Characterization of the Globule Size Distribution of Cyclosporine Ophthalmic Emulsion to Establish Bioequivalence of a Test Formulation

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Purpose
Globule size distribution (GSD) of cyclosporine ophthalmic emulsion is a critical physicochemical attribute that influences drug release, turbidity, viscosity and consequently bioavailability of the formulation. Hence, a Test cyclosporine ophthalmic emulsion product should have similar GSD as that of the reference listed drug (RLD). Since GSD of the commercial cyclosporine emulsion product has not been comprehensively investigated, this study sought to characterize the globule size and report a suitable method for comparing a Test product to the RLD.

Methods
Commercially available 0.05% cyclosporine emulsion product was procured. GSD was measured by laser diffraction (LD) technique using Malvern Mastersizer 3000. For dynamic light scattering (DLS) technique, two instruments – Malvern Zetasizer Nano ZS and Microtrac NANO-flex were used. The effect of sample preparation was investigated by serial dilution of the sample from 10- to 1000-fold with 10 mM NaCl. In addition, Transmission Electron Microscopy (TEM; JEM 1400 by JEOL USA) – both negative staining TEM and cryo-TEM – was used to measure globule size of the formulation.

Results
A multimodal size distribution was observed for cyclosporine ophthalmic emulsion product when measured using LD technique with reported D10, D50 and D90 being 61, 109 and 433 nm, respectively. For DLS measurement using Microtrac NANO-flex, a multimodal size distribution with high PdI was observed with the mean intensity globule size of 569 nm for undiluted sample. Variation of GSD was observed between the measurements of the same sample. Following dilution, the mean intensity globule size was 216, 275, and 278 nm with 10-, 100-, and 1000-fold dilution, respectively. Similar to the Microtrac system, a multimodal size distribution was also observed with Malvern Zetasizer with a mean Z-average size and PdI of 301 nm and 0.557, respectively. The size distribution histogram for each sample varies considerably within the measurements when plotted using narrow mode algorithm; the Z-average size and PdI, however, remained constant between the measurements. Dilution of the sample with 10 mM NaCl markedly reduced the size of the globules – mean Z-average size of 104, 101, and 111 nm with 10-, 100- and 1000-fold dilution, respectively, compared with 301 nm for undiluted sample. The histogram of size distribution was also altered as the larger globules disappeared upon dilution. The observed effect of dilution was in agreement with previous report by Rahman et al (2014). The negative staining TEM and the cryo-TEM images further confirmed a polydispersed distribution with globules of varying size (Fig. 1).

Conclusion
Cyclosporine ophthalmic emulsion is composed of globules of a broad size range. Since dilution affects globule size, undiluted samples are informative when characterizing globule size of the test formulations. Hence, the size measurement techniques that involve dilution of sample should be avoided. If DLS is used, comparison of particle size distribution should be based on Z-average size and PdI , instead of D50 and SPAN, since these are the reporting parameters for DLS as recommended by ISO 13321:1996 and ISO 22412:2008. Comparable size distribution profiles upon serial dilution of the test and reference products are important when comparing the effect of dilution on the test products. Analysis mode used for globule size measurement, which influences the histogram, should also be described.

Fig. 1: Particle size characterization of cyclosporine ophthalmic emulsion by (A) dynamic light scattering (narrow mode), (B) laser diffraction, (C) negative staining (methanol:urea:toluene) TEM, and (D) Cryo-TEM