

PURPOSE

Presently there is no specific standard assay for dissolution for topical ophthalmic emulsion formulations. Durezol®, a topical emulsion of difluprednate is used for the treatment of postoperative inflammation and pain. This study aimed to develop and validate *in vitro* dissolution methods to different formulations of DFBA (Difluoroprednisolone Butyrate Acetate), and methods that can differentiate the product and formulation variables of difluprednate emulsion.

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

Formulation	Particle size (nm)	PDI	Zeta Potential (mv)
Durezol	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

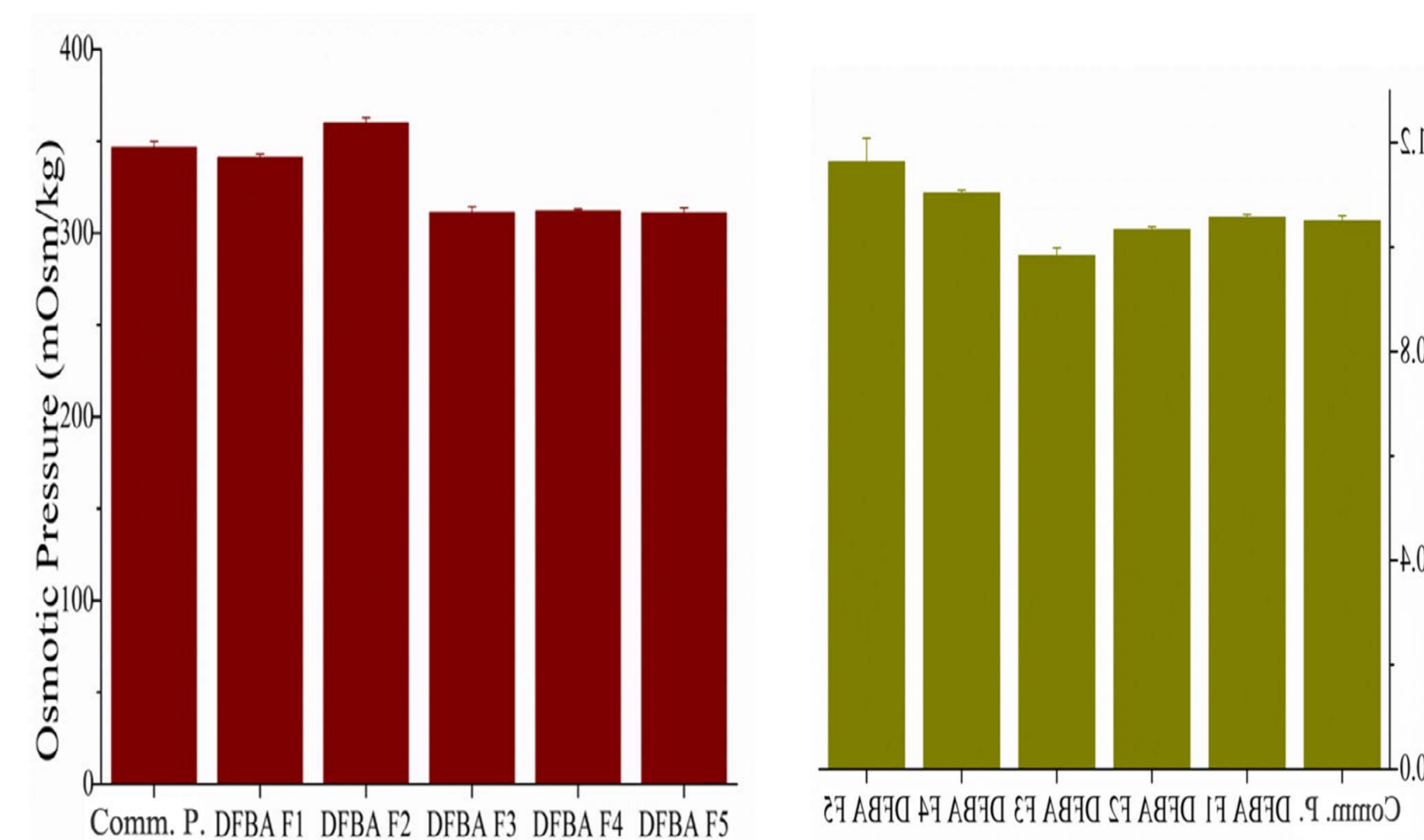


Fig. 1. Determination of osmotic pressure for different DFBA emulsions. (By Micro Osmometer)

Fig. 2. Determination of viscosity for different DFBA emulsions. (By Brookfield Rheometer)

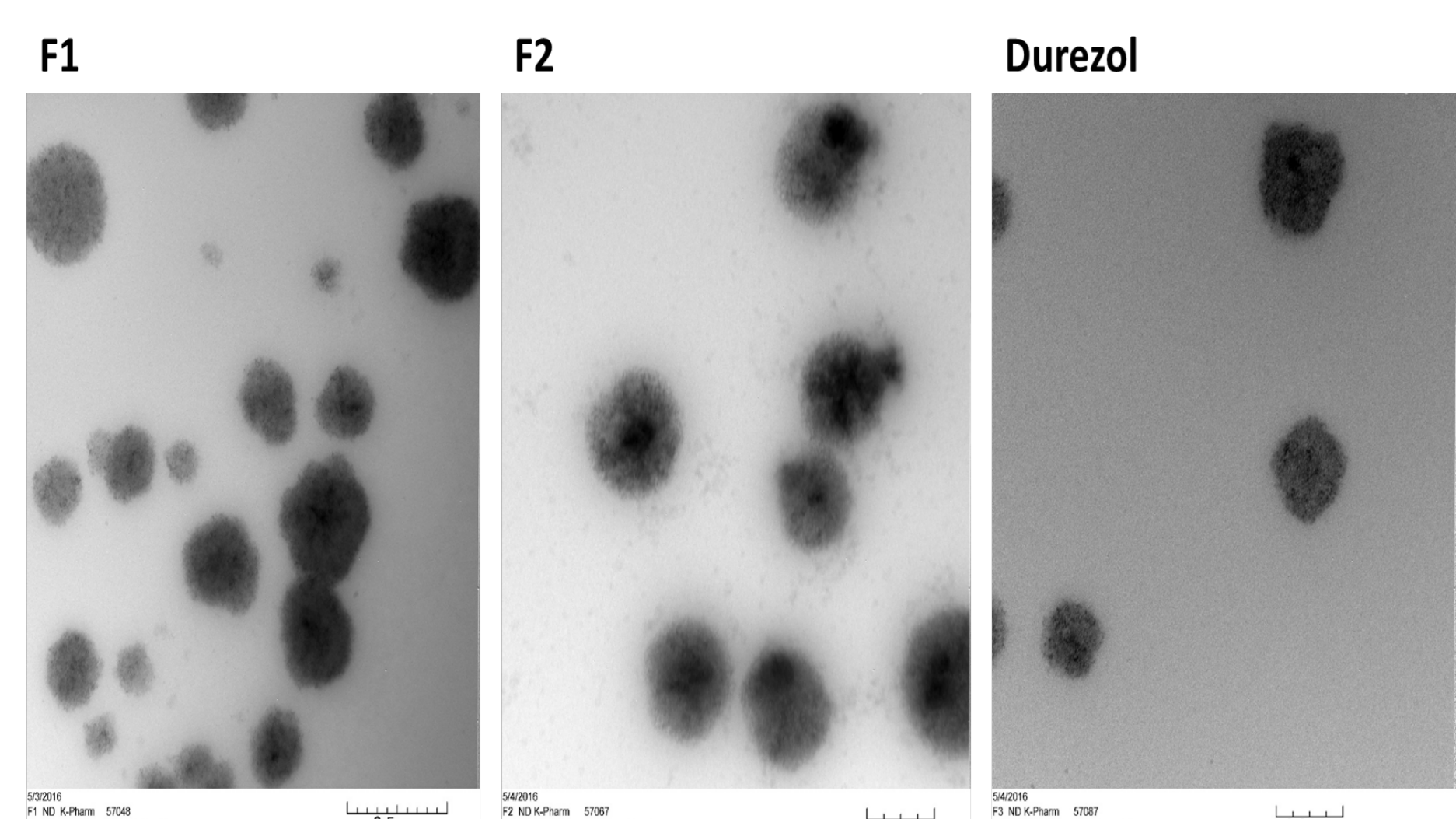


Fig. 3. TEM images of DFBA Emulsions (F1 & F2) and Durezol (by JEM-1400Plus Transmission Electron Microscope).

METHOD

A two step method was used. As the first step, the difluprednate coarse-emulsion containing 0.05% difluprednate, castor oil as an oil phase and polysorbate 80 as an emulsifying agent was produced with mixture system at 70 °C and 12000 rpm for 1 h. Then the coarse-emulsion was subjected to high-pressure emulsification at 10,000 and 30,000 psi pressure for 10 volume cycles. Two emulsion formulations F1 and F2 were prepared. The particle size and zeta-potential of the emulsions were characterized. *In vitro* release behaviors of different emulsions were investigated by following methods:

Dialysis method: Dialysis membranes of different nature (Cellulose Ester and Regenerated Cellulose) and different molecular weight cut off was investigated with 0.05% sodium lauryl sulphate (SLS) in phosphate buffered saline (PBS) as the dissolution medium. One mL of emulsion diluted with simulated tear fluid (STF) at a 1:4 ratio and was accurately placed into the dialysis bag and the bag was suspended in 75 mL of the dissolution medium.

Gel Chromatography: After equilibrating the column with 50 ml of running PBS 0.1 ml DFBA emulsions was loaded onto the PD-10 column separately, and flushed with PBS to separate the free drug from the emulsion. The eluent was collected into test tubes that each contained 0.5 ml eluent. Then the emulsion eluent fractions were combined into a 10 ml volumetric flask and made up to mark with acetonitrile.

Microdialysis and retro-microdialysis: This study was performed with a CMA High Cut-Off 4mm probe and molecular weight cut off (100 kD) with 0.15% and 0.5% SLS in PBS as the dissolution medium. One mL of emulsion was diluted with 0.5% SLS in PBS at 1:4 ratio and placed into the syringe. The probe was dipped in 10 mL of the dissolution medium, flow rate 5.5 µL/ml, stirring rate 150 rpm and temperature 37± 0.5 °C. A 50 µL sample of dissolution medium was withdrawn at different time intervals up to 2 h and replaced with the same volume of release medium. In retro-microdialysis all the conditions were the same except dissolution medium placed into the syringe and probe was dipped in 1 mL of emulsion.

The concentrations of difluprednate in the samples were determined by a HPLC method.

RESULTS

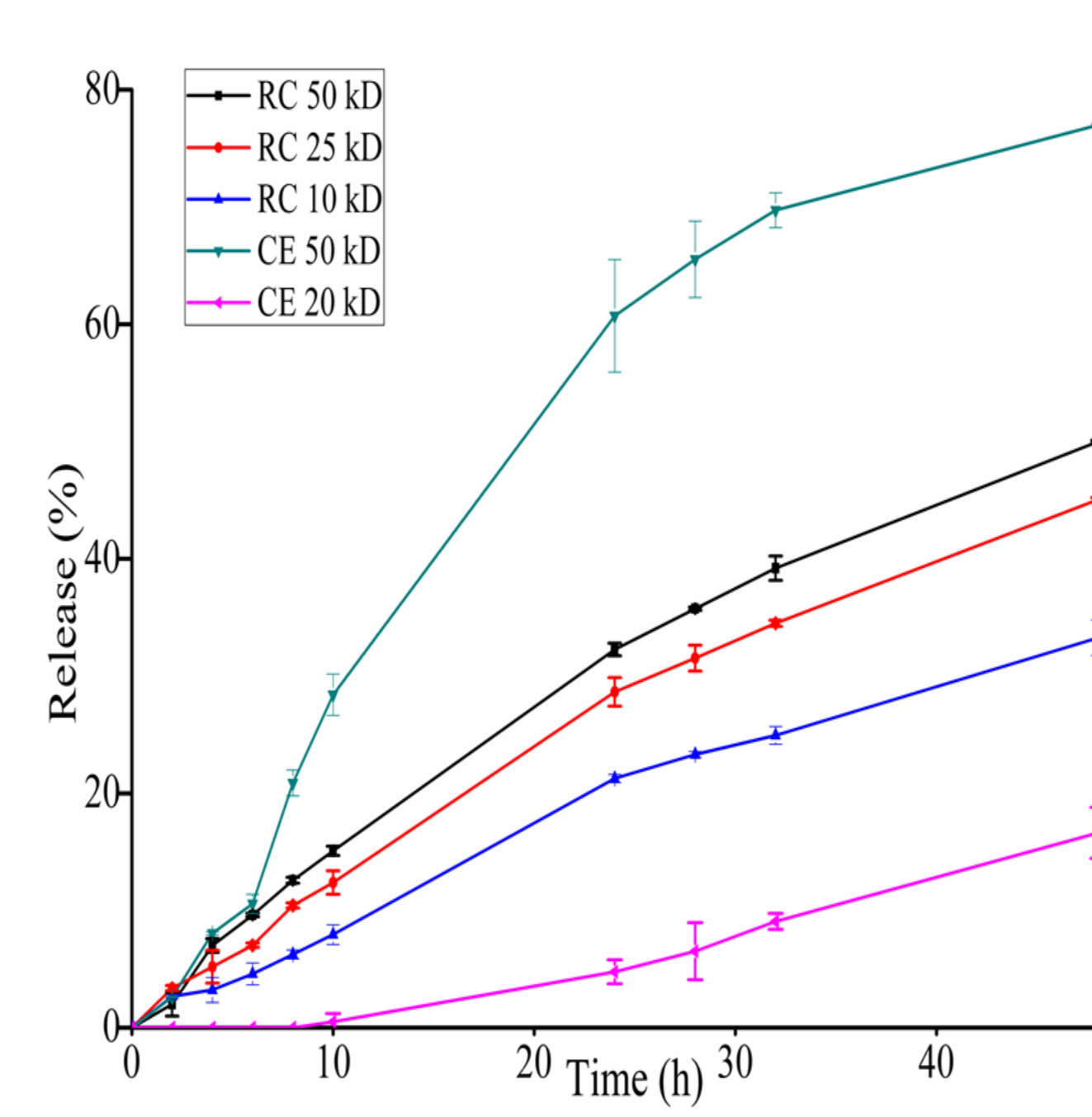


Fig. 4. Effect of different nature (CE and RC) and molecular weight cut off (10, 25 and 50 kD) of dialysis membranes on the release profiles of DFBA F1.

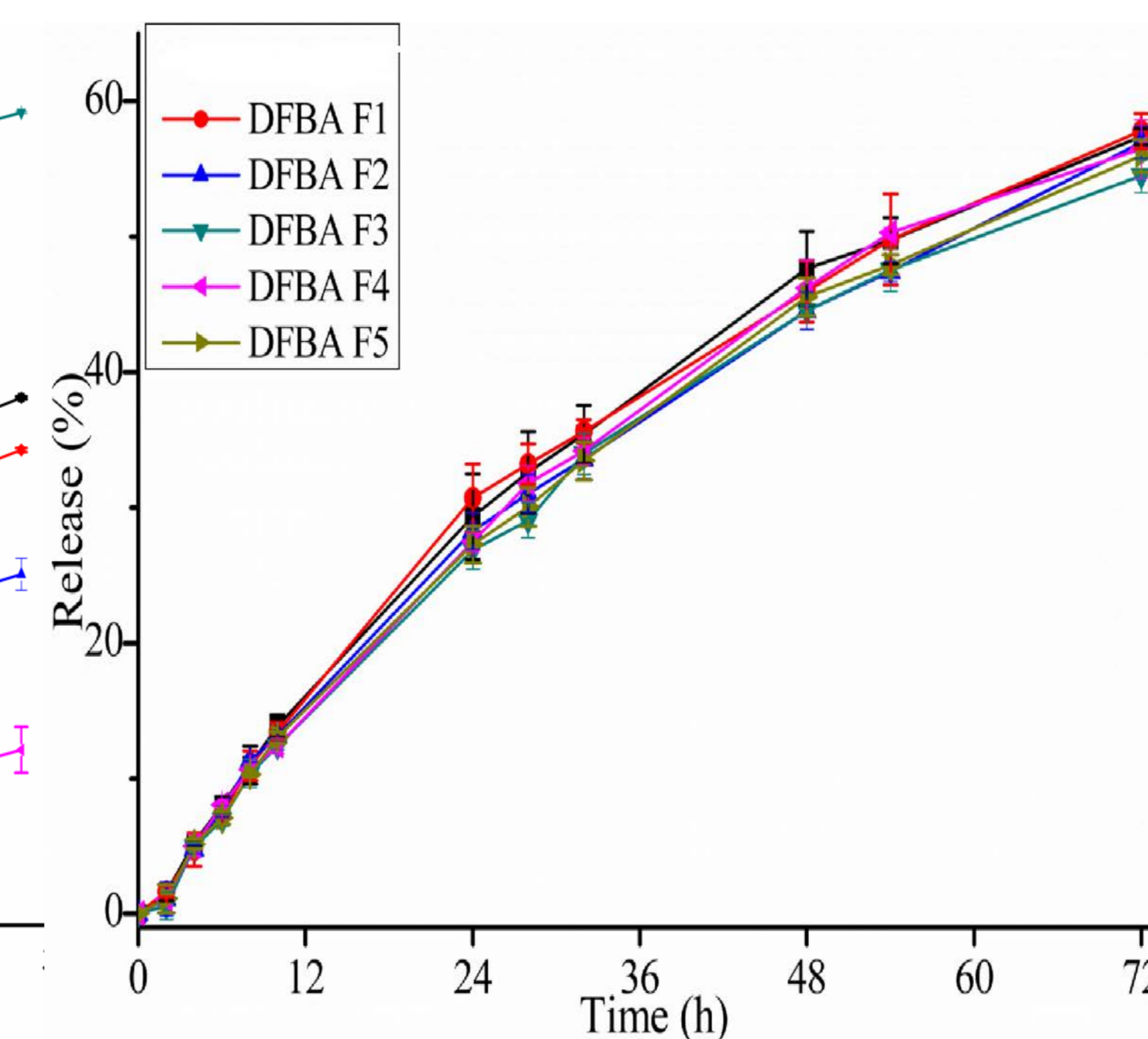


Fig. 5. *In vitro* release profiles of different DFBA emulsion using 25 kD RC dialysis membrane

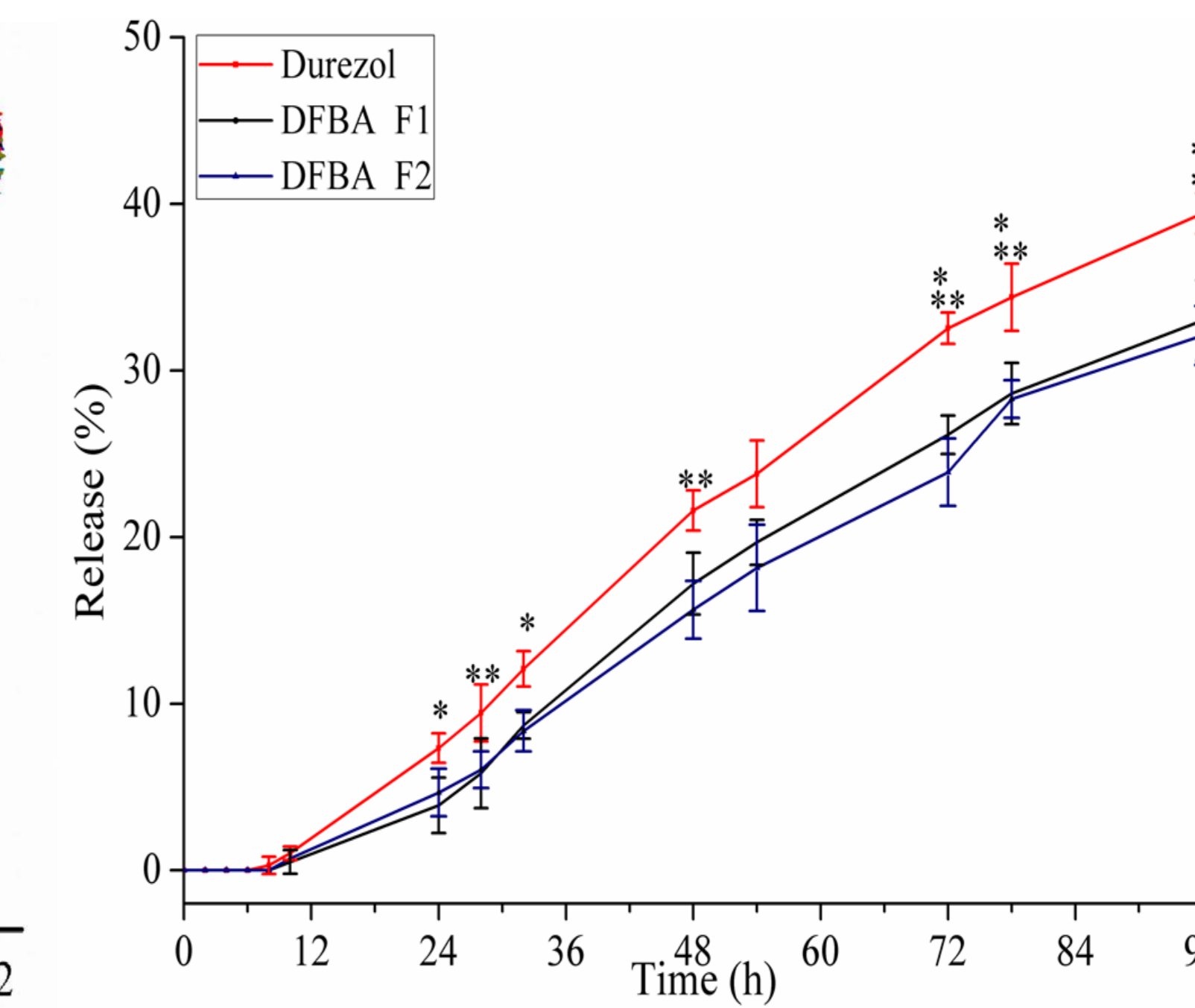


Fig. 6. The comparison of different difluprednate emulsions release profiles using 20 kD CE dialysis membrane. Statistical analysis was performed with Student's t test. *, ** and *** indicate p < 0.05 for Durezol versus F1, Durezol versus F2 and F1 versus F2.

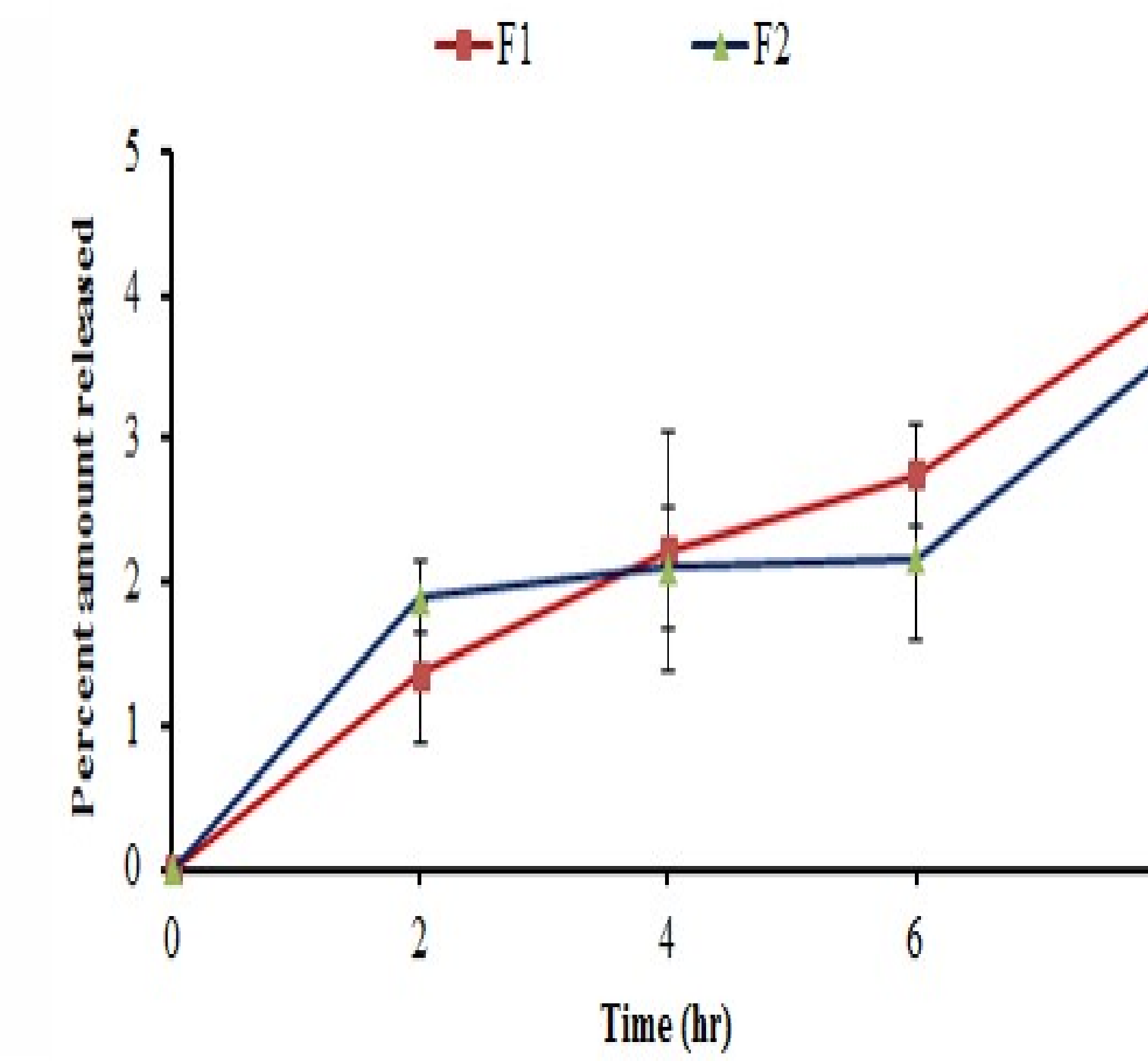


Fig. 7. Release Profiles for DFBA Emulsions (F1 & F2) in using 0.5% SLS as dissolution medium PD-10 Sephadex gel G25 column.

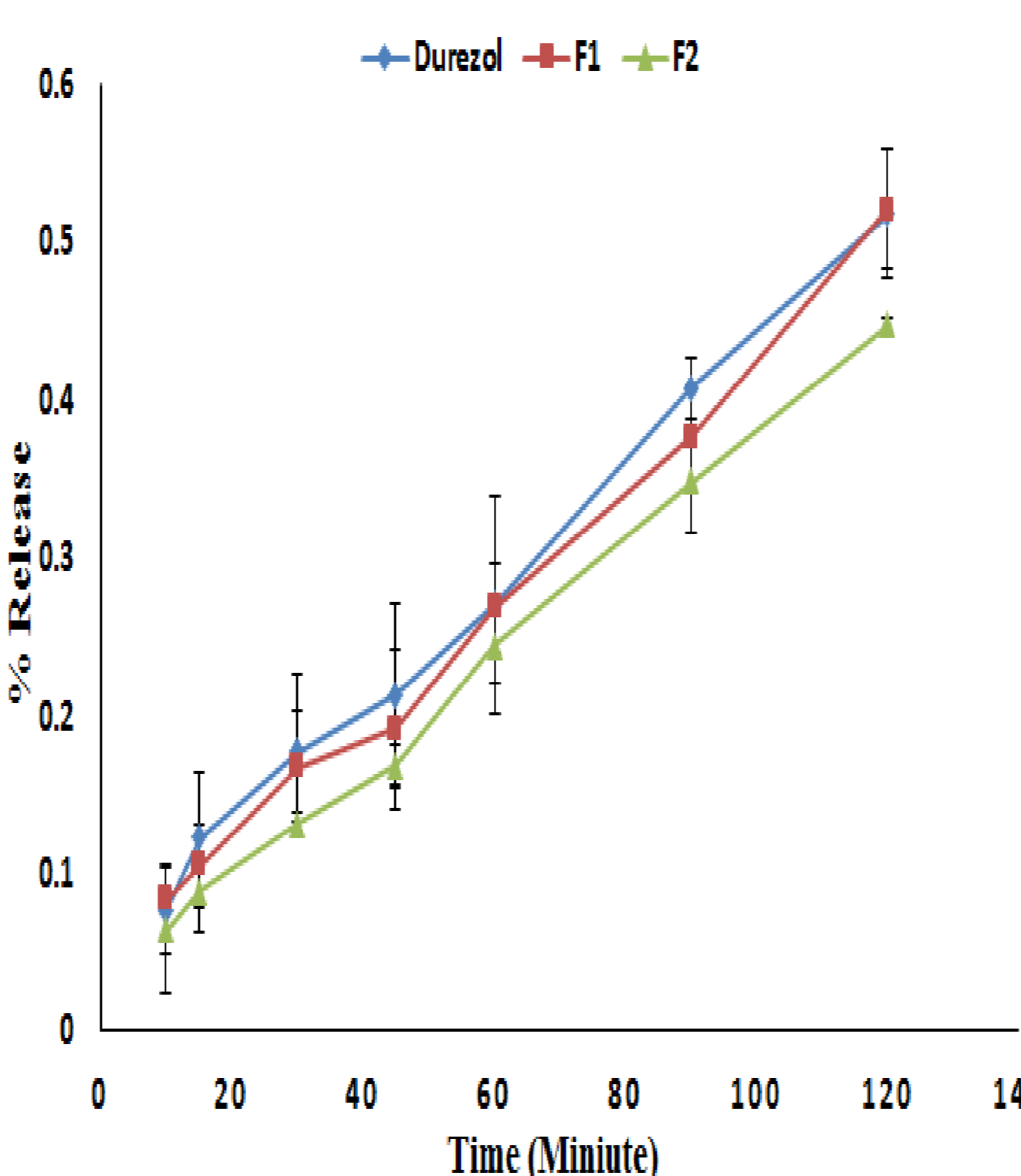


Fig. 8. Release Profiles for Durezol and DFBA Emulsions (F1 & F2) in using 0.5% SLS as dissolution medium using microdialysis method.

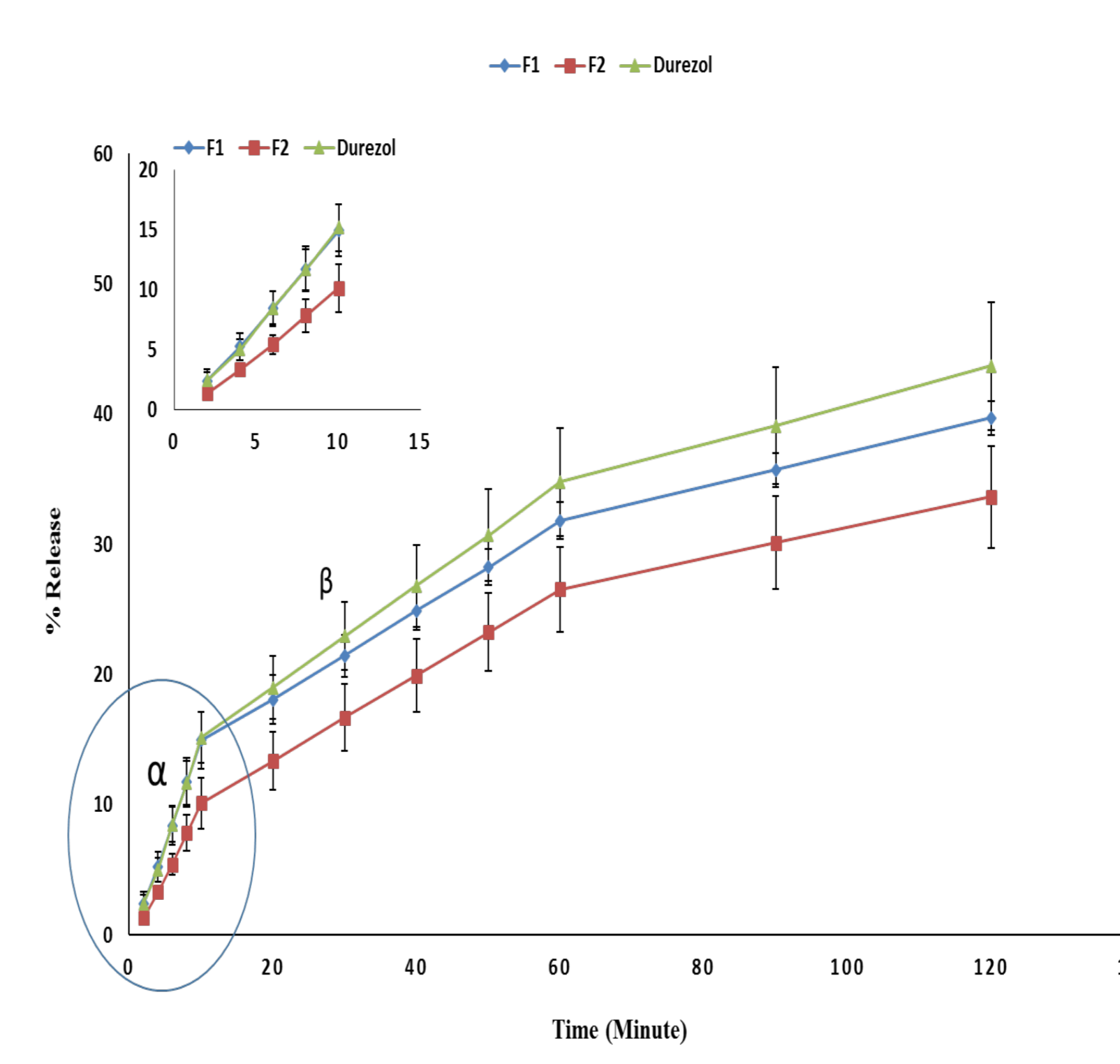


Fig. 9. Release Profiles for Durezol and DFBA Emulsions (F1 & F2) in using 0.5% SLS as dissolution medium using retro-microdialysis method.

Formulation	α	β
Durezol	1.66 ± 0.23	0.4 ± 0.05
F1	1.49 ± 0.14	0.35 ± 0.005
F2	1.18 ± 0.32	0.33 ± 0.03

Table 2. Different α and β values for DFBA Emulsions (F1 & F2) and Durezol

Formulation	Zero Order (R ²)	First Order (R ²)	Higuchi Model (R ²)	Korsmeyer-Peppas Model (R ²)
F1	0.8791	0.9184	0.9769	0.9755
F2	0.9034	0.9315	0.9878	0.9615
Durezol	0.8892	0.9309	0.9820	0.9707

Table 3. Different rate kinetic for DFBA Emulsions (F1 & F2) and Durezol

CONCLUSION

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 of the membrane from 25 kD to 50 kD. A statistically significant difference in release profile was seen between F1 and F2 formulations when CE 20 kD membrane was used, however, the difference was marginal. No significant difference was observed in the *in-vitro* drug release profile from DFBA, F1 and F2 formulations in 0.5% SLS in PBS using PD 10 Sephadex Gel column. Microdialysis results have shown differences between the release profiles of F1, F2 and marketed formulations in 0.5 % SLS in PBS. A significant difference in % drug release was seen between F1 & Durezol vs F2 using ANOVA at p<0.01, but not at P<0.001 in retro-microdialysis using 0.5 % SLS in PBS. Studies are in progress to further differentiate the release profiles from F1 and F2 formulations using a variety of other methods such as permeation across fresh cornea by Ussing diffusion chamber and permeation across polarized rabbit corneal epithelial (RCE) monolayer

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