Measuring Drug Partitioning and Release to Support In Vitro Bioequivalence of Generic Ophthalmic Emulsion Drugs

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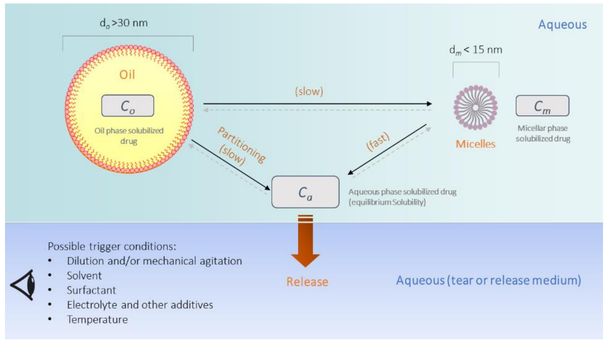
Purpose

FDA recently approved the first generic difluprednate ophthalmic emulsion (08/09/2021) and cyclosporine ophthalmic emulsion (02/02/2022) drug products. FDA recommends two options to demonstrate bioequivalence (BE) for these complex ophthalmic emulsion products: (1) an in vivo pharmacokinetic or clinical endpoint study, or (2) in vitro studies with comparative physicochemical characterization.[1, 2] As the in vitro option relies on demonstrating sameness to the Reference Listed Drug (RLD) product, FDA has conducted several studies to assess the critical quality attributes (CQAs) of these products as well as methods and best practices to measure them. Of considerable interest and challenge is measuring drug partitioning (i.e., the amount of drug in the aqueous vs oil phase of the formulation) and the rate of drug release from the emulsion oil phase.



Materials and Methods

A systematic approach was adopted to identify the CQAs of these products as well as analytical method considerations. Difluprednate and cyclosporine emulsion formulations manufactured as micelles or with small, medium, or large globule size distributions (GSD) were used to assess drug partitioning and the drug release profiles. Drug release profiles from conventional low surface area to volume methods, such as dialysis, were compared to novel higher surface area to volume methods, such as pulsatile microdialysis (PMD) and adaptive perfusion (AP).

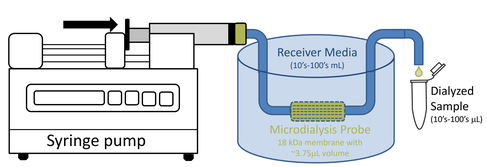
Conventional reverse dialysis was done using Float-A-Lyzer G2, regenerated cellulose, 100-300kD MWCO tubes loaded with approximately 1 mL of formulation placed in a USP 2 apparatus with 200 mL of an 80:20 v/v ratio of 10 mM pH 7.4 phosphate buffer and ethanol.

Regenerated cellulose PMD probes with a molecular weight cutoff of 18 kDa, 200 µm diameter, and 10 cm length were purchased from Spectrum Lab (Rancho Dominguez, CA). A HARVARD Model PHD 2000 syringe pump was used to pump formulation into the PMD probe and allowed to rest for 0, 20, 40, 60, 90, 120, 180, 300, or 600 seconds, before being collected and analysis by reversed-phase HPLC (Agilent 1100 Series).[3]

AP studies used MicroKros® mPES membranes with 100kD MWCO and 20 cm^2 surface area from Spectrum Lab (Rancho Dominguez, CA). A feed flow rate of 200 mL/min with a backpressure setting of 2.7 was used as this condition gave rise to the maximum flux for the difluprednate emulsion samples tested. Difluprednate concentrations were measured by UPLC using a Waters Acquity I-Class (Milford, MA).[4]

Results and Discussion

Pulsatile Microdialysis



PMD probes are a segment of dialysis membrane tubing (the probe window connected to impermeable tubing with a programmable syringe pump and sample collection at each end. Formulation is pumped into the probe window, stopped for selected resting times, then pumped out for collection and analysis. The large surface area to volume ratio of the probe window (~20) in combination with the easily achieved sink conditions, due in part to the significant receiver media to dialysis volume, gives rise to a rapid and relatively large fraction of drug release compared to dialysis bag methods.

Sample	GSD**	PDI**	Drug in Aqueous phase***
RLD (Restasis)	~160 nm	0.417	20.4 ± 1.6 µg/mL (4%)
Polysorbate 80 Micelles*	~15 nm	-	200 µg/mL (100%)
Medium GSD	~163 nm	0.244	52.5 ± 3.5 µg/mL (10%)
Large GSD	~225 nm	0.318	32.0 ± 1.9 µg/mL (6%)

* Micelle formulation was made with 200 µg/mL of cyclosporine in 1% w/w polysorbate 80 in water
** Cyclosporine ophthalmic emulsions formulations referencing Restasis are polydisperse non-monomodal systems that may not be accurately described by conventional GSD descriptors such as Z-ave and polydispersity index (PDI).
*** Concentration of drug substance measured in the aqueous phase of the formulation prior to IVRT

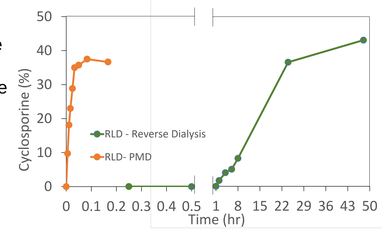


Figure 1. Drug release profiles for cyclosporine emulsion RLD (Restasis) using reverse dialysis and PMD. Both methods gave rise to a similar amount of drug released, but PMD rate was significantly faster

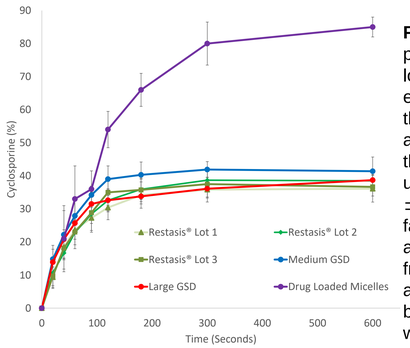
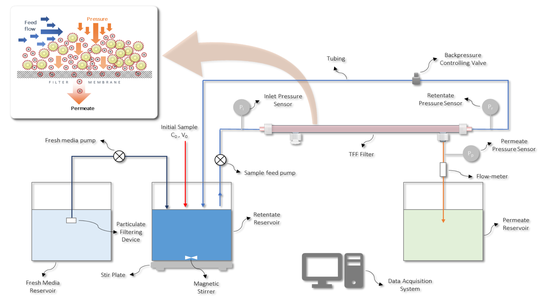


Figure 2. Drug release profiles for cyclosporine loaded micelles and from emulsion samples with the same GSD (Medium) and larger GSD (Large) than the RLD (Restasis) using PMD (n=3, mean ± sd). PMD shows a faster rate and higher amount of drug release from micelles, but is not able to distinguish between formulations with different GSDs.

Adaptive Perfusion



Based on the principle of tangential flow filtration, the AP IVRT method can be used to measure the rate and extent of the drug release from drug solution, drug loaded micelles, and emulsions via adjustment of the filter molecular weight cutoff, feed flow rate, and back-pressure. The tangential flow across parallel membranes gives rise to a high surface area with simultaneous size-based separation and sample dilution that helps maintain continuous sink conditions.

Sample	GSD	PDI	Drug in Aqueous phase***
RLD (Durezol)	123 ± 2.9 nm	0.073	~175 µg/mL 35%
Polysorbate 80 Micelle	~15 nm	-	100 µg/mL (100%)
Small GSD	78.5 nm	0.206	-
Large GSD	152 nm	0.181	-

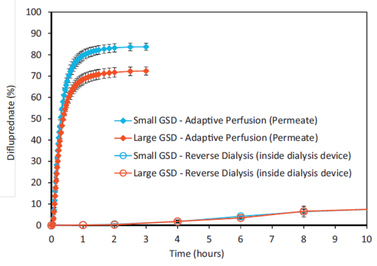
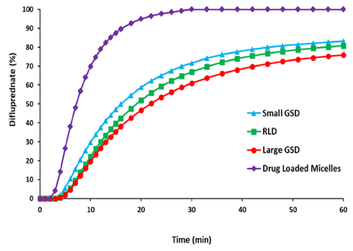


Figure 3. Drug release profiles for difluprednate emulsion samples with small and large GSD using the AP and reverse dialysis methods (n=3, mean ± sd). AP gives rise to a faster rate and higher amount of drug release compared to reverse dialysis

Figure 4. Drug release profiles from an automated AP system (n=1). Illustrating the faster rate and higher amount of drug release from micelles as well as ability to distinguish between formulations with different GSDs.



Factor change		Rate (to aq.)	Extent (to aq.)
Formulation associated variables*	Surfactant (e.g., Tween 80) conc. ↑	↑↑↑	↑↑↑
	Carbomer conc. ↑	↑	↑
	Glycerin conc. (viscosity) ↑	Cyclosporine ↓ Difluprednate –	Cyclosporine ↓ Difluprednate –
	Total surface area (i.e., globule size) ↑	↑↑	– (underestimating due to curvature effect)
Release associated variables	Dilution ↑	Depends on medium	↑↑↑
	Surfactant (e.g., SDS) conc. ↑	↑↑↑	↑↑↑
	Solvent (e.g., ethanol) ↑	↑↑	↑↑
	Electrolyte (e.g., ionic strength) ↑	Cyclosporine ↓ Difluprednate –	Cyclosporine ↓ Difluprednate –
	Temperature ↑	Cyclosporine ↓ Difluprednate ↑	Cyclosporine ↓ Difluprednate –
	Agitation† ↑	↑↑↑	–

Drug release can be further optimized via selection of the release media composition and IVRT conditions. The impact of surfactant, solvent, electrolyte, and temperature on the rate and extent of cyclosporine and difluprednate drug substance diffusion from a castor oil phase to aqueous phase was measured using a model two-phase oil and water system. SDS and ethanol were found to have the biggest impact on the rate and extent of diffusion of both drug substances from oil to aqueous phases.[5]

Conclusion

High surface area to volume methods for measuring drug release from emulsion drug products helps address critical challenges in the development and assessment of complex formulations such as emulsions. PMD requires very small sample volumes and was able to discriminate drug release from micelle and emulsion formulations as well as amount of drug in the different phases of the formulation. Although drug release is measured on the order of seconds the overall measurement time is significantly longer and can have low reproducibility. AP gave rise to better discrimination of the different formulations, but requires significant set-up optimization that benefits from additional automation of the method .

Disclosure / Disclaimer

All authors are employees of the U.S. Food and Drug Administration and do not have any financial or commercial relationships that may create a conflict of interest. This poster reflects the views of the authors and should not be construed to represent FDA's views or policies. Pulsatile microdialysis was conducted by Physical Pharmaceutica LLC under U.S. Food and Drug Administration Contract HHSF223201610105C.

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2. *Draft Guidance on Cyclosporine Ophthalmic Emulsion* (Revised Oct 2016)
3. Bellantone, Robert., et al. *Inter J of Pharmaceutics* 615 (2022): 121521
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