

Evaluating Viscosity and Tear Breakup Time of Contemporary Commercial Ocular Lubricants on an In Vitro Eye Model

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Purpose: To evaluate the link between the viscosity of ophthalmic formulation and tear film stability using a novel in vitro eye model.

Methods: The viscosities and noninvasive tear breakup time (NIKBUT) of 13 commercial ocular lubricants were measured to evaluate the correlation between viscosity and NIKBUT. The complex viscosity of each lubricant was measured three times for each angular frequency (ranging from 0.1 to 100 rad/s) using the Discovery HR-2 hybrid rheometer. The NIKBUT measurements were performed eight times for each lubricant using an advanced eye model mounted on the OCULUS Keratograph 5M. A contact lens (CL; ACUVUE OASYS [etafilcon A]) or a collagen shield (CS) was used as the simulated corneal surface. Phosphate-buffered saline was used as a simulated fluid.

Results: The results showed a positive correlation between viscosity and NIKBUT at high shear rates (at 10 rad/s, $r = 0.67$) but not at low shear. This correlation was even better for viscosities between 0 and 100 mPa*s ($r = 0.85$). Most of the lubricants tested in this study also had shear-thinning properties. OPTASE INTENSE, I-DROP PUR GEL, I DROP MGD, OASIS TEARS PLUS, and I-DROP PUR had higher viscosity in comparison to other lubricants ($P < 0.05$). All of the formulations had a higher NIKBUT than the control (2.7 ± 1.2 seconds for CS and 5.4 ± 0.9 seconds for CL) without any lubricant ($P < 0.05$). I-DROP PUR GEL, OASIS TEARS PLUS, I-DROP PUR MGD, REFRESH OPTIVE ADVANCED, and OPTASE INTENSE had the highest NIKBUT using this eye model.

Conclusions: The results show that the viscosity is correlated with NIKBUT, but further work is necessary to determine the underlying mechanisms.

Translational Relevance: The viscosity of ocular lubricants can affect NIKBUT and tear film stability, so it is an important property to consider when formulating ocular lubricants.

Introduction

Dry eye disease (DED), characterized by inadequate tear lubrication, affects hundreds of millions of people worldwide.¹ The changes in lifestyle and work trend, which increasingly rely on the use of digital

devices such as mobile phones, tablets, and computers, have led to an increased prevalence of the disease.² A study has shown that as many as two of three office workers who utilize smartphones or computers have dry eye.² Patients with DED often have tear film instability, which can be assessed clinically by measuring the noninvasive tear breakup time (NIBUT).^{3,4} Lower

values for tear breakup correspond to higher tear film instability, with values less than 10 seconds are indicative for DED.^{3,5}

One method to measure NIBUT is using the Keeler Tearscope-Plus (Keeler, Windsor, UK), which enables a clinician to visualize the lipid layer interference pattern on the eye and determine the breakup time.^{6,7} However, this method is subjective as it relies on the experience of the clinician to properly determine the time for tear breakup.^{6,8} A more objective method to measure NIBUT can be achieved using a keratograph such as the Oculus Keratograph 5M (OCULUS Optikgeräte, Wetzlar, Germany). This device projects a set of placido rings on the eye using infrared illumination and then measures the time for these rings to break up to determine the NIKBUT.⁶

The most common treatment for dry eye disease is the application of artificial tears (ocular lubricants) because of their relatively low cost, ease of use, and minimal adverse effects.⁹ The long-term use of ocular lubricants has shown positive outcomes in healing the ocular surface in patients with DED.^{10–12} Eyecare practitioners and patients have a wide selection when it comes to commercially available topical ocular lubricants. Manufacturers can achieve various unique properties of their lubricants by varying the concentration of key ingredient(s) and formulation of ingredients, including the choice of wetting agents and preservatives.¹² The challenge for manufacturers and researchers is understanding how certain properties of a lubricant can affect tear film stability and improve efficacy.

Upon instillation on the eye, the lubricants are rapidly removed from the surface of the eye by several mechanisms that are in place to protect the eye, such as tear drainage and blinking.¹³ There is also a limit to how much volume can be placed on the eye, as the cul-de-sac can only accommodate approximately 30 μ L of tear fluid.^{14,15} One well-known strategy to help increase the residence time of topical ocular lubricants on the ocular surface is to increase the viscosity of the formulation.^{16–18}

Several factors should be considered in regard to increasing the viscosity of an ocular lubricant formulation. First, a higher viscosity in the ocular lubricant compared with natural tears is important since the formulation will be diluted once instilled in the eye.^{19,20} However, increasing the viscosity of ocular lubricant formulations above a threshold can lead to ocular discomfort and irritation^{17,19,21} due to the increased friction between the ocular surface and the eyelid during blinking.²² Viscous formulations can also cause visual blurring, which can last several minutes.²¹ Ocular lubricants should also be able to mimic the shear-

thinning properties of natural tear fluid, which is a non-Newtonian fluid. At high shear rates, such as during the blink motion, the viscosity of non-Newtonian fluids decreases drastically,²³ thereby reducing the frictional forces between the sliding surfaces.

The link between the viscosity of ophthalmic formulation and tear film stability is not yet well understood, mainly due to the difficulty of obtaining appropriate stability measurements in a controlled manner. Clinical measurements of tear film stability (such as those assessed via NIKBUT values) can be affected by a wide variety of both patient and environmental factors,²⁴ and assessing NIKBUT using in vitro methods would overcome such variability. Previously, our group developed in vitro eye models for in vitro testing the NIKBUT over the anterior surface of contact lenses,^{25–27} and these models could be adapted for in vitro measurements of NIKBUT of topical ocular lubricants. The aim of this study was to evaluate the link between the viscosity of ophthalmic formulation and tear film stability, as measured by NIKBUT, using a novel in vitro eye model.

Materials and Methods

Ocular Lubricants

The topical ocular lubricants evaluated in this study are listed in [Table 1](#). The Formulation Information was Obtained From the Commercial Product Label. A Wide Range of Formulations was Chosen to Provide a Range of Viscosities Ranging From Low to High. The Viscosity of Each ocular Lubricant was Measured and Then Compared to Their NIKBUT Values Using an in Vitro Eye Model.

Viscosity Measurements

The complex viscosity of the ocular lubricants ($n = 3$) was measured using the Discovery HR-2 hybrid rheometer (Waters, Newcastle, DE, USA) fitted with a 40-mm (radius), 1.005-degree aluminum cone and Peltier plate assembly. In brief, 300 μ L of a given ocular lubricant formulation was tested by both strain sweep ($n = 1$) and frequency sweep analysis ($n = 3$). Strain sweeps were conducted at 25°C to determine the range of the linear viscoelastic region. The complex viscosity (mPa*s) for each ocular lubricant formulation was measured by frequency sweep analysis at 25°C, with angular frequencies ranging from 0.1 to 100 radians/s.

Two reference viscosities were selected that were physiologically relevant, one corresponding to the

Table 1. Ingredients of Topical Ocular Lubricants Tested in This Study

Commercial Name	Manufacturer	Ingredients
SYSTANE COMPLETE	Alcon	Propylene glycol 0.6%, boric acid, dimyristoyl, phosphatidylglycerol, edetate disodium, hydroxypropyl guar, mineral oil, polyoxyl 40 stearate, polyquaternium-1, sorbitan tristearate, sorbitol, and purified water
REFRESH OPTIVE ADVANCED	AbbVie	Carboxymethylcellulose sodium 0.5, glycerin 1%, polysorbate 80 0.5%, boric acid, castor oil, erythritol, levocarnitine, carbomer copolymer type A, purified water, and PURITE
REFRESH OPTIVE MEGA-3 HYDROCELL	Allergan	Carboxymethylcellulose sodium 0.5%, glycerin 1%, polysorbate 80 0.5%, boric acid, butylated utylated hydroxyl toluene, carbomer copolymer type A, castor oil, erythritol, flaxseed oil, levocarnitine, polyoxyl 40 stearate, trehalose, and purified water
REFRESH OPTIVE MEGA-3 FLAXSEED	Allergan	Carboxymethylcellulose sodium 0.5%, glycerin 1%, polysorbate 80 0.5%, boric acid, butylated utylated hydroxyl toluene, castor oil, erythritol, flaxseed oil, levocarnitine, pemulen TR-2, polyoxyl 40 stearate, trehalose, and purified water
FRESHKOTE	Eyevance Pharmaceuticals	Polyvinyl alcohol 2.7%, povidone 2.0%, boric acid, edetate disodium, ethanal, glycerin, phospholipids, polysorbate 80, potassium chloride, purified water, and sodium chloride
BLINK TEARS	Johnson & Johnson	Polyethylene glycol 400 0.25%, boric acid, calcium chloride, magnesium chloride, potassium chloride, purified water, sodium borate, sodium chloride, and sodium hyaluronate
I-DROP PUR	I-MED Pharma	0.18% viscoadaptive sodium hyaluronate, sodium chloride, glycerine, sodium phosphate, and water
I-DROP MGD	I-MED Pharma	0.2% viscoadaptive sodium hyaluronate, phosphorylcholine polymer, sodium chloride, glycerin, sodium phosphate, and water
I-DROP PUR GEL	I-MED Pharma	0.3% viscoadaptive sodium hyaluronate, sodium chloride, glycerin, sodium phosphate, and water
THEALOZ DUO	Labtician Thea	Trehalose 3%, hyaluronic acid 0.15%, carbomer 0.25%, sodium chloride, trometamol, hydrochloric acid, and water
OASIS TEARS PLUS	OASIS Medical Inc.	Glycerin 0.25%, sodium chloride, sodium hyaluronate, sodium phosphate dibasic, sodium phosphate monobasic, and water
RETAIN E MGD	OcuSOFT	Mineral oil 0.5%, cetalkonium chloride, glycerol, poloxamer 188, tris hydrochloride, tromethamine, tyloxapol, and water
OPTASE INTENSE	Scope Health Inc.	Glycerin, sodium hyaluronate, sorbitol, trometamol, hydrochloric acid, and water

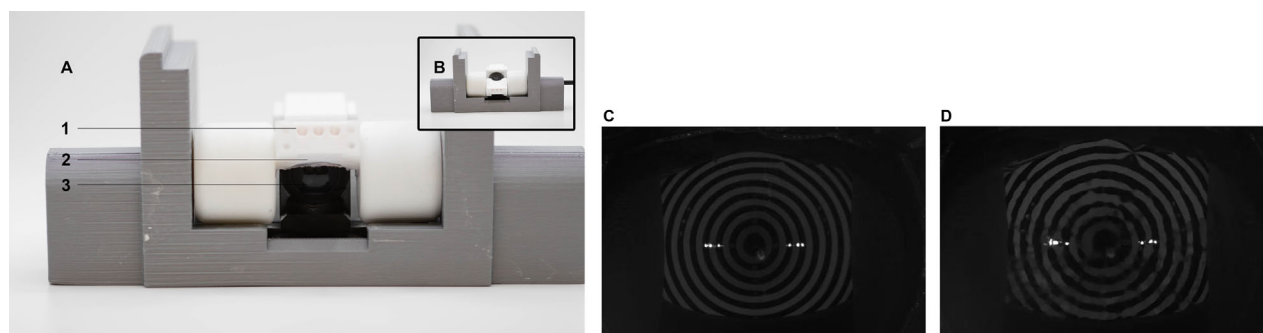


Figure 1. The OcuBlink system in the open (A) and the closed eye positions (B). PBS is delivered to the system via three inlets at the top of the eyelid (1). The eyelid (2) then spreads the tear fluid to create an artificial tear film with each blink motion (3). (C) A representative image of the tear film on the OcuBlink as imaged using a keratograph (OCULUS Keratograph 5M; OCULUS Optikgeräte, Wetzlar, Germany). (D) A representative image of a broken tear film on the OcuBlink.

“open eye” condition and the second one corresponding to the “blinking” condition. The reported physiologic blink speeds in a human at peak velocity are approximately $243 \pm 9 \text{ mm s}^{-1}$ and $157 \pm 5 \text{ mm s}^{-1}$ during the closing and opening phase, respectively.²⁸ The average speed for closing and opening of the eyelid is approximately $134 \pm 4 \text{ mm s}^{-1}$ and $26 \pm 2 \text{ mm s}^{-1}$, respectively.²⁸ Thus, for this study, 0.1 rad/s (4 mm s^{-1}) was selected as the “open eye” condition and 10 rad/s (400 mm s^{-1}) as the “blinking” state. The conversion from rad/s to mm s^{-1} was calculated using 40 mm as the radius.

In Vitro Eye Model

The eye model used in this study was adapted from a previous iteration of a model used to measure NIKBUT over contact lenses (Fig. 1).²⁵ In brief, the blinking mechanism of the model was controlled by a motor and an Arduino board. For this study, phosphate-buffered saline (PBS) was used as the representative tear solution. PBS was delivered to the system using a syringe pump (PHD ULTRA; Harvard Apparatus, Holliston, MA, USA) via three inlets located at the top of the eyelid. With each blink, the eyelid is designed to spread a layer of artificial tear film layer over the ocular surface.

The corneal area of the eye has a $300\text{-}\mu\text{m}$ cutout, 15 mm in diameter, to allow for a contact lens or a collagen shield to be mounted. The corneal surface “mimic” in this study comprised either etafilcon A (ACUVUE OASYS; Johnson & Johnson, Jacksonville, FL, USA) or a collagen shield (VET SHIELD; OASIS Medical Inc., Glendora, CA, USA). Both of these mounting materials were selected due to their respective advantages and disadvantages in simulating the corneal surface. The collagen shield was selected because it is

made of collagen, which is also found on the cornea; however, it was prone to dehydration and degradation. On the other hand, the contact lens had a smoother surface and was less prone to dehydration or degradation but was made from synthetic material.

Prior to use, both the contact lens and collagen shield were soaked in 5 mL PBS for 1 hour . The tear flow rate over these corneal mimics was $0.5 \mu\text{L}/\text{min}$ and $2 \mu\text{L}/\text{min}$ for the contact lens and collagen shield, respectively (the collagen shield dried up very quickly and thus required a higher flow rate). The model was equilibrated with tear flow for 20 minutes before measurement, with a blink interval of 7 seconds . The humidity of the ambient environment was kept above 50% during the study using a room humidifier.

Noninvasive Keratographic Breakup Time Measurements

The eye model was mounted on the OCULUS Keratograph 5M (OCULUS Optikgeräte, Wetzlar, Germany) to measure NIKBUT. The NIKBUT was measured for each ophthalmic formulation ($n = 8$). Two controls were used in the study: (1) NIKBUT of the contact lens and collagen shield without any addition of a drop and (2) NIKBUT with the addition of $10 \mu\text{L}$ PBS. NIKBUT measurements were analyzed using the standard protocol for the keratograph. The eyeball was first centered and focused, and then $10 \mu\text{L}$ of the lubricant drop was placed at the center of the corneal surface using a microliter pipette. The NIKBUT was then measured after three consecutive blinks ($1\text{--}2 \text{ seconds}$ apart) and analyzed manually with the assistance of the Oculus computer software. After each measurement, there was a washout period for 3 minutes (blink interval of 7 seconds) to reequilibrate

the system before the addition of another eye drop. Each ocular lubricant was measured eight times and averaged for analysis. The maximum NIKBUT for the machine was 25 seconds, and any values above this threshold were recorded as 25 seconds. A fresh contact lens or collagen shield was used for each ocular lubricant.

Statistical Analysis

Data analysis was performed using GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA). A one-way analysis of variance was used to test differences in NIKBUT, complex viscosity, and shear thinning across different ocular lubricant formulations. A post hoc multiple comparisons analysis was used to determine where those differences occurred. A P value of <0.05 was considered statistically significant.

To determine a potential correlation between viscosity and NIKBUT, the NIKBUT was plotted against the viscosity at 0.1 rad/s and 10 rad/s. The Pearson correlation coefficient r was used to determine the relationship between the two variables.

Results

The complex viscosities (mPa*s) for the lubricants between 0.1 and 100 rad/s are shown in Figure 2. Table 2 shows the viscosity of the lubricants at various angular speeds.

Figure 3 shows the viscosity of the lubricants at 0.1 and 10 rad/s. OPTASE INTENSE (Scope Health Inc., Dublin, Ireland; I-DROP PUR GEL (I-MED Pharma, Saint-Laurent, QC, Canada; I DROP MGD (I-MED Pharma, Saint-Laurent, QC, Canada), OASIS TEARS PLUS (OASIS Medical Inc., CA, USA and I-DROP PUR (I-MED Pharma, Saint-Laurent, QC, Canada) had higher complex viscosities at 0.1 and 10 rad/s in comparison to other lubricants ($P < 0.05$). SYSTANE COMPLETE (Alcon, Fort Worth, TX, USA had a very high viscosity at 0.1 rad/s, but the viscosity significantly dropped at 10 rad/s. It was also observed that SYSTANE COMPLETE also had variable viscosities at low shear rates, suggesting nonhomogeneity of the formulation. At 10 rad/s, OPTASE INTENSE had the highest viscosity value, at 147.8 ± 1.7 mPa*s, and RETAIN MGD (OcuSOFT, Rosenberg, TX, USA had the lowest value at 1.6 ± 0.4 mPa*s ($P < 0.05$). Most of the lubricants tested had shear-thinning properties, which was indicated by a higher viscosity at 0.1 rad/s compared to 100 rad/s (see Table 2). Only THEALOZ DUO (Labtician Thea, Oakville, ONT, Canada) and FRESHKOTE (Eyevance Pharmaceuticals, Fort Worth, TX, USA) did not show shear thinning.

The NIKBUTs of the lubricants on the contact lens and the collagen shield are shown in Figure 4 and Table 3. All the formulations had a higher NIKBUT than the control ($P < 0.05$) for both the contact lens and collagen shield. For the collagen shield, all of the formulations also had a higher NIKBUT than the control ($P < 0.05$) with the exception of RETAIN MGD and BLINK TEARS (Johnson & Johnson,

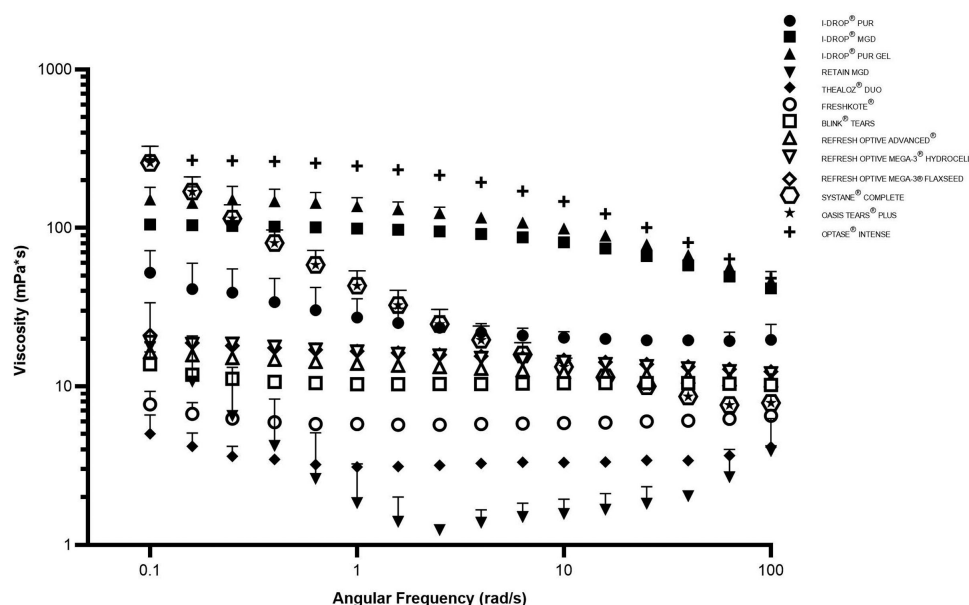


Figure 2. Viscosity profile of lubricants between 0.1 and 100 rad/s on a logarithmic scale showing the mean $\pm$ standard deviation ($n = 3$).

Table 2. Viscosity of Lubricants Measured in mPa*s

Angular Frequency (rad/s)	I-DROP PUR	I-DROP MGD	I-DROP PUR GEL	RETAIN MGD	THEALOZ DUO	FRESHKOTE	BLINK TEARS	REFRESH OPTIVE ADVANCED	REFRESH OPTIVE MEGA-3 HYDROCELL	REFRESH OPTIVE MEGA-3 FLAXSEED	SYSTANE COMPLETE	OASIS TEARS PLUS	OPTASE INTENSE
0.1	52.3 ± 19.6	105.3 ± 5	151.1 ± 29.6	17.8 ± 15.9	5.0 ± 1.6	7.7 ± 1.6	13.8 ± 2.8	16.5 ± 2.5	19.1 ± 0.6	21.1 ± 1.4	257.0 ± 70.1	72.4 ± 3.7	270.2 ± 3.1
1	27.3 ± 8.4	99.3 ± 1.6	137.0 ± 18.0	1.8 ± 1.4	3.1 ± 0.1	5.8 ± 0.1	10.3 ± 0.1	13.8 ± 0.6	16.6 ± 0.3	16.7 ± 0.1	43.2 ± 10.4	69.7 ± 4.2	246.7 ± 3.1
10	20.3 ± 1.8	81.3 ± 0.3	99.00 ± 4.2	1.6 ± 0.4	3.3 ± 0.2	5.9 ± 0.1	10.5 ± 0.2	12.3 ± 0.5	14.3 ± 0.4	14.7 ± 0.1	13.3 ± 1.8	64.0 ± 3.7	147.8 ± 1.7
100	19.7 ± 4.9	41.6 ± 0.4	46.2 ± 6.9	3.9 ± 2.4	4.1 ± 0.3	6.4 ± 0.1	10.2 ± 0.1	10.9 ± 0.2	12.1 ± 0.3	12.4 ± 0.1	7.9 ± 0.8	37.2 ± 1.5	48.3 ± 1.8
(0.1 vs. 100) (P < 0.05)	Yes	Yes	Yes	Yes	NS	NS	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Results are reported as mean ± deviation (n = 3). NS, not significant.

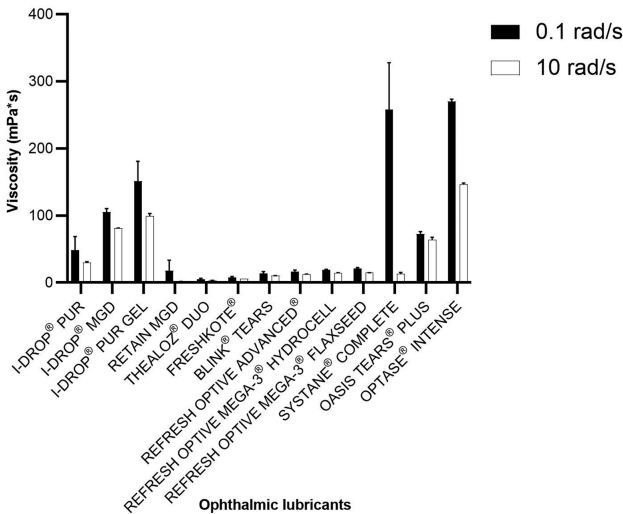


Figure 3. Viscosity profile of lubricants at 0.1 and 10 rad/s.

Jacksonville, FL, USA) ($P > 0.05$). For the contact lens, RETAIN MGD, FRESHKOTE, REFRESH OPTIVE MEGA-3 HYDROCELL (AbbVie, North Chicago, IL, USA), and SYSTANE COMPLETE had similar NIKBUT to the PBS control ($P > 0.05$). The results overall show that the NIKBUTs of the ocular lubricants were similar between the contact lens and the collagen shield, but there were some notable differences. The NIKBUT was significantly different between the collagen shield and contact lens for the control, PBS, THEALOZ DUO, and BLINK TEARS ($P < 0.05$). I-DROP PUR GEL, OASIS TEARS PLUS, I-DROP MGD, REFRESH OPTIVE ADVANCED (AbbVie, North Chicago, IL, USA), and OPTASE INTENSE had the highest NIKBUT for both representative corneal surfaces.

There was no correlation found for viscosity and NIKBUT at low shear rates (0.1 rad/s) (Pearson $r = 0.27$). However, there was indeed a good positive correlation for viscosity at higher shear. At 10 rad/s, the viscosity versus NIKBUT had a Pearson $r = 0.67$. This correlation was even better when considering only the viscosities between 0 and 100 mPa*s ($r = 0.85$) versus NIKBUT, as shown in Figure 5.

Discussion

For most of the lubricants tested, increasing the angular frequencies was correlated with a reduction in viscosity, indicating the formulations have shear-thinning properties.^{29,30} This study also showed that there were significant differences in the complex viscosities of the various commercial lubricants tested, which also corresponded to differences in NIKBUT.

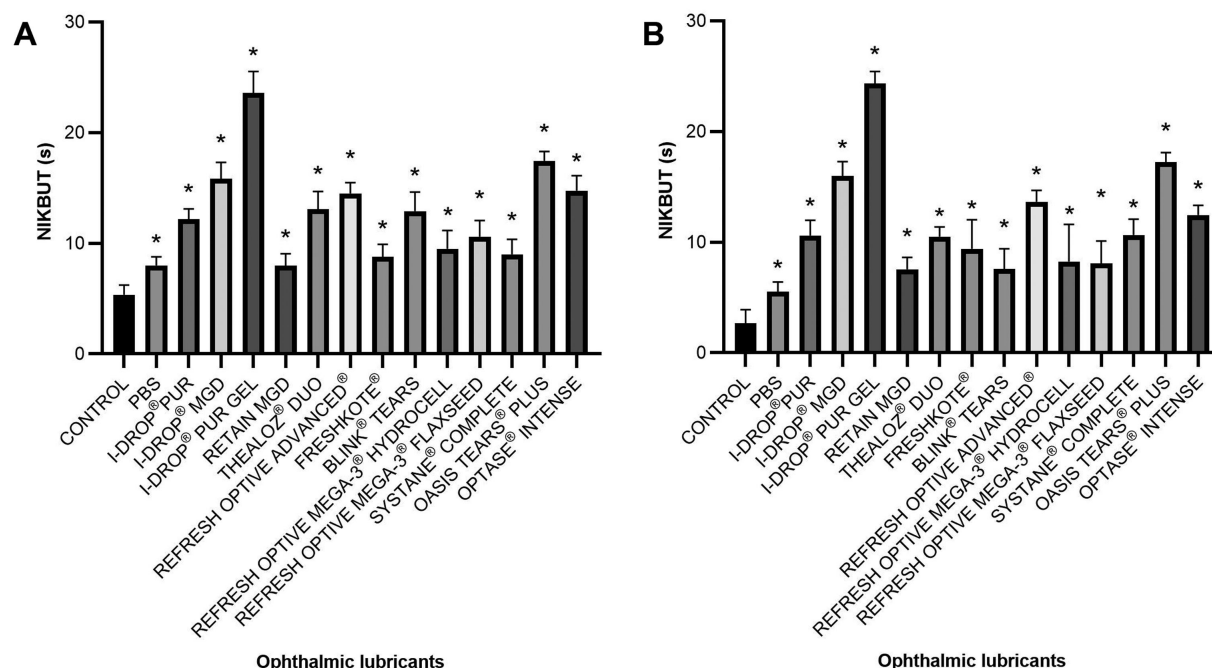


Figure 4. Noninvasive keratographic tear breakup time of lubricants over (A) etafilcon A and (B) collagen shield as the mean $\pm$ standard deviation of $n = 8$. All lubricants had a higher NIKBT than the control ($P < 0.05$) in both conditions.

Overall, the NIKBT data suggest that almost all formulations performed better than the control (without any lubricants) and PBS instillation. The top five highest NIKBTs for both representative surfaces were I-DROP PUR GEL, OASIS TEARS PLUS, I-DROP MGD, REFRESH OPTIVE ADVANCED, and OPTASE INTENSE.

A successful ocular lubricant should ideally mimic viscosities and shear-thinning properties to that of natural tear fluid. However, to our knowledge, few studies have attempted to measure the viscosity of the natural tear film.^{30–32} This is largely due to the difficulty of collecting sufficiently large samples for analysis using a commercial rheometer; thus, tear samples have to be pooled.^{30,32} The natural tear film has a reported dynamic viscosity of 65.5 mPa*s and 10.1 mPa*s at low and high rotational frequencies.³⁰

It has been suggested that ocular lubricants should have viscosities of at least 10 to 15 mPa*s or higher for improved retention time on the eye.^{17,18} Some lubricants tested in this study, notably I-DROP PUR, I-DROP MGD, I-DROP PUR GEL, OASIS TEARS PLUS, and OPTASE INTENSE, have viscosities above the recommended values of 15 mPa*s in both low (open eye) and high (blinking) shear rates, so it is expected that these lubricants will have long-lasting effects on the eye. Too high a viscosity, however, may cause initial patient discomfort and visual blurring,³³ which need to be evaluated in further clinical studies.

We initially hypothesized that there would be a correlation between viscosity and NIKBT. These results suggest that there was no correlation between viscosities at velocities corresponding to the “open eye” condition and NIKBT. There was, however, a positive correlation between viscosities measured at 10 rad/s, corresponding to the blink velocities, and NIKBT up until a threshold value of 100 mPa*s ($r = 0.85$). After 100 mPa*s, increasing viscosity did not seem to continue to have a positive effect on NIKBT.

Formulations with low viscosity have a faster washout on the ocular surface, potentially leading to lower NIKBT. This was indeed observed for some lubricants, such as FRESHKOTE and RETAIN MGD, with viscosities lower than 10 mPa*s at 10 rad/s. On the other hand, we hypothesize that formulations with extremely high viscosities, such as OPTASE INTENSE, may create uneven viscous regions in the tear film that may lead to faster NIKBT. However, this hypothesis warrants further investigation as we were unable to observe this visually using the current experimental setup. It is also important to note that the maximum value for NIKBT is 25 seconds, a limit of the keratograph’s software, which creates an asymptote to which increasing viscosity would, in theory, have diminishing returns for NIKBT as it approaches 25 seconds.

The correlation between viscosity and NIKBT in this study may be affected by the differences in viscosity

Table 3. Noninvasive Breakup Time (s) of Lubricants

Surfaces	Control	PBS	I-DROP		I-DROP		I-DROP		RETAIN		THEALOZ		FRESHKOTE		BLINK		REFRESH		REFRESH OPTIVE		REFRESH OPTIVE		OASIS		OPTASE	
			PUR	MGD	PUR	MGD	PUR GEL	MGD	DUO	MGD	MGD	DUO	TEARS	ADVANCED	OPTIVE	HYDROCELL	MEGA-3	FLAXSEED	COMPLETE	PLUS	PLUS	INTENSE				
Collagen	2.7 ± 1.2	5.5 ± 0.9	10.6 ± 1.4	16.0 ± 1.3	24.3 ± 1.1	7.6 ± 1.1	10.5 ± 0.9	9.4 ± 2.6	7.6 ± 1.8	13.7 ± 1.1	8.3 ± 3.3	8.1 ± 2.0	10.7 ± 1.4	17.2 ± 0.9	12.5 ± 0.9											
Contact lens	5.4 ± 0.9	8.0 ± 0.8	12.2 ± 0.9	15.8 ± 1.5	23.3 ± 2.0	8.0 ± 1.1	13.5 ± 1.6	8.8 ± 1.3	12.9 ± 1.7	14.5 ± 1.0	9.5 ± 1.7	10.6 ± 1.5	9.1 ± 1.4	17.5 ± 0.9	14.7 ± 1.3											
<i>P</i> < 0.05	Yes	Yes	NS	NS	NS	NS	Yes	NS	Yes	NS	NS	NS	NS	NS	NS											

Results are reported as mean ± deviation (n = 8). NS, not significant.

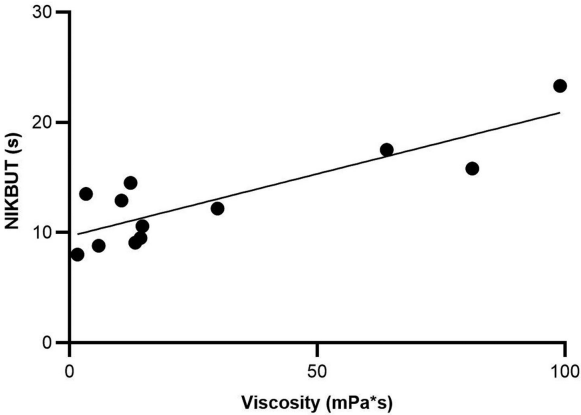


Figure 5. Positive correlation between noninvasive tear breakup time and average viscosity at 10 rad/s from 0 to 100 mPa as determined by the Pearson correlation coefficient ($r = 0.85$).

enhancers used in different formulations (see Table 1). Each lubricant drop used a unique combination of viscosity enhancers, including sodium hyaluronate, propylene glycol, carboxymethylcellulose, glycerin, polyvinyl alcohol, and mineral oil. We hypothesize that each type of viscosity enhancer could have different physical and chemical interactions with the tear film that could enhance NIKBUT and warrants their own investigations in future studies.

The base of the eyeball was three-dimensionally printed using a resin material, which is highly hydrophobic and thus could not be used to measure NIKBUT. Therefore, two hydrogel surfaces with different material compositions were used as representative surfaces for the eye model, one of which was a conventional hydrogel contact lens while the other was a biodegradable collagen shield. We hypothesized that these two surfaces may provide different NIKBUT values for the same ocular lubricant, but the overall trends regarding which formulations have higher or lower NIKBUT should be similar. The results were in good agreement with the initial hypothesis. For instance, both the contact lens and the collagen shield showed that I-DROP PUR GEL had the highest NIKBUT of all the formulations tested. For both materials, all the formulations also had a higher NIKBUT than the control without any lubricant ($P < 0.05$). As expected, there were also differences in NIKBUT values for some formulations (PBS, THEALOZ DUO, and BLINK TEARS), depending on which corneal mimic material was used.

Overall, measuring NIKBUT on the contact lens was much easier than on the collagen shield. The collagen shield was prone to drying during the measurements and would also deteriorate after several measurements. It should be noted that the two surfaces used

to test NIKBUT in this study are only representative and lack some of the critical components of the corneal surface that could affect “real” clinical NIKBUT. For instance, the presence of mucins on the surface could interact differently with the various ingredients in the lubricants. Hyaluronic acid, present in several of the ophthalmic formulations (BLINK TEARS, I-DROP PUR, I-DROP MGD, I-DROP PUR GEL, THEALOF DUO, OASIS TEARS PLUS, OPTASE INTENSE), can also bind to hyaladherins protein receptors found on the ocular surface.³⁴ Additionally, PBS was used as the simulated tear fluid in this study, which does not contain any of the complex salts, proteins, and lipids found in human tear fluid,³⁵ all of which could also interact with components in the ocular lubricant formulation. For future studies, NIKBUT should be tested across other polymeric biomaterials that are more appropriate corneal mimics and investigate a more physiologically relevant artificial tear solution. Another important consideration is that the study was conducted at 25°C and that NIKBUT and viscosity values could change significantly at ocular temperatures (35°C). Previous studies have already shown that viscosity changes significantly with temperature.²⁹

Despite the aforementioned limitations of the model, the results of this in vitro study were fairly comparable to a previous clinical study evaluating NIKBUT after instillation with I-DROP MGD and THEALOF DUO.³⁶ In vivo NIKBUTs for I-DROP MGD and THEALOF DUO were reported as 14.2 ± 5.1 seconds and 10.5 ± 5.7 seconds, respectively, in the clinical study,³⁶ which are similar to the in vitro results for this study. The NIKBUTs for I-DROP MGD were 16.0 ± 1.3 seconds (collagen shield) and 15.8 ± 1.5 seconds (contact lens), whereas the NIKBUTs for THEALOF DUO were 10.5 ± 0.9 seconds (collagen shield) and 13.5 ± 1.6 seconds (contact lens). Overall, these results support that the in vitro model could be a potential tool to measure NIKBUT, but further in vitro–in vivo validation work is necessary.

The results of this study show that the developed eye model, with further validation, could potentially be used to test in vitro NIKBUT of different lubricant formulations. However, future models will need to incorporate surfaces that better represent the cornea, as well as a more complex artificial tear fluid, in order to properly simulate the interactions of the ocular lubricants with the tear film and ocular surface. The results show that the viscosities of the lubricants tested at high shear are positively correlated to NIKBUT, but too high a viscosity did not seem to have an effect or a negative effect on NIKBUT. Based on the results, lubricants with a viscosity up to approximately 100 mPa*s at 10 rad/s showed a high positive correlation with

NIKBUT. It is also important to consider the quality and properties of the viscosity enhancers used in the formulation. Future studies will examine the effects of increasing the concentration of a specific viscosity enhancer and evaluate the effects on NIKBUT. It would also be of interest to study the effects of NIKBUT after a certain period of time postinstillation (i.e., 15, 30, and 60 minutes) to evaluate the long-term effects of the lubricants on the ocular surface. With additional work, viscosity and NIKBUT measurements may provide insights into compositions that will improve future topical lubricant formulations.

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References

1. Craig JP, Nelson JD, Azar DT, et al. TFOS DEWS II report executive summary. *Ocul Surf*. 2017;15:802–812.
2. Uchino M, Yokoi N, Uchino Y, et al. Prevalence of dry eye disease and its risk factors in visual display terminal users: the Osaka study. *Am J Ophthalmol*. 2013;156:759–766.
3. Stapleton F, Alves M, Bunya VY, et al. TFOS DEWS II epidemiology report. *Ocul Surf*. 2017;15:334–365.
4. Vidas Pauk S, Petriček I, Jukić T, et al. Noninvasive tear film break-up time assessment using handheld lipid layer examination instrument. *Acta Clinica Croatica*. 2019;58:63–71.
5. Mengher LS, Bron AJ, Tonge SR, Gilbert DJ. A non-invasive instrument for clinical assessment of the pre-corneal tear film stability. *Curr Eye Res*. 1985;4:1–7.
6. Markoulli M, Duong TB, Lin M, Papas E. Imaging the tear film: a comparison between the subjective keeler tearscope-plus and the objective oculus keratograph 5M and LipiView interferometer. *Curr Eye Res*. 2018;43:155–162.
7. Guillon JP. Non-invasive Tearscope Plus routine for contact lens fitting. *Cont Lens Anterior Eye*. 1998;21(suppl 1):S31–S40.

8. Nichols JJ, Nichols KK, Puent B, Saracino M, Mitchell GL. Evaluation of tear film interference patterns and measures of tear break-up time. *Optom Vis Sci*. 2002;79:363–369.
9. Arif FAC, Hilmi MR, Kamal KM, Ithnin MH. Evaluation of 18 artificial tears based on viscosity and pH. *Malay J Ophthalmol*. 2020;2:96–111.
10. Ousler G, III, Devries DK, Karpecki PM, Ciolino JB. An evaluation of Retaine ophthalmic emulsion in the management of tear film stability and ocular surface staining in patients diagnosed with dry eye. *Clin Ophthalmol*. 2015;9:235.
11. Simmons PA, Liu H, Carlisle-Wilcox C, Vehige JG. Efficacy and safety of two new formulations of artificial tears in subjects with dry eye disease: a 3-month, multicenter, active-controlled, randomized trial. *Clin Ophthalmol*. 2015;9:665–675.
12. Stonecipher KG, Torkildsen GL, Ousler GW, III, Morris S, Villanueva L, Hollander DA. The IMPACT study: a prospective evaluation of the effects of cyclosporine ophthalmic emulsion 0.05% on ocular surface staining and visual performance in patients with dry eye. *Clin Ophthalmol*. 2016;10:887–895.
13. Patel A, Cholkar K, Agrahari V, Mitra AK. Ocular drug delivery systems: an overview. *World J Pharmacol*. 2013;2:47–64.
14. Van Santvliet L, Ludwig A. Determinants of eye drop size. *Surv Ophthalmol*. 2004;49:197–213.
15. Mishima S, Gasset A, Klyce SD, Jr, Baum JL. Determination of tear volume and tear flow. *Invest Ophthalmol*. 1966;5:264–276.
16. Salzillo R, Schiraldi C, Corsuto L, et al. Optimization of hyaluronan-based eye drop formulations. *Carbohydr Polym*. 2016;153:275–283.
17. Zhu H, Chauhan A. Effect of viscosity on tear drainage and ocular residence time. *Optom Vis Sci*. 2008;85:715–725.
18. Rupenthal ID, Green CR, Alany RG. Comparison of ion-activated in situ gelling systems for ocular drug delivery. Part 2: Precorneal retention and in vivo pharmacodynamic study. *Int J Pharm*. 2011;411:78–85.
19. Oechsner M, Keipert S. Polyacrylic acid/polyvinylpyrrolidone bipolymeric systems. I. Rheological and mucoadhesive properties of formulations potentially useful for the treatment of dry-eye-syndrome. *Eur J Pharm Biopharm*. 1999;47:113–118.
20. Lamble JW, Gilbert D, Ashford JJ. The break-up time of artificial pre-ocular films on the rabbit cornea. *J Pharm Pharmacol*. 1976;28:450–451.
21. Adler C, Maurice D, Paterson M. The effect of viscosity of the vehicle on the penetration of fluorescein into the human eye. *Exp Eye Res*. 1971;11:34–42.
22. Pult H, Tosatti SG, Spencer ND, Asfour JM, Ebenhoch M, Murphy PJ. Spontaneous blinking from a tribological viewpoint. *Ocul Surf*. 2015;13:236–249.
23. Picchi D, Poesio P, Ullmann A, Brauner N. Characteristics of stratified flows of Newtonian/non-Newtonian shear-thinning fluids. *Int J Multiphase Flow*. 2017;97:109–133.
24. Borchman D, Foulks GN, Yappert MC, Mathews J, Leake K, Bell J. Factors affecting evaporation rates of tear film components measured in vitro. *Eye Contact Lens*. 2009;35:32–37.
25. Phan CM, Walther H, Qiao H, Shinde R, Jones L. Development of an eye model with a physiological blink mechanism. *Transl Vis Sci Technol*. 2019;8:1.
26. Walther H, Subbaraman LN, Jones L. Novel in vitro method to determine pre-lens tear break-up time of hydrogel and silicone hydrogel contact lenses. *Cont Lens Anterior Eye*. 2019;42:178–184.
27. Walther H, Chan VW, Phan C-M, Jones LW. Modelling non-invasive tear break-up times of soft lenses using a sophisticated in vitro blink platform. *Invest Ophthalmol Vis Sci*. 2019;60:6328.
28. Kwon KA, Shipley RJ, Edirisinghe M, et al. High-speed camera characterization of voluntary eye blinking kinematics. *J R Soc Interface*. 2013;10:20130227.
29. Kapadia W, Qin N, Zhao P, et al. Shear-thinning and temperature-dependent viscosity relationships of contemporary ocular lubricants. *Transl Vis Sci Technol*. 2022;11:1.
30. Tiffany JM. Viscoelastic properties of human tears and polymer solutions. *Adv Exp Med Biol*. 1994;350:267–270.
31. McDonnell A, Lee JH, Makrai E, Yeo LY, Downie LE. Tear film extensional viscosity is a novel potential biomarker of dry eye disease. *Ophthalmology*. 2019;126:1196–1198.
32. Tiffany JM. The viscosity of human tears. *Int Ophthalmol*. 1991;15:371–376.
33. McKenzie B, Kay G, Matthews KH, Knott R, Cairns D. Preformulation of cysteamine gels for treatment of the ophthalmic complications in cystinosis. *Int J Pharm*. 2016;515:575–582.
34. Lardner E, van Setten G-B. Detection of TSG-6-like protein in human corneal epithelium. Simultaneous presence with CD44 and hyaluronic acid. *J Fr Ophthalmol*. 2020;43:879–883.

35. Lorentz H, Heynen M, Kay LM, et al. Contact lens physical properties and lipid deposition in a novel characterized artificial tear solution. *Mol Vis*. 2011;17:3392–3405.
36. Ng A, Dantam J, Woods J, McEwen B, Jones L. Examining symptomatic relief and kinetic tear film stability of I-DROP MGD eye drops. Contact lens update; 2022, <https://contactlensupdate.com/2022/11/14/examining-symptomatic-relief-and-kinetic-tear-film-stability-of-i-drop-mgd-eye-drops/>. Accessed April 21, 2023.