Toward Development of Sensitive In Vitro Drug Release Methods for Difluprednate Ophthalmic Emulsion
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Purpose
Presently there is no compendial dissolution method for topical ophthalmic emulsion formulations. Durezol®, a topical emulsion of difluprednate, is used for the treatment of postoperative inflammation and pain. Difluprednate (DFBA) is insoluble in water and its systemic absorption was shown to be very limited. The aim of the present study was to prepare test Q1/Q2 formulations of Durezol® and develop a drug release method that can discriminate the process variability in the production of the test formulations.

Methods
Difluprednate coarse-emulsion containing 0.05% difluprednate, castor oil as an oil phase and polysorbate 80 as an emulsifying agent was produced with PolyTron mixture system at 70 0C and 12000 rpm for 1 h. The coarse-emulsion was then subjected to a high-pressure emulsification (Microfluidizer M-110P) at 10,000 and 30,000 psi for 10 volume cycles to prepare two nanoemulsions (F1, 134.5±2.5 nm; F2, 219±4.3 nm). Physicochemical properties (size, zeta potential, viscosity, osmolarity and surface tension) of the nanoemulsions were compared with other submicron emulsions (F3:370 nm; F4:434 nm; F5:782 nm) which were prepared by also varying the pressure during emulsification. In vitro release of Durezol® and the test formulations were investigated by dialysis method, gel chromatography and microdialysis. The dialysis method was studied using Spectrum dialysis membranes of different types (CE, Cellulose Ester; and RC, Regenerated Cellulose) and molecular weight cut offs (10, 25 and 50 KD) with 0.05% sodium lauryl sulfate (SLS) in phosphate buffered saline (PBS) as the dissolution medium. A 1 mL of emulsion diluted with simulated tear fluid (STF) at a 1:4 ratio was accurately placed into the dialysis bag and the bag was suspended in 75 mL of the dissolution medium. A 1 mL of dissolution medium was withdrawn at predetermined time intervals up to 72 h and replaced with the same volume of fresh release medium to maintain a constant volume. For the gel chromatography method, PD-10 column was equilibrated with 50 ml of running PBS, then 0.1 ml DFBA emulsions was loaded onto the column separately, and eluted with PBS to separate the free drug from the emulsion. The eluent fractions were collected into a 10 ml volumetric flask and made up to mark with acetonitrile, filtered through a 0.45 μm filter membrane and drug concentration was analyzed by HPLC method. For microdialysis and reverse-microdialysis method, 1 mL of emulsion diluted with 0.5% SLS in PBS at a 1:4 ratio was accurately placed into the syringe and passed through the CMA High Cut-Off microdialysis probe at 5.5 μL/min. The probe was suspended in 10 mL of the dissolution medium under stirring at 150 rpm and maintained at 37± 0.5 °C. A 50 μL of dissolution medium was withdrawn at different time intervals up to 2 h and replaced with the same volume of fresh release medium to maintain a constant volume. In reverse-microdialysis the dissolution medium was passed through a probe dipped in 1 mL of emulsion diluted with 0.5% SLS in PBS at a 1:4 ratio. The concentration of difluprednate in the samples was determined by HPLC method. The statistical analysis was performed with Student’s t test. P<0.05 was considered statistically significant.

Results
Mean droplet size of Durezol® was found to be 137.8±2.8 nm. F1 emulsion had an average droplet size of 134.5±2.5 nm, and a zeta potential of -6.7±1.5 mV. Size and zeta potential of F2 was 219±4.3 nm and -6.4±1.9 mV, respectively. Whilst F1 and F2 were stable for at least 3 months, other submicron emulsions were stable for 72 h only. Nature of the dialysis membrane and the molecular weight cut off of the dialysis bag has a significant effect on the release profile of the different difluprednate emulsions. Increase in molecular weight cut off of dialysis membrane (RC and CE) increased the percent drug released from the emulsion. A statistically significant difference in release profile was seen between F1 and F2 formulation when CE 20 KD membrane was used (p<0.05). The rate of drug release from the emulsion (flux) increased proportionally with increase in the drug loading. No significant difference was observed between the F1 and F2 formulations in 0.5% SLS in PBS using PD 10 Gel column chromatography and the microdialysis and reverse-microdialysis methods.

Conclusion
DFBA ophthalmic emulsion formulations with different droplet sizes were prepared and their stability was characterized. Dialysis method using CE 20 KD membrane could differentiate the release profiles of F1 and F2 formulations. However, no statistical difference between the drug release profiles of Durezol, F1 and F2 was observed using the microdialysis method. Further optimization of the microdialysis