Measuring Globule Size Distribution of Cyclosporine Ophthalmic Emulsion by Cryogenic Electron Microscopy

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In the draft guidance on cyclosporine ophthalmic emulsion, US Food and Drug Administration (FDA) recommends an in vitro option in which a Test product should (i) be \( Q_1/Q_2 \) the same as the reference listed drug (RLD), (ii) have comparable physicochemical characteristics, and (iii) have comparable in vitro drug release rate to that of RLD. One of the physicochemical characteristics that is recommended for evaluation is similarity in the globule size distribution (GSD) between test and reference products. Since the GSD of the commercial cyclosporine ophthalmic emulsion has not been comprehensively investigated, we measured its globule size distribution using light scattering, laser diffraction and cryogenic electron microscopy.

Data interpretation from light scattering and laser diffraction techniques is model dependent and complicated by broad size distributions with multiple particle size groups. In contrast to these indirect sizing techniques, electron microscopy (EM) provides much higher resolution compared to other optical techniques and has a broad range of operational magnifications for direct visualization of emulsion particles. By rapid freezing, cryo-EM can measure emulsions in a frozen hydrated state, which forms thin vitrified ice to physically immobilize emulsion particles in their native solution. Image interpretation from cryo-EM is often straightforward.

The cryo-transmission electron microscopy (TEM) images confirmed a polydispersed distribution with globules of varying size. Under cyro-TEM, spherical cyclosporine ophthalmic emulsion particles (stock solution and 10x dilution in deionized water) were visualized with dark contrast against the vitrified ice layer (Figure 1). These emulsion particles dispersed through the ice layer, with a heterogeneous population in size range of 11.0-202.2 nm (stock solution) and 15.0-177.2 nm (10x dilution)(Figure 2). As expected, the emulsion rendered lower particle numbers after 10x dilution (Figure 1). Besides the population density difference, the stock emulsion and the diluted sample exhibited very similar number-averaged sizes, 38.9 nm and 39.6 nm, respectively. Cryo-TEM revealed no significant size change caused by the dilution.
Figure 1. Cryo-TEM micrographs of cyclosporine ophthalmic emulsion A) stock solution and B) 10X dilution in di-water.

Figure 2. Cryo-TEM particle size distribution of cyclosporine ophthalmic emulsion A) stock solution and B) 10 X dilution in di-water.