Preparation and Evaluation of Difluprednate Topical Ocular Emulsions

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SUMMARY

Currently there is no specific compendial assay for dissolution for topical ophthalmic emulsion formulations. A topical nanoemulsion formulation of difluprednate (glucocorticoid), was approved for the treatment of inflammation and pain associated with post-operative inflammation following cataract surgery, and for the treatment of endogenous anterior uveitis. DFBA emulsions with same ingredients (Q1), and with same concentration of the ingredients (Q2) to that of the marketed product were prepared under different process conditions. This study sought to develop *in vitro* testing methods to compare *drug release from* different emulsion formulations of DFBA, and be able to predict the clinical efficacy of the product. Results suggest that, at given conditions, no significant difference between F1 formulation and the marketed product, and the method was sensitive to drug concentration in the formulation.

METHODOLOGY

Preparation the difluprednate emulsion:

The difluprednate emulsion formulation 1 and 2 (F1 and F2) were prepared in two steps. In the first step, the difluprednate coarse-emulsion containing 0.05% difluprednate, caster oil as an oil phase and polysorbate 80 as an emulsifying agent was produced with PolyTron System at 70 ℃ and 12000 rpm for 1 h. The coarse-emulsion was subjected to a high-pressure emulsification (Microfluidizer M-110P) at 10,000 and 30,000 psi pressure for 10 volume cycles. The difluprednate emulsion formulations 3, 4 and 5 (F3, F4 and F5) were prepared with PolyTron mixture system at different temperature, rotor speed and time conditions.

Characterization of DFBA emulsion:

Particle size, polydispersity indexe (PDI) and zeta potential of all the prepared DFBA emulsions were determined by dynamic light scattering (DLS) analysis using ZetaPALS zeta potential analyzer (Brookhaven, NY). The osmotic pressure and viscosity measurements were made by 5004 Micro-OSMETTE automatic osmometer (Precision System, MA) and Brookfield DV3T Rheometer (Brookfield, MA).

Determination of DFBA in emulsion:

The DFBA in emulsion was analyzed using a Shimadzu HPLC system equipped with a LC 20 AB solvent pump, SIL-20A HT autosampler, CTO-20A column temperature oven and a SPD-20A UV/vis detector. Separations were accomplished on Waters Atlantis (250×4.6 mm id, 5 µm particles) at 40 ℃. The mobile phase consisted of acetonitrile and water (60: 40) at a flow rate of 1.0 mL.min^{-1}. The injection volume was 10 µL, the absorbance was measured at a wavelength of 245 nm.

In vitro DFBA drug release:

Dialysis method: *In vitro* release profile of the prepared difluprednate emulsions (F1, F2, F3, F4 and F5) and the commercial product were investigated by dialysis method using Spectrum dialysis membranes of different nature (CE, Cellulose Ester; and RC, Regenerated Cellulose) and molecular weight cut off (10, 25 and 50 KD) with 0.05% sodium lauryl sulphate (SLS) in phosphate buffered saline (PBS) as the dissolution medium. A 1 mL of emulsion was diluted with simulated tear fluid (STF) at 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. The concentration of difluprednate in the samples was determined by HPLC.

Ultrafiltration Method:

In vitro release profiles of the prepared difluprednate emulsions (F1, F2, F3, F4, F5) and the marketed product were also investigated by ultrafiltration method using ultrafiltration tube of different nature (PES: Polyethersulfone; RC: Regenerated Cellulose) and molecular weight cut off (10, 30 and 50 KD). A 1.5 mL of the emulsion diluted with simulated tear fluid (STF) at 1:4 ratio was accurately placed into the filter device of ultrafiltration tube and centrifuged at different speed and time. Aliquot of the filtrate was collected from filtrate collection tube and diluted to 2 ml with methanol. The concentration of difluprednate in the samples was determined by a HPLC method.

RESULTS

Table 1. Particle size and zeta potential measurements of various DFBA emulsions (data represent mean±SD, *n*=3).

	Particle size (nm)	PDI	Zeta Potential (mv)
Comm. P.	136.9±3.3	0.033±0.004	-6.24±2.53
DFBA F1	136.9±1.4	0.106±0.012	-6.97±1.94
DFBA F2	207.4±1.7	0.165±0.025	-6.97±1.45
DFBA F3	372.2±8.1	0.335±0.041	-9.25±1.18
DFBA F4	433.9±5.8	0.310±0.036	-6.97±2.88
DFBA F5	781.2±24.4	0.327±0.028	-10.41±1.41

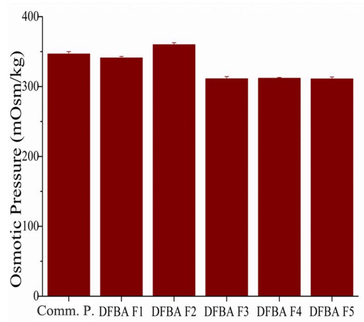

Fig.1. Determination of osmotic pressure for different DFBA emulsions.

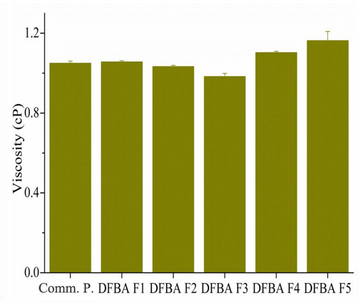

Fig. 2. Determination of viscosity for different DFBA emulsions.

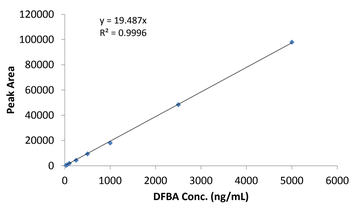

Fig.3. Calibration curve of DFBA in the concentration range of 0.025-5.0 µg/mL.

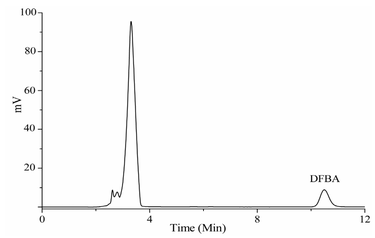

Fig.4. A representative HPLC chromatogram for the dissolution sample of DFBA emulsion .

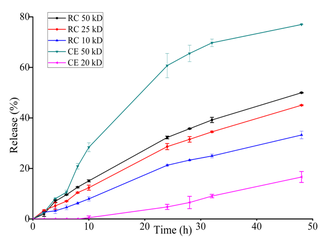

Fig. 5. Effect of different nature (CE and RC) and molecular weight cut off (10, 25 and 50 KD) of dialysis membranes on the release profiles of DFBA F1 .

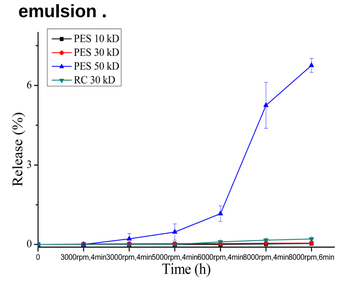

Fig. 6. Effect of different nature (PES and RC) and molecular weight cut off (10, 30 and 50 KD) of ultrafiltration tube on the release profiles of DFBA F1 .

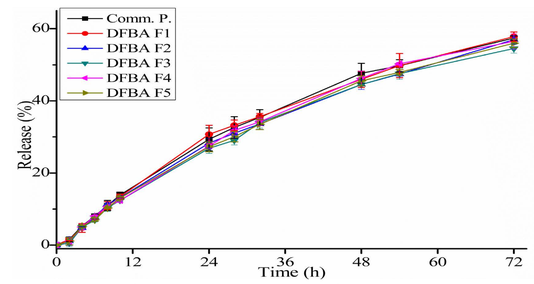

Fig. 7. *In vitro* release profiles of different DFBA emulsion using 25 kD RC dialysis membrane.

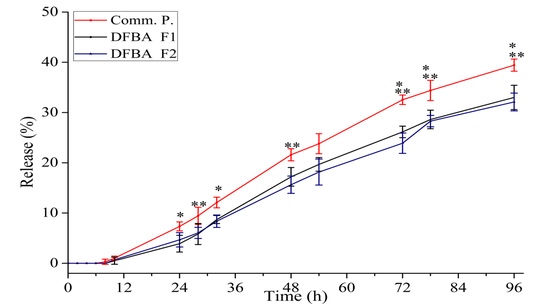

Fig.8. The comparison of different difluprednate emulsions release profiles using 20 kD CE dialysis membrane. Statistical analysis was performed with Student's t test. *, ** and * indicate p < 0.05 for Durezol versus F1, Durezol versus F2 and F1 versus F2.**

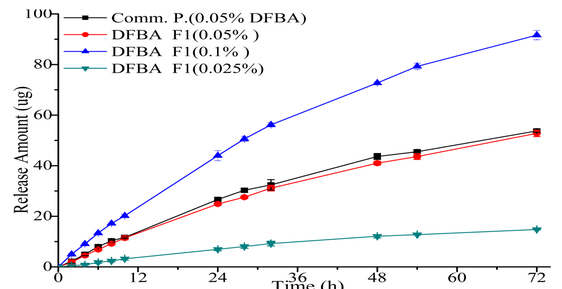

Fig. 9. Comparison the release profiles for F1 with different content of DFBA (0.025, 0.05 and 0.1%) using RC25 kD (diluted with STF).

DISCUSSION

Rate of drug release from the formulations was higher with dialysis membrane made of cellulose ester (CE) as compared to regenerated cellulose (RC). Rate of drug release also increased with increase in MWCO from 25 KD to 50 KD. A statistically significant difference in release profile was seen between F1 and F2 formulation when a dialysis method using CE 20 kD membrane was used. However, the difference was marginal. Ultrafiltration method was not suitable for the discrimination of different emulsions. As at the low centrifuge speed no drug release was observed, whilst the whole emulsion will come out at the high centrifuge speed. Studies are in progress to differentiate the permeability of the above selected DFBA emulsions using fresh rabbit corneal epithelium, and pharmacokinetic study in a rabbit model.

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