Physicochemical Characterization of Tobradex and Tobradex ST Under Physiological Conditions

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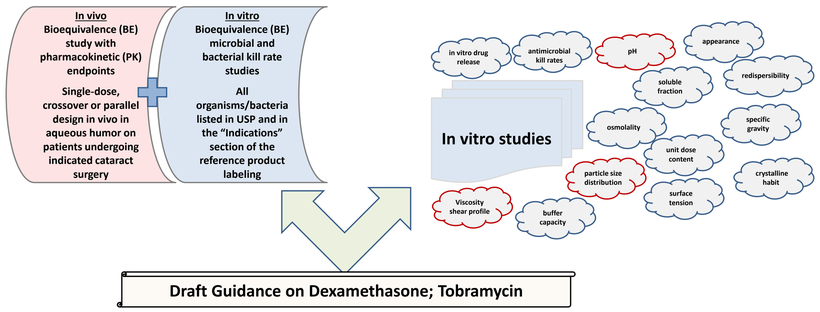
BACKGROUND

- FDA currently recommends an in vitro only option to compare generics to Tobradex and Tobradex ST (tobramycin/dexamethasone ophthalmic suspension) when all of the following criteria are met*:
  - The test and reference listed drug (RLD) formulations are qualitatively and quantitatively the same (Q1/Q2).
  - Acceptable comparative physicochemical characterizations of the Test and RLD formulations on at least three exhibit batches for each.
  - Acceptable comparative in vitro drug release of dexamethasone
  - Acceptable comparative in vitro antimicrobial kill rates



- Tobradex and Tobradex ST contain different viscosity modifiers. Tobradex uses hydroxyethyl cellulose and Tobradex ST uses xanthan gum.
- Differences in physicochemical properties of ophthalmic suspensions may affect ocular bioavailability.
- Viscosity profiles and particle size distributions of both products were measured to investigate differences in physicochemical properties.

*Draft guidance on Dexamethasone; Tobramycin ophthalmic suspension (https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM199633.pdf)

OBJECTIVES

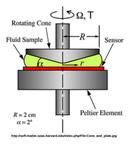
1. Investigate key physicochemical properties that may affect ocular bioavailability.
2. Assess suitability of particle size techniques for measuring the size of tobramycin/dexamethasone suspensions.
3. Evaluate the effect of pH, dilution, and temperature on viscosity measurements.

MATERIAL AND METHODS

- Simulated tear fluid and phosphate buffered saline were prepared and used as dispersants for viscosity and size measurements. Dispersants were buffered to a pH of 7.4 to simulate the pH of the eye.

Simulated Tear Fluid	
Component	**Concentration**
Sodium Bicarbonate, $NaHCO_3$	1.924 mg/mL
Potassium Chloride, KCl	1.11 mg/mL
Calcium Chloride, $CaCl_2$	0.0229 mg/mL
Sodium Chloride, NaCl	6.728 mg/mL
Glucose	0.025 mg/mL
Tris/HCl/NaOH	to pH to 7.4
Albumin (omitted)	6.69 mg/mL

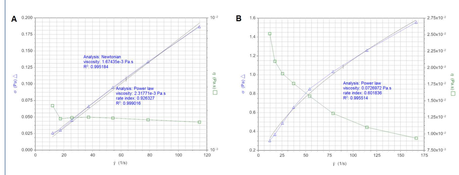
- A 1° cone-and-plate geometry was used to obtain full rheological profiles of the drug formulations at 37 °C and different dilutions, pH values, and shear rates.



- Particle size measurement was performed using light scattering and diffraction instruments to evaluate changes in particle size upon dilution.
- Optical and scanning electron microscopy was performed to confirm results and evaluate particle morphology.

RESULTS

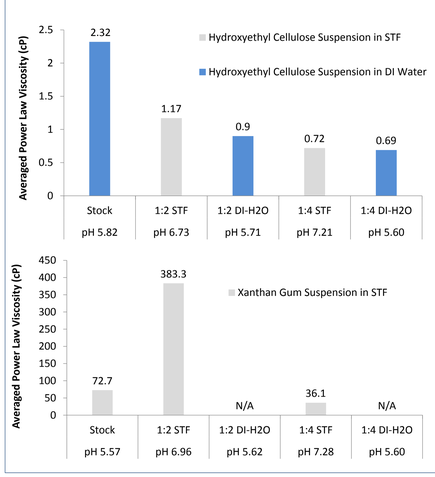
Viscosity with Applied Shear



A. Tobradex is opaque and has a Newtonian viscosity of 1.67 cP. At a stock pH of 5.82 and at 37°C it is a slightly shear thinning fluid, but is approximated as Newtonian with a power law rate index of (0.92).

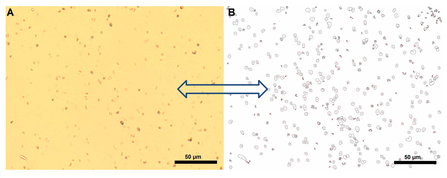
B. Tobradex ST is much more viscous with an approximated viscosity of 72.7 cP for shear rates 10-200 s-1. The rate index is 0.6, indicating a shear thinning fluid. The stock had a pH of 5.57 at 37°C.

Viscosity with Dilution

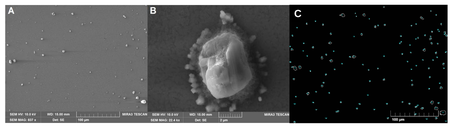


- Tobradex diluted with both DI water and STF produces a viscosity decrease upon dilution (approaching that of water, 0.69 cP at 37°C) as seen in the figure on the left.
- STF is buffered to have a physiologically relevant pH of 7.4; upon dilution with STF pH increases towards 7.4, while dilution in distilled water decreases pH.
- Diluting Tobradex ST in DI water formed clumps during measurements causing errors.
- Diluting Tobradex ST with pH 7.4 STF increased average viscosity at 1:2 (stock:STF), indicating a response to change in pH from 5.57 of the stock to pH 6.96 of the STF dilution. The viscosity of the stock solution was 72.7 cP and increased over 5 times to 383 cP, at 1:2 parts STF. Further dilution with 1:4 parts STF caused the pH to increase to 7.28, but decreased the measured apparent viscosity to 36.1 cP, roughly to half of the stock viscosity (72.7 cP).

Size and Morphology with Microscopy

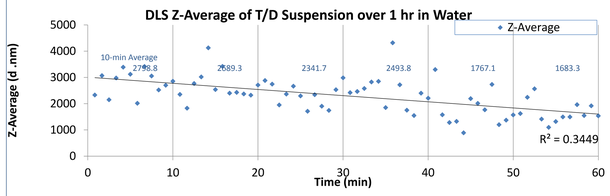


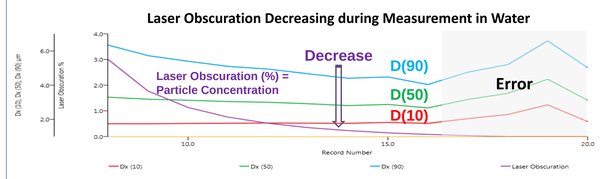
- (A) Polarized image of Tobradex generated with BX51P optical microscope shows dexamethasone particles in solution.
- (B) The same image after threshold adjustment during ImageJ analysis. Mean Feret diameter was found to be 3.45 ± 1.15 µm.



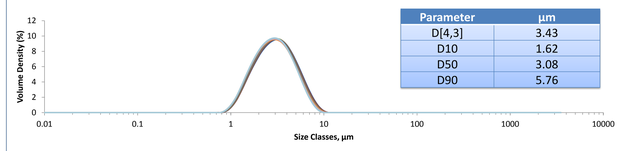
- Scanning electron micrographs of Tobradex at (A) 837X, (B) 22,400X magnifications, and (C) outlines of particles using a threshold function in ImageJ revealing a mean Feret diameter of 2.9 ± 1.7 µm.

Particle Size in Suspension



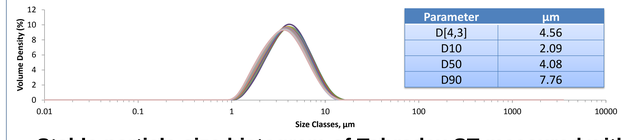


- Light Scattering Methods such as DLS that require sample dilution for unrestricted Brownian motion may not be suitable due to particle size instability.
- Average size of dexamethasone particles in suspensions decreases when using either DLS or Laser Diffraction, due to particles dissolving and/or settling out.
- To limit dissolution, 1X PBS saturated with dexamethasone was used as a dispersant after 100 nm filtration.
- A method that does not require dilution is ideal



Parameter	µm
D[4,3]	3.43
D10	1.62
D50	3.08
D90	5.76

- Stable particle size histogram of Tobradex measured with laser diffraction in dexamethasone saturated 1X DPBS dispersant.



Parameter	µm
D[4,3]	4.56
D10	2.09
D50	4.08
D90	7.76

- Stable particle size histogram of Tobradex ST measured with laser diffraction in dexamethasone saturated 1X DPBS dispersant.

DISCUSSION

- A full viscosity profile in the presence of shear is necessary to demonstrate any differences in the viscosity modifying excipient.
- When the drug product (pH 5.6) comes in contact with tears (pH 6.5 – 7.6), the xanthan gum-tobramycin ionic bond is interrupted, increasing viscosity to that of an analogous solution of xanthan gum.
- Dexamethasone particles are unstable when diluted for measurements; aqueous solubility of 0.08-0.1 mg/mL vs. tobramycin 1000 mg/ml.

CONCLUSIONS

- Xanthan gum in Tobradex ST creates a very different shear thinning and more viscous profile than Tobradex which uses hydroxyethyl cellulose (72.7 cP vs. 1.67 cP, respectively). When the formulations are diluted in half with tear fluid, the viscosity of Tobradex ST increases over 5-fold, while the viscosity of Tobradex decreases by half.
- Laser diffraction is more suitable and reproducible than DLS, but steps to prevent particle dissolution during measurement must be taken.
- Optical imaging of solutions was more suited for size measurement than SEM because of drying artifacts, but high resolution SEM was more suitable for morphology examination.

DISCLAIMER

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