Tear Film Thickness (TFT) is the Most Sensitive Measure to Capture Differences in the Critical Quality Attributes of a Formulation Using Anterior Segment Optical Coherence Tomography (AS-OCT)

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Introduction

The purpose of this study was to obtain TFT and menisci measurements on Dutch Belted (DB) rabbit ocular surface post instillation of cyclosporine ophthalmic emulsion formulations with varying viscosity and globule size distribution. This information will support the development of a mechanistic absorption model to better understand and predict formulation effects on the bioavailability of cyclosporine ~~to~~ in ~~the~~ human cornea and ~~the~~ conjunctiva ~~in humans~~ [1-5].

Methods

A total of 416 experiments were conducted on 16 DB rabbits. Tear variable measurements were obtained using Heidelberg Spectralis® Eye Explorer (HEYEX) software on the scans captured with anterior segment optical coherence tomography (AS-OCT). Inter-operator and inter-occasion reproducibility of HEYEX was also established. Formulations tested included, five cyclosporine emulsion formulations (EMULCYA-F1 to F5), three placebos (P1, P2 and P3) which were compositionally identical to Restasis® but had different physicochemical characteristics, along with three lots of the reference listed drug (RLD) Restasis, the artificial tear product Refresh Liquigel®, and sterile water. Each formulation was tested for tear variables like central tear film thickness (TFT), upper tear film meniscus cross-sectional area (UMA) and lower tear film meniscus cross-sectional area (LMA) [6].

Table 1: Globule Size Distribution (GSD) (intensity weighted; 10x diluted with DI water) of various cyclosporine emulsions (Mean ± SD, n=3).

FDA Test Formulation	Sample	Z-Average (d.nm)	PdI	Di(10) (nm)	Di(50) (nm)	Di(90) (nm)
FDA Formulation 1	EMUL-CYA-F1	112.3 ± 1.2	0.298 ± 0.007	57.4 ± 5.3	143.4 ± 5.5	299.6 ± 18.6
FDA Formulation 2	EMUL-CYA-F3	92.2 ± 1.4	0.290 ± 0.007	49.0 ± 4.2	115.7 ± 4.7	243.7 ± 12.6
FDA Formulation 3	EMUL-CYA-F4A	117.3 ± 2.3	0.306 ± 0.020	64.3 ± 8.5	148.0 ± 6.5	315.1 ± 22.6
FDA Formulation 4	EMUL-CYA-F4B	120.1 ± 1.4	0.317 ± 0.015	59.5 ± 8.0	158.5 ± 3.2	329.8 ± 21.1
FDA Formulation 5	EMUL-CYA-F5	204.4 ± 6.1	0.323 ± 0.036	110.0 ± 10.9	257.7 ± 16.2	562.7 ± 84.3
Restasis[ǂ]	Restasis	117.9 ± 2.0	0.35 ± 0.04			

*GSD encompasses size and size distribution.
ǂ DLS values reported in *International Journal of Pharmaceutics* 550.1-2 (2018): 229-239 for 10x dilution[3]

Table 2: Viscosity of various cyclosporine emulsions (Mean ± SD, n=3).

FDA Test Formulation	Sample	Apparent Viscosity (mPa.s)	Rate index
FDA Formulation 1	EMUL-CYA-F1	643.8 ± 20.0	0.460 ± 0.004
FDA Formulation 2	EMUL-CYA-F3	238.6 ± 3.7	0.572 ± 0.000
FDA Formulation 3	EMUL-CYA-F4A	246.6 ± 2.0	0.572 ± 0.004
FDA Formulation 4	EMUL-CYA-F4B	140.6 ± 7.5	0.635 ± 0.005
FDA Formulation 5	EMUL-CYA-F5	249.6 ± 3.5	0.566 ± 0.009
Placebo P1	EMUL-CYA-P1	667 ± 24	0.457 ± 0.000
Placebo P2	EMUL-CYA-P2	508 ± 70	0.493 ± 0.027
Placebo P3	EMUL-CYA-P3	1548 ± 29	0.442 ± 0.002
Restasis[ǂ]	Restasis	170.94 ± 13.61	0.52 ± 0.01

ǂ DLS values reported in *International Journal of Pharmaceutics* 550.1-2 (2018): 229-239 for 10x dilution[3]

Results

Verification of Inter-Operator and Intra-Operator Accuracy and Precision for AS-OCT Measurements Using Contact Lenses with Known Thickness and a Micrometer Device with Known Scale:

Figure 1: (AS-OCT) scans through the center of each type of contact lens and of the micrometer

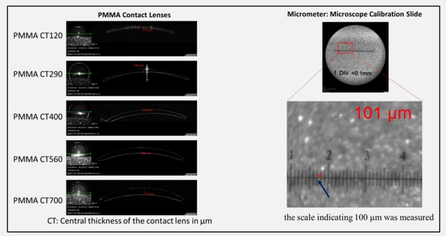


Table 1: Inter-Operator Accuracy

Operator 1						
Lens	Micrometer	CT120	CT290	CT400	CT560	CT700
Overall Accuracy (% of Nominal)	104.8%	96.5%	95.8%	100.9%	101.5%	102.6%
Mean Intra-Assay CV	1.7%	2.0%	0.2%	0.4%	0.4%	0.4%
Inter-Assay CV	5.2%	0.2%	0.8%	0.4%	0.1%	0.6%
Operator 2						
Lens	Micrometer	CT120	CT290	CT400	CT560	CT700
Overall Accuracy (% of Nominal)	101.0%	88.9%	95.2%	100.8%	101.2%	102.3%
Mean Intra-Assay CV	2.3%	1.5%	0.3%	0.6%	0.5%	0.4%
Inter-Assay CV	1.4%	0.9%	1.0%	0.9%	0.04%	0.5%
Inter-Operator						
Lens	Micrometer	CT120	CT290	CT400	CT560	CT700
Inter-Operator CV	2.6%	5.8%	0.5%	0.1%	0.2%	0.2%

CV: coefficient of variation

Table 2: Inter-Operator Precision

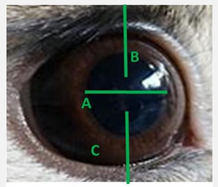
	Micrometer	CT120	CT290	CT400	CT560	CT700
Operator 1 Inter-Assay Mean (µm)	104.8	115.8	277.8	403.7	568.7	718.0
Operator 2 Inter-Assay Mean (µm)	101.0	106.7	276.0	403.0	566.8	716.2
Inter-Operator Mean	102.9	111.3	276.9	403.3	567.8	717.1
Inter-Operator Std Dev	2.7	6.5	1.3	0.5	1.3	1.3
Inter-Operator CV	2.6%	5.8%	0.5%	0.1%	0.2%	0.2%

CV: coefficient of variation

- The data showed good accuracy, with mean measurements within 100±5% of nominal thickness for most subjects and within 100±12% for all subjects.
- Intra-operator (both intra-run and inter-run) and inter-operator reliability were high, with intra-assay coefficients of variation (CV) <3%, inter-assay CV <6%, and inter-operator CV <6%.

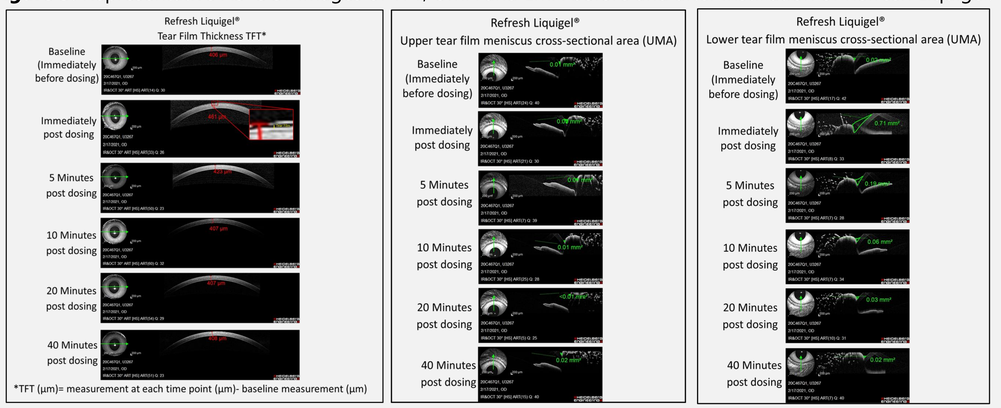
Measurement of Tear Film Thickness and Meniscus Cross-Sectional Area on the Ocular Surface of Dutch Belted~~}~~ Rabbits after Instillation of Cyclosporine Ophthalmic Emulsion:

Figure 2: Schematic Representation of AS-OCT Scan Types



A- Center of cornea
B- Upper Meniscus
C- Lower Meniscus

Figure 3: Representative AS-OCT images of TFT, UMA and LMA before and after instillation of Refresh Liquigel.



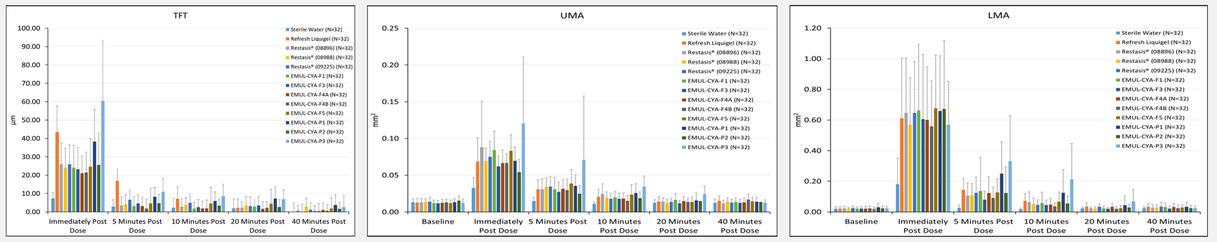


Figure 3. Tear variable measurements were taken at baseline (just before test article instillation), immediately after instillation, and at 5, 10, 20, and 40 minutes post instillation for each formulation. Data expressed as Mean ± SD, n=.

- Overall, instillation of all 13 formulations resulted in an immediate increase in the levels of tear variables like TFT, UMA and LMA. TFT and LMA levels ~~were followed by~~ showed a sharp decrease by 10 minutes and then a more gradual decrease. UMA levels ~~were followed by~~ showed a sharp decrease by 5 minutes post instillation.
- The study showed that physicochemical characteristics such as viscosity and GSD are CQAs, which impacted the tear variables, albeit in different ways.
- Among all tear variables, change in TFT appears to be the most sensitive measure to capture differences in GSD and viscosity as compared to UMA and LMA.
- In addition, TFT data revealed that earlier time points within 10 minutes post instillation should enable a sensitive comparison among formulations that vary in GSD and viscosity.
- Different lots of Restasis showed similar tear variables. This further supports the idea that observed differences in tear variables can be attributed to changes in the critical quality attributes (e.g., GSD, viscosity) of the formulations.

Conclusion

- Results suggest that for cyclosporine ophthalmic emulsions, CQAs such as GSD and viscosity had a direct impact on TFT.
- These study data will be utilized for the validation of a previously developed in silico model[7] along with rabbit pharmacokinetics data, to examine the influence of certain physicochemical properties on the predicted local bioavailability of cyclosporine ophthalmic emulsion and thereby help inform CQA-specific bioequivalence (BE) limits.

References

1. Rahman et al., *Molecular Pharmaceutics* (2014); 11.3: 787-99.
2. Petrochenko et al., *International Journal of Pharmaceutics* (2018); Vol: 550, Issue:1: 229-39.
3. Dong et al., *Journal of Controlled Release* (2019); 313: 96-105.
4. Dong et al., *Journal of Controlled Release* (2020); 327: 360-70.
5. Hu et al., *The AAPS Journal* (2018); 20.3: 62.
6. *Wang et al., Arch Ophthalmol. (2008);126.5: 619-25.*
7. Walenga et al., *Journal of Pharmaceutical Sciences* (2019);108.1: 620-9.

DISCLAIMER:

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