Determination of Globule Size Distribution of Cyclosporine Ophthalmic Emulsions using Asymmetric Flow Field Flow Fractionation

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Abstract

Cyclosporine ophthalmic emulsion (Restasis®) is a complex formulation, with drug that may be distributed across different phases. Among many physicochemical properties, globule size distribution (GSD) is one of the key properties recommended to be characterized if a generic drug product applicant intends to demonstrate the bioequivalence of the drug product through an in vitro option. Previous data on DLS and cryo-TEM suggest that cyclosporine emulsions has a relatively broad size distribution, ranging from tens to a few hundred nanometers. The aim of this study is to develop a method using asymmetric flow field flow fractionation (AF4) coupled with multiple online detectors to separate and determine the globules size distribution of cyclosporine ophthalmic emulsion. Restasis® was used as a model drug product for method development.

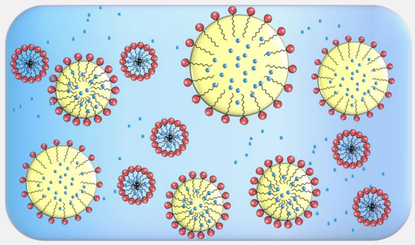

Figure 1. Schematic illustration of microstructure of cyclosporine ophthalmic emulsions

Method

Instrumentation

Agilent 1260 liquid chromatography and Wyatt Eclipse DualTec AF4 system coupled with UV, MALS, DLS and RI detectors.

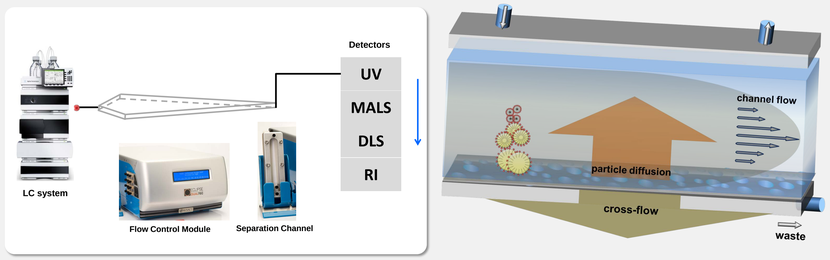

Figure 2. AF4 system components (left); and separation principles inside the channel (right).

AF4

- **Channel: short with 350 µm spacer**
- **Membrane: regenerated cellulose, 10 kDa**
- **Mobile phase: 1 mM NaCl**
- **Focus flow: 1 mL/min**
- **Detector flow: 1 mL/min**

Processing condition for in-house formulation

Formulation	Temperature (°C)	Microfluidization Pressure, Cycles
F1	70	20 Kpsi, 6 cycles
F2	70	20 Kpsi, 2 cycles
F3	70	10 Kpsi, 6 cycles

Results

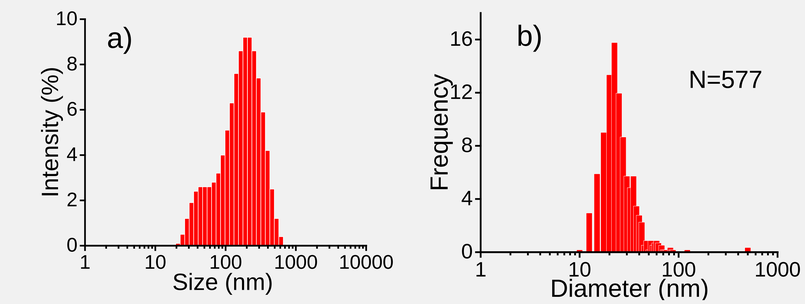

Figure 3. Globule size distribution analysis of Restasis® by a) DLS and b) cryo-TEM

- Cyclosporine ophthalmic emulsions is a polydispersed system
- Large run-to-run variation for un-diluted samples, partly due to viscosity effect
- Z-average values are relatively stable for diluted samples
- Cryo-TEM is able to accurately determine the size of individual particles
- Large number of particles from different viewing are needed to avoid counting bias through cryo-TEM

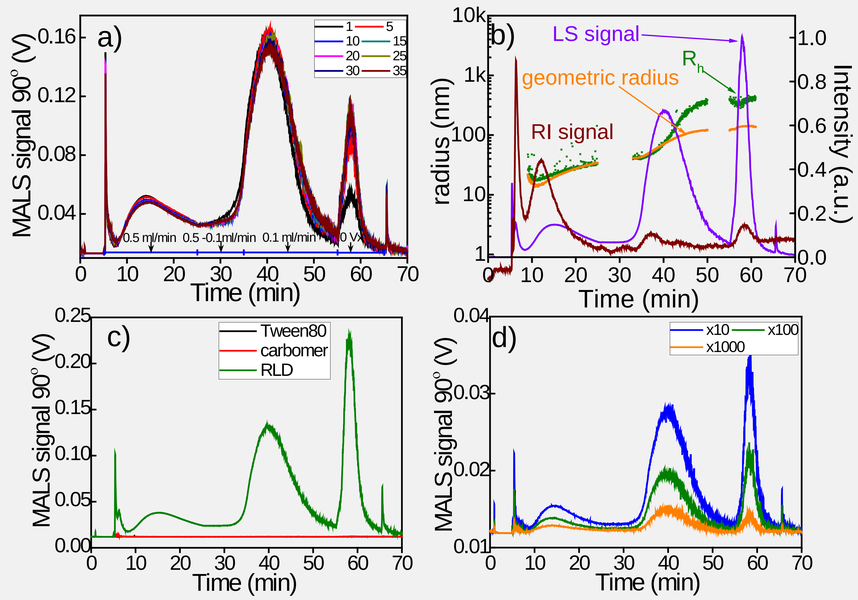

Figure 4. a) 35 consecutive injections of cyclosporine ophthalmic emulsion under optimized condition. b) Globule size distribution of Restasis® obtained by AF4. c) individual components in the cyclosporine ophthalmic emulsion formulation. d) Restasis at different dilution factors

- The optimized method exhibits good repeatability in terms of retention time and signal intensity
- Geometric size, hydrodynamic size and the relative concentration of each size fraction can be determined in a single run
- Individual components do not contribute significantly to the light scattering signal
- Dilution does not cause observable effect to the stability of globule size

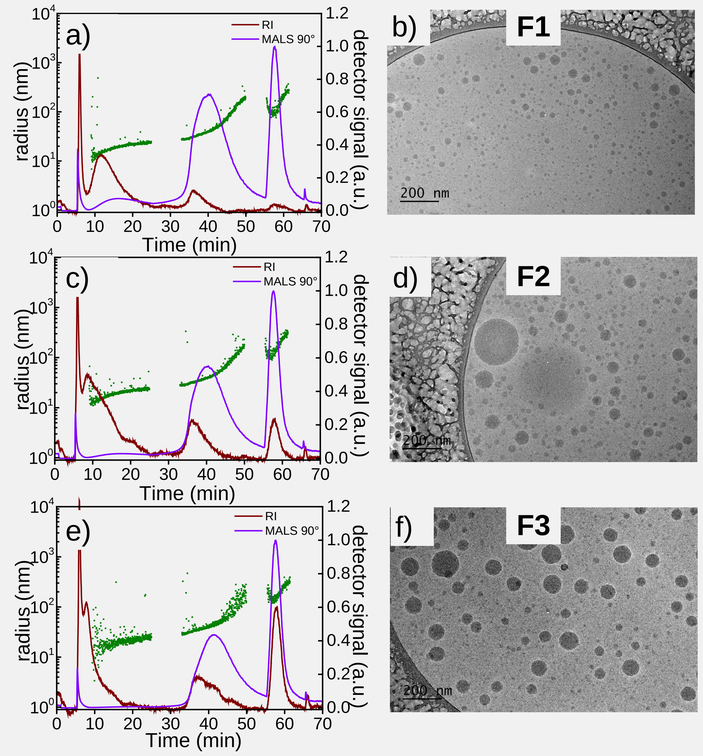

Figure 5. AF4 and cryo-TEM characterization of in-house cyclosporine emulsions

- Varying the process condition, i.e. applied shear force, has significant effect on the globule size distribution
- Difference in globule size distribution caused by changes to the manufacturing process can be detected by the AF4 method.

Conclusions

- **A high resolution method based on AF4 technique was developed to characterize globule size distribution of cyclosporine ophthalmic emulsions**
- **Globule size distribution obtained by AF4 method is consistent with cryo-TEM**
- **Dilution during the AF4 analysis does not adversely impact oil globule stability**
- **Differences in the oil globule size distribution caused by changes to the manufacturing process were successfully detected by the AF4 method**
- **AF4 may become a valuable technique for other complex drug products**

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