C), which was thought to be due to environmental factors such as dust and make up, or to various tear film components, such as mucins and proteins. Occasionally, a tear sample would appear to be hazy, almost as if there was a film on its surface (Figure 3.2B). Upon biomicroscopic examination of participants whose tears had such an appearance, investigators noticed that these participants had particularly oily tear films. Therefore, investigators postulated that the haze visible in the osmometer images was due to the presence of high levels of tear film lipid in the sample. The imaging software had difficulty detecting the freezing point of the hazy samples, as the difference in light transmission between the frozen and un-frozen samples was not always obvious, and could not easily be detected by the instrumentation.

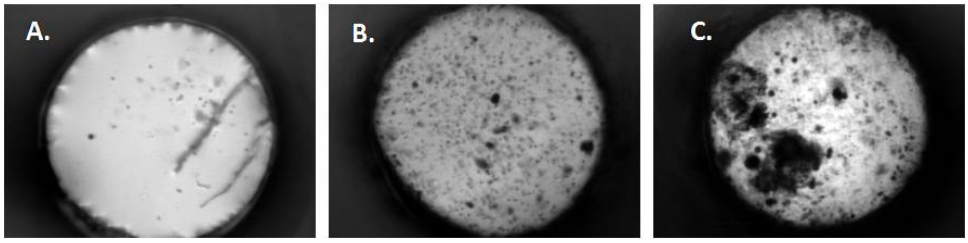



**Figure 3-2: Appearance of various tear samples analyzed with the Advanced Instruments Model 3100 Nanolitre Osmometer; A) a clear sample, B) a sample with small amounts of debris, and C) a sample with large pieces of debris.**

All of the participants (10 males and 30 females aged 18-56 years old) completed the study. Contact lens wear was permitted during this study, and there were 22 non-contact lens wearers involved, 16 soft contact lens wearers, and 2 gas permeable contact lens wearers.

Of the 240 individual tear samples collected, 12 samples (5% of the total samples taken) were lost during the sample loading or measurement processes. Reasons for this included poorly loaded samples, software crashes, or an inability of the optical system of the instrument to properly detect the freezing or melting point of the samples.

The mean tear film osmolality of the 228 remaining samples was 298.7±11.4mOsm/Kg (range: 284.0 – 312.0mOsm/Kg) (Figure 3.2). The population was sub-divided into whether they wore contact lenses and their lens type. All types of contact lens wear (soft lenses and gas permeable lenses) were grouped together, and compared to the non-contact lens wearing group. The non-contact

lens wearing group had a tear film osmolality of 298.9±11.5mOsm/Kg (range: 286.2 – 312.0mOsm.Kg) and the contact lens wearing group had a tear film osmolality of 298.5±11.2mOsm/Kg (range: 284.0 – 307.6mOsm/Kg). There was no significant difference between the tear film osmolalities between the non-contact wearing group and the contact lens wearing group ($p>0.05$). There were no significant differences in the mean tear film osmolalities of either group compared to the total population mean either ($p>0.05$) (Figure 3.2).

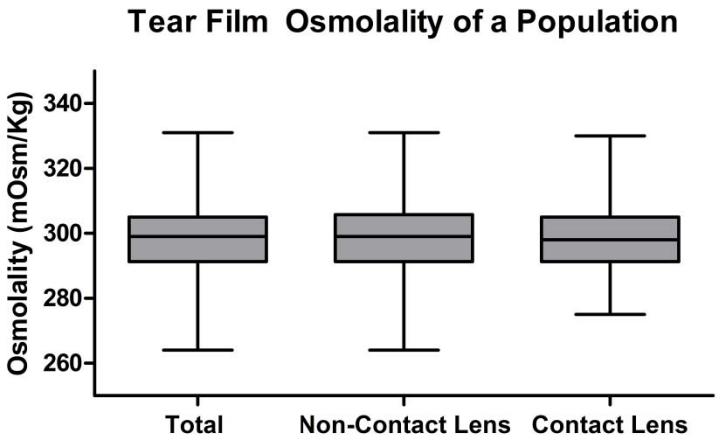



**Figure 3-3: Tear film osmolality measured with the Advanced Instruments Model 3100 Nanolitre Osmometer. Groups include: total population, non-contact lens wearers, and contact lens wearers. Boxes indicate population means; error bars indicate max and min values.**

## 3.4 Discussion

Tear film osmolality in a normal population is reported to be approximately 305mOsm/Kg, but reported values fall in the range of 297 – 318mOsm/Kg.[3, 5-14] The tear film osmolality of this particular population sample was 298.7 ± 11.4mOsm/Kg (range: 284.0 – 312.0mOsm/Kg). There was no significant difference between tear film osmolality of the contact lens wearers and non-contact lens wearers. The mean tear film osmolality measured in this study is lower than the reported mean in previous studies, but it still falls within the range of previously reported values. Therefore, it appears that the Advanced Instruments Model 3100 Nanoliter Osmometer is capable of measuring tear film osmolality using a 0.5µL tear sample.

One possible explanation for the lower than average tear film osmolality value measured in this study is that reflex tear samples may have been collected instead of basal tear samples. The solute

concentration of reflex tears is lower than it is in basal tears, as it is diluted by the large amount of aqueous fluid created during the process of reflex tearing. The bright light of the biomicroscope has the potential to stimulate the production of reflex tear by increasing the evaporation of basal tears from the ocular surface.[1] Reflex tearing can also be stimulated during the tear collection process by touching the eyelids or the conjunctival surface. Although every effort was made by the investigator to avoid inducing reflex tearing, this was not always possible. The investigator was trained in tear collection prior to the commencement of this study, but they did not have a vast amount of experience with the procedure. The lack of experience of the investigator may have lead to the reflex tearing in some individuals as well.

Much of the previous research measuring tear film osmolality was preformed with instruments that required sample volumes of 5-10μL.[1] With sample volumes this large, it is possible for significant tear film evaporation to occur from the ocular surface, providing that reflex tearing has not occurred. During the process of collecting basal tear samples, participants are required keep their eyes open for relatively long periods of time while upwards of 70% of the tear volume (approximately 7μL[2]) is collected. Evaporation primarily effects the aqueous component of the tear film and causes a relative increase in the tear film solute concentration which in turn can artificially increase tear film osmolality. Theoretically, smaller samples should take less time to collect, thus being less affected by evaporation of during the collection process, and may have lower osmolality values as a result. As more instruments capable of measuring tear film osmolality in small samples become available, it is plausible that the reported normal tear film osmolality could decrease. Further investigation is needed to determine if reflex tearing was a significant factor in the lower than normal tear film osmolality found in this population, or if the lower than normal tear film osmolality measured is merely a result of improved measurement techniques.

As stated previously, the sample process time for the Advanced Instruments Model 3100 Nanolitre Osmometer is approximately 10-15minutes. The instrument is fairly easy to use, once the loading technique is mastered, and it does not require a highly trained laboratory technician to obtain measurements. One drawback of the Advanced Instruments Model 3100 Nanolitre Osmometer is that its sample process time is longer than some of the other commercially available, larger volume osmometers. The ability of the Advanced osmometer to measure small sample volumes outweighs this drawback though, as a small sample size is of huge clinical benefit, especially when working with individuals who have dry eye disease.

5% of the samples collected were lost due to either loading errors or software issues, but this is not unreasonable. Loading issues became less of a problem as the experience of the individual using the instrument increased. Newer versions of the software are being developed as well, in hopes of addressing some of the problems currently experienced with the technology. Hopefully, with increased experience and improved software, the percentage of samples lost will be further decreased.

Considering its ability to measure small sample volumes, and its relative ease of use, the Advanced Instruments Model 3100 Nanolitre Osmometer is a valuable clinical instrument for the determination of tear film osmolality. Further work needs to be done to examine the repeatability of the instrument and the reproducibility of measurements over multiple days. The stability of tear film osmolality over the course of a day needs to be examined, as do the relationships between tear film osmolality, ocular surface comfort and other clinical tests used in the examination of the tear film in the diagnosis of dry eye disease. Finally, more work designed to investigate differences in tear film osmolality between normal and dry eyed populations is also necessary.

# Chapter 4
# Repeatability of the Advanced Instruments Model 3100 Nanolitre Osmometer

## 4.1 Introduction

It is important to determine if measurements obtained with new clinical diagnostic instrumentation are repeatable. Repeatability is a measure of the variability in the results of multiple measurements taken by the same instrument (or person), under the same conditions, on the same sample. The lower the variability that exists within the results, the higher the repeatability of the procedure.

The Advanced Instruments Model 3100 Nanolitre Osmometer is capable of measuring human tear film osmolality in a normal population using a sample volume of only 0.5µL, as shown in Chapter 3. However, how does it compare with other commercially available osmometers? Do multiple measurements of the same sample all produce the same result? These are some of the questions to be addressed in this Chapter.

Stahl *et al.* recently presented data from a study which compared the Advanced Instruments Model 3100 Nanolitre Osmometer and the Wescor Vapor Pressure Osmometer using human tear samples.[1] Wescor Vapor Pressure Osmometers are commonly used commercial osmometers with various sample volume requirements – in this case the required sample volume was 1µL. Tear film osmolality readings with the Advanced Instruments Model 3100 Nanolitre Osmometer were found to be on average 1.5mOsm/Kg higher than those measured with the Wescor Vapor Pressure Osmometer, although this difference was not significant ($p=0.13$).[1] It appears that the Advanced Instruments Model 3100 Nanolitre Osmometer is comparable to the Wescor Vapor Pressure Osmometer for the measurement of small volume human tear samples.

The following study was designed to look at the repeatability of the new instrument over multiple measurements on the same sample and over multiple days. Additionally, two different collection techniques were also compared. During the first technique (Collection 1) a large volume sample (3µL) was collected and multiple measurements were made, while the second technique (Collection 2) required multiple small volume samples (1µL) to be taken. A single measurement was taken on each of the small volume samples gathered during the second collection.

## 4.2 Methods

The protocol for this study was approved by the Office of Research Ethics at the University of Waterloo (ORE#14862), prior to the commencement of the study, and informed consent was obtained from all participants. 10 volunteer participants were recruited from the students, staff and faculty, at the School of Optometry, University of Waterloo and enrolled in this study.

### 4.2.1 Inclusion Criteria

Participants were eligible for entry into the study if they:

1. Were at least 17 years of age and had full legal capacity to volunteer.
2. Had read and signed an information consent letter.
3. Were willing and able to follow instructions and maintain the appointment schedule.
4. Had not used artificial tear lubricants 48 hours prior to any of the study visits.
5. Had clear corneas and no signs of active ocular disease.
6. Had an ocular examination in the last two years.
7. Were a non-contact lens wearer*.

*Non-contact lens wear was defined as less than three full (eight hour) days of wear per month with no contact lens wear for at least seven days prior to study visits.

### 4.2.2 Exclusion Criteria

Participants were ineligible for entry into the study if they:

1. Wore any form of contact lenses*.
2. Had used artificial tear lubricants for 48 hours prior to any of the study visits.
3. Had any active ocular disease.
4. Had any systemic disease affecting ocular health.
5. Were using any systemic or topical medications that may affect ocular health.
6. Were pregnant or lactating.
7. Were participating in any other type of clinical or research study.

*Contact lens wear was defined as more than three full (eight hour) days of wear per month or contact lenses worn less than seven days prior to a study visit.

### 4.2.3 Study Visits

Participants were required to attend two study visits per day, on three separate days (six study visits in total). The first study visit of each day was scheduled between 0900 and 1200 hours, the second visit of each day was scheduled between 1300 and 1600 hours. Participants' visits were scheduled at the same two times on all three days, and had a 4 hour break between appointments (i.e. 0900 and 1300, or 1100 and 1500hours).

### 4.2.4 Study Procedures

Tears were collected from one eye only during this study - participants being randomly assigned to have tears collected from either their right or left eyes. During the morning visit, 1 x 3µL sample of tears was taken from either the right or left eye (Collection 1). At the afternoon visit 3 x 1µL samples were taken, one immediately after the other, from the same eye tested in the morning.

Tears were collected by a single experienced clinical investigator who used single use, disposable glass capillary tubes (Drummond Scientific Company, Broomall, PA, USA) for all tear collections. Participants were reclined in a chair during the procedures, and care was taken to ensure that the lid margin and corneal surfaces were not touched. Participants were also asked to look in a superior-nasal direction to further protect the corneal surface.

Tear samples were aliquoted into small 0.2mL PCR tubes (Axygen Scientific Inc., Union City, CA, USA) prior to being transferred to the Advanced Instruments Model 3100 Nanolitre Osmometer sample loading tip. All of the PCR tubes were chilled prior to their use, and samples were kept frozen at -4°C between measurements, in order to minimize evaporation effects. Tear samples were stored for no longer than 1hour, as this was the time needed to complete multiple measurements on each sample. After measurements were completed, tear samples were immediately disposed of. The 3µL samples were aliquoted into a single PCR tube, while the 1µL samples were aliquoted into three separate tubes and were not pooled.

### 4.2.5 Instrument Calibration

The calibration of the Advanced Instruments Model 3100 Nanolitre Tear Osmometer was checked daily. The osmolality of a 304mOsm/Kg standard reference solution (Advanced Instruments Inc,

Norwood, MA, USA) was measured a minimum of three times per day, whenever the instrument was being used. The calibration of the machine was considered to be acceptable if the mean ± standard deviation of the reference samples were within 304 ± 4mOsm/Kg. If the mean ± standard deviation of the reference samples did not fall within this range, than the instrument was immediately recalibrated before any tear samples were measured.

### 4.2.6 Statistical Analysis

Comparison of the variability between groups vs. the variability within groups was assessed with a mean Intraclass Correlation. The software for this analysis is freely available on the web from the Department of Obstetrics and Gynaecology, at The Chinese University of Hong Kong (http://department.obg.cuhk.edu.hk/researchsupport/statstesthome.asp). The intraclass correlation coefficient is representative of concordance, thus "1" is considered to be perfect agreement and "0" to be no agreement at all. In the analysis of variance, the F value for between raters tests whether the raters significantly differ in their assessment or not.[2]

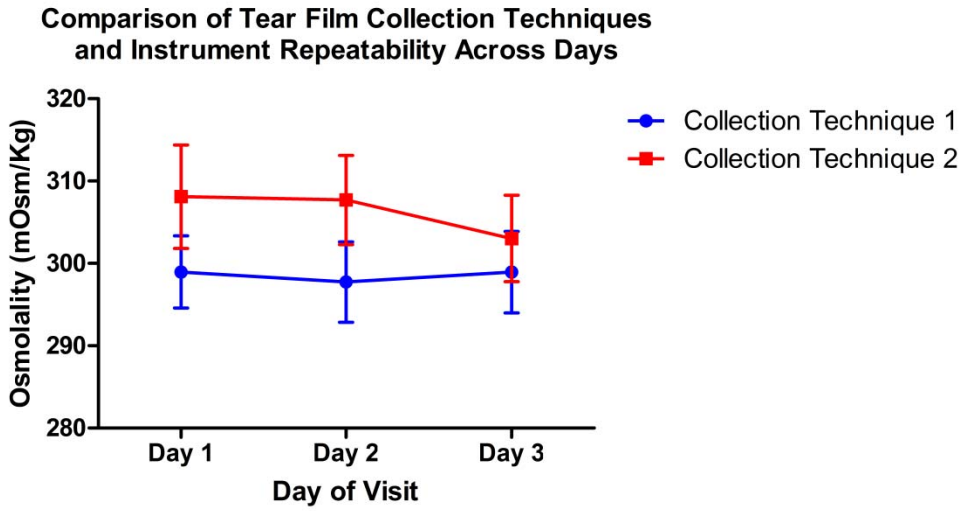
Repeated measures ANOVAs were used to determine if there were any significant differences between collection techniques or between the days of the study.

## 4.3 Results

For analysis purposes, each group of three tear film measurements was referred to as a "cluster". A single cluster was collected from each participant at every visit, for a total of 10 clusters per visit. 10 clusters were successfully collected on all of the visits except for two. At the afternoon visit on Day 2 and the afternoon visit on Day 3 only 8 and 9 clusters were collected respectively. At both of these visits, some of the individual 1µL tear samples were lost during the sample loading and measurement processes and could not be recollected. Two of the clusters from the Day 2 visit, and one of the clusters from the Day 3 visit did not consist of three repeated measurements of tear film osmolality, and were subsequently excluded from the analysis.

There was not a significant difference ($p=0.366$) in tear film osmolality between any of the days, but tear samples collected during Collection 2 (3 x 1µL samples, each measured 1 time) did have a significantly higher ($p<0.001$) mean tear film osmolality value, than tear samples collected during Collection 1 (1 x 3µL samples, each measured three times) (Figure 4.1). Tear samples collected during Collection 1 had a mean tear film osmolality of 298.5mOsm/Kg, while tear samples collected in Collection 2 had a mean tear film osmolality of 306.4mOsm/Kg.

Table 4.1 details the mean tear film osmolality values and the mean intraclass correlations coefficients found on each day of this study. Collection 1 refers to the 3µL samples collected at the morning visits, and Collection 2 refers to the three 1µL samples collected at the afternoon visits.




**4-1: Comparison of mean tear film osmolality values obtained using Collection 1 (3µL sample, measured three times) and Collection 2 (3 x 1µL samples, each measured one time) across Days 1, 2, and 3.**

**Table 4-1: Mean intraclass correlation coefficients for the comparison of measurements taken in Collection 1 (3µL sample, measured three times) and Collection 2 (3 x 1µL samples, each measured one time), compared across Days 1, 2, and 3.**

| | Day 1 | Day 2 | Day 3 |
|---|---|---|---|
| **Collection 1 – Mean** | 299.0 ± 6.12mOsm/Kg | 297.7 ± 6.80mOsm/Kg | 298.9 ± 6.93mOsm/Kg |
| **Collection 2 – Mean** | 308.1 ± 8.77mOsm/Kg | 307.7 ± 7.56mOsm/Kg | 303.6 ± 7.36mOsm/Kg |
| **Collection 1 (3µL)** | 0.8347 (F = 0.7797) | 0.8883 (F = 0.9377) | 0.6497 (F= 0.0582) |
| **Collection 2 (3 x 1µL)** | 0.8707 (F = 5.0643) | 0.9550 (F = 0.5893) | 0.6733 (F = 0.3017) |

The intraclass correlation coefficients for Collection 1 were as follows: Day 1 = 0.8347, Day 2 = 0.8883 and Day 3 = 0.6497. All of the measurements showed reasonable concordance, although concordance on day three was lower than it was on either day 1 or day 2.

A similar trend can be seen in the Collection 2 measurements, with concordance again being higher on days 1 and 2 than it was on day 3 (Day 1 = 0.8707, Day 2 = 0.9550 and Day 3 = 0.6733).

Potential causes of the difference between the measurements on Day 3 and those taken on Days 1 and 2 will be explored in the following discussion.

A statistical difference within the measurements taken at each visit was not found. This suggests that the Advanced Instruments Model 3100 Nanolitre Osmometer demonstrates good repeatability when measuring osmolality of small volume tear samples.

## 4.4 Discussion

The Advanced Instruments Model 3100 Nanolitre Osmometer has been previously shown to be comparable to other commercially available osmometers for the measurement of tear film osmolality,[1] and the above results indicate that there is reasonably good concordance between measurements of tear film osmolality taken with this instrument. Therefore the Advanced Insturments Model 3100 Nanolitre Osmometer, as a clinical instrument, appears to be capable of successfully measuring tear film osmolality on small sample volumes.

The collection technique used in Collection 2 (three small samples), had slightly higher concordance values than samples obtained using the Collection 1 technique (one large sample). There are a few possible explanations for this. Firstly, tears gathered in Collection 1 are aliquoted into a single Eppendorf tube, which had to be re-opened, and re-exposed to a pipette every time a measurement was taken. Repeated re-opening of the vial, and re-exposure of the sample to the pipette and the environment, increases the potential for evaporation to occur in these samples. Evaporation, if significant, could produce some variability in the results of multiple measurements taken on the same sample, as the sample would effectively be different from one measurement to the next. Samples from Collection 2 were aliquoted into individual vials, thus they were only opened and exposed to the environment and the pipette once, which decreased the potential for evaporation to occur.

A second possible cause of the slightly higher variability between the samples gathered during Collection 1 could be the repeated freezing/thawing that these samples were exposed to during the measurement process. All of the samples were stored at -4°C until just before they could be measured, but the samples in Collection 1 were re-frozen between measurements (and re-thawed before every measurement) in order to try and minimize evaporative effects. The samples in

Collection 2, on the other hand, were only frozen and thawed once. It is possible that the repeated freezing and thawing of the samples from Collection 1 may have had the effect of mildly increasing in the variability in the measurement of their tear film osmolality. This being said, the concordance within both measurement techniques is quite similar, and quite high, so the effects of evaporation and temperature changes on measurement variability is likely to be minimal.

As discussed previously (Chapter 3), loading samples into the Advanced Instruments Model 3100 Nanolitre Osmometer is a significant potential source of error. This is a delicate technique that requires a user to be quite skilled to minimize sample damage or loss. Some of the samples taken during Collection 2 were lost during the loading process and had to be ignored for analysis purposes. This may have lead to an artificially higher or lower concordance between the samples of Collection 2, depending upon how significantly these missing samples varied from the mean. Unfortunately, there is no way to predict the effect these samples would have had on the intraclass correlation coefficients, as the sample loss during the measurement process was completely random.

One advantage to taking a larger sample volume than needed (i.e. the 3μL sample in Collection 1), is that it is easier to re-measure a sample if a problem occurs during the loading process. A disadvantage to this type of sample collection though, is that it limits the number of samples one can feasibly collect during a day before significantly increasing the risk of reflex tearing. Choosing an appropriate sample size becomes a trade off between the skill of the investigator and the number of measurements that need to be taken, and the most appropriate sample size needed will vary accordingly.

As mentioned earlier, a marked reduction in the intraclass correlation coefficient was noticed with both collection techniques on Day 3. Investigators have noticed that the calibration of Advanced Instruments Model 3100 Nanolitre Osmometer remains quite stable when the humidity only changes gradually ($\leq$5% per day) over a day and recalibration is rarely necessary. However, when the humidity changes drastically (>10% per day) the instrument calibration does not remain stable, and the machine often requires daily recalibration. Frequent unnecessary recalibration has been shown to introduce inaccuracies to results obtained with the Advanced Instruments Model 3100 Nanolitre Osmometer,[3] and it is for this reason that the investigators feel that the concordance was lower on Day 3 of this study than it was on Days 1 and 2.

This study started towards the end of January, when the weather was quite cold and the humidity is quite low, but it did not end until early March, when the weather and the humidity were changing

dramatically. As the weather was warming up, the humidity could change by as much as 20-30% within a 12 hour time period. It was during this time period when the humidity was fluctuating dramatically, that most of the Day 3 measurements were being taken. Daily recalibration was necessary during this time, and the investigators feel that this may have significantly lowered the repeatability of these measurements. The inaccuracies in the instrumentation caused by frequent recalibration were thought to be primarily responsible for the decreased concordance between the measurements taken on Day 3 when compared with those taken on Days 1 and 2.

Despite the increased internal variability in measurements taken on Day 3, there was no significant difference in mean tear film osmolality measurements taken on different days for either of the collection techniques. This further suggests that the Advanced Instruments Model 3100 Tear Osmometer is indeed capable of repeatedly measuring tear film osmolality. It also suggests that tear film osmolality measurements do not vary significantly between days in individuals, or that tear film osmolality appears to be relatively constant when measured at the same time on different days. Interestingly tear samples collected during Collection 2 (3 x 1µL samples, each measured one time) had a significantly higher mean tear film osmolality than those collected during Collection 1 (1 x 3µL sample, measured three times).

It is possible that by collecting smaller samples, as in Collection 2, there was a smaller chance of inducing reflex tearing because smaller volumes of tears were being removed from the ocular surface at any one time. The high intraclass correlation coefficients found with this method suggest that reflex tearing was not induced with the multiple collections. Unfortunately, working with smaller sample volumes increases the risk that the samples will be affected by evaporation during the storage and transfer processes. Although every attempt was made by the investigators to minimize sample evaporation, it is possible that the higher mean tear film osmolality found with this collection technique was due to sample evaporation.

Samples collected during Collection 1 had a significantly lower mean tear film osmolality value compared with samples collected in Collection 2. It is possible that the samples collected in Collection 1 could have been affected by reflex tearing, which may have been an induced during the 3µL sample collection due to the larger volume of tears being collected in one attempt. If this were the case, it would not have been detected by the intraclass correlation analysis, as each of the three readings were taken on the same sample and would have been affected by reflex tearing equally. It is possible though, that samples obtained during Collection 1 provide a truer representation of mean tear

film osmolality, as evaporation would be less of an issue with the storage and transfer of this larger volume sample.

Further work is needed to determine if the evaporative effects of sample storage and transfer can significantly affect osmolality measurements or not. This work could be done with either standardized saline solutions or with artificial tear solutions of known osmolality, and should investigate various sample transfer and storage techniques to determine the effects evaporation may have on the measurement of osmolality in small volume samples such as human tears.

A potential criticism of this study would be that the possibility of a diurnal variation in tear film osmolality was not considered when the study was designed, as similar samples were all collected at the same time of day. Previous work has shown that the concentration of some tear film solutes, such as proteins, lipids, mucins and salts, demonstrate circadian rhythms.[4] As these solutes are responsible for tear film osmolality, it is possible that a diurnal variation in tear film osmolality could occur. A diurnal variation in tear film osmolality could also account for the significant difference between mean tear film osmolality measurements collected during Collections 1 and 2. All of the Collection 1 measurements took place in the morning, while all of the Collection 2 measurements took place in the afternoon. This possibility of tear film osmolality measurements being affected by a diurnal variation requires further investigation and will be examined in the following chapter of this thesis.

# Chapter 5
# Diurnal Variation in Tear Film Osmolality

## 5.1 Introduction

Dry eye disease is one of the most frequently diagnosed ocular problems.[1] As a condition, its severity ranges from the minor discomfort reported by people who use visual display terminals and contact lenses to the extremely devastating damage and pain experienced by people with end-stage ocular pemphigoid and Sjogren's syndrome.

Tear film osmolality has been reported to be the "gold standard" diagnostic test for the evaluation of dry eye disease, as a distinct separation between tear film osmolalities in normal and dry-eyed populations has become evident.[2-5] Research suggests that a hyperosmotic tear film is a trait common to all forms of dry eye, and may be the driving force causing the discomfort, ocular surface damage and inflammation found in both evaporative and tear deficient forms of dry eye disease.[2]

Tear film osmolality is a product of the varying concentrations of dissolved solutes (proteins, lipids, mucins and salts) in the tear fluid. Previous studies have demonstrated that the concentration of these solutes can vary during the day, suggesting that their concentration may demonstrate a circadian rhythm.[6] Therefore, it is not unrealistic to hypothesize that tear film osmolality may be affected in a similar way.

In 1978, Terry and Hill measured diurnal variations in tear film osmolality with a precision thermocouple hygrometer in six non-contact lens wearing subjects (three males, three females). Measurements were taken every hour between 9am and 10pm over a five day period from each of the subjects, and they were taken immediately upon eye opening (after a period of six to eight hours of sleep) in five of six subjects.[7] A 5μL sample volume was required for each measurement. Certain subjects were found to demonstrate a greater variability in their tear film osmolalities than others, but overall a significant diurnal variation in tear film osmolality was not recorded.[7] No differences between male and female subjects were noted, nor were any correlations with food and fluid intake detected.[7] Interestingly, the measurements taken immediately upon waking were found to be significantly lower than the measurements taken when the eyes were open. It was suggested that a reduced rate of evaporation and tear clearance during eye closure was responsible for the considerable decrease in the osmotic pressure of the tear film compared with the pressure measured when the eyes were open.[7]

Benjamin and Hill re-visited the diurnal variation in tear film osmolality concept in 1982. This time, they worked with a freezing point depression osmometer capable of measuring 200nL samples.[8] Six healthy young adults were enrolled in the study, and their tear film osmolalities were measured every 10minutes for 8.5hours.[8] An overall trend of increasing tear hypertonicity towards the end of the day was found and tear film osmolality was estimated to increase by approximately 1.43mOsm/Kg•$s^{-1}$. However, two subjects actually demonstrated a mild decrease in tear film osmolality over the day.[8] The smaller sample volume (200nL) was deemed to be advantageous in measuring tear film osmolality, as it minimized the risk of reflex tearing and made the rapid collection of reliable samples feasible, thereby making it possible to collect samples at frequent time intervals without significantly depleting the normal tear volume.[8]

Although this previous research determined that a significant diurnal variation in tear film osmolality does not exist in a normal population, they are not entirely conclusive. Both of these studies involved a very low number of participants (n=6), therefore they may not be representative of the larger population. The measurements in both studies were taken with extremely sophisticated laboratory equipment. While this is not a problem in specialized research centers, this equipment would not be practical to use in a regular optometry clinic. Measurements taken with specialized equipment may, or may not be, comparable to the type of measurements which could be obtained in a normal clinical setting.

The purpose of this study was to measure the diurnal variation in tear film osmolality on a larger population, and in a normal clinical setting, using the Advanced Instruments Model 3100 Nanolitre Osmometer. The Advanced Instruments Model 3100 Nanolitre Osmometer requires a small sample volume (0.5µL or 500nL), thus it is an appropriate instrument for taking repeated measurements of the tear film over the course of a day. As this was a clinical study, it was designed to mimic, as accurately as possible, the conditions of a typical optometry clinic. Participants' environments were not restricted in any way, nor were participants pre-selected based on their ocular surface comfort.

The study consisted of two phases – an initial and a secondary phase. During the initial phase, monocular measurements were taken, and restrictions were made only relating to the use of artificial tears. In the secondary phase of the study, participants' artificial tear use and contact lens wear were restricted, and all testing was undertaken binocularly. Binocular testing was used to compare tear film osmolality between participants' eyes and to examine the effect of tear collection on the tear film osmolality of the contralateral eye.

## 5.2 General Methods

This study consisted of two phases, and was designed to investigate diurnal variation of tear film osmolality in a normal population. The protocol for both phases were approved by the Office of Research Ethics at the University of Waterloo (ORE# 12350 (phase 1) and 14862 (phase 2)), prior to their commencement. An informed consent was obtained from all participants prior to their enrolment in the studies.

## 5.3 Diurnal Variation in Tear Film Osmolality – Phase 1

### 5.3.1 Methods

40 volunteer participants were recruited from the students, staff and faculty at the School of Optometry, University of Waterloo, and enrolled in the first phase of this study.

#### 5.3.1.1 Inclusion Criteria

Participants were eligible for entry into the study if he or she:

1. Was at least 18 years of age and had full legal capacity to volunteer.
2. Had read and understood the Statement of Informed Consent.

#### 5.3.1.2 Exclusion Criteria

Participants were ineligible for entry into the study if he or she:

1. Had applied artificial tear lubricants in the preceding 6 hours.

#### 5.3.1.3 Study Visits

Participants were required to attend a total of six study visits over two days (three visits per day). Study visits were scheduled at nine am, twelve pm, and four pm; a time tolerance of ± one hour per visit was acceptable. Participants' visits were scheduled at the same time on both days, and all participants had an equal time interval between visits (i.e. all participants had a three hour break between the morning and mid-day visits, and a four hour break between the mid-day and afternoon visits).

#### 5.3.1.4 Study Procedures

Tear samples (0.5-1.0μL) were collected from the inferior temporal canthus of the left eye of every participant at each of the study visits. Tears were collected using a single use, disposable, flexible polycarbonate capillary tube (Advanced Instruments Inc, Norwood, MA, USA), while participants were seated at a biomicroscope. Care was taken to ensure that the lid margin and corneal surfaces were not touched, and participants were asked to look in a superior-nasal direction to further protect the corneal surface. Corneal anaesthesia was not used.[9] If participants wore their contact lenses on the day of the study, the lenses were not removed. Tear collection was performed in the same manner as without lenses (from the inferior temporal canthus) while participants sat at a biomicroscope.

Immediately after collection, tear samples were transferred to the Advanced Instruments Model 3100 Nanolitre Osmometer. Tear samples were disposed of immediately after osmolality measurements had been completed.

#### 5.3.1.5 Instrument Calibration

The calibration of the Advanced Instruments Model 3100 Nanolitre Tear Osmometer was checked daily. The osmolality of a 304 mOsm/Kg standard reference solution (Advanced Instruments Inc, Norwood, MA, USA) was measured a minimum of three times per day, whenever the instrument was being used. The calibration of the machine was considered to be acceptable if the mean ± standard deviation of the reference samples were within 304 ± 4mOsm/Kg. If the mean ± standard deviation of the reference samples did not fall within this range, than the instrument was immediately recalibrated before any tear film samples were measured.

#### 5.3.1.6 Statistical Analysis

The data from all participants was pooled in order to determine a population mean and standard deviation. Repeated measures ANOVAs were used to determine if there were any significant differences in the population tear film osmolality between visits or between days.

### 5.3.2 Results

All 40 participants (10 males, 30 females) completed the entire series of study visits. There were 22 non-contact lens wearers and 18 contact lens wearers. Of the contact lens wearers, 16 participants wore soft contact lenses and 2 wore gas permeable contact lenses. For analysis purposes the soft

contact lens wearers and gas permeable contact lens wearers were pooled into a single group, henceforth referred to as "contact lens wearers".

The mean osmolality of the entire population (all time points pooled) was 298.7±11.4mOsm/Kg. The contact lens wearing group (298.5±11.2mOsm/Kg) did not have a significantly different mean tear film osmolality than either the non-contact lens wearing group (298.9±11.5mOsm/Kg) or the total population ($p>0.05$). The mean (± standard deviation) tear film osmolalities of the total population at each individual time point are listed in Table 5.1. There was no significant diurnal change in tear film osmolality over the course of a day ($p=0.33096$), although there was a significant increase in tear film osmolality on day 2 when compared to day 1, $p<0.001$ (Figure 5.1).

**Table 5-1: Mean tear film osmolality (mOsm/Kg) of the population at each measurement time point.**

| Osmolality (mOsm/Kg) | Visit 1 (9am ± 1hour) | Visit 2 (12pm ± 1 hour) | Visit 3 (4pm ± 1 hour) |
|---|---|---|---|
| **Day 1** | 295.8 ± 9.4 | 295.7 ± 11.6 | 297.9 ± 12.8 |
| **Day 2** | 301.7 ± 11.1 | 299.0 ± 11.6 | 301.7 ± 10.4 |

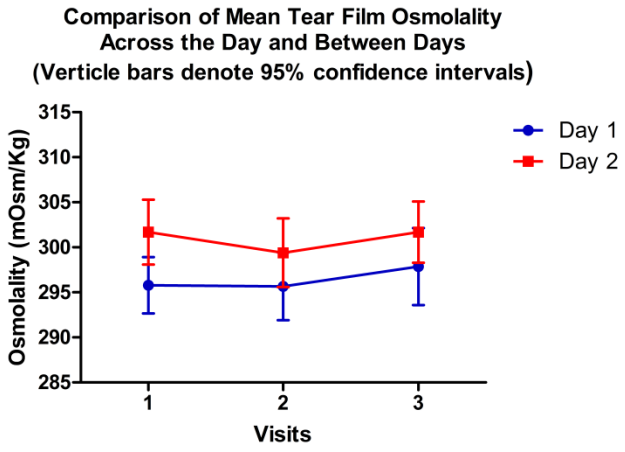



**Figure 5-1: Comparison of mean tear film osmolality (mOsm/Kg) across the day and between days (all participants pooled).**

The significant increase in tear film osmolality from day 1 to day 2 was believed to be due to one of three things:

1) there was some variability within the instrument itself,
2) participants underwent an adaptation process between the two days of the study,
3) the investigator's ability to collect tears improved over the course of the study.

As shown previously, the Advanced Instruments Model 3100 Nanolitre Tear Film Osmometer is repeatable over multiple days, so it is unlikely that the difference in tear film osmolality between days 1 and 2 was an instrument effect (Chapter 4).

Tear collection with a capillary tube may have acted as a stimulus for the initiation of reflex tearing, especially as most of the participants had never had tears collected previously. In order to determine if there was a process of ocular surface adaptation occurring between measurements taken on day 1 and day 2, participants were split into two groups depending upon their contact lens wear. The contact lens wearing group was considered to have an "adapted" ocular surface, due to the reduction in corneal sensitivity that occurs with long term contact lens wear.[10-13] If a participant adaptation to tear film collection was occurring during the study, it was hypothesized that the non-contact lens group would have a higher tear film osmolality (less reflex tearing) on day 2, while the there would be no change in the tear film osmolality of the contact lens wearing group.

As demonstrated in Figure 5.2 there was a significant increase ($p=0.033$) in the mean tear film osmolality on the second day for both groups. There was no significant difference in mean tear film osmolality between the non-contact lens wearing and contact lens wearing groups at any of the individual visits ($p>0.05$), nor was there a significant diurnal change in tear film osmolality found in either group ($p>0.05$).

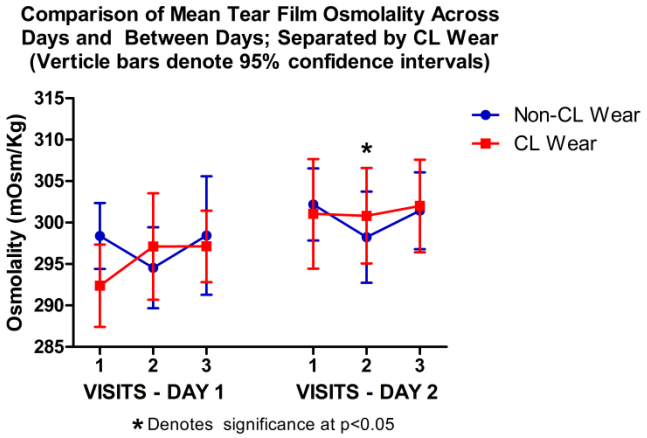



**Figure 5-2: Comparison of mean tear film osmolality (mOsm/Kg) across the day and between days; participants separated into non-contact lens wearing and contact lens wearing groups.**

An unskilled investigator may accidentally stimulate reflex tearing in participants through contact with the lid margins and/or the conjunctival or corneal surfaces. In order to determine if the difference between the mean tear film osmolality on day 1 and day 2 was indeed due to a learning process for the investigator, the first and last ten participants to complete the study were compared. If there was no investigator learning effect taking place, it was hypothesized that the difference between the day 1 and day 2 measurements would be present in both groups. If there was a learning effect for the investigator, than it was hypothesized that the difference between the day 1 and day 2 measurements would only be found in the first ten participants. The last ten participants would have been enrolled in the study after the learning process had taken place, and no difference in tear film osmolality between the days should be observed.

Figure 5.3 demonstrates the difference in mean tear film osmolality between day 1 and day 2 for the first and last ten participants to complete the study. The first ten participants demonstrated a significant increase in tear film osmolality between day 1 and day 2 measurements ($p<0.05$), but there was no significant change in mean tear film osmolality between day 1 and day 2 for the last ten participants ($p=0.843$).

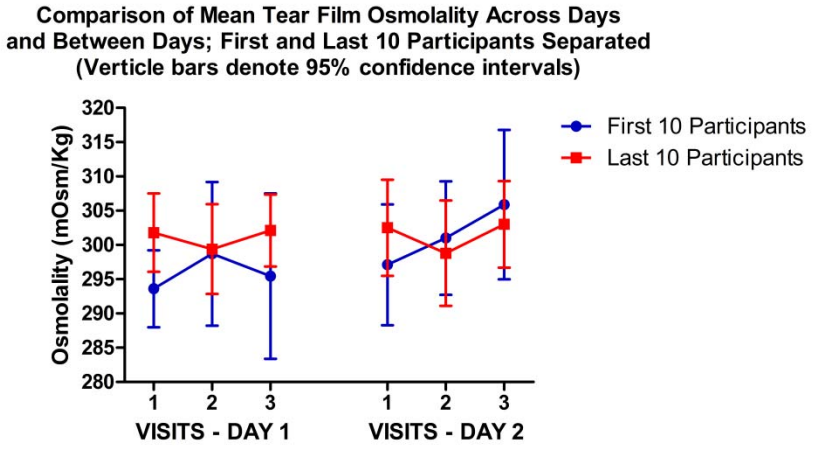



**Figure 5-3: Comparison of mean tear film osmolality (mOsm/Kg) across the day and between days; first ten participants to complete the study compared with the last ten participants to complete the study.**

### 5.3.3 Discussion

The mean tear film osmolality of the population studied was 298.7±11.4mOsm/Kg, and ranged from approximately 296 – 302mOsm/Kg over the entire series of time points studied. No significant diurnal variation in tear film osmolality was found to occur over the course of a normal working day. This is in agreement with previously published work by Terry and Hill (1978)[7] and Benjamin and Hill (1981)[8], who both found that there was no significant diurnal variation in tear film osmolality over the course of the day. Terry and Hill (1978) did find that tear film osmolality was significantly lower immediately upon waking, but measurements of this nature were not taken during this current study, and comparisons between the previous and current studies cannot be confirmed at this time.

22 non-contact lens wearers and 18 contact lens wearers (16 soft lens wearers, 2 gas permeable lens wearers), completed this study. Contact lens wear has been previously shown to increase tear film osmolality in individuals,[14, 15] due to decreased tear secretion resulting from reduced corneal sensitivity.[16] The results of this study do not agree with this earlier research, as the mean tear film osmolality of the contact lens wearing group (298.5±11.2mOsm/Kg) was not significantly higher than the non contact lens wearing group (298.9±11.5mOsm/Kg) in this study. Contact lens wear was not found to have a significant effect on diurnal changes in mean tear film osmolality either. However, this study was not designed to look for differences in tear film osmolality between contact lens wearers and non-contact lens wearers, thus these findings cannot be considered to be conclusive.

Further investigation into the effect of contact lens wear on tear film osmolality is still needed at this time.

Mean tear film osmolality was found to be significantly higher on day 2 compared to day 1 in this study. This may be due to either variability in the instrument, a participant adaptation process, or an improvement in investigator technique. As the instrument had been previously shown to be repeatable (Chapter 4), participant adaptation or the improvement in investigator technique would have been a more likely cause.

Many of the participants had never had tears collected before and the sensations experienced during the tear collection procedure could have triggered reflex tearing in some individuals. In order to determine if this was the case, participants were divided into two groups based upon their contact lens wear. The group of contact lens wearers were considered to be previously "adapted" and less likely to reflex tear. The non contact lens wearers were considered to be "non-adapted" and to have a higher potential for producing a reflex response. If the difference in the day 1 and day 2 measurements was only present in the non-contact lens wearers, this would be a good indication of a participant adaptation process taking place. Alas, the difference in the day 1 and day 2 measurements was found to be present in both groups (Figure 5.2). Therefore, it is unlikely that a participant adaptation effect was responsible for the higher mean tear film osmolality found on day 2 compared with day 1.

An inexperienced individual would have a greater chance of directly contacting the lid margins and/or the conjunctival or corneal surfaces when collecting tears, and this could increase the amount of reflex tearing occurring. To investigate this, the first and last ten participants to enroll and complete the study were compared. One would expect that if the inexperience of the investigator collecting tears was the cause of the difference between the day 1 and day 2 measurements, then the difference in these measurements would be more pronounced in the first 10 participants when compared with the last 10 participants. Indeed, this was found to be the case. The first ten participants demonstrated a significant increase in mean tear film osmolality between day 1 and day 2 measurements, but no difference was seen in the measurements taken on the last ten participants (Figure 5.3). This highlights the importance of clinician experience when performing delicate procedures such as tear film collection, as the normal state of the ocular surface can be easily disrupted. Further investigation with an experienced clinician is needed to confirm that the difference in the day 1 and day 2 measurements was an artefact of investigator experience, rather than a true effect.

## 5.4 Diurnal Variation in Tear Film Osmolality – Phase 2

### 5.4.1 Methods

40 volunteer participants were recruited from the students, staff and faculty, at the School of Optometry, University of Waterloo, and enrolled in the second phase of this study.

#### 5.4.1.1 Inclusion Criteria

Participants were eligible for entry into the study if he or she:

1. Were at least 17 years of age and had full legal capacity to volunteer.
2. Had read and signed an information consent letter.
3. Were willing and able to follow instructions and maintain the appointment schedule.
4. Had not used artificial tear lubricants 48 hours prior to any of the study visits.
5. Had clear corneas and no signs of active ocular disease.
6. Had had an ocular examination in the last two years.
7. Were a non-contact lens wearer*.

*Non-contact lens wear was defined as less than three full (eight hour) days of wear per month with no contact lens wear for at least seven days prior to study visits.

#### 5.4.1.2 Exclusion Criteria

Participants were ineligible for entry into the study if he or she:

1. Wore any form of contact lenses*.
2. Had used artificial tear lubricants for 48 hours prior to any of the study visits.
3. Had any active ocular disease.
4. Had any systemic disease affecting ocular health.
5. Were using any systemic or topical medications that may affect ocular health.
6. Were pregnant or lactating.
7. Were participating in any other type of clinical or research study.

*Contact lens wear was defined as more than three full days of wear (minimum of eight hours per day) per month, or contact lenses worn less than seven days prior to a study visit.

#### 5.4.1.3 Study Visits

Participants were required to attend a total of six study visits over two days (three visits per day). Study visits were scheduled at nine am, twelve pm, and four pm; a time tolerance of ± one hour per visit was acceptable. Participants' visits were scheduled at the same time on both days, and all participants had an equal time interval between visits (i.e. all participants had a three hour break between the morning and mid-day visits, and a four hour break between the mid-day and afternoon visits).

#### 5.4.1.4 Study Procedures

Tear samples (1 – 2µl) were collected from both eyes of every participant at each of the study visits by one of two experienced investigators. Tears were collected using a single use, disposable glass capillary tube (Drummond Scientific Company, Broomall, PA, USA), while participants were reclined in a chair. A randomization table was used to determine which eye would be used first for tear collection. The first eye measured was the same for all of the subsequent visits. Care was taken to ensure that the lid margin and corneal surfaces were not touched. Participants were asked to look in a superior-nasal direction to further protect the corneal surface.

Immediately after collection, tear samples were transferred to the Advanced Instruments Model 3100 Nanolitre Osmometer. Data was collected from each eye individually; tear samples were not pooled. Tear samples were disposed of immediately after osmolality measurements had been completed.

#### 5.4.1.5 Instrument Calibration

The calibration of the Advanced Instruments Model 3100 Nanolitre Tear Osmometer was checked daily. The osmolality of a 304mOsm/Kg standard reference solution (Advanced Instruments Inc, Norwood, MA, USA) was measured a minimum of three times per day, whenever the instrument was being used. The calibration of the machine was considered to be acceptable if the mean ± standard deviation of the reference samples were within 304 ± 4mOsm/Kg. If the mean ± standard deviation of the reference samples did not fall within this range, than the instrument was immediately recalibrated before any tear film samples were measured.

#### 5.4.1.6 Statistical Analysis

Statistical analysis was performed using STATISTICA 7 (StatSoft ®, Tulsa Oklahoma, www.statsoft.com) and all graphing analysis was completed using Graph Pad Prism 5 Software (Graph Pad Software Inc., www.graphpad.com).

The data from all participants was pooled in order to determine a population mean and standard deviation. A Sign test was used to determine if there was any difference in tear film osmolality between the first and second eye measurements. The Sign test was also used to determine if there was any difference in tear film osmolality between the right and left eyes of participants.

Repeated measures ANOVAs were performed to determine if there were any significant differences in the population tear film osmolality between visits or between days. Spearman correlations were used to determine if there was any association between tear film osmolality and the number of hours a participant had been awake at the time of the tear film osmolality measurement.

### 5.4.2 Results

All 40 participants (14 males, 26 females) completed the entire series of study visits. All participants were non-contact lens wearers and had a mean age of 33.1 ± 11.1 years. 21 participants had tears collected from their right eye first, while the remaining 19 had tears collected from their left eye first.

The mean tear film osmolalities of the first eye (right or left, 298.9 ± 9.0mOsm/Kg) and the second eye (298.5 ± 8.0mOsm/Kg) tested were not significantly different ($p>0.05$) (Figure 5.4). There was no significant difference ($p>0.05$) between the mean tear film osmolality of the right or left eyes of participants (Figure 5.5). For all future analyses, only first eye data was used. It was felt that even though there was no difference between first and second eye measurements, the first eye data was less likely to have been affected by confounding factors such as excessive tear film evaporation or reflex tearing during the tear collection process.

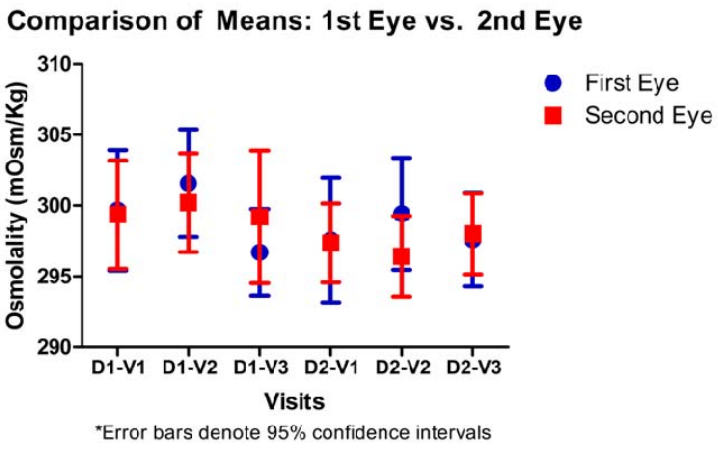



**Figure 5-4: Comparison of mean tear film osmolality (mOsm/Kg) between the first and second eyes of each participant measured, at each study visit. The D-V labels on the x-axis refer to the day and visit number at which the measurements were taken (D1-V1 refers to the measurement taken on Day 1 at Visit 1, D1-V2 is Day 1-Visit 2, etc.)**

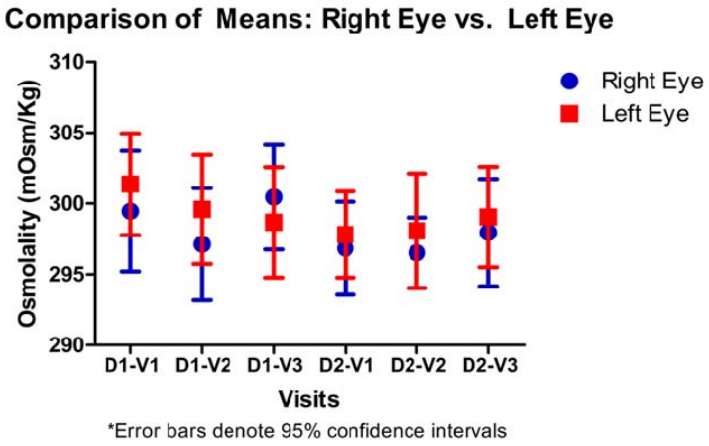



**Figure 5-5: Comparison of mean tear film osmolality (mOsm/Kg) between the right and left eyes of all participants, at each study visit. The D-V labels on the x-axis refer to the day and visit number at which the measurements were taken (D1-V1 refers to the measurement taken on Day 1 at Visit 1, D1-V2 is Day 1-Visit 2, etc.)**

The mean osmolality of the entire population (all time points pooled, first eye data only) was 298.9±9.0mOsm/Kg. The mean (± standard deviation) tear film osmolalities of the total population at each individual time point are listed in Table 5.2. There was no significant diurnal change in tear film

osmolality over the course of a day (p=0.827), and there was no significant difference in tear film osmolality between day 1 and day 2 measurements, p=0.743 (Figure 5.6).

**Table 5-2: Mean tear film osmolality (mOsm/Kg) of the population at each measurement time point.**

| Osmolality (mOsm/Kg) | Visit 1 (9am ± 1hour) | Visit 2 (12pm ± 1 hour) | Visit 3 (4pm ± 1 hour) |
|---|---|---|---|
| **Day 1** | 299.7±13.3 | 301.6±11.7 | 296.7±9.5 |
| **Day 2** | 297.6±13.7 | 299.4±12.0 | 297.6±10.3 |

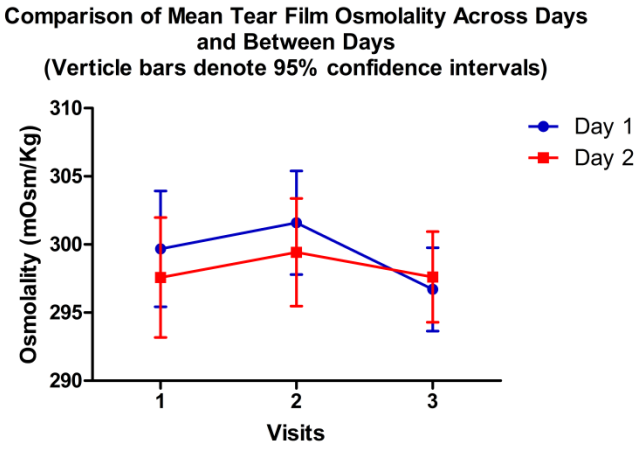



**Figure 5-6: Comparison of mean tear film osmolality (mOsm/Kg) across the day and between days (all participants pooled, first eye data only).**

On average, participants were awake 1.9 ± 0.8hours at their first visit (range: 0.25 – 4.5hours), 4.9 ± 0.8hours at their second visit (range: 3.25 – 7.5hours) and 8.9 ± 0.8hours at their final visit (range: 7.25 – 11.5hours). When tear film osmolality was compared with the number of hours participants had been awake, no significant correlation (Spearman r=0.07044) was found (Figure 5.7).

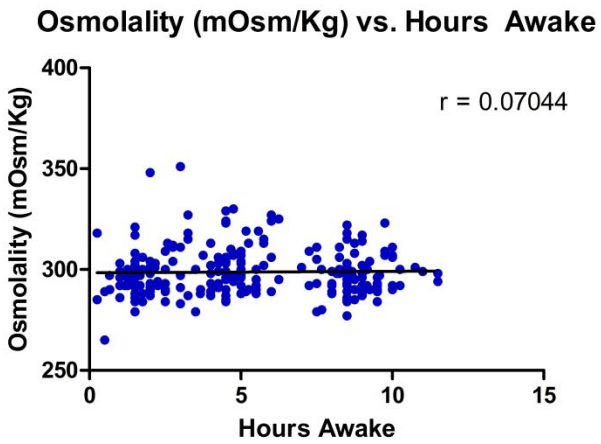



**Figure 5-7: Comparison of tear film osmolality (mOsm/Kg) with the number of hours participants were awake at the time of measurement (all participants pooled, first eye data only).**

### 5.4.3 Discussion

The mean tear film osmolality of this non-contact lens wearing population was 298.9±9.02mOsm/Kg, (range: 297 – 302mOsm/Kg), and no significant diurnal variation in tear film osmolality was observed over the course of a normal working day. These results are very similar to those found in the first phase of this study, and they are in agreement with previously published research.[7, 8] When tear film osmolality was compared with the number of hours participants had been awake, there was still no significant diurnal change in tear film osmolality detected.

A significant difference in mean tear film osmolality measurements taken on days 1 and 2 was not found in this study. Contact lens wear was not permitted in the second phase of this study, as it was thought to alter the normal state of the ocular surface tear film, through the reduction of corneal sensitivity that has been shown to occur with contact lens wear. [14-16] Many of the participants enrolled in the second phase of this study had never experienced tear collection before, and in this regard they strongly resembled the participants who took place in the initial phase of the study. As there was no difference between the day 1 and day 2 measurements in this phase of the study, it appears that participant adaptation does not have a significant effect on the measurement of tear film osmolality.

The individuals collecting tears in the second phase of this study had a great deal more experience than the individual responsible for collecting tears in the first phase of this study. This was felt to be the most likely reason that the significant difference in mean tear film osmolality found in the first phase of the study between the day 1 and day 2 measurements was not present in the second phase of the study.

With respect to collection technique there was not a significant difference in tear film osmolality between tear samples collected with participants seated at a biomicroscope (part 1, mean: 298.7±11.4mOsm/Kg) and tear samples collected with participants reclined in a chair (part 2, mean: 298.90±9.02mOsm/Kg). Although both techniques produce similar results, we recommend that the second technique, with participants reclined in a chair, be used, as participants typically found this more comfortable. Reclining the participants in a chair eliminates the risk of the illumination source of the biomicroscope acting as a stimulus for reflex tearing and/or causing any tear film evaporation during the collection process as well.

Interestingly, there was no significant difference in tear film osmolality between the first and second eyes measured, nor was there any difference between measurements taken on the right and left eyes. This suggests that the collection of 1 – 2μL of tears with a glass capillary tube does not act as a strong stimulus for reflex tearing in either the donating or contralateral eyes, as long as care is taken not to touch the lid margins or the conjunctival and/or corneal surfaces. This is important for two reasons – first, it confirms that tears can be safely collected without inducing a reflex tearing response, and secondly, it appears that tear samples collected from one eye may be representative of the tear film osmolality of both eyes. Conclusions

The Advanced Instruments Model 3100 Nanolitre Osmometer is capable of measuring tear film osmolality in a clinical setting. Work with this instrument has demonstrated that despite the diurnal variation of some tear film proteins,[6] tear film osmolality does not change significantly over the course of a day. Although this had been demonstrated previously,[7, 8] these studies use highly specialized laboratory equipment on small populations.

Unlike intraocular pressure (IOP) measurements, which must be taken at multiple times over the day in individuals highly suspect for glaucoma,[17, 18] tear film osmolality needs only to be measured once to obtain data which is representative of the habitual state of the tear film. Clinically, this increases the speed and efficiency of tear film osmolality assessment when it is used as a diagnostic test for dry eye disease. The efficiency of the tear film osmolality measurement is further increased

by the fact that it appears to be similar between eyes. Thus, it may not be necessary to take a measurement from both eyes.

In the population studied during the first phase of this work, contact lens wear did not appear to have an effect on tear film osmolality, but previous research has reported that contact lens wear increases tear film osmolality.[14-16] Further work, specifically designed to look for differences in tear film osmolality between contact lens wearers and non-contact lens wearers is still needed.

The usefulness of tear film osmolality as a clinical test will be ultimately limited by its diagnostic capabilities. The remainder of this thesis will examine how tear film osmolality measurements relate to other commonly used clinical tests of dry eye disease, and how tear film osmolality differs between normal and dry eyed populations.

# Chapter 6
# Comparison of Osmolality with Other Common Clinical Tests of Dry Eye Syndrome

## 6.1 Introduction

Dry eye disease, or dry eye syndrome, has most recently been defined as a "multifactorial ocular surface disease diagnosed by symptoms of discomfort and signs of visual disturbance, tear film instability and ocular surface damage, accompanied by increased osmolarity of the tear film and ocular surface inflammation".[1]

Although tear film osmolality has reported to be the "gold standard" diagnostic test for the evaluation of dry eye disease,[2-5] it has not been used to a large extent clinically, due to the lack of available instrumentation. Osmometers designed for use in optometric practice, have only recently started to appear in the marketplace. Historically, clinicians have had to rely on many other techniques and instruments to evaluate patients with dry eye disease. To date there is not a single, definitive test for the evaluation of dry eye disease, and clinicians often find themselves using one or more of the various tests available to evaluate patients' symptoms and the health of their ocular surfaces. Some of these tests include patient histories, validated questionnaires, linear analogue comfort scales, fluorescein or non-invasive tear break-up times, measurements of ocular surface redness, corneal and/or conjunctival staining (fluorescein, lissamine green or rose bengal), tear ferning, Schirmer Strips and Phenol Red Threads. [1, 3, 6-8] With so many diagnostic tests available, many studies have reported an absence of correlation between patient symptoms and signs in dry eye disease.[9-13]

Patient histories, questionnaires and comfort scales are techniques used by clinicians to evaluate patients' subjective symptoms, while the other tests are designed for the purpose of assessing the ocular surface damage caused by dry eye disease. Fluorescein and non-invasive tear break-up times are measures of tear film stability,[14, 15] and ocular surface redness is often thought to be a marker of ocular surface inflammation.[11, 16] Corneal and conjunctival staining techniques are used to assess the integrity of the ocular surface cells, [1, 3, 16-18] while Schirmer Strips and Phenol Red Thread tests are thought to be indicators of tear volume. [1, 3, 16, 19-21] Tear ferning is a simple technique often used to assess the quality of the tear film.[22-24]

The purpose of this study was to compare tear film osmolality measurements with various other commonly used tests of dry eye disease and to classify the relationships existing between them. As this study was run in conjunction with the second phase of the diurnal variation in tear film osmolality study (see Chapter 5), we were also able to measure the diurnal variations in some of the commonly used clinical tests. The tests chosen for use in this study were the Single Item Dry Eye Questionnaire (SIDEQ),[6] the Ocular Surface Disease Index (OSDI),[25, 26] and the McMonnies Questionnaire,[7, 8] a linear analogue comfort scale, non-invasive tear break-up time, subjective and objective measurements of ocular surface redness, and tear ferning.

## 6.2 Methods

This study was designed for the investigation of relationships between tear film osmolality measurements, and various other techniques used for the assessment of dry eye disease. It was also designed to explore the diurnal variations in commonly used clinical tests over the course of a routine working day. The protocol for this study was approved by the Office of Research Ethics at the University of Waterloo (ORE# 14862) prior to its commencement.

40 volunteer participants were recruited from the students, staff and faculty, at the School of Optometry, University of Waterloo, and enrolled in this study. An informed consent was obtained from all participants prior to their enrollment in the study.

### 6.2.1 Inclusion Criteria

Participants were eligible for entry into the study if they:

1. Were at least 17 years of age and had full legal capacity to volunteer.
2. Had read and signed an information consent letter.
3. Were willing and able to follow instructions and maintain the appointment schedule.
4. Had not used artificial tear lubricants 48 hours prior to any of the study visits.
5. Had clear corneas and no signs of active ocular disease.
6. Had undergone an ocular examination in the last two years.
7. Were a non-contact lens wearer*.

*Non-contact lens wear was defined as less than three full (eight hour) days of wear per month with no contact lens wear for at least seven days prior to study visits.

### 6.2.2 Exclusion Criteria

Participants were ineligible for entry into the study if they:

1. Wore any form of contact lenses*.
2. Had used artificial tear lubricants for 48 hours prior to any of the study visits.
3. Had any active ocular disease.
4. Had any systemic disease affecting ocular health.
5. Were using any systemic or topical medications that may affect ocular health.
6. Were pregnant or lactating.
7. Were participating in any other type of clinical or research study.

*Contact lens wear was defined as more than three full (eight hour) days of wear per month or contact lenses worn less than seven days prior to a study visit.

### 6.2.3 Study Visits

Participants were required to attend a total of six study visits over two days (three visits per day). Study visits were scheduled at nine am, twelve pm, and four pm; a time tolerance of plus/minus one

hour per visit was acceptable. Participants' visits were scheduled at the same time on both days, and all participants had an equal time interval between visits (i.e. all participants had a three hour break between the morning and mid-day visits, and a four hour break between the mid-day and afternoon visits). Participants were examined by one of two experienced clinicians at every visit.

### 6.2.4 Participant Randomization

At the first study visit, a randomization table was used to determine which eye (right or left) would be tested first for all of the procedures. The first eye measured remained the same for all of the subsequent visits.

### 6.2.5 Study Procedures

#### 6.2.5.1 Case History

A short case history was taken before participants were enrolled in the study. The case history asked questions about participants' age, general health, medication use, allergies, and artificial tear use. In addition, participants were asked what time they had woken up at their first visits on day 1 and day 2.

#### 6.2.5.2 Dry Eye Questionnaires

The Single Item Dry Eye Questionnaire (SIDEQ),[6] the Ocular Surface Disease Index (OSDI), and the McMonnies Questionnaire[7, 8] are all validated questionnaires designed for the evaluation of ocular surface comfort. Each of these questionnaires has a pre-defined scoring system – participants' final scores were compared with their mean tear film osmolality values to determine if a relationship was present. These questionnaires were administered at the initial study visit only, prior to any testing taking place. Participants were asked to complete the questionnaires as honestly as possible, and to use the previous 1 month (30 days) as a time reference when answering the questions.

Linear analogue scales were administered at every study visit, prior to any clinical measurements being taken. Linear analogue scales are simple linear scales ranging from 0 to 100 (see Figure 2.1), where 0 represented complete ocular surface discomfort and 100 represented complete ocular surface comfort. Participants were asked to record, by placing a slash mark or an "x" on the scale, what they felt the comfort of each of their eyes was at that particular moment. Participants were always asked to rate the comfort of their designated "first eye" first. Comfort scores were expressed as a percentage of ocular surface comfort, and were compared with tear film osmolality results. Comfort

scores were analyzed to determine if there was any change in participant comfort over the course of the day.

#### 6.2.5.3 Non-Invasive Tear Break-Up Time

Non-invasive tear break-up time (NITBUT) was the first objective tear film test performed as it was felt to be the least disruptive to the normal state of the ocular surface. It was measured with an Atlas Topographer, (Carl Zeiss Canada Ltd. Toronto, ON, Canada). The chin rest and head rest of the instrument were cleaned using alcohol swabs (Isopropyl alcohol 70%, Becton and Dickinson Canada Inc. Oakville, Ontario) prior to all measurements taken.

Participants were asked to place their chin on the appropriate chin rest and to fixate on a single red light directly in front of them. They were then asked to blink completely three times and hold their eye open as long as possible. This process was repeated for each measurement and three measurements were taken per eye.

The investigator watched the reflected concentric rings for the first sign of distortion or disruption in their pattern (see Figure 2.6) – this was considered to be equivalent to a disruption of the tear film surface, or tear film break-up.[14] The time to the first disruption of the image was measured in seconds, to the nearest 0.1 seconds. For each eye, all three measurements were averaged; the average score was considered to be the tear film break-up time. Tear film break-up time was compared with tear film osmolality and examined for diurnal variation in its values.

#### 6.2.5.4 Subjective Grading of Temporal Bulbar Conjunctival Redness

Subjective grading of temporal bulbar conjunctival redness was the next procedure completed after non-invasive tear break-up time measurements. All measurements were taken on the temporal bulbar conjunctiva of the right and left eyes using a biomicroscope. The chin rest and head rest of the biomicroscope were cleaned prior to use with an alcohol swab (Isopropyl alcohol 70%, Becton and Dickinson Canada Inc. Oakville, Ontario). Participants were initially asked to look straight ahead, and then to direct their gaze to either the left (for right eye measurements) or right (for left eye measurements).

Temporal bulbar redness was graded based upon a modified CCLRU 0-100 scale, where 0 was considered to be negligible redness, and 100 was considered to be severe redness (25 was trace, 50

was mild, and 75 was moderate).[27-29] Subjective redness scores were compared with tear film osmolality and over the course of the day.

#### 6.2.5.5 Objective Grading of Temporal Bulbar Conjunctival Redness

Objectively, temporal bulbar redness was determined using the SprectraScan PR650© Spectrophotometer (Photo Research Inc., Chatsworth, CA, USA) (see Figure 2.7). This was done immediately after subjective grading of bulbar redness was completed.

Prior to measurements being taken, the instrument chin and head rests were cleaned using an alcohol swab (Isopropyl alcohol 70%, Becton and Dickinson Canada Inc. Oakville, Ontario). Participants sat at the photometer and their head position was adjusted until it was aligned with the photometer. They were asked to look at fixation lights, on either their left or their right, in order to align their temporal bulbar conjunctiva with the instrumentation. Looking through the eye piece, the examiner positioned a black circle (with an area of approximately 19.63mm$^2$) over the area of the temporal conjunctiva that was to be measured. The area measured in this study was approximately 2mm from the temporal limbus, centred vertically between the upper and lower lids on the temporal bulbar conjunctiva (see Figure 2.8).

The spectrophotometer was not turned on until just before measurements were taken and it was turned off immediately after, in order to minimize the amount of time the ocular surface was exposed to the heat given off by the bulb. This was done in order to minimize tear film evaporation caused by the increased ambient temperature during the procedure. One measurement was taken per eye at each visit as it was felt that multiple measurements also had the potential to increase tear film evaporation un-necessarily, there-by interfering with the other tear film measurements being taken in the same visit.

Objective measurements of bulbar conjunctival redness were compared with tear film osmolality and over the course of the day.

#### 6.2.5.6 Tear Film Collection

Tear samples (1 – 2µl) were collected from both eyes of every participant at each of the study visits by one of two experienced investigators. Tears were collected using a single use, disposable glass capillary tube (Drummond Scientific Company, Broomall, PA, USA), while participants were reclined in a chair. Care was taken to ensure that the lid margin and corneal surfaces were not

touched. Participants were asked to look in a superior-nasal direction to further protect the corneal surface.

#### 6.2.5.7 Tear Ferning

Tear ferning was performed by taking a small (0.3μL) droplet of a tear sample, placing it on a clean, grease-free microscope slide, and then leaving it to dry at room temperature for five to seven minutes. The tear ferning patterns were microscopically examined and photographed at a magnification of x40 to x100. The images were graded by a masked examiner on a 1-4 scale. Tear ferning grades were compared with tear film osmolality results and they were examined for a diurnal variation.

#### 6.2.5.8 Tear Film Osmolality

Immediately after collection, tear samples were transferred to the Advanced Instruments Model 3100 Nanolitre Osmometer. Data was collected from each eye individually; tear samples were not pooled. Tear samples were disposed of immediately after osmolality measurements had been completed.

### 6.2.6 Instrument Calibration

The calibration of the Advanced Instruments Model 3100 Nanolitre Tear Osmometer was checked daily. The osmolality of a 304mOsm/Kg standard reference solution (Advanced Instruments Inc, Norwood, MA, USA) was measured a minimum of three times per day, whenever the instrument was being used. The calibration of the machine was considered to be acceptable if the mean ± standard deviation of the reference samples were within 304 ± 4mOsm/Kg. If the mean ± standard deviation of the reference samples did not fall within this range, than the instrument was immediately recalibrated before any tear film samples were measured.

### 6.2.7 Statistical Analysis

Statistical analysis was performed using STATISTICA 7 (StatSoft ®, Tulsa Oklahoma, www.statsoft.com) and all graphing analysis was completed using Graph Pad Prism 5 Software (Graph Pad Software Inc., www.graphpad.com).

Data from all participants was pooled in order to determine a population mean and standard deviation for each clinical measurement. A Sign test was used to determine if there was any difference between the first and second eye measurements. The Sign test was also used to determine if there was any difference between the right and left eyes of participants.

Repeated measures ANOVAs were performed to determine if there were any significant differences in test measures between visits or between days. Spearman correlations were used to determine if there was any association between the test measures and tear film osmolality results.

Sensitivity and specificity values were calculated to determine the diagnostic potential of tear film osmolality as an indicator of dry eye disease, using 317mOsm/Kg as a cutoff value[30] (i.e. individuals with tear film osmolalities ≥317mOsm/Kg were classified as having an abnormal result). The diagnostic potential of tear film osmolality was compared against the diagnostic capabilities of the individual validated questionnaires completed during this study (SIDEQ, OSDI and McMonnies) and some of the clinical tests (NIBUT and subjective redness) which have clinically accepted dry eye diagnostic criteria. For the purposes of this analysis, participants were classified as having either dry eye disease or not (normal) based upon a single criteria which was either the pre-defined, validated scoring criteria of a questionnaire or the accepted cutoff value for a clinical test.

## 6.3 Results

There was no significant difference between first eye and second eye values for all of the measurements taken ($p>0.05$), nor was there a significant difference between measurements taken on participants' right and left eyes ($p>0.05$). Investigators felt that first eye measurements had the least chance of being influenced by extraneous factors during the data collection processes, therefore all of the following results presented are first eye measurements only.

### 6.3.1 Analysis of Participant Symptoms

Participant's ocular surface comfort was assessed using four different techniques. Three validated questionnaires were used for the assessment of overall ocular surface comfort, while a linear analogue comfort scale was used to measure immediate comfort. The results are presented below.

#### 6.3.1.1 Single Item Dry Eye Questionnaire (SIDEQ)

The SIDEQ (Chapter 2, Appendix 1) is a single item, self assessment questionnaire that allows participants to rate their ocular surface comfort on a 0 to 4 scale.[6] A score of "0" corresponds to no discomfort (no dry eye disease), while a score of "4" corresponds to severe symptoms of ocular surface discomfort, which have often been associated with advanced dry eye disease.[6]

The mean SIDEQ score of this population was $1.1 \pm 0.93$. A score of 1.0 is classified as "trace" dry eye symptoms and it can be seen that our population, on average, did not have any significant dry

eye symptoms. The breakdown of the SIDEQ results for the entire population can be seen in Table 6.1. One participant did not complete a SIDEQ questionnaire, therefore only the data from the remaining 39 participants was included in Table 6.1 and analyzed in these results. For the purposes of this study, individuals with a score of 0 (none) or 1 (trace), were considered to be normal.

**Table 6-1: Results of the SIDEQ questionnaire – population is classified based upon their symptom score. Scores of 0-1 were considered to be normal, 2 were mild, 3 were moderate, and 4 were severe ocular surface discomfort symptoms.**

| Classification | Mean Score | Number of Participants |
|---|---|---|
| **Normal (0-1)** | 0.62 | 29 |
| **Mild (2)** | 2 | 6 |
| **Moderate (3)** | 3 | 4 |
| **Severe (4)** | -- | 0 |

The SIDEQ was used to gain an understanding of the population's general ocular surface comfort, rather than their immediate comfort, and was only administered at the initial study visit. Individual's SIDEQ score was compared with their mean tear film osmolality, and analyzed with a Spearman correlation (Figure 6.1). A Spearman correlation of r = 0.60 was considered to be clinically significant.[16] The correlation between SIDEQ score and mean tear film osmolality was not significant (r = 0.1347).

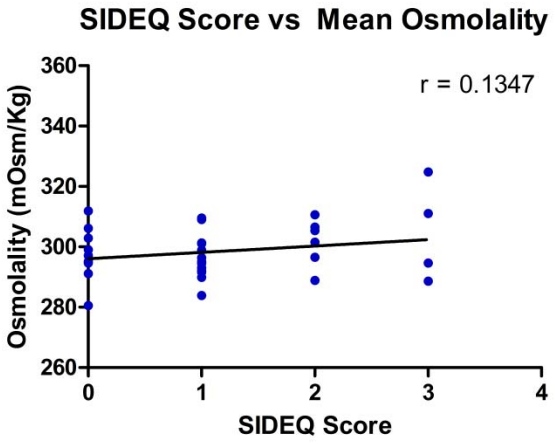



**Figure 6-1: SIDEQ scores plotted as a function of mean tear film osmolality (mOsm/Kg). Spearman correlation (r = 0.1347) was not significant for these variables.**

When our population was classified as being either dry eyed (SIDEQ score ≥2) or normal (SIDEQ score <2), tear film osmolality was found to have a sensitivity of 10%. The specificity of tear film osmolality was much higher (100.0%), which suggests that tear film osmolality as a test may be more useful in correctly identifying normal individuals rather than individuals with dry eye disease.

#### 6.3.1.2 Ocular Surface Disease Index (OSDI)

The OSDI is a 12-item quality of life questionnaire designed to measure the severity of ocular surface disease and its impact on vision related functions.[25, 26] Participants were asked to evaluate each of the items on the instrument on a 5-point Likert scale (all of the time, most of the time, half of the time, some of the time, none of the time). The 12 items were divided into three subgroups and the scores for each of the subgroups were summed to get the total OSDI score between 0-100. The higher a participants score, the greater the disability participants experienced (see Chapter 2, Appendix 2).[25, 26]

The mean OSDI score of this population was 6.70 ± 8.04 which is quite low. As seen in the SIDEQ, our population did not suffer from significant dry eye symptoms. Detailed results of the OSDI can be seen in Table 6.2.

**Table 6-2: Results of the OSDI questionnaire – population is classified based upon their symptom score. Scores of 0-12 were considered to be normal; scores of 13-22 were indicative of mild dry eye, 23-32 of moderate dry eye, and 33-100 of severe dry eye disease.**

| Classification | Mean Score | Number of Participants |
|---|---|---|
| **Normal (0-12)** | 3.3 | 32 |
| **Mild (13-22)** | 15.9 | 5 |
| **Moderate (23-32)** | 27.8 | 3 |
| **Severe (33-100)** | -- | 0 |

Much like the SIDEQ, the OSDI was used to understand the population's overall ocular surface comfort, rather than their immediate comfort. As such it was only administered at the initial study visit. OSDI scores were compared with individuals' mean tear film osmolality, and analyzed with a Spearman correlation (Figure 6.2). A Spearman correlation of r = 0.60 was considered to be clinically significant.[16] The correlation between OSDI score and mean tear film osmolality was not significant (r = 0.0331).

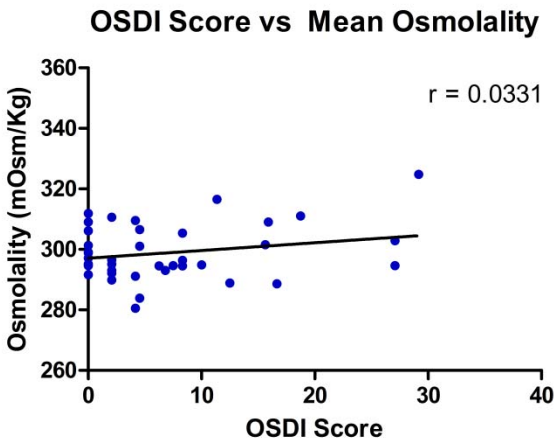



**Figure 6-2: OSDI scores plotted as a function of mean tear film osmolality (mOsm/Kg). Spearman correlation (r = 0.0331) was not significant for these variables.**

When our population was classified as either dry eyed (OSDI score ≥13) or normal (OSDI score <12), based upon the OSDI criteria we identified 8 individuals who had primarily mild dry eye disease. The sensitivity of tear film osmolality as a clinical diagnostic test in this population was 11.1%, while the specificity of tear film osmolality was much higher at 96.8%.

#### 6.3.1.3 McMonnies Questionnaire

The McMonnies Questionnaire is made up of 15 questions, 14 of which focus on clinical "risk factors" for dry eye disease derived from the literature.[30] It uses a weighted-scale scoring algorithm, to obtain an overall "Index" score. The Index score can fall between 0 and 45, and can be used to categorize participants based on their severity of dry eye disease; higher scores are indicative of greater dry eye disease (see Chapter 2, Appendix 3).[7, 8]

The mean McMonnies questionnaire score of this population was 7.13 ± 4.69, which is considered to be normal. As with the SIDEQ and OSDI questionnaires, the McMonnies questionnaire indicated that our population did not suffer from significant dry eye symptoms. The McMonnies questionnaire results are shown in Table 6.3.

**Table 6-3: Results of the McMonnies questionnaire – population is classified based upon their symptom score. Scores of 0-10 were considered to be normal, scores of 11-19 were suggestive of borderline dry eye, and scores of 20 or greater, were indicative of dry eye disease.**

| Classification | Mean Score | Number of Participants |
|---|---|---|
| **Normal (0-10)** | 5.2 | 30 |
| **Borderline (11-19)** | 13.8 | 10 |
| **Dry Eye (≥20)** | -- | 0 |

The McMonnies questionnaire was used in the same fashion as both the SIDEQ and OSDI questionnaires, because it was used to examine individuals' overall ocular surface comfort rather than their immediate comfort. It was only completed at the initial study visit, and Index scores were compared with mean tear film osmolality using a Spearman correlation (Figure 6.3). A Spearman correlation of 0.60 was considered to be clinically significant.[16] The correlation between McMonnies Index score and mean tear film osmolality was not significant (r = 0.2727).

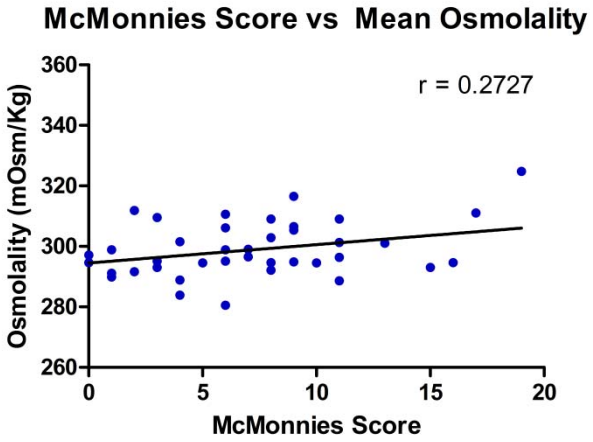



**Figure 6-3: McMonnies questionnaire scores plotted as a function of mean tear film osmolality (mOsm/Kg). Spearman correlation (r = 0.2727) was not significant for these variables.**

When the McMonnies questionnaire criteria was used to classify our population as either dry eyed (McMonnies score ≥11) or normal (OSDI score <11), we identified 10 individuals who had borderline dry eye. The remaining 30 individuals in our study did not have dry eye disease. When calculated based upon the McMonnies questionnaire criteria, the sensitivity of tear film osmolality as a clinical diagnostic test in this population was 10.0%, and its' specificity was much higher at 96.7%.

### 6.3.1.4 Linear Analogue Comfort Scales

Linear analogue comfort scales are another way of assessing ocular surface comfort,[31] although they have yet to be validated. They are simple linear scales ranging from 0 to 100. Participants were asked to record on the scale, with a slash or an "x", what they felt the comfort of their eyes were at that particular moment. In the particular scale used in this study, 0 was defined as complete ocular surface discomfort and 100 as complete ocular surface comfort. Scores were expressed as a percentage. They were examined for diurnal fluctuations, and were compared with mean tear film osmolality values to determine if a relationship existed between the two measures. A Spearman correlation of r = 0.60 was considered to be clinically significant.[16]

Although some participants reported increased discomfort towards the end of the day, the overall change in ocular surface comfort of the entire population was not significant (p=0.16). There was also no difference in subjective assessment of ocular surface comfort using a linear analogue scale between days (p=0.91) (Figure 6.4).

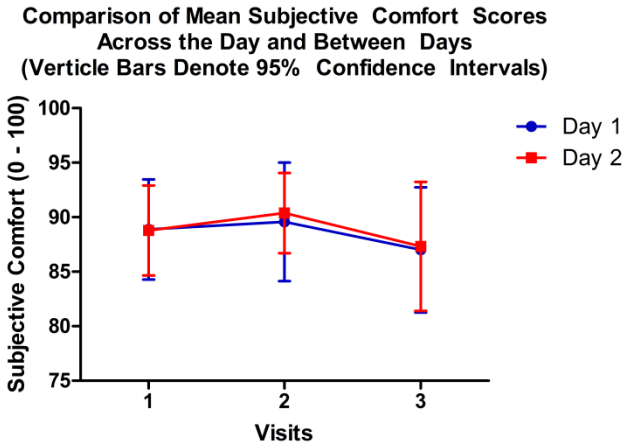



**Figure 6-4: Comparison of mean subjective comfort, expressed as a percentage across the day and between days (all participants pooled).**

In the entire population, subjective comfort scores did not significantly correlate with tear film osmolality at any time point during the study. Table 6.4 summarizes the correlations found between subjective comfort and tear film osmolality at each study visit. Figure 6.5 is a graph of mean subjective comfort scores plotted as a function of mean tear film osmolality – a significant Spearman correlation is not seen here either (r = -0.1622).

**Table 6-4: Spearman correlation (r) values for the comparison of subjective comfort score (percentage) and tear film osmolality (mOsm/Kg) at each study visit. Statistically significant correlations are shown in *italics*. A clinically significant correlation (r = 0.60) was not found at any of the time points.**

| | Day 1: 9am± 1hour | Day 1: 12pm± 1hour | Day 1: 4pm± 1hour | Day 2: 9am± 1hour | Day 2: 12pm± 1hour | Day 2: 4pm± 1hour |
|---|---|---|---|---|---|---|
| **Spearman Correlation (r)** | -0.0932 | -0.1749 | -0.1339 | -0.2166 | 0.0804 | -0.0710 |

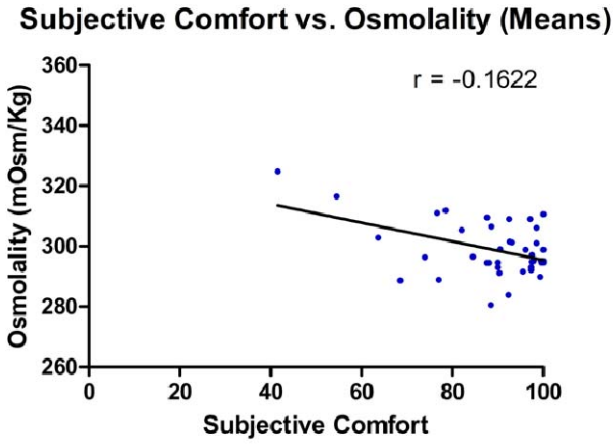



**Figure 6-5: Mean subjective comfort score (percentage) plotted as a function of mean tear film osmolality (mOsm/Kg). A significant Spearman correlation was not present (r=-0.1622).**

End of day discomfort has been previously associated with dry eye disease, particularly in contact lens wearers.[32, 33] Therefore investigators thought it was worthwhile to split this population into two groups – a group who did not experience end of day discomfort and a group who did experience end of day discomfort. Subjective comfort scores were then again compared with tear film osmolality to determine if a relationship existed between tear film osmolality and subjective comfort in individuals with symptoms of end of day discomfort.

End of day discomfort was defined as a 5% or greater decrease in comfort between the initial morning measurement and the final measurement at the end of the day. Participants included in the

end of day discomfort group had to have demonstrated a 5% decrease in ocular surface comfort on at least one of the study days. 16 of 40 participants were classified as having end of day discomfort. The average decrease in comfort experienced was 10.5 ± 16.1% from morning to evening measurements.

Subjective comfort scores in both groups were compared with tear film osmolality using Spearman correlations (Figure 6.6). No correlation was found between tear film osmolality and subjective comfort score in the group of participants who did not experience end of day dryness (r=0.04081). The group of participants who experienced end of day dryness did not demonstrate any significant correlation between tear film osmolality and subjective comfort scores (r=-0.1572) either, although a weak negative trend (decreasing osmolality with increasing comfort) was present. It is possible that this trend may become more significant if a larger population was tested, or if the participants had had more severe symptoms of end of day dryness.

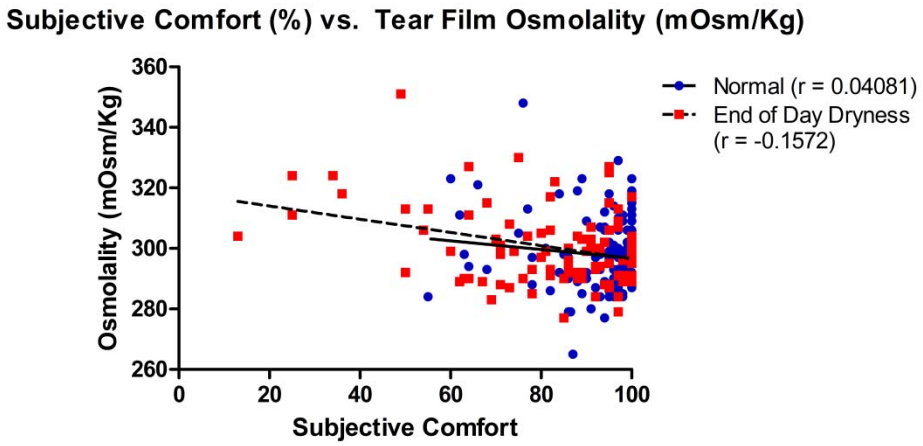



**Figure 6-6: Subjective comfort score (percentage) plotted as a function of tear film osmolality (mOsm/Kg). Participants were divided into two group based upon their end of day discomfort. Significant correlations were not found between tear film osmolality and subjective comfort scores in either group (normal: r = 0.04081, end of day dryness group: r = -0.1572), but a weak trend to decreasing osmolality with increasing comfort was present in the group of participants who experienced end of day dryness.**

### 6.3.2 Analysis of Participant Signs

Participant's ocular surfaces were assessed using four different clinical procedures. Non-invasive tear break-up time was used to measure the stability of the tear film, bulbar conjunctival redness, an indication of ocular surface inflammation, was measured both subjectively and objectively, and tear ferning was undertaken to examine the quality of the tear film. The results of these clinical procedures are presented below.

#### 6.3.2.1 Non-Invasive Tear Break-Up Time (NIBUT)

NITBUT was measured with the Atlas Topographer, (Carl Zeiss Canada Ltd. Toronto, ON, Canada). Participants were asked to blink completely three times, and then to hold their eyes open for as long as possible. The clinician started timing tear film stability the moment they eye was opened, and stopped the measurement when the first sign of distortion or disruption in the reflected ring pattern was noticed (see Figure 2.6).[14] Tear break-up time was measured to the nearest 0.1 seconds. Three measurements were taken per eye and the times were averaged in order to calculate an individual's tear break-up time. Tear break-up time was compared with tear film osmolality values, and a Spearman correlation of $r = 0.60$ was considered to be clinically significant.[16]

The mean NIBUT of the population tested was $13.4 \pm 17.6$s (range of 1.6 – 160.5s). A significant change in NIBUT over the day was not found ($p=0.317$), but there was a significant difference in NIBUT between days ($p<0.05$) (Figure 6.7). Reasons for this difference will be speculated upon in the ensuing discussion.

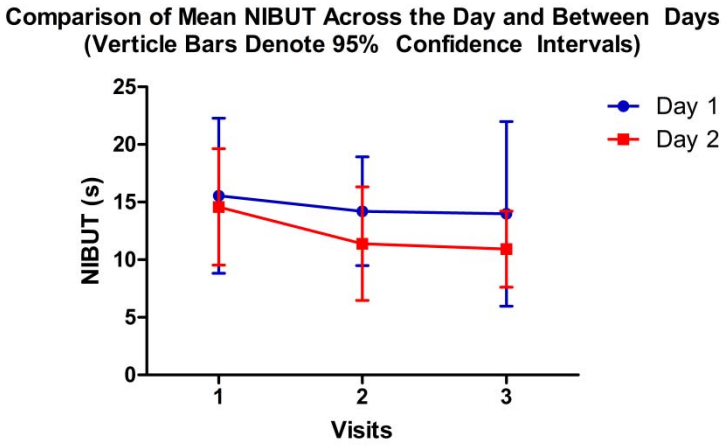



**Figure 6-7: Comparison of mean NIBUT (s) across the day and between days (all participants pooled).**

NIBUT was found to significantly correlate with tear film osmolality at only one time point (Day 1, morning visit) during the study. All of the other correlations were insignificant. Table 6.5 summarizes the correlations found between NIBUT and tear film osmolality at each study visit. Although a significant negative correlation was found between NIBUT and tear film osmolality measures at one visit, this correlation is quite weak, and is not considered clinically significant. Figure 6.8 is a graph of mean NIBUT plotted as a function of mean tear film osmolality – a significant Spearman correlation is not seen here either (r = -0.2280).

**Table 6-5: Spearman correlation (r) values for the comparison of NIBUT (s) and tear film osmolality (mOsm/Kg) at each study visit. Statistically significant correlations are shown in *italics*. A clinically significant correlation (r=0.60) was not found at any of the time points, although a statistically significant correlation was found at the morning visit on Day 1.**

| | Day 1: 9am± 1hour | Day 1: 12pm± 1hour | Day 1: 4pm± 1hour | Day 2: 9am± 1hour | Day 2: 12pm± 1hour | Day 2: 4pm± 1hour |
|---|---|---|---|---|---|---|
| **Spearman Correlation (r)** | *-0.3323* | -0.1628 | -0.1206 | -0.1621 | -0.0990 | 0.0955 |

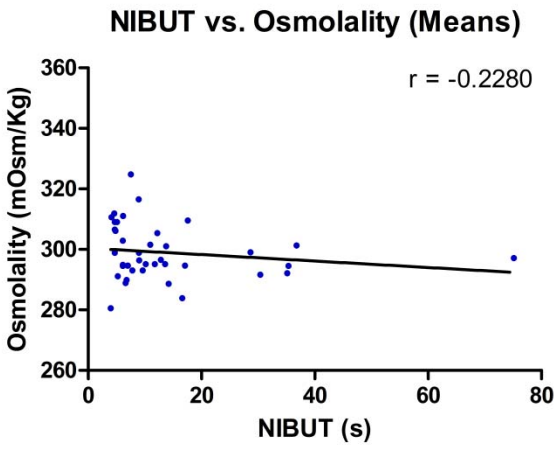



**Figure 6-8: Mean NIBUT (s) plotted as a function of mean tear film osmolality (mOsm/Kg). A significant Spearman correlation was not present (r = -0.2280).**

Classification of our population into a dry eyed and a normal group was done using a NIBUT cutoff value of 10s. Individuals with NIBUT <10s were considered to have dry eye disease and individuals with NIBUT >10s were considered to be normal.[16] Based upon this criterion 22 individuals in our study were classified as having dry eye and 18 individuals were classified as not having dry eye disease. The sensitivity of tear film osmolality (cutoff 317mOsm/Kg)[30] in this population was 9.1%, while the specificity of tear film osmolality was 100%, suggesting again that tear film osmolality may be more useful for identifying normal individuals rather than for identifying individuals with dry eye disease.

#### 6.3.2.2 Subjective Redness

Subjective redness measurements were taken from the temporal bulbar conjunctiva while participants looked to either the left or the right. Redness was graded by one of two experienced investigators, using a modified CCLRU 0-100 scale. A grade of 0 was considered to be negligible redness, 25 was trace, 50 was mild, and 75 was moderate and 100 was considered to be severe redness.[27-29] Subjective redness scores were compared over the course of the day, and with tear film osmolality values. A Spearman correlation of r = 0.60 was considered to be clinically significant.[16]

Subjective redness was found to change significantly over the course of a day ($p<0.05$), and was higher in the afternoon but it did not change between days ($p=0.744$). The increase in redness over the course of the day was more marked on day 1 than on day 2 (Figure 6.9). Although subjective

redness scores changed significantly over the day, the magnitude of the increase was less than 5 units, therefore this difference is not likely clinically significant.

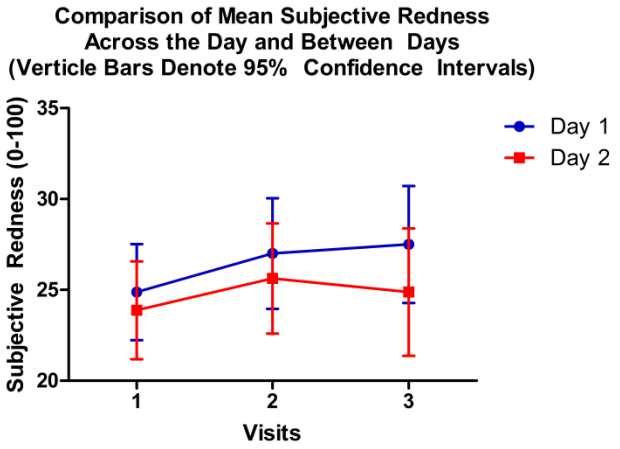



**Figure 6-9: Comparison of mean subjective redness grades (0-100 scale) across the day and between days (all participants pooled).**

Table 6.6 summarizes the correlations found between mean tear film osmolality and mean subjective redness grades at each visit. Overall there was no correlation (r = -0.2280) between mean tear film osmolality and mean subjective redness scores (Figure 6.10).

**Table 6-6: Spearman correlation (r) values for the comparison of subjective redness scores (0-100 scale) and tear film osmolality (mOsm/Kg) at each study visit. Statistically significant correlations are shown in *italics*. A clinically significant correlation (r=0.60) was not found at any of the time points.**

| | Day 1: 9am± 1hour | Day 1: 12pm± 1hour | Day 1: 4pm± 1hour | Day 2: 9am± 1hour | Day 2: 12pm± 1hour | Day 2: 4pm± 1hour |
|---|---|---|---|---|---|---|
| **Spearman Correlation (r)** | 0.1575 | 0.1437 | 0.1323 | 0.2045 | -0.0004 | 0.0941 |

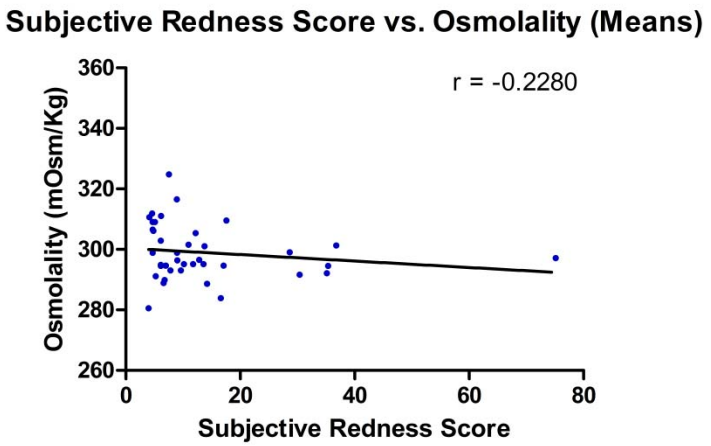



**Figure 6-10: Mean subjective redness score (0-100 scale) plotted as a function of mean tear film osmolality (mOsm/Kg). A significant Spearman correlation was not present (r= - 0.2280).**

Investigators felt that a subjective redness score of 50, comparable to grade 2 redness on a typical 0-4 clinical scale, was an abnormal result. In this population then, subjective redness scores ≥50 were thought to be indicative of dry eye disease, while subjective redness scores <50 were considered to be normal. When these classification criteria were applied to our population we had 1 individual with dry eye disease and 39 individuals who were normal. The sensitivity of tear film osmolality (cutoff 317mOsm/Kg)[30] in this population as 0%, while the specificity of tear film osmolality was 95.0%, sObjective Redness

The SprectraScan PR650© Spectrophotometer (Photo Research Inc., Chatsworth, CA, USA) (Figure 2.7) was used to objectively measure temporal bulbar redness. It measures luminance and chromaticity values through the measurement of absolute intensity at each wavelength of light and then uses these values (luminance and chromaticity) to calculate the equivalent CIE u' (Commision Internationale d'Eclairage) value. u' is one of two chromaticity coordinates (u', v') used to describe the position of a colour in the CIE colour space diagram (1976) and does not have a specified unit. Higher u' values have been shown to correspond with greater bulbar conjunctival redness.[29, 34]

Objective measurements of bulbar conjunctival redness were examined for diurnal changes and they were also correlated with tear film osmolality values.

Bulbar conjunctival redness was not found to change significantly over the day ($p=0.70$) when measured objectively, but it was found to be significantly different between days ($p<0.05$). In this

case, the statistics may be deceptive, as the magnitude of the difference in u' values between days 1 and 2 is less than 0.01units. Clinically this is likely insignificant.

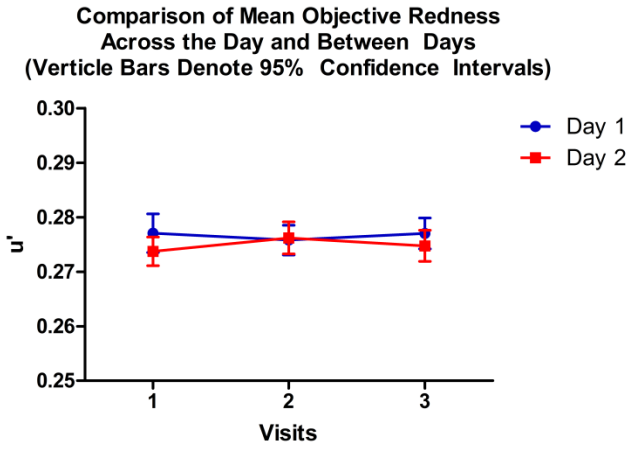



**Figure 6-11: Comparison of mean objective redness (u') across the day and between days (all participants pooled).**

Table 6.7 provides a summary of all of the correlations found between mean tear film osmolality and mean objective redness values at each visit. A significant correlation between mean tear film osmolality and mean objective redness was not found (r = 0.1233) (Figure 6.12).

**Table 6-7: Spearman correlation (r) values for the comparison of objective redness (u') and tear film osmolality (mOsm/Kg) at each study visit. Statistically significant correlations are shown in *italics*. A clinically significant correlation (r = 0.60) was not found at any of the time points.**

| | Day 1: 9am± 1hour | Day 1: 12pm± 1hour | Day 1: 4pm± 1hour | Day 2: 9am± 1hour | Day 2: 12pm± 1hour | Day 2: 4pm± 1hour |
|---|---|---|---|---|---|---|
| **Spearman Correlation (r)** | 0.0268 | -0.0697 | 0.1306 | -0.0219 | -0.0846 | 0.0256 |

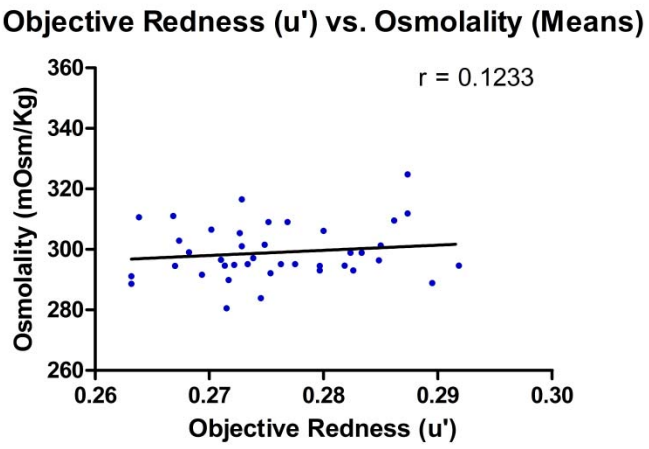



**Figure 6-12: Mean objective redness value (u') plotted as a function of mean tear film osmolality (mOsm/Kg). A significant Spearman correlation was not present (r= 0.1233).**

#### 6.3.2.3 Tear Ferning

Tear ferning patterns were photographed and then graded by a masked examiner on a 1-4 scale. A grade of 1 or 2 has been reported to be indicative of a normal tear film, while a grade of 3 or 4 is indicative of an abnormal tear film or dry eye disease.[22, 23] Diurnal changes in tear ferning grades were examined over the course of the day, and over both days. Tear ferning grades were also compared with tear film osmolality results, in an attempt to determine if a relationship existed between them.

When compared over the day and between days, no significant changes in tear ferning grade was found (over a day: p=0.31, between days: p=0.39) (Figure 6.13).

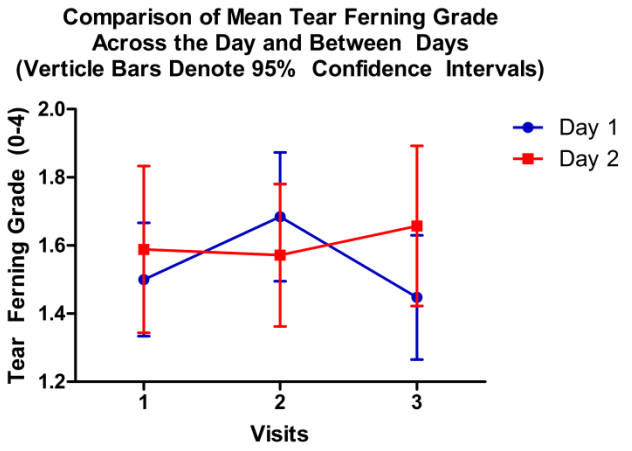



**Figure 6-13: Comparison of mean subjective redness grades (0-100 scale) across the day and between days (all participants pooled).**

Statistically significant positive correlations were found between tear ferning grades and tear film osmolality at the midday (12pm±1hour) (r = 0.3162) and evening visits (4pm±1hour) (r = 0.5312) on Day 2 (Table 6.8). Although neither of these values is clinically significant, the correlation found at the evening visit on day 2 does approach the level of clinical significance. A weak positive correlation was also found between mean tear ferning grade and mean tear film osmolality (r = 0.3978) (Figure 6.12).

**Table 6-8: Spearman correlation (r) values for the comparison of tear ferning grades (1-4 scale) and tear film osmolality (mOsm/Kg) at each study visit. Statistically significant correlations are shown in italics. A clinically significant correlation (r = 0.60) was not found at any of the time points, although the correlation at the evening visit on day 2 does approach the level of clinical significance.**

| | Day 1: 9am± 1hour | Day 1: 12pm± 1hour | Day 1: 4pm± 1hour | Day 2: 9am± 1hour | Day 2: 12pm± 1hour | Day 2: 4pm± 1hour |
|---|---|---|---|---|---|---|
| **Spearman Correlation (r)** | 0.2995 | 0.2379 | 0.2574 | 0.2811 | *0.3162* | *0.5312* |

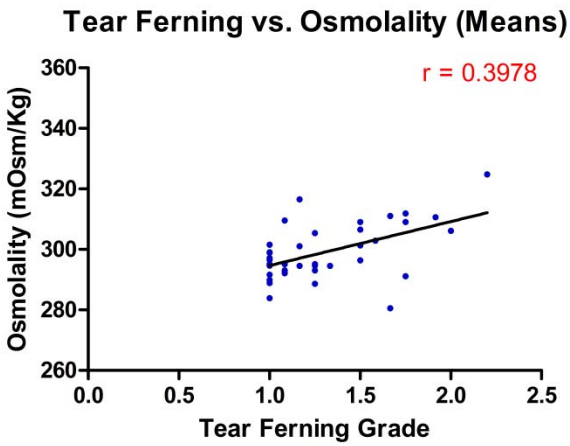



**Figure 6-14: Mean tear ferning grade (1-4) plotted as a function of mean tear film osmolality (mOsm/Kg). A significant positive Spearman correlation was present (r= 0.3978).**

## 6.4 Discussion

This study was designed to explore the relationships that may exist between tear film osmolality and other commonly used clinical instruments used for the assessment of dry eye disease. Some of these instruments, such as validated questionnaires and linear comfort scales are designed to assess patients' dry eye symptoms, while others, such as NIBUT, ocular surface redness measures and tear ferning are designed to assess the health of an individuals' tear film and ocular surface.

Research has suggested that tear film osmolality may be the new "gold standard" test for dry eye disease,[2-5] in that it has been shown to be associated with patients' symptoms, and it is thought to be the driving factor behind many of the processes causing the ocular surface damage commonly found in patients with dry eye disease.[2]

The population we studied was a normal, healthy population composed of primarily non-dry eyed individuals. Participants were non-contact lens wearers and could not use artificial tears for at least 48 hours prior to any of their study visits. Although one may expect to find a higher percentage of individuals with dry eye disease in a non-contact lens wearing population (due to self selection and contact lens dryness), the inability to use artificial lubricants probably counteracted this. Anyone with severe dry eye symptoms would probably find it quite difficult to not use drops for a 48 hour period of time.

Approximately 10% of this population had moderate dry eye symptoms, as assessed by the SIDEQ, OSDI and McMonnies questionnaires, but no one had severe symptoms of dry eye disease. In this population no correlations were found between tear film osmolality values and any of the SIDEQ, OSDI or McMonnies questionnaire scores. Therefore, it is apparent that in normal individuals with mostly mild symptoms of dry eye disease, tear film osmolality does not correlate well with patient symptoms. If this study were repeated on a population that consisted primarily of moderate to severe dry eyed individuals these results may be different, but more investigation is needed for this to be determined.

The sensitivity (approximately 10%) and specificity (approximately 98%) of tear film osmolality (cutoff 317mOsm/Kg)[30] as a clinical diagnostic test for dry eye disease were very similar in our population, regardless of which specific questionnaire (SIDEQ, OSDI or McMonnies) was used to classify participants. Unfortunately our population did not contain a significant number of individuals with dry eye disease, and the clinical application of these sensitivity and specificity results is limited as a result. Further work is needed in a larger population, with more significant symptoms of dry eye disease, before the sensitivity and specificity of tear film osmolality can be truly understood.

Linear analogue comfort scales were used to assess participants' immediate comfort in order to determine if immediate comfort had a stronger correlation with tear film osmolality than the more "general" ocular surface comfort assessment (questionnaires). A significant diurnal variation in subjective comfort assessed with linear analogue scales was not present, nor was a significant correlation between comfort scores (expressed as a percentage) and tear film osmolality values at any of the time points assessed during the study. This supports the previous finding that in a normal population tear film osmolality does not correlate well with individuals' ocular surface comfort assessments.

Of the 40 participants who participated in this study, 16 were defined as having end of day discomfort symptoms. End of day discomfort is a popular topic in dry eye research, particularly in the investigation of contact lens associated dry eye disease.[32, 33] For the purposes of this study, end of day discomfort was defined as a decrease of 5% or greater in ocular surface comfort between the morning and evening visits on at least one day. When tear film osmolality values were compared with comfort scores in the populations who experienced end of day discomfort, a stronger trend to decreasing comfort with increasing tear film osmolality was found. Unfortunately this trend was not

significant, as the correlation was still very weak. Perhaps if this type of study could be repeated in participants with more severe symptoms of dry eye disease and end of day discomfort, the correlation may grow stronger. Further investigation is needed for this to be established.

Tear break-up time is a commonly used clinical test in the evaluation of patients complaining of dry eye symptoms. It can be done through the instillation of sodium fluorescein (NaFl) and subsequent examination under cobalt blue light at a biomicroscope (NaFl TBUT or commonly, TBUT),[35] or it can be measured non-invasively through the reflection of a series of rings off the corneal surface (NIBUT).[14] In this study, tear break up time was measured non-invasively, as we did not want to disrupt the tear film through the instillation of NaFl prior to the measurement of tear film osmolality and tear ferning.

NIBUT did not change significantly over the course of a day, but it was found to be significantly different on Day 2 compared with Day 1. Looking at Figure 6.7, it is apparent that the 95% confidence interval at visit three on Day 2 is very large – at this time point in the study, one individual demonstrated an exceptionally long NIBUT (>100sec), which had not been seen at any other time point, or in any other participant in the study. Removing this individual from the analysis, eliminated the significant difference found between mean NIBUT measured on Days 1 and 2 ($p=0.2789$), but investigators felt that this individual's NIBUT could provide important information in the tear film osmolality correlation analysis and chose not to remove this data from the previously presented results.

NIBUT was found to correlate significantly with tear film osmolality at only one visit during the study (Day 1, morning visit). This correlation was a very weak, negative correlation suggesting that as tear break-up time increased, osmolality decreased. Theoretically, this is what we would have expected to happen. Unfortunately, this correlation was not clinically significant ($r = -0.3323$, $r<0.60$), and no other significant correlations between NIBUT and tear film osmolality were found at any of the study visits. When mean NIBUT was compared with mean tear film osmolality values, a weak negative trend was noticed, but again this correlation was not significant ($r = -0.2280$).

Ocular surface inflammation is associated with dry eye disease.[36] Typically, bulbar conjunctival redness is measured, as it is thought to be a good indicator of the inflammatory processes taking place on the ocular surface.[7, 11] Bulbar conjunctival, or ocular surface, redness can be measured in one of two ways, either subjectively using a standardized grading scale, or objectively using a spectrophotometer which measures the absolute intensity at each wavelength of light and calculates

an equivalent CIE u' (Commision Internationale d'Eclairage) value. Increasing values of u' have been shown to be associated with increasing ocular surface redness.[29, 34]

Subjective redness in this study was graded on the temporal bulbar conjunctiva while participants were seated at a biomicroscope by an experienced investigator. A modified CCLRU (0-100) scale was used as the standardized scale for this process.[27-29] Mean subjective redness was found to have significantly increased by the end of the day ($p<0.05$), particularly on Day 1, although no significant difference was found between overall measurements taken on Days 1 and 2. The increase in redness that was found to be significant was equal to less than a 5 unit change on the grading scale used, therefore clinical significance of this statistically significant increase remains to be seen.

It is questionable as to whether or not an individual would be able to detect a change in redness of this magnitude, as most grading scales are built in steps of 4 and a single step change on a grading scale with 4 levels of severity would be equivalent to a 25 unit change on the modified CCLRU 0-100 scale used.[27-29] Recently, a 0-100 scale has been developed which is divided into steps of 10 units[37] – it is possible that a clinician who is very experienced with this scale may be able to perceive half step, or 5 unit changes, in ocular surface redness, but further research into perceivable changes and what they mean clinically is still needed.

Subjective redness was not found to correlate with tear film osmolality at any time point during the study. Inspection of Figure 6.9 shows that there is a weak negative trend occurring ($r=-0.2280$). If tear film hyperosmolality is one of the forces driving the ocular surface inflammation,[2] than one would expect to find a positive correlation between these two measurements (ocular surface redness increases with increasing tear film osmolality), which was not the case in our study.

The sensitivity (approximately 5%) and specificity (approximately 97%) of tear film osmolality (cutoff 317mOsm/Kg)[30] as a clinical diagnostic test for dry eye disease in populations defined by either their NIBUT ($<10$)[16] or their subjective redness scores ($\geq 50$) suggest that tear film osmolality may be a more useful test for defining normal individuals rather than individuals with dry eye disease. Clinically, the significance of these findings needs greater investigation as our study was limited by both its relatively small sample size, and by the fact that the vast majority of our participants did not have any symptoms of dry eye disease. Only two individuals had osmolality values greater than 317mOsm/Kg, and only one individual had a subjective redness score >50. A great deal more research is needed into understanding the diagnostic potential of tear film osmolality and other clinical measures, when used either as stand-alone tests or in a series of diagnostic procedures.

When measured objectively, temporal bulbar conjunctival redness was not found to change significantly over the course of the day, but it was found to be significantly different between days ($p<0.05$). Previous work with the spectrophotometer has demonstrated that bulbar conjunctival redness measured with this technique does in fact increase over the course of the day. [34] Our study may be limited in its ability to detect this difference, simply by the length of day we studied. We did not take measurements in the early hours of the morning or in the late hours of the evening, and as such we may not have missed detecting significant changes in objectively measured ocular surface redness. Although there was a statistically significant difference between measurements taken on Day 1 and Day 2, the magnitude of this difference is on the order of 0.01 units and it is unlikely to be clinically significant. Determining the clinical significance of this difference, if there is one, warrants further investigation.

As with subjectively measured ocular surface redness, no correlation was found to exist between objectively measured ocular surface redness and tear film osmolality at any of the time points investigated in this study. Tear film osmolality does not appear to correlate well with any of the clinical measures investigated thus far, at least in a normal population. If this study were repeated in a population comprised of primarily moderate to severe dry-eyed individuals, perhaps the results would be different.

The final clinical measure investigated in this study was tear ferning. Although not performed as often clinically, tear ferning is a simple, novel method capable of providing important information about the quality of the tear film.[22, 23] Tear ferning grades did not vary significantly over the day and they were consistent between both days of the study. Interestingly, tear ferning appeared to have the greatest potential for correlation with tear film osmolality measurements. Significant positive correlations ($r > 0.3$, $p<0.05$) were noticed between these two tests at both the midday and afternoon visits on Day 2. At all of the other time points, the correlations approached statistical significance. The correlation between mean tear ferning grade and mean tear film osmolality ($r=0.3978$) was not clinically significant ($r<0.60$), but it was statistically significant, and a definite positive trend was present. That is, tear ferning grade increased as tear film osmolality increased. Tear ferning and tear film osmolality measures are both dependent upon the concentrations of dissolved solutes in the tear film, thus the relationship that appears to exist between them would not have been unrealistic to expect. Further investigation of tear ferning patterns and tear film osmolality in a population which

has more significant symptoms of dry eye disease is required to obtain greater details regarding the relationship between these two tests. Perhaps tear ferning will become a more important clinical test in the evaluation of dry eye disease and incorporated into routine clinical practice as a result.

Overall, tear film osmolality did not correlate well with any of the commonly used clinical tests used in the evaluation of dry eye syndrome. Although there was a weak positive correlation between tear film osmolality and tear ferning results, all of these relationships need to be investigated in more detail, in a population with greater symptoms and signs of dry eye disease.

# Chapter 7
# Comparison of Tear Film Osmolality between Individuals with Mild to Moderate Dry Eye Disease and Individuals without Dry Eye Disease

## 7.1 Introduction

Dry eye disease is one the most frequently diagnosed ocular problems in optometry clinics throughout the world.[1] The most recent definition of dry eye disease comes from the proceedings of the Dry Eye Workshop in 2007. It states that dry eye is a *"multifactorial ocular surface disease diagnosed by symptoms of discomfort and signs of visual disturbance, tear film instability and ocular surface damage, accompanied by increased osmolarity of the tear film and ocular surface inflammation"*.[2] Clinically, this condition ranges from the minor discomfort reported by people who use visual display terminals and contact lenses to the extremely severe condition seen in end-stage ocular pemphigoid and Sjogren's syndrome. It is exactly this wide range of symptoms and disease presentations that can make dry eye disease a challenge to diagnose and manage – clinicians must use a wide variety of tests, often in combination, in order to make a diagnosis.[2-4] Ideally, if a single test could be used, the diagnosis of dry eye disease could be greatly simplified.

Tear film osmolality is reported to be the "gold standard" for the evaluation of dry eye,[3, 5-7] as a distinct separation between the osmolality of the normal and dry-eyed populations has become evident. Research suggests that a hyperosmotic tear film is a trait common to all forms of dry eye, and may be the driving force causing the discomfort, ocular surface damage and inflammation found in both evaporative and tear deficient dry eye.[3]

The purpose of this study was to compare tear film osmolality values in two populations – one with moderate to severe symptoms of dry eye, and a second of age matched, asymptomatic controls – in order to determine if a difference in tear film osmolality exists and can be measured with the Advanced Instruments Model 3100 Nanolitre Osmometer (Advanced Instruments Inc, Norwood, MA, USA). Secondly, this study was designed to look for any relationships between dry eye symptoms and tear film osmolality in a population which was determined to have moderate to severe dry eye disease, based upon a clinical examination.

## 7.2 Methods

The protocol for this study was approved by the Office of Research Ethics at the University of Waterloo (ORE#13990), prior to the commencement of the study, and informed consent was obtained from all participants. 40 volunteer participants were actively recruited through the Center for Contact Lens Research, University of Waterloo. Participants received a monetary sum as remuneration for their participation in this study. This study was funded in part by a grant provided to the Center for Contact Lens Research from Alcon Laboratories, Fort Worth, TX.

### 7.2.1 Participants

Two groups of 20 participants were recruited for this study (n=40 participants). The first group of 20 individuals consisted of people with moderate to severe dry eye disease. The second group consisted of 20 asymptomatic age and gender matched control participants. Eligibility was determined using the inclusion and exclusion criteria detailed below.

### 7.2.2 Moderate to Severe Dry Eye Group

#### 7.2.2.1 Inclusion Criteria (Dry Eye Group)

A person was eligible for this study, in the dry eye group, if he/she:

1. Had moderate or severe dry eye symptoms based on a clinical examination and half of the time wanted to use eye drops for dry eye symptoms.
2. Had read, understood and signed an information consent letter.
3. Were willing and able to follow instructions and maintain the appointment schedule.
4. Had an ocular examination in the last two years.

#### 7.2.2.2 Exclusion Criteria (Dry Eye Group)

A person was excluded from this study if he/she:

1. Wore contact lenses.
2. Had any clinically significant blepharitis.
3. Had undergone corneal or refractive surgery.
4. Were aphakic.
5. Had any active ocular disease other than dry eye disease.
6. Were using any topical or systemic medications that may affect ocular health.
7. Had a known sensitivity to the diagnostic pharmaceuticals used in the study.
8. Were participating in any other type of clinical or research study.

### 7.2.3 Asymptomatic Control Group

#### 7.2.3.1 Inclusion Criteria

A person was eligible for entry to this study in the asymptomatic control group, if he/she:

1. Had read, understood and signed an information consent letter.
2. Were willing and able to follow instructions and maintain the appointment schedule.
3. Had clear corneas and no active ocular surface disease.
4. Had an ocular examination in the last two years.

#### 7.2.3.2 Exclusion Criteria

A person was ineligible for this study if he/she:

1. Had rheumatoid arthritis, diabetes or Sjögren's syndrome or any other systemic disease affecting health.
2. Were using any systemic or topical medications (other than eye drops for occasional dry eye symptoms) that may affect ocular health and neuro-endocrine system function.
3. Had undergone corneal or refractive surgery.
4. Were apahkic.
5. Had any active ocular surface disease.
6. Had a known sensitivity to the diagnostic pharmaceuticals used in the study.
7. Were participating in any other type of clinical research study.
8. Wore contact lenses.
9. Had blepharitis.

### 7.2.4 Study Visits

Data and observations were collected at a total of one scheduled appointment (screening combined with the study visit). The screening procedures included a case history, a white light biomicroscopy exam, the phenol red thread test and corneal staining assessment. Study procedures included the further administration of the Single Item Dry Eye Questionnaire (SIDEQ),[8] the Ocular Surface Disease Index (OSDI)[9, 10] and the McMonnies questionnaires,[11, 12] as well as the measurement of tear film osmolality. A decision was made by the experienced clinician conducting the exam as to whether or not a participant had dry eye disease. Their decision was based upon the results of the screening procedures (case history, white light biomicroscopy examination, phenol red thread test and corneal staining assessment). The clinician was unaware of participants SIDEQ, OSDI and McMonnies scores and their tear film osmolality results when the diagnosis of dry eye was made.

### 7.2.5 Study Procedures

#### 7.2.5.1 Case History

The investigating clinician took a detailed case history from each of the participants, as part of the screening process. This case history included questions about participants' age, general health, medication use, systemic conditions and artificial tear usage.

#### 7.2.5.2 Questionnaires

Participants were asked to complete the Single Item Dry Eye Questionnaire (SIDEQ),[8] the Ocular Surface Disease Index (OSDI),[9, 10] and the McMonnies Questionnaire,[11, 12] using the previous week (7 days) as a time reference (see Appendices 1, 2 and 3, Chapter 2). The results of all three questionnaires were compared with tear film osmolality in order to determine if a relationship existed between patient symptoms and tear film osmolality values, particularly in the dry eye group.

#### 7.2.5.3 Biomicroscopy without Corneal Staining

Prior to tear film collection, a biomicroscopy exam was performed using white light only. No vital dyes were used as part of this screening procedure. The clinician was instructed to look for any signs of ocular surface disease and blepharitis. They also measured bulbar conjunctival redness and limbal hyperemia in all quadrants (superior, inferior, nasal and temporal) using a modified CCLRU grading scale (0-100).[13-15]

#### 7.2.5.4 Phenol Red Thread

The Phenol Red Thread (PRT) test (ZONEQUICK, Showa Yakuhin Kako Co., Ltd. Tokyo, Japan), was used during the screening process as a diagnostic test for dry eye disease. This test was used to measure tear volume in all of the participants. Phenol Red Threads change colour from yellow to red when they are wet by human tears (see Figure 2.9). The length of the thread that changes colour within 15 seconds of exposure to the tear fluid (measured in millimeters) is considered to be an indicator of tear volume. Participants were considered to have a dry eye test result if <10mm of the thread had changed colour (was wet) in the 15 second time limit.[2, 16-18]

#### 7.2.5.5 Tear Film Collection and Osmolality Measurement

Tear samples (0.5-1.0μL) were collected from both of the participants' eyes; tears were always collected from the right eye first. Tear samples were collected without anesthesia, by one of two

experienced investigators who used single use, disposable glass capillary tubes (Drummond Scientific Company, Broomall, PA, USA) for the collection. Participants were reclined in a chair for all of the tear collections. Care was taken to ensure that the lid margin and corneal surfaces were not touched, and participants were asked to look in a superior-nasal direction to further protect the corneal surface.

Tear samples were aliquoted into small 0.2mL PCR tubes (Axygen Scientific Inc., Union City, CA, USA) prior to being transferred to the Advanced Instruments Model 3100 Nanolitre Osmometer sample loading tip. Tear film osmolality measurements were taken as quickly as possible after tear collection and tear samples were disposed of immediately after measurements were taken.

#### 7.2.5.6 Biomicroscopy with Corneal Staining

Corneal staining was performed after tears had been collected for tear film osmolality measurements. Sodium fluorescein (NaFl) ophthalmic strips (Fluorets®, Bausch & Lomb) were used for this procedure in all participants. The strip was wet with saline (Bausch & Lomb Sensitive Eyes Saline, Bausch & Lomb), and the dye strip was then touched to the lower tarsal conjunctiva, with care being taken to avoid touching the surfaces of both the cornea and bulbar conjunctiva.

Corneal staining was assessed over the entire corneal surface in three separate categories: depth, extent and type. In each of these categories staining was graded on a 0-100 scale, where a grade of zero meant there was no corneal staining observed. Each of the three categories was graded individually; these grades can be summed to give a total corneal staining score (Chapter 2, Appendix 4).

At the time the clinician made a decision regarding participants' levels of corneal staining, they were unable to calculate a corneal staining score. Rather they were asked to judge whether or not the corneal staining appeared to be significant for dry eye disease. The clinician was instructed that some levels of corneal staining can occur in normal individuals without dry eye disease (Chapter 2, Figure 2.10). The clinician had experience in the examination of patients with dry eye disease and was deemed capable of making this decision. Only one clinician performed all of the examinations, therefore there were no inter-observer biases in the assessment of corneal staining.

### 7.2.6 Instrument Calibration

The calibration of the Advanced Instruments Model 3100 Nanolitre Tear Osmometer was checked daily. The osmolality of a 304mOsm/Kg standard reference solution (Advanced Instruments Inc,

Norwood, MA, USA) was measured a minimum of three times per day, whenever the instrument was being used. The calibration of the machine was considered to be acceptable if the mean ± standard deviation of the reference samples were within 304 ± 4mOsm/Kg. If the mean ± standard deviation of the reference samples did not fall within this range, than the instrument was immediately recalibrated before any tear film samples were measured.

### 7.2.7 Statistical Analysis

Statistical analysis was performed using STATISTICA 7 (StatSoft ®, Tulsa Oklahoma, www.statsoft.com) and all graphing analysis was completed using Graph Pad Prism 5 Software (Graph Pad Software Inc., www.graphpad.com).

Mean ± standard deviations were calculated for all of the test parameters (questionnaire scores and tear film osmolality) for both of the test groups. Mann-Whitney U tests were used to determine if there were differences in questionnaire scores and tear film osmolality measurements between the dry eyed and asymptomatic control groups. Spearman correlations were performed to examine the relationships between questionnaire scores and tear film osmolality values in both groups.

## 7.3 Results

41 participants completed this study – 20 were classified as having dry eye and 21 were asymptomatic controls. One of the control participants was initially classified as being dry eyed, but upon file review was re-classified as being asymptomatic. The data collected from this participant was felt to be important, and as such they were not removed from the analysis. Table 7.1 summarizes the demographics of the two groups of participants examined in this study.

**Table 7-1: Summary of participant demographics for both the dry eyed and asymptomatic control groups.**

| | Dry Eye Group | Asymptomatic Control Group |
|---|---|---|
| **Age** | 56.4 ± 14.8 years | 53.1 ± 12.4 years |
| **Gender** | 17 females, 2 males | 19 females, 2 males |

### 7.3.1 Questionnaire Results

The dry eye group of participants had significantly higher questionnaire scores than the asymptomatic control group ($p<0.001$) on all three of the questionnaires administered.

#### 7.3.1.1 Single Item Dry Eye Questionnaire (SIDEQ)

Participants who were classified as having dry eye based on the screening exam were found to have a significantly higher mean SIDEQ score than the asymptomatic control group ($p<0.001$). Not all of the dry eye participants experienced moderate to severe dry eye symptoms as defined by the SIDEQ questionnaire, but none of the asymptomatic participants experienced moderate or severe dry eye symptoms. The mean score of the dry eye group fell in the "mild dry eye" category, while the mean score of the control group fell in the "normal" category (see Table 7.2). It is not uncommon for some participants who have dry eye disease to have minimal or no symptoms of the disease, while other individuals may have many symptoms and very few signs.[4] This is one of the challenges that using tear film osmolality as a diagnostic test for dry eye disease is trying to overcome.

**Table 7-2: Breakdown of SIDEQ scores for each study group.**

| Classification | Dry Eye Group (Number of Participants) | Asymptomatic Control Group (Number of Participants) |
|---|---|---|
| **Normal (0-1)** | 6 | 11 |
| **Mild (2)** | 3 | 4 |
| **Moderate (3)** | 8 | 6 |
| **Severe (4)** | 3 | 0 |
| **Mean Score ± SD** | 2.2 ± 1.4 | 0.8 ± 0.9 |

#### 7.3.1.2 Ocular Surface Disease Index (OSDI)

The mean OSDI score of participants who were classified as having dry eye based on the screening exam was significantly higher than that of the asymptomatic control group ($p<0.001$). Some of the participants graded as being "dry eye" reported not having any (3), or only having mild (6) symptoms, although the majority of them reported having moderate to severe symptoms (11). A few of the asymptomatic participants experienced moderate symptoms, and one normal participant reported having severe symptoms. Although, similar to the SIDEQ questionnaire, most of the normal

participants did not experience any significant dry eye symptoms as defined by the OSDI questionnaire. Table 7.3 summarizes the OSDI questionnaire data and includes the number of participants who fell into each symptom category, as well as the mean questionnaire score for each clinical group (dry eye or normal).

**Table 7-3: Breakdown of OSDI scores for each study group.**

| Classification | Dry Eye Group (Number of Participants) | Asymptomatic Control Group (Number of Participants) |
|---|---|---|
| **Normal (0-12)** | 3 | 13 |
| **Mild (13-22)** | 6 | 3 |
| **Moderate (23-32)** | 4 | 3 |
| **Severe (33-100)** | 7 | 1 |
| **Mean Score ± SD** | 25.3 ± 12.8 | 10.5 ± 11.0 |

#### 7.3.1.3 McMonnies Questionnaire

The mean McMonnies score of the dry eye participants (11.1 ± 4.6) was significantly higher ($p<0.001$) than that of the asymptomatic control group (5.2 ± 4.2). Unfortunately, much like the results of the SIDEQ and OSDI questionnaires, there is some overlap in patient symptoms between the groups. Some of the dry eye participants reported having no symptoms of dryness, while one asymptomatic control patient was found to have borderline symptoms of dry eye. Interestingly, when the McMonnies scoring criteria was applied to this population, none of the participants, even those diagnosed with dry eye disease, were classified as having dry eye. Instead our dry eye participants were primarily classified as having borderline dry eye disease. Table 7.4 summarizes the McMonnies questionnaire data, including the number of participants who fell into each category and the mean questionnaire score for each group.

**Table 7-4: Breakdown of McMonnies scores for each study group.**

| Classification | Dry Eye Group (Number of Participants) | Asymptomatic Control Group (Number of Participants) |
|---|---|---|
| **Normal (0-10)** | 8 | 20 |

| | | |
|---|---|---|
| **Borderline (11-19)** | 12 | 1 |
| **Dry Eye (≥20)** | 0 | 0 |
| **Mean Score ± SD** | 11.1 ± 4.6 | 5.2 ± 4.2 |

### 7.3.2 Tear film osmolality Results

Tear film osmolality measurements were taken from both eyes of all patients, starting with the right eye every time. Tear film osmolality values were not significantly different between participants eyes for either group (p=0.32), as shown in Figure 7.1, indicating that there were no interocular differences between subjects.

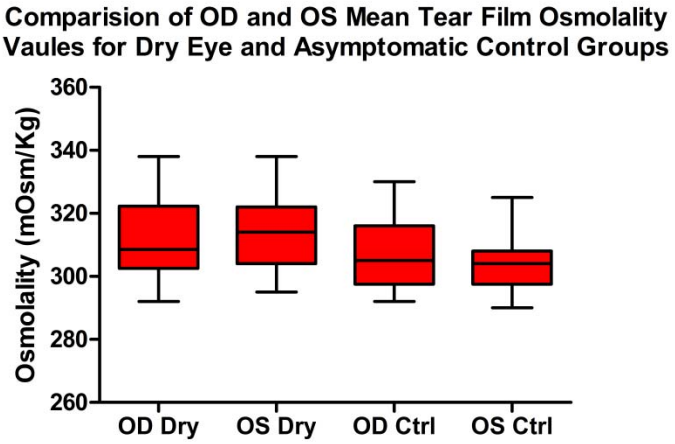



**Figure 7-1: Mean tear film osmolality (mOsm/Kg) values (right eye (OD) and left eye (OS)) for both the dry eye and asymptomatic control groups. There was no significant difference between eyes for either group (p=0.32).**

Mean tear film osmolality values were found to be numerically higher in both right and left eyes of the dry eye participants when compared to the asymptomatic controls. However, the right eye measurements were not statistically significantly different (p=0.21) between the dry eye (311.1±12.4mOsm/Kg) and control groups (306.2±11.2mOsm/Kg) (see Figure 7.2). There was a statistically significant difference (p<0.01) between the measurements made in the left eye (dry eye = 313.2±11.9mOsm/Kg, control = 304.0±7.5mOsm/Kg) (see Figure 7.3).

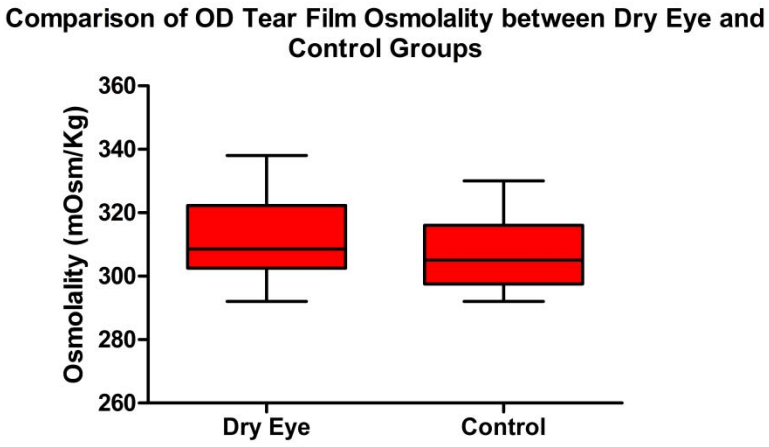



**Figure 7-2: Right eye (OD) mean tear film osmolality (mOsm/Kg) values for both the dry eye (311.1±12.4mOsm/Kg) and asymptomatic control groups (306.2±11.2mOsm/Kg). There was no significant difference between groups (p=0.21).**

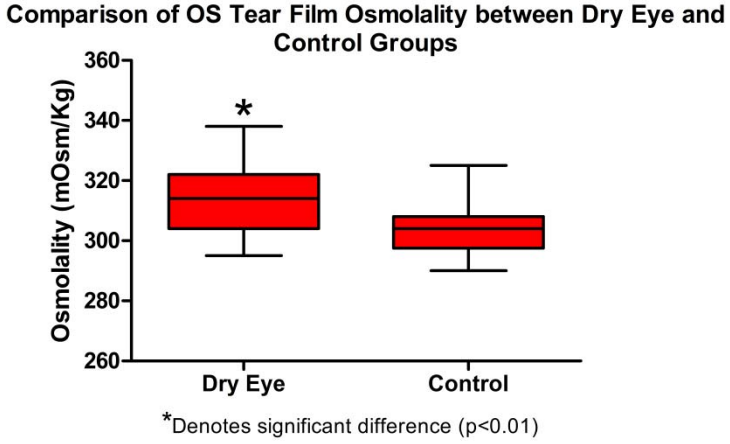



**Figure 7-3: Left eye (OS) mean tear film osmolality (mOsm/Kg) values for both the dry eye (313.2±11.9mOsm/Kg) and asymptomatic control groups (304.0±7.5mOsm/Kg). There was a significant difference between groups (p<0.01).**

### 7.3.3 Comparison of questionnaire results with tear film osmolality results

Previously, in the experiment discussed in Chapter 6, tear film osmolality values of normal, non-contact lens wearers were compared with SIDEQ, OSDI and McMonnies questionnaire results. No correlation was found between the questionnaire results and the tear film osmolality values, but investigators felt that this needed to be re-examined in a population with dry eye disease. Therefore,

the tear film osmolality values of the dry eye participants who participated in this study were compared with their results on the SIDEQ, OSDI and McMonnies questionnaires.

### 7.3.3.1 Tear Film Osmolality and the Single Item Dry Eye Questionnaire (SIDEQ)

The SIDEQ is a single item, self assessment questionnaire that allows participants to rate their ocular surface comfort on a 0 to 4 scale.[8] A score of "0" corresponds to no discomfort or dry eye disease, while a score of "4" corresponds to severe symptoms of ocular surface discomfort, often associated with advanced dry eye disease (see Chapter 2, Appendix 1). On average the dry eyed participants in this study had a SIDEQ score of 2.2 ± 1.4 which is defined as being 'mild' dry eye symptoms.

Tear film osmolality values for both the right and left eyes were compared with participant's SIDEQ scores, and Spearman correlations were used to evaluate the quality of their relationship. Neither the right (r = 0.1978) nor left (r = -0.1042) eye tear film osmolality measurements were found to correlate significantly with SIDEQ scores in this dry eye population (see Figure 7.4).

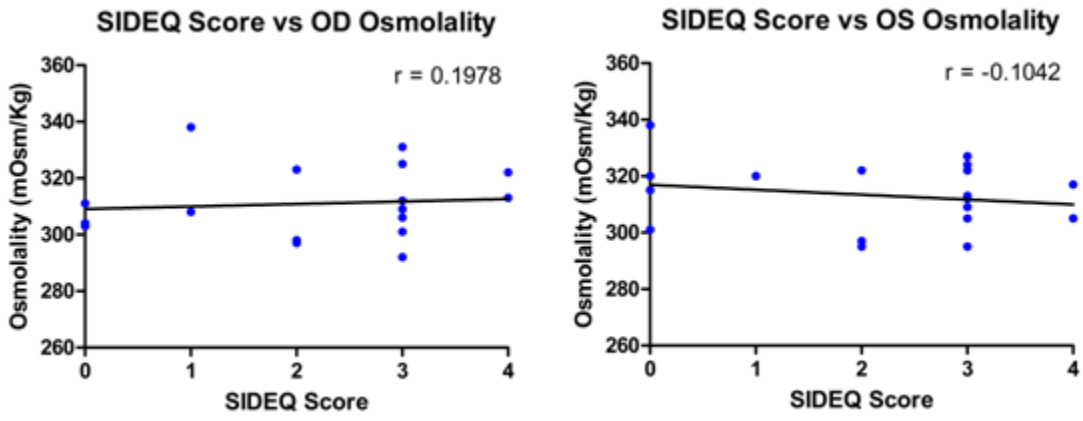



**Figure 7-4: Comparison of SIDEQ scores for right (OD) eye (A) and left (OS) eye (B) tear film osmolality measurements in dry eyed individuals. A Spearman correlation of p>0.60 was considered to be clinically significant.**

### 7.3.3.2 Tear Film Osmolality and the Ocular Surface Disease Index (OSDI) Questionnaire

The OSDI is a 12-item quality of life questionnaire designed to measure the severity of ocular surface disease, and its impact on vision related functions.[10, 19] Participants were asked to evaluate each of the items on the instrument on a 5-point Likert scale (all of the time, most of the time, half of the time, some of the time, none of the time). The 12 items are divided into three subgroups and the

scores for each of the subgroups were summed to get the total OSDI score between 0-100. The higher a participants score, the greater the disability they experience (see Chapter 2, Appendix 2).[10, 19]

The mean OSDI score of the dry eye population examined in this study was 25.3 ± 12.8 which corresponds to moderate dry eye symptoms as defined by this questionnaire's scoring system. A statistically and clinically significant positive correlation (r = 0.6075) was found between right eye tear film osmolality measurements and OSDI scores, but a similar correlation did not exist between left eye tear film osmolality measurements and OSDI scores (r = -0.0016) (see Figure 7.5).

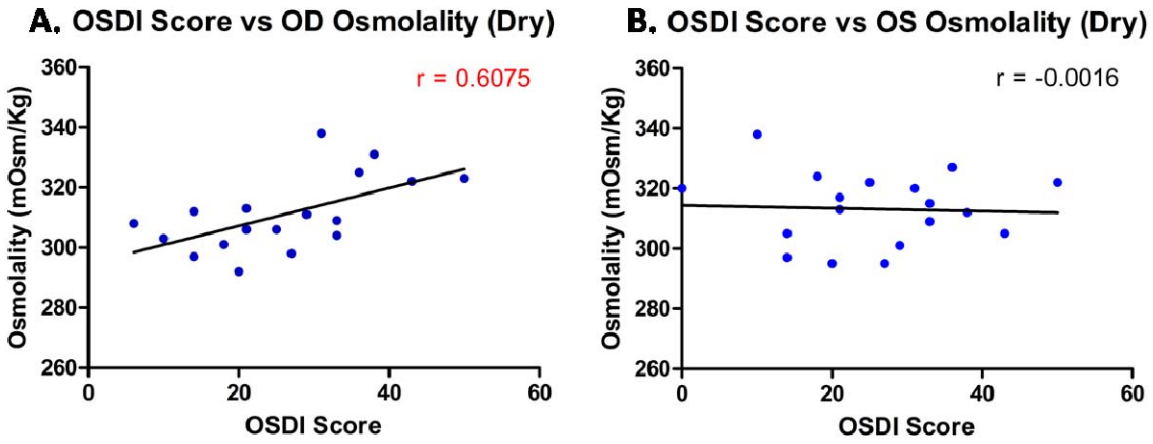



**Figure 7-5: Comparison of OSDI scores for right (OD) eye (A) and left (OS) eye (B) tear film osmolality measurements in dry eyed individuals. A Spearman correlation of p>0.60 was considered to be clinically significant.**

#### 7.3.3.3 Tear Film Osmolality and the McMonnies Questionnaire

The McMonnies Questionnaire is made up of 15 questions, 14 of which focus on clinical "risk factors" for dry eye disease, which have been derived from the literature.[20] It uses a weighted-scale scoring algorithm, to obtain an overall "Index" score. The Index score can fall between 0 and 45, and can be used to categorize participants based on their severity of dry eye disease; higher scores are indicative of greater dry eye disease (see Chapter 2, Appendix 3).[11, 12]

According to the McMonnies classification criteria, our dry eye population on average had symptoms which were considered to be indicative of borderline dry eye disease (mean score 11.1 ± 4.6). When McMonnies scores were compared with tear film osmolality values a significant correlation did not exist in either eye (see Figure 7.6).

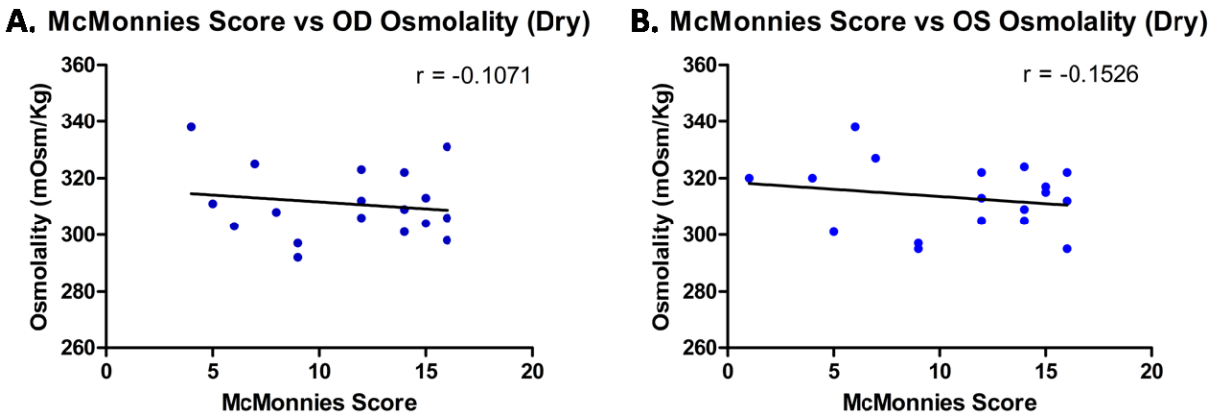



**Figure 7-6: Comparison of McMonnies questionnaire scores for right (OD) eye (A) and left (OS) eye (B) tear film osmolality measurements in dry eyed individuals. A Spearman correlation of p>0.60 was considered to be clinically significant.**

## 7.4 Discussion

Participants in this study were classified as being either dry eyed or asymptomatic age-matched controls based on a clinical exam undertaken by a single, experienced examiner. The clinical exam included a detailed case history, white light biomicroscpy examination, phenol red thread test and sodium fluoroscein (NaFl) corneal staining assessment.

The dry-eyed participants had significantly higher ($p<0.001$) symptom scores on all three of the validated questionnaires administered (SIDEQ, OSDI and McMonnies). Ideally, we were hoping to recruit dry eye patients with moderate to severe dry eye disease, and asymptomatic controls with no symptoms of dry eye disease. Unfortunately, we ended up recruiting a dry eye population that would be better classified as having mild to moderate dry eye disease, and an asymptomatic population that would be better classified as having none to mild dry eye disease. This is one of the challenges of assessing dry eye disease clinically, as patient symptoms and clinical signs rarely correlate well.[4] Earlier we found that tear film osmolality and symptoms did not correlate well in a normal non-contact lens wearing population (see Chapter 6). This study was designed to evaluate tear film osmolality in a dry eyed population, and also to compare tear film osmolality values with questionnaire scores in the same population.

Tear film osmolality was found to be higher in both the right and left eyes of the dry eyed population, compared to the asymptomatic controls, but this difference was only significant ($p<0.01$)

between measurements taken on the left eye. These results support the current literature[3, 5-7] as our population of dry eyed participants did have a tear film which was hyperosmotic compared to the normal, asymptomatic population. If the two populations studied had exhibited less overlap of their symptoms, it is possible that the difference in tear film osmolality would have been greater and found to be significantly different in both eyes, rather than in only the left eye. Unfortunately, recruitment of dry eye participants for research remains a prominent issue – if criteria are too strict, than participants cannot be recruited, and yet if criteria are more lax, there seems to be the issue of overlap between normal and dry eye participants, which makes differences harder to identify.[4, 21]

Tear film osmolality was compared with dry eyed participants SIDEQ, OSDI and McMonnies' questionnaire scores, in an attempt to gain an understanding of the relationship between patient symptoms and osmolality values. Significant correlations were not found between both the SIDEQ or McMonnies questionnaire scores and tear film osmolality values for either the right or left eyes of participants. Interestingly, a clinically significant, positive correlation ($r = 0.6079$) was found between OSDI scores and tear film osmolality values in the right eye of the dry eye participants. Theoretically, this is what we would have predicted to happen, based upon the research that suggests that a hyperosmotic tear film is the driving force between the discomfort and ocular surface damage caused in dry eye disease.[2, 6] Unfortunately, this correlation was not found at all between dry eyed participants OSDI scores and tear film osmolality values in their left eyes ($r = -0.0016$). While this is interesting from a statistical standpoint, and with further research, may provide us with further insight into the sensitivity of using one or both of these as diagnostic measures for dry eye disease, clinically, it has made it impossible to draw conclusions about the relationship between tear film osmolality, OSDI scores and other measures of patient comfort. If further research were done, using a dry eyed population with more severe forms of dry eye disease, results may be less confusing.

Based on the results of this study, it can be said that tear film osmolality is higher (although not necessarily significantly higher) in a population of participants with mild to moderate symptoms of dry eye disease when compared with a population of asymptomatic, age matched controls. The potential for a correlation between participants dry eye symptoms and their tear film osmolality measurements exists – especially with symptoms as assessed by the OSDI – but a significant amount of work is still needed in this area. There is also a need for a classification system which will enable researchers to recruit dry eye patients with the desired severity of disease, in order for this research to be continued.

# Chapter 8
# Discussion

Normal tear film dynamics, including the distribution, turnover and drainage, evaporation, and absorption of tears, require adequate tear production, retention on the ocular surface and balanced elimination.[1] Changes in these processes occur with dry eye disease, and tear film osmolality measurements are thought to represent the end product of these changes,[2] which is one of the reasons that tear film osmolality is thought of as being an attractive index for dry eye diagnosis.[3] Another reason is that previous research has demonstrated that a hyperosmotic tear film is a common trait of all forms of dry eye, [4-7] possibly acting as the driving force that causes the discomfort, ocular surface damage and inflammation found in both evaporative and tear deficient forms of dry eye disease.[4] Therefore tear film osmolality is thought to have the potential to be the "gold standard" diagnostic test for the clinical evaluation of dry eye disease.[4-7]

Unfortunately, clinical measurement of tear film osmolality in everyday optometric practice has been limited by the lack of simple, easy to use instrumentation. Much of the early research in the area of tear film osmolality was completed using a Clifton Nanolitre osmometer, [8-15] which is a delicate and extremely complicated laboratory instrument to use. This instrument requires a trained laboratory technician to run it, and was not practical for use in a clinical setting. The recent advent of relatively simple, easy to use clinical instrumentation such as the Advanced Instruments Model 3100 Nanolitre Tear Film Osmometer and the OcuSense, has made the measurement of tear film osmolality in a clincial setting possible, and is driving the dry eye research community's renewed interest in tear film osmolality.

If tear film osmolality is ever to become a commonly used clinical test, there are a number of questions that need to be addressed. Some of these questions include: can these new instruments accurately measure tear film osmolality? Are measurements of tear film osmolality affected by diurnal variations, just as other clinical measures such as intraocular pressure are? Once it can be established that the instruments truly work, the nature of the relationships between tear film osmolality and other commonly used clinical tests of dry eye disease need to be investigated. Although previous research has demonstrated that a hyperosmotic tear film is associated with dry eye disease,[4-7] this needs to be re-examined with the newly available instrumentation.

The purpose of this thesis was to evaluate the Advanced Instruments Model 3100 Nanolitre Tear Film Osmometer as a clinical instrument and to begin the re-investigation of the association between tear film osmolality and dry eye disease.

## 8.1 Evaluation of the Advanced Instruments Model 3100 Tear Osmometer as a Clinical Instrument

The studies completed in Chapters 3 and 4 of this thesis were designed to investigate the feasibility of using the Advanced Instruments Model 3100 Nanolitre Tear Film Osmometer in clinical practice. In Chapter 3 it was determined that the Advanced Instruments Model 3100 Nanolitre Tear Film Osmometer was capable of measuring tear film osmolality in a normal population. Although the mean tear film osmolality value for the population studied (298.7±11.4mOsm/Kg) was lower than the average mean tear film osmolality previously reported (305mOs/kg), it still fell within the range of reported normal tear film osmolalities (297 – 318mOsm/Kg).[8, 9, 11-19]

Earlier research by Stahl *et al*., had shown that the Advanced Instruments Model 3100 Nanolitre Osmometer was comparable to other commercially available osmometers for the measurement of tear film osmolality.[20] In Chapter 4 the Advanced Instruments Model 3100 Nanolitre Osmometer was found to demonstrate good repeatability in measuring tear film osmolality when environmental conditions are stable. Investigators noticed that the calibration of Advanced Instruments Model 3100 Nanolitre Osmometer remained stable when the humidity changed less than 5% per day, but fluctuated dramatically when the humidity changed more than 10% per day. When the humidity was changing rapidly, the instrument required frequent, often daily, recalibration, but frequent unnecessary recalibration has been shown to introduce inaccuracies to results obtained with the Advanced Instruments Model 3100 Nanolitre Osmometer.[21] This is an issue that needs to be addressed either in the design of the instrument, or by placing the instrument in a humidity controlled environment, in order to minimize the need for recalibration. Further investigation is needed to determine which solution would be easier to implement both commercially and clinically.

Four tear film collection techniques – the collection of tears at a biomicroscope vs. the collection of tears with participants reclined in a chair (Chapter 3) and the collection of multiple small samples vs. the collection of one large sample (Chapter 4) were compared in this thesis. No difference in tear film osmolality values measured from tears collected at either a biomicroscope or with participants reclined in a chair, but investigators recommend collecting tears when participants are reclined in a

chair. Participants appeared to be more relaxed when reclined in a chair, and this technique eliminates the possibility that tear film osmolality could be artificially elevated by the heat given off from the biomicroscope light source and the possibility of the light source could act as a stimulus for reflex tearing. During the comparison of what volume of tears (one large sample or multiple small samples) is appropriate to collect, the collection of multiple smaller samples was found to have a slightly higher concordance value in this particular study, but investigators felt both techniques were comparable. In a clinical setting, it is unlikely that multiple samples would be collected and run immediately following each other, thus the collection of a sample that is slightly larger than needed is advantageous, as it helps to minimize the risk of sample loss during the sample loading process. Further work looking specifically at tear film collection, storage, and loading techniques would be valuable as it would help to maximise the efficiency of this instrument in clinical practice and research environments.

Overall, investigators felt that the Advanced Instruments Model 3100 Tear Osmometer was relatively easy to use and could be implemented in clinical practice with little difficulty.

## 8.2 Evaluation of the Advanced Instruments Model 3100 Tear Osmometer as a Diagnostic Test

Some commonly used clinical tests, such as intraocular pressure (IOP) measurements, have been shown to be affected by diurnal variations, and must be taken multiple times over the day in individuals highly suspect for glaucoma.[22, 23] There has been speculation as to whether or not tear film osmolality could be affected in a similar manner. Some of this speculation has arisen from the vast number of studies which have looked for circadian rhythms in tear film protein concentrations. Some of these studies have detected diurnal changes in tear film protein concentrations, [24, 25] although the results are inconclusive, as others have demonstrated that there is no change in tear film protein concentrations.[26-29] Tear film osmolality, being the product of the varying concentrations of dissolved solutes (proteins, lipids and mucins) in the tear fluid,[176] would be significantly affected by diurnal variations in the concentrations of some or all of these solutes.

Previous research which looked at diurnal variations in tear film osmolality specifically have shown that tear film osmolality measurements, unlike intraocular pressure measurements, do not appear to be affected by a diurnal variation.[11, 18] Unfortunately, these studies were completed on very small populations (n=6), and may not be representative of the population as a whole. They were also

completed with the Clifton Nanolitre osmometer, which is no longer available, and further investigation is needed to determine if a diurnal variation in tear film osmolality can be measured using the new clinical instrumentation instead.

Chapter 5 of this thesis was dedicated to the investigation of the diurnal variation in tear film osmolality in a larger population using the Advanced Instruments Model 3100 Nanolitre Osmometer. Two studies, designed to investigate tear film osmolality over the course of a normal working day when patients would routinely present to an optometric clinic, were completed. In both studies, tear film osmolality was not found to change significantly over the course of a day in a normal, primarily asymptomatic population.

One of the major short comings of all of the tear film osmolality diurnal research conducted thus far is that it has been completed on normal individuals without dry eye disease. The clinically significant fluctuation in IOP measurements was demonstrated in individuals highly suspect for glaucoma,[22, 23] not normal individuals. Tear film osmolality may resemble IOP measurements, in that significant fluctuations are only present in individuals highly suspect for, or who have, dry eye disease. Further work is needed to investigate diurnal variations in tear film osmolality in populations of individuals who have dry eye disease. Until these are completed, one cannot rule out the possibility of a diurnal variation in tear film osmolality being present.

The study of dry eye disease can be challenging, as we currently lack a set of simple, concise, globally accepted diagnostic criteria[30] defining not only the disease itself, but also its accepted levels of severity. Currently, symptoms of discomfort, tear film instability, tear film hyperosmolality, ocular surface inflammation and ocular surface damage are thought to be characteristics common to most forms of dry eye disease,[5, 31] but patient symptoms and clinical signs do not correlate well, if at all,[32-36] and this has made defining diagnostic criteria extremely difficult.

To date there is not a single, definitive test for the evaluation of dry eye disease, and clinicians often find themselves using one or more of the various tests available to evaluate patients' symptoms and ocular surface health. Some of these tests include patient histories, validated questionnaires, linear analogue comfort scales, fluorescein or non-invasive tear break-up times, measurements of ocular surface redness, corneal and/or conjunctival staining (fluorescein, lissamine green or rose bengal), tear ferning, Schirmer Strips and Phenol Red Threads. [5, 31, 37-39] The purpose of the study completed in Chapter 6 was to compare tear film osmolality with various other clinical tests commonly used in the evaluation of dry eye disease. The tests included measures of participant

comfort (assessed with the Single Item Dry Eye Questionnaire (SIDEQ), the Ocular Surface Disease Index (OSDI), the McMonnies questionnaire and a linear analogue scale), and measures of ocular surface health (non-invasive tear break-up time (NIBUT), ocular surface redness and tear ferning).

No correlations were found between tear film osmolality and patient comfort with any of the instruments used (SIDEQ, OSDI, McMonnies questionnaire scores or linear analogue scales). The population studied was a predominantly normal, asymptomatic population – only 10% of participants had moderate dry eye symptoms and none had severe symptoms of dry eye. It is possible that tear film osmolality may have a stronger correlation with patient comfort in individuals with more significant symptoms of dry eye disease, and studies targeting these populations are needed before the relationship between tear film osmolality and patient comfort can be completely understood.

When compared with other clinical signs of dry eye disease, tear film osmolality values did not correlate significantly with NIBUT or ocular surface redness measured either subjectively (with a grading scale) or objectively (with a photometer). Interestingly, tear film osmolality and tear ferning were found to have a weak positive correlation (tear ferning grade increased as tear film osmolality values increased) that was statistically significant ($r=0.3978$), although not clinically significant ($r<0.60$). Tear ferning and tear film osmolality measures are both dependent upon the concentrations of dissolved solutes in the tear film, thus the relationship that appears to exist between them is not unrealistic to expect. Further investigation of tear ferning patterns and tear film osmolality in populations with more significant symptoms of dry eye disease is required in order to completely understand this relationship. Investigation of the relationships between tear film osmolality and NIBUT and ocular surface redness in populations with significant dry eye symptoms are also recommended.

The final investigational chapter of this thesis (Chapter 7) was dedicated to the measurement of tear film osmolality in clinically defined populations of normal and dry eyed individuals. Tear film osmolality has been shown to be higher in individuals with dry eye disease when compared with normal individuals[4-7] but the vast majority of this work was completed using the Clifton osmometer. The purpose of our study was to determine if this difference could still be measured with the Advanced Instruments Model 3100 Tear Film Osmometer.

Age and gender matched participants, with and without dry eye disease, were recruited for this particular study. They were classified as being either dry eyed or asymptomatic controls based on a clinical exam (case history, white light biomicroscopy, phenol red thread test, and sodium fluorescein

(NaFl) corneal staining assessment) performed by a single, experienced examiner. Tear film osmolality measurements were collected from both eyes, and patients completed the SIDEQ, OSDI and McMonnies questionnaires, enabling us to investigate the relationship of tear film osmolality and participant symptoms in a dry eyed population as well.

The dry-eyed participants had significantly higher ($p<0.001$) symptom scores on all three of the questionnaires (SIDEQ, OSDI and McMonnies), but their symptoms were classified as mild to moderate, rather than severe. Our normal population was found to have none to mild symptoms of dry eye disease, and as such there was some overlap in symptoms between the two groups.

Tear film osmolality measurements were higher in both the right and left eyes of the dry eyed population compared to the asymptomatic controls, unfortunately the difference was only significant ($p<0.01$) between measurements taken on the left eye. Despite the lack of a significant difference in the right eye, the author believes that this study supports the current osmolality literature,[4-7] as our population of dry eyed participants did have a tear film which was hyperosmotic compared to the normal, asymptomatic population. Had there been less overlap in symptoms between the two populations, one would hypothesize that the difference in tear film osmolality measurements between the groups would increase. Further investigation of tear film osmolality using the newly available clinical instrumentation is still needed in individuals with dry eye disease, particularly in those with severe symptoms and/or ocular surface damage.

No correlation was found between tear film osmolality values and patient symptoms (SIDEQ, OSDI and McMonnies questionnaires) in our dry eyed population. The lack of correlation between clinical signs and patient symptoms is a common problem both clinically and in dry eye research.[32-36] It has been postulated that tear film osmolality has the potential to be a single "gold-standard" test for dry eye disease, but at this time further investigation into the usefulness of tear film osmolality as a diagnostic measure, especially in dry eyed individuals, is still required.

## 8.3 Conclusions

The Advanced Instruments Model 3100 Nanolitre Osmometer is capable of measuring tear film osmolality in a clinical setting and in a normal population tear film osmolality does not appear to be affected by diurnal variations.

In normal individuals, tear film osmolality results do not correlate well with many other clinical tests of patients symptoms and ocular surface damage, but tear film osmolality does appear to be higher in individuals with mild to moderate dry eye disease.

A significant amount of work, focusing primarily on measuring tear film osmolality, its diurnal variation and its relationships with other commonly used clinical tests in patients with moderate to severe dry eye disease, is needed in order to decide if tear film osmolality is truly the “gold-standard” test for the diagnosis of dry eye disease.

# Appendix A

## Single Item Dry Eye Questionnaire

SINGLE ITEM DRY EYE QUESTIONNAIRE

Please evaluate your ocular discomfort due to the symptom of "Dryness" on a scale of 0 (none) to 4 (severe). You may use the following descriptions to assist in your score.

| | | |
|---|---|---|
| None (0) | = | I do not have this symptom |
| Trace (1) | = | I seldom notice this symptom, and it does not make me uncomfortable. |
| Mild (2) | = | I sometimes notice this symptom, it does make me uncomfortable, but it does not interfere with my activities. |
| Moderate (3) | = | I frequently notice this symptom, it does make me uncomfortable, and it sometimes interferes with my activities. |
| Severe (4) | = | I always notice this symptom, it does make me uncomfortable, and it usually interferes with my activities. |

# Appendix B

# Ocular Surface Disease Index

## OCULAR SURFACE DISEASE INDEX©

**Please answer the following questions by checking the box that best represents your answer.**

**Have you experienced any of the following during the last week:**

| | All of the time | Most of the time | Half of the time | Some of the time | None of the time |
|---|---|---|---|---|---|
| **1. Eyes that are sensitive to light?** | | | | | |
| **2. Eyes that feel gritty?** | | | | | |
| **3. Painful or sore eyes?** | | | | | |
| **4. Blurred vision?** | | | | | |
| **5. Poor vision?** | | | | | |

**Have problems with your eyes limited you in performing any of the following during the last week:**

| | All of the time | Most of the time | Half of the time | Some of the time | None of the time | N/A |
|---|---|---|---|---|---|---|
| **6. Reading?** | | | | | | |
| **7. Driving at night?** | | | | | | |
| **8. Working with a computer or bank machine (ATM)?** | | | | | | |
| **9. Watching TV?** | | | | | | |

**Have your eyes felt uncomfortable in any of the following situations during the last week**:

| | All of the time | Most of the time | Half of the time | Some of the time | None of the time | N/A |
|---|---|---|---|---|---|---|
| **10. Windy conditions?** | | | | | | |
| **11. Places or areas with low humidity (very dry)?** | | | | | | |
| **12. Areas that are air conditioned?** | | | | | | |

# Appendix C

# McMonnies Questionnaire

## McMonnies Questionnaire

Please answer the following by underlining the appropriate response;

1. **Female** **Male**
2. **Under 25 years** **25 to 45 years** **Over 45 years**
3. **No CL wear** **Wear soft CL** **Wear hard CL**
4. **Have you ever had drops prescribed, or other treatment, for dry eyes?**
   Yes No Uncertain
5. **Do you ever experience any of the following eye symptoms?**
   Soreness Scratchiness Dryness
   Grittiness Burning
6. **How often do your eyes have these symptoms?**
   Never Sometimes Often Constantly
7. **Are you eyes *unusually* sensitive to cigarette smoke, smog, air conditioning, or central heating?**
   Yes No Sometimes
8. **Do your eyes easily become very red and irritated when swimming**
   Yes No Sometimes Not applicable
9. **Are your eyes dry and irritated the day after drinking alcohol?**
   Yes No Sometimes Not applicable
10. **Do you take any of the following?**
    Antihistamine Diuretics Sleeping tablets Tranquilizers
    Oral contracept. HBP meds Ulcer meds
11. **Do you suffer from arthritis?**
    Yes No Uncertain
12. **Do you experience dryness of the nose, mouth, throat, chest or vagina?**
    Never Sometimes Often Constantly
13. **Do you suffer from thyroid abnormality?**
    Yes No Uncertain
14. **Are you known to sleep with your eyes partly open?**
    Yes No Sometimes
15. **Do you have eye irritation as you wake from sleep?**
    Yes No Sometimes

# Appendix D
# Corneal Staining Determination

The CCLR method for assessing corneal staining is based on the assessment of 4 peripheral quadrants (nasal, temporal, superior and inferior) as well as the central region (i.e. 5 zones in total).[220] The grading is undertaken for each zone using the CCLRU Photographic Scale[216] as a reference, in which a "severity/type" score and "area" of staining is recorded for each zone independently. Severity is recorded using a scale of 0 (negligible fluorescein staining) to 100 (severe fluorescein staining) and an area score for each zone is recorded from 1-100%, where 100% indicates a zone that is stained over the entire extent of the zone.

The staining score for each zone is calculated as the product of severity score and percent corneal coverage. The staining scores for all 5 zones of the cornea are then summed to provide a Global Staining Score for the cornea. For each visit the average of both eyes are typically used for statistical analysis.

To provide some background data to explain the corneal staining results and to provide some context, it is worth considering some examples to put the data into perspective. As explained above, each of the 5 corneal zones could potentially exhibit a staining score of 100 (severity) x 100 (total zone stained), resulting in a maximum score per zone of 100 x 100=10,000. If the entire cornea exhibited such staining then it is theoretically possible that the cornea would exhibit a maximum Global Staining Score of 5 x 10,000=50,000. As the mean of the 2 eyes are reported then the theoretical staining scores range from 0 (no zones in either eye exhibiting any staining) to 50,000 (both eyes exhibiting dense staining across all possible zones). The latter of these values would not be expected to be seen in a contact lens wearer and would probably only be seen in a case in which the epithelium of both corneas had been severely affected, for example in bilateral acid or alkali burns. In most recent reports we also report the "Mean Global Staining Score", which is this value divided by 5 to give a max value of 10,000. It is made clear in the report which of these is reported. In some instances an average score is more useful and in some (where there is a marked zonal difference) the overall summed score is more relevant.

Values representative of those seen in contact lens studies could be envisaged using the following 4 examples (one eye only described):

- Inferior grade 2 (0-4) SMILE staining. If we assume that the severity was 35 (0-100) across the 2 inferior zones and in each zone the staining occurred in 15% (1-100%) of each zone's area, the Global Staining Score (GSS) for the cornea would be [(35x15) + (35x15)]=1050. Mean GSS would be 1050/5 = 210. This would be considered insignificant as a Mean GSS number, but based on the fact that it is grade 2 staining in the inferior quadrant could be considered to be clinically significant and the clinician may decide that intervention is required to minimize that staining by trying to reduce dehydration, giving blinking exercises, rewetting drops etc.
- Micropunctate staining in the inferior nasal zone only. If the severity is assumed to be 25 over 15% of that 1 zone then the GSS would be 25x15=375. Mean GSS = 375/5 = 75. Clinically insignificant.
- Light staining representative of that seen in studies investigating corneal staining with solutions used with silicone hydrogels. Light punctate staining over the 4 peripheral zones with less staining centrally. Assume the peripheral zones exhibited a severity of 25 over 60% of the zone and centrally 15 over 20% of the zone, then GSS = [(25x60) + (25x60) + (25x60) + (25x60) + (15x20)] = 6300. Mean GSS = 6300/5 = 1260. Borderline significance. Some practitioners may feel that this is irrelevant. Others may wish to change solution and eliminate it. Also may depend upon time of the day. If after 2 hours then may decide that, as will reduce after 2-3 hours that this is entirely acceptable. If after 8 hours then may decide to review again after 2 hours, as may be worse then and then would warrant management by changing regimens.
- Heavy staining representative of that seen in studies investigating corneal staining with solutions used with silicone hydrogels. Moderate punctate staining over the 4 peripheral zones with less staining centrally. Assume the peripheral zones exhibited a severity of 45 over 70% of the zone and centrally 25 over 30% of the zone, then GSS = [(45x70) + (45x70) + (45x70) + (45x70) + (25x30)] = 13350. Mean GSS = 13350/5 = 2670. Almost certainly will change solution. But, also time dependent, as pointed out above.

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# Appendicies

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