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Effect of castor oil emulsion eyedrops on tear film composition and stability☆

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ABSTRACT

Purpose: An emulsion eyedrop containing castor oil has been shown to modify the tear film lipid layer and increase tear film stability. The primary objectives of this investigation were to measure the prevalence of castor oil in the tear fluid over time and quantify the effects on the lipid layer. A secondary objective was to quantify the initial effects on ocular symptomatology.

Methods: The investigation was an open label pilot study on 5 normal and 10 dry eye subjects. A single eyedrop (Castor oil emulsion, Allergan) was instilled in each eye; the tear film appearance and composition were monitored for 4 h via in vivo visualisation using the Tearscope™ and post in vivo tear samples analysis by HPLC.

Results: Combined results for both normal and dry eye subjects showed that castor oil was detected up to 4 h after a single eyedrop instillation and associated with an increase in the level of tear film lipid. The relative amount of various lipid families was also changed. An increase in tear lipid layer thickness was significant up to one hour post-instillation for the symptomatic sub-population. The changes in tear film characteristics were associated with significantly lower symptoms up to four hours post-instillation for the symptomatic sub-population.

Conclusion: This pilot investigation showed that castor oil eyedrops achieved a residence time of at least four hours post-instillation, producing a more stable tear film and an associated significant decrease in ocular symptoms over the entire follow-up period for the symptomatic subjects.

1. Introduction

Dry eye is defined as a disorder of the tear film due to a deficiency in aqueous tear production and/or increased evaporative loss that leads to irritation of the ocular surface and is associated with symptoms of discomfort [1].

The prevalence rates reported in the literature are highly dependent upon the selection criteria used to diagnose dry eye subjects. Estimates in the prevalence of dry eye syndrome ranged from 14.4% to 34.0% [2–9] depending upon population biases and selection criteria. Additionally, an increased prevalence of dry eye with age [3,6], in women [3,6,10] and in contact lens wearers with estimates from 43% to 50.1% [2,3,11] has also been observed.

Treatments have been formulated to either restore tear volume or to increase tear film stability hence reducing tear evaporation. The most commonly used treatment for dry eyes consists of topically applied artificial tears and lubricants in the forms of eyedrops, gel or ointments. In a 2000 study by Nelson et al. [12], 87% of dry eye patients were reported to have used medications for dry eye in the previous 3 months, 56% reported using lubricant drops and 40% using lubricant ointments. The main active agents in traditional artificial tears products are viscosity enhancing agents used in a range of concentrations, in preserved or unpreserved formulations. Such products have been used in practice to help in the relief of the symptoms present in mild dry eye conditions, with more viscous products dedicated to more pronounced symptoms [13,14].

In the last few years, as a result of a better understanding of the complex aetiology of dry eye syndrome, more targeted, specialised treatments have emerged, either pharmacological compounds aimed at decreasing inflammation, improving lipid production and/or stimulating mucin and aqueous secretions from the ocular surface or treatments formulated to mimic the structure and function of natural tears.

A new emulsion eyedrop developed by Allergan, containing 1.25% castor oil stabilised within an aqueous demulcent formula, was initially used as a vehicle for cyclosporine ophthalmic emulsion 0.05% (Restasis®, Allergan), a pharmaceutical compound used to modulate inflammatory components in KCS and severe dry

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eye cases. It is available in the US in slightly modified form as an artificial tear emulsion (Refresh Endura®, Allergan). This artificial tear solution falls into the category of eyedrops with targeted efficacy, aiming at treating all three layers of the tear film. Upon release the oil is thought to interact with the superficial lipid layer stabilising the tear film and reducing tear film evaporation, while the aqueous demulcent enhances the aqueous and mucin layers [15].

Interestingly, in clinical studies of Restasis® cyclosporine ophthalmic emulsion, the castor oil vehicle alone was reported to reduce some signs and symptoms of dry eye [16,17] and a pre-market study on the emulsion itself as an artificial tear on 73 mild to moderate dry eye subjects reported a significant increase in tear break-up time compared to baseline together with some improvements in signs and symptoms of dry eye after 90 days of usage [18].

Di Pascuale et al. [19] in a study on 5 normals and 10 aqueous tear deficient subjects reported a significant increase in tear lipid layer thickness and improved tear film spread time following the use of 1.25% castor oil emulsion eyedrop. Khanal et al. [20] measured reduced tear film evaporation with use of the 1.25% castor oil emulsion eyedrop, greater than that with a conventional aqueous drop. Further, Goto et al. [21] reported improved symptoms scores, increased tear break-up time and decreased tear evaporation after 2 weeks of six times daily treatment of homogenised castor oil compared to placebo for patients with non-inflamed obstructive meibomian gland dysfunction.

Castor oil eyedrops are lipid eyedrops which beneficial effects are thought to be associated with a modification of the lipid layer properties. The objectives of this pilot study were primarily to measure the prevalence of castor oil in the tear fluid over time and quantify the effects of the castor oil eyedrops on the tear film lipid layer of normal and dry eye subjects. A secondary objective was to quantify the initial effects of the emulsion eyedrop on ocular symptomatology.

2. Materials and methods

2.1. Test products

The test product was an investigative formula of a new emulsion eyedrop containing a polar oil (castor oil) within a aqueous demulcent formula. The castor oil primarily consists of the triglyceride of ricinoleic acid. The demulcent aqueous phase consists of polysorbate 80 (demulcent and emulsifier), carbomer 1342 (gelling agent and emulsifier) and glycerin (demulcent and tonicity agent). The non-preserved formula was dispensed in unit-dose plastic ampoules. The modality of use of the test product was a single instillation by the investigator. The test product, which was an investigational product, was used under a clinical trial exemption (CTX) from the Medicines and Healthcare products Regulatory Agency.

2.2. Subjects

Non-contact lens wearers were randomly enrolled in this research study. The test population included both normal subjects ($n = 5$) and subjects who complained of dry eye ($n = 10$). The McMonnies questionnaire was used to assess the symptomatology of the subjects at the enrolment visit [22]. The dry eye group (Symptomatic group) was defined as those subjects with a score ≥ 40 and the remainder were classified as normal (Asymptomatic group).

Subjects were excluded if they showed signs of ocular infection or anomaly and if ocular medication was currently being used. Systemic diseases, general medications and systemic allergy with subjects signed an informed consent and experimental procedures were reviewed and approved by an ICH-GCP independent ethics committee.

2.3. Clinical test procedures

The *in vivo* evaluation of the tear film characteristics was carried out using a slit-lamp observation system with the Tearscope™ lighting system allowing the different layers of the tear film to be visualised non-invasively.

The lipid layer was observed over the whole corneal surface; the mixing patterns observed within the lipid layer were classified upon their appearance. Lipid mixing patterns, that are transient or of the open meshwork type, are considered to be of poor efficacy and characteristics of a thin lipid layer (~15 nm), close meshwork layer mixing patterns are viewed as average in efficacy and flow and subsequent layer mixing patterns, characteristics of a thick lipid layer (~30–80 nm) are considered optimal [23].

The Non-Invasive Break-Up Time (NIBUT) was taken as the quantification of the pre-ocular tear film stability. Three successive measurements of the NIBUT were recorded; the smallest value recorded (Minimum NIBUT), representing the worst case, and the median value (Median NIBUT) were used for statistical analysis.

The tear prism height was measured as an indication of tear volume pre- and post-eyedrop instillation. The measurement was made immediately below the central part of the inferior cornea using the graduated slit opening on the biomicroscope.

Subjective tolerance and satisfaction were evaluated in terms of ocular comfort, subjective vision and ocular symptomatology during four hours post-instillation. Ocular comfort and subjective vision were recorded on dedicated continuous 50-point scales with the following descriptive anchors (0 = Very poor; 8 = Poor; 17 = Less than satisfactory (below average); 25 = Satisfactory; 33 = Better than satisfactory; 42 = Good; 50 = Excellent). Ocular symptomatology was monitored in terms of ocular dryness, grittiness, burning sensation, scratchiness and itchiness. All symptoms were recorded on continuous 50-point scales with anchors (0 = Constantly; 8 = Very Often; 17 = Often; 25 = Sometimes; 33 = Rarely; 42 = Very rarely; 50 = Never) [24,25].

The other parameters recorded during the clinical examination were not efficacy parameters but were carried out for legal and safety purposes. Visual acuity measurement and safety slit-lamp biomicroscopy with sodium fluorescein and lissamine green vital stains instillation were carried out before eyedrop instillation and four hours post-eyedrops instillation.

2.4. Laboratory procedures

Tear samples were collected at the test visit at regular intervals before and after single eyedrop instillation (15 min, 1 h and 4 h) from both the right and left eyes. The tear samples were collected from the lower tear prism of each eye using sterile disposable surgical eye sponges for the overall lipid profiling and glass microcapillaries for the quantification of castor oil by the investigators, trained in the techniques and who paid particular attention not to stimulate reflex tearing. Sampling of tear in the left eye took place after sampling in the right eye. Approximately 2 μl of tears was collected from each eye.

The tear samples from the right and left eyes were analysed by two different High Performance Liquid Chromatography (HPLC) methods. The amount of castor oil present in the tear film was quantified from the right eye tear samples which were analysed by HPLC, using a technique optimised for fatty acids/triglyceride separation with a reverse phase column and UV detection at 205 nm. The height and area of the peak characteristic of castor oil

collected at the various time points was used as an indicator of the emulsion eyedrop residency time. Quantification was carried out by calibration of the HPLC with castor oil samples of known concentrations.

The endogenous lipid profile and its possible changes over time were analysed from the left eye tear samples by HPLC on a normal phase silica column (LiChrospher SI60) with UV and fluorescence detectors. Five main lipid classes, ranked in increasing retention times, were separated from the tear fluid: Cholesterol esters, Phospholipids/Triglycerides, Fatty acids, Monoglycerides, and Cholesterol. The data was reported for each tear sample in terms of the total amount of lipids detected and the individual amount for each of the lipid classes detected (in absolute amount and in percentage (%) of total lipids).

2.5. Study design

The study was a prospective, open label, interventional study introducing a single eyedrop in each eye. Each subject was required to attend for one enrolment visit and one test visit. The test visit involved the use of the investigational eyedrop in both eyes and a four hours follow-up post-instillation. The eyedrops were instilled by the investigator. The primary end points were the objective measurement of tear lipid biochemical and biophysical characteristics 15 min, one hour and four hours post-eyedrop instillation.

2.6. Data analysis

The relative performance over time of the eyedrop was compared to baseline for each time point using paired statistics, for the overall population and both the symptomatic and normal asymptomatic sub-populations. Non-parametric data was compared by Wilcoxon Signed Rank Exact Test and parametric data was compared using Paired Samples *T*-test or Repeated Measures ANOVA with Time post-instillation & eye as factors.

3. Results

Fifteen subjects completed the study with no observed adverse events. The demographics of the population are presented in Table 1. Out of the fifteen subjects, ten subjects were classified as symptomatic according to the McMonnies questionnaire with an average score of 58.2 ranging from 40 to 88; the remaining five subjects were representative of normal asymptomatic patients with an average McMonnies score of 24.4 ranging from 16 to 34.

Conjunctival hyperaemia, rated using a 5-point scale, was low; on average over the bulbar and limbal areas, hyperaemia was graded as slight or less in 30–100% of cases prior to eyedrop instillation and in 55–100% of cases four hours post-instillation. Hyperaemia was never worse than Mild (Grade 2.5). Corneal staining, recorded on a 0–5-point scale, and conjunctival staining, recorded on a 0–4 point scale, were most commonly rated as Absent (Grade 0) or Slight (Grade 1), both prior and following the use of the eyedrops. The safety data gathered revealed an overall good tolerance of the test eyedrop by the ocular tissues.

Table 1
Demographics of cohort population ($n = 15$).

Overall ($n = 15$)	Age (mean ± SD) [range]	43.7 ± 19.4 [18–72] years
	Sex (male:female)	3:12
Symptomatic ($n = 10$)	Age (mean ± SD) [range]	47.4 ± 20.8 [18–72] years
	Sex (male:female)	3:7
Asymptomatic ($n = 5$)	Age (mean ± SD) [range]	36.2 ± 15.3 [25–62] years
	Sex (male:female)	0:5

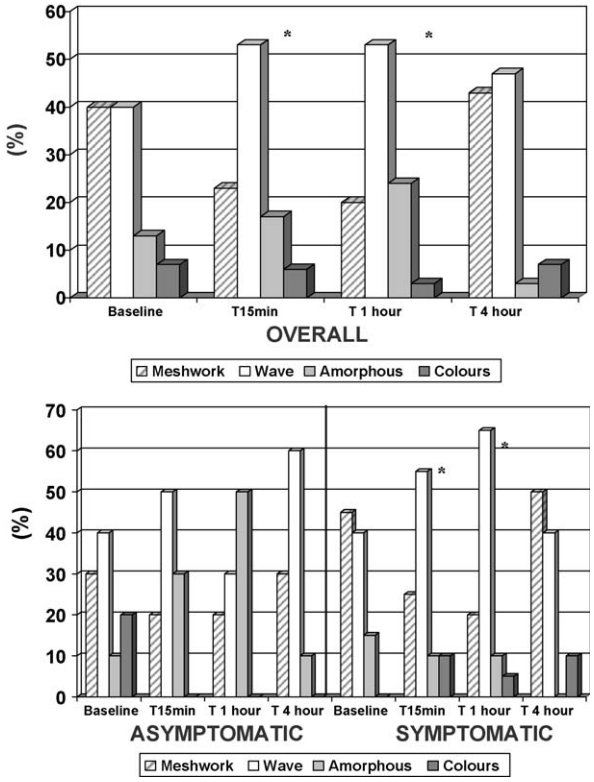

Fig. 1. POTF lipid layer distribution for the overall population and each sub-population at various time point ($^{*}p \leq 0.05$).

The lipid mixing patterns of the POTF lipid layer were most commonly meshwork or wave patterns at all time points pre- and post-eyedrop instillation (Meshwork: 20–43%, Wave 40–53%) (Fig. 1). For the overall population the effect of the eyedrops on the lipid pattern was statistically significant and limited to the first hour post-instillation (15 min, $p = 0.050$; 1 h, $p = 0.035$) (Fig. 1). The effect was, however, more marked for the symptomatic sub-population; the eyedrops significantly modified the patterns observed for the symptomatic sub-population, producing a thicker lipid layer both 15 min ($p = 0.013$), and one hour ($p = 0.006$) post-instillation. The changes were characterised by less open meshwork patterns (Thinnest layers) and more wave patterns (Thick layers) post-instillation compared to pre-instillation (Fig. 1) (Pre: Open Meshwork = 20%, Wave = 40%; 15 min: Open Meshwork = 0%, Wave = 55%; One hour: Open Meshwork = 0%, Wave = 65%; Four hours: Open Meshwork = 10%, Wave = 40%). Neither a change in patterns distribution over time, nor statistically significant changes were observed in the asymptomatic population (Fig. 1).

The POTF NIBUTs (Median and Minimum) tended to increase post-eyedrop instillation compared to baseline. The increase was initially slow and only observed from one hour post-instillation when the NIBUT was longest (Fig. 2). One and four hours post-instillation, the differences in average response observed in the symptomatic subpopulation were always superior to 2 s and considered to be clinically significant (average increase >15% of the mean value) for both the Minimum and Median NIBUTs (Minimum NIBUT Pre: 8.2 s, One hour: 10.5 s, Four hours: 10.4 s; Median NIBUT Pre: 12.6 s, One hour: 16.8 s, Four hours: 15.3 s). However, due to the small sample size in this pilot study, even though the improvement in mean amplitude was large (2.2–4.2 s), it failed to reach statistical significance ($p = 0.061$–0.100). No statistically significant differences were observed for the asympto-

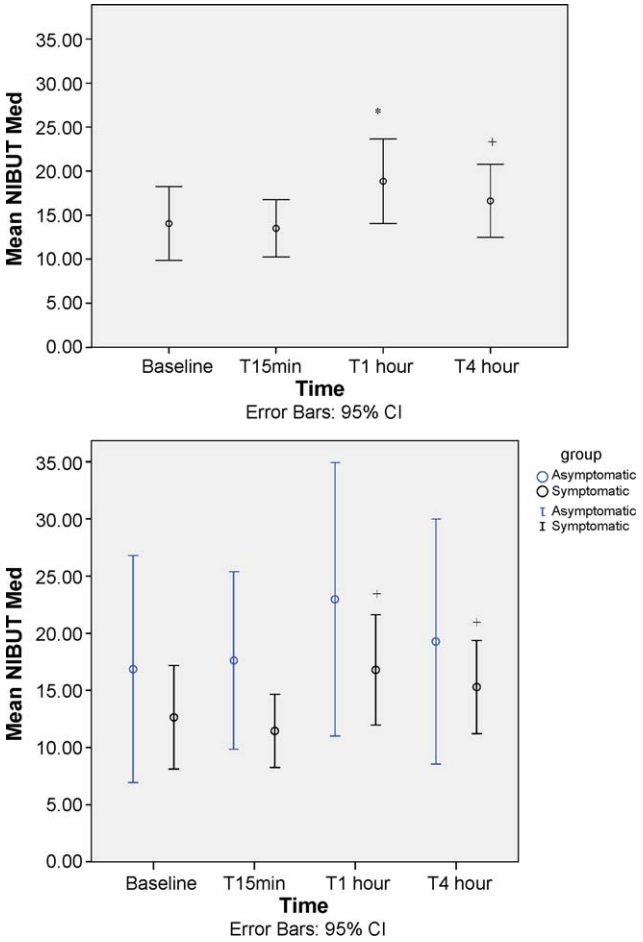

Fig. 2. POTF Median NIBUT over time—overall and for each population subgroups ($^{*}p < 0.05$, $^{+}p < 0.1$).

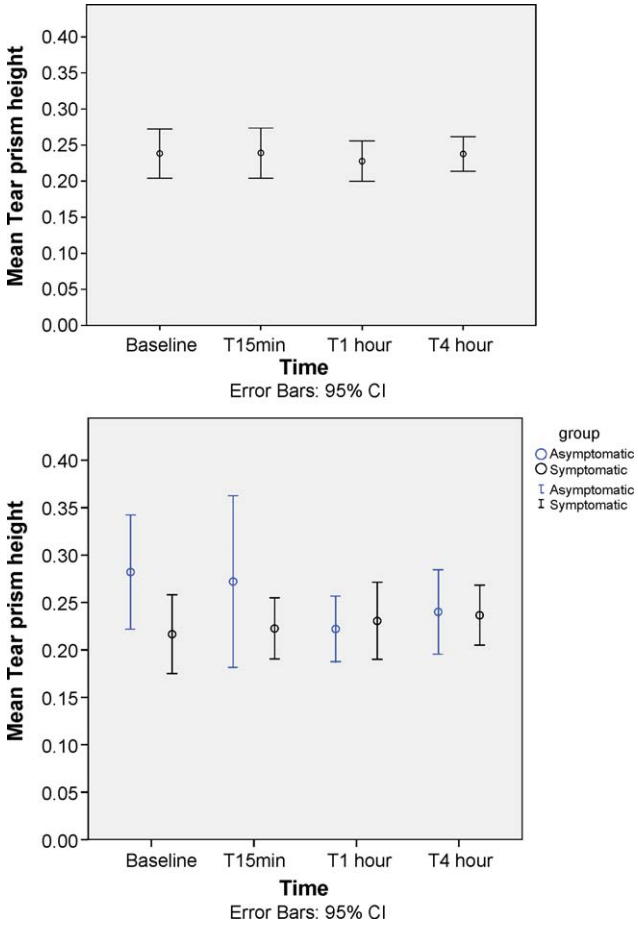

Fig. 3. Tear prism height over time—overall and for each population subgroups.

One hour: 13.2 s, Four hours: 10.6 s; Median NIBUT Pre: 16.9 s, One hour: 23.0 s, Four hours: 19.3 s). In the overall population, the differences in average response observed were more variable (0.8–4.9 s) and statistically significant for the median NIBUT after 1 h ($p = 0.020$).

The tear film volume, which was evaluated in terms of Tear Prism Height, was statistically and clinically similar at all times post-instillation and unchanged ($p = 0.157$–0.484) from pre-instillation (Pre-instillation Asympt = 0.28 mm, Sympt = 0.22 mm, Overall = 0.24 mm; Post-instillation Asympt = 0.22–0.27 mm, Sympt = 0.22–0.24 mm, Overall = 0.23–0.24 mm) with the exception of one hour ($p = 0.010$) and four hours ($p = 0.060$) post-instillation when the tear prism height measured for the asymptomatic sub-population was smaller than baseline measurements (Fig. 3).

Table 2
Comfort scores overtime.

	Baseline	*T* 15 min	*T* 1 h	*T* 4 h
Asymptomatic ($n = 5$)	43.6 ± 8.2 (30 → 50)	45.8 ± 4.0 (40 → 50)	43.0 ± 6.3 (35 → 50)	45.2 ± 3.7 (40 → 50)
p (*vs. Baseline*)	–	$p = 0.121$	$p = 0.404$	$p = 0.208$
Symptomatic ($n = 10$)	36.0 ± 6.7 (30 → 50)	33.8 ± 11.5 (20 → 50)	41.6 ± 5.6 (30 → 50)	41.0 ± 10.8 (10 → 50)
p (*vs. Baseline*)	–	$p = 0.232$	$p = 0.003$	$p = 0.063$
Overall ($n = 15$)	38.5 ± 8.0 (30 → 50)	37.8 ± 11.2 (20 → 50)	42.1 ± 5.8 (30 → 50)	42.4 ± 9.4 (10 → 50)
p (*vs. Baseline*)	–	$p = 0.368$	$p = 0.012$	$p = 0.041$

The ocular comfort recorded on the 50-point continuous scale was on average reported as "better than satisfactory" to "excellent" prior to eyedrop instillation. Clinically significantly higher mean scores were achieved prior to the eyedrop instillation by the asymptomatic than the symptomatic sub-population (Table 2). Post-instillation the increase in comfort scores from the pre-instillation baseline was statistically significant one hour ($p = 0.012$) and four hours ($p = 0.041$) post-eyedrop instillation for the overall population (Fig. 4). Similarly, a statistically and clinically significant improvement in comfort scores was observed for the symptomatic population one hour post-instillation ($p = 0.003$); after four hours the improvement was at the limit of statistical significance ($p = 0.063$). No statistically significant changes in comfort were observed at any time for the asymptomatic population ($p = 0.121$–0.404) (Table 2).

For the overall population a clinically significant decrease in symptomatology was observed up to four hours post-instillation (Fig. 5). The improvement was across all the symptoms recorded: dryness ($p < 0.001$–0.003), grittiness ($p = 0.002$–0.010), itchiness ($p < 0.001$), burning ($p < 0.001$–0.006) and scratchiness ($p = 0.002$–0.003). For the symptomatic sub-population, the decrease in symptoms scores from the pre-instillation values were also very marked, with differences in average score ranging from 7 to 21 points. For all symptoms recorded, the scores at one hour ($p < 0.001$–0.003) and four hours post-instillation ($p < 0.001$–0.019) were statistically significantly different than those recorded at baseline. For the asymptomatic sub-population, the change in the average scores recorded post-eyedrop instillation

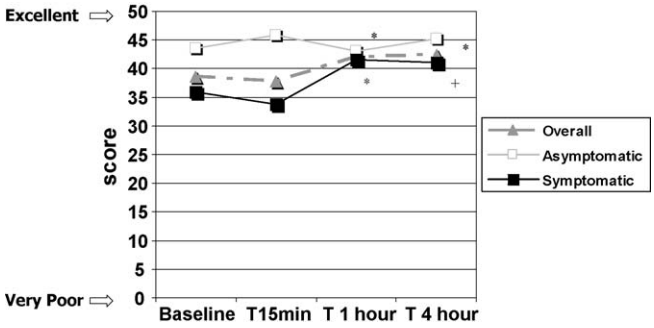

Fig. 4. Comfort pre- and post-eyedrop instillation—overall population ($^{*}p < 0.05$, $^{+}p < 0.1$).

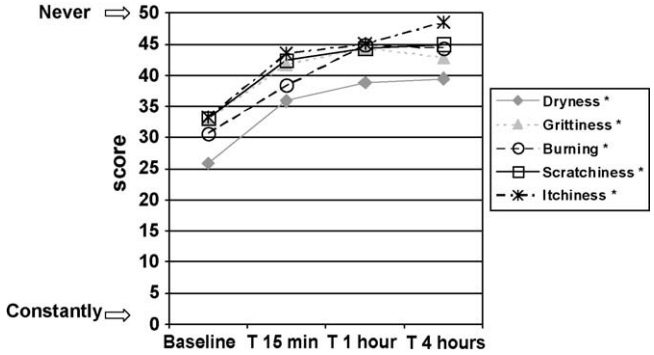

Fig. 5. Ocular symptomatology pre- and post-eyedrop instillation—overall population ($^{*}p < 0.05$ at all time points).

differences were observed but were limited to the symptoms of burning at all time points (Mean score: Baseline = 39.0, 15 min = 48.0, One hour = 46.0, Four hours = 47.4, $p = 0.007$–0.027) and to scratchiness symptoms at 15 min ($p = 0.049$) and four hours ($p = 0.049$) post-instillation (Mean score: Baseline = 40.0, 15 min = 48.0, One hour = 44.0, Four hours = 48.0).

Post-instillation the subjective vision recorded on a 50 points continuous scale was on average reported as "good" to "excellent" for the overall population. The eyedrops did not adversely affect vision for either of the sub-populations and on the contrary, at most time points post-instillation, the subjective vision scores recorded were statistically significantly higher, e.g. better vision than at baseline (Baseline Mean score: Overall = 37.2, Asymptomatic = 40.3, Symptomatic = 31.0; Mean score post-instillation: Overall = 43.4–45.4 ($p < 0.001$–0.011), Asymptomatic = 43.2–44.0 ($p = 0.014$–0.021), Symptomatic = 43.5–46.1 ($p = 0.009$–0.133)).

Castor oil was detected in 67% of tear samples up to one hour post-instillation and in 53% up to four hours (Table 3). On average the level of castor oil measured was similar 15 min and one hour post-instillation and equivalent to 0.17 μl of eyedrop on average; four hours post-instillation the level was on average equivalent to 0.11 μl (Table 4). The presence of castor oil was associated with a more stable tear film as shown by the longer NIBUT for the subjects

Table 3
Castor oil emulsion eyedrop prevalence over time ($n = 15$).

	Incidence of cases with detectable amount of castor oil *n*[%]		
	At 15 min	At 1 h	At 4 h
Asymptomatic	3[60%]	5[100%]	3[60%]
Symptomatic	7[70%]	5[50%]	5[50%]
Overall	10[67%]	10[67%]	8[53%]

Table 4
Castor oil emulsion eyedrop—volume detected overtime ($n = 15$).

	Volume detected (in μl eyedrop equivalent) mean [SD]		
	At 15 min	At 1 h	At 4 h
Asymptomatic	0.12[0.12]	0.22[0.13]	0.09[0.10]
Symptomatic	0.21[0.26]	0.13[0.16]	0.12[0.19]
Overall	0.18[0.22]	0.16[0.15]	0.11[0.16]

Table 5
Effect of castor oil presence on NIBUT.

	At 15 min	At 1 h	At 4 h
No castor oil detected			
NIBUT Minimum (mean ± SD) (s)	9.3 ± 6.1	7.7 ± 3.3	10.9 ± 6.9
NIBUT Median (mean ± SD) (s)	13.0 ± 7.6	16.7 ± 13.7	16.5 ± 7.1
Castor oil detected			
NIBUT Minimum (mean ± SD) (s)	11.4 ± 8.0	13.7 ± 8.8	11.9 ± 9.7
NIBUT Median (mean ± SD) (s)	16.4 ± 9.8	22.4 ± 12.6	19.0 ± 15.1

with detectable levels of emulsion eyedrops in the tear film than for those without (Table 5). The differences were maximal one hour post-instillation (Mean NIBUT Increase: Minimum = 6.0 s (78.5%), Median = 5.7 s (34%)). The difference in tear film stability was still present four hours post-instillation but of limited amplitude (Mean NIBUT Increase: Minimum = 0.9 s (9%), Median = 2.5s (15%)). Despite their amplitude, the differences above, failed to reach statistical significance ($p = 0.084$–0.417).

A trend towards an increased volume of total lipids in the tear film post-instillation was observed for both sub-populations. The effect was noticeable from one hour post-instillation ($p = 0.162$) and most marked four hours ($p = 0.086$) post-instillation (5× increase) but never reached statistical significance.

Overall, no statistically significant differences were observed in the level of any individual lipid family. However, the following trends were observed ($p < 0.1$): a twofold increase in the level of fatty acids ($p = 0.082$) and a higher level of triglycerides compared to baseline ($p = 0.085$) four hours post-instillation. Similar results were obtained for the proportion of each lipid class in the tear film. A significantly lower proportion of cholesterol ester (18.9% *vs.* 26.8%, $p = 0.045$) and higher proportion of triglycerides at the limit of statistical significance (28.5% *vs.* 16.1%, $p = 0.061$) compared to baseline were recorded four hours post-instillation.

4. Discussion

The primary laboratory objective of this investigation was to measure the residence time of castor oil emulsion eyedrops in the tear film. Castor oil presence was detected up to 4 h after a single eyedrop instillation. The use of the emulsion eyedrop was also associated in some cases with a significant increase in the total volume of lipids presents in the tear film. Such increase was most marked for the triglycerides family at one and four hours post-instillation. However, overall due to the small sample size in this pilot study, statistical significance was not achieved. The elevated level of lipids observed post-instillation could be due to the previously reported enhanced meibomian gland secretion with homogenized castor oil [13]. However, the level of triglycerides recorded was also significantly higher than average population levels (at least ×10 times) suggesting that some of the triglycerides sampled were non-endogenous and most likely have originated from the eyedrop itself.

The primary clinical objectives were to quantify the effect of the eyedrops on the tear film. Firstly it is important to note that the tear film volume, evaluated in terms of Tear Prism Height, was

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