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Pitfalls and Challenges of Analyzing Particle Size Distribution in Ophthalmic Suspensions Using Laser Diffraction

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PURPOSE

Particle size distribution (PSD) is a critical quality attribute of ophthalmic suspensions as it can affect not only the dose uniformity, physical stability, and dissolution properties of the product, but may also affect bioavailability and impact the determination of bioequivalence. To measure the PSD in ophthalmic suspensions, laser diffraction (LD) is the most commonly used particle sizing technique, as most of the commercial products exhibit size in the range of few hundred nanometers to a few microns. However, presence of heterogeneous polymeric excipients can interfere with the PSD analysis and lead to potentially erroneous interpretation of the results. Using two case studies (loteprednol and brinzolamide ophthalmic suspensions), we intend to demonstrate the challenges and nontypical solutions to eliminate the material influences to allow accurate and precise measurement of the PSD in ophthalmic suspensions.

METHODS

Commercially available loteprednol and brinzolamide ophthalmic suspensions were used as model systems, as both products contain similar polyacrylic acid polymers (e.g., carbomer or polycarbophil). The impact of polymer on the LD measurement results was determined using one factor at a time experimental design approach, e.g., Table 1, whereas NIST (National Institute of Standards and Technology) traceable size standards were used as a reference.

A Malvern Mastersizer 3000 equipped with a Hydro-MV dispersion unit was used to measure sample PSD based on diffraction of both red and blue laser lights. Particle free water or a saturated solution of loteprednol or brinzolamide was used as a dispersant.

Samples were dispersed using the built-in stirrer along with an external bath sonicator or the in situ sonicator. Image-based particle size analysis using polarized light microscopy (Olympus BX51) coupled with ImageJ software was used as an orthogonal technique to check the suitability of the developed method.

Table 1: Example study design (leave-one-out) using the placebo formulations

No.	Ingredient	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Polycarbophil	+	+	+	+	+	+	+	-	+
2	Boric acid	-	+	+	+	+	+	+	+	+
3	Glycerin	+	-	+	+	+	+	+	+	+
4	Propylene glycol	+	+	-	+	+	+	+	+	+
5	Tyloxapol	+	+	+	-	+	+	+	+	+
6	Benz. Cl	+	+	+	+	-	+	+	+	+
7	NaCl	+	+	+	+	+	-	+	+	+
8	EDTA	+	+	+	+	+	+	-	+	+
9	0.9 µm STD	+	+	+	+	+	+	+	+	+
10	NaOH	q.s.								
11	HCl	q.s.								
12	Water	q.s.								

RESULTS

Identification of the Excipients Interference on PSD Analysis Using Size Standards

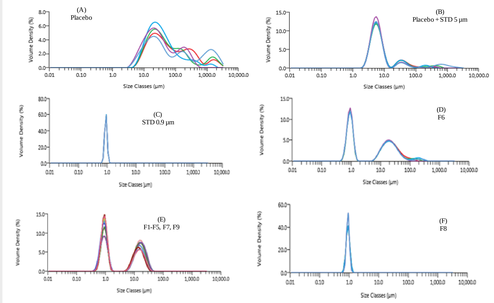

Figure 1. PSD histograms of (A) Placebo (B) Placebo spiked with 5 µm size standard, (C) 0.9 µm size standard, (D) F6 (without NaCl) (E) F1-F5, F7, F9, (F) F8 (without polycarbophil).

- *Interference by the excipients was in micrometer range (Figure 1E).*
- *Polycarbophil generated laser diffraction signals, and could interfere with the PSD measurement of the suspensions (Figure 1B vs 1F).*
- *Presence of sodium chloride reduced the interferences (Figure 1D vs. 1E).*

Table 2: Particle size analysis results of formulation (mean±sd, n=5)

Formulation		D_{10}, µm	D_{50}, µm	D_{90}, µm	SPAN	D[4,3], µm
Population 1 (Size standard)	F1	0.66 ± 0.00	0.90 ± 0.00	1.20 ± 0.00	0.606 ± 0.000	0.91 ± 0.00
	F2	0.60 ± 0.00	0.87 ± 0.00	1.24 ± 0.00	0.742 ± 0.000	0.90 ± 0.00
	F3	0.65 ± 0.02	0.90± 0.00	1.20 ± 0.00	0.611 ± 0.006	0.91 ± 0.00
	F4	0.60 ± 0.00	0.87 ± 0.00	1.24 ± 0.00	0.743 ± 0.000	0.90 ± 0.00
	F5	0.67 ± 0.00	0.89 ± 0.00	1.18 ± 0.00	0.577 ± 0.000	0.91 ± 0.00
	F6	0.66 ± 0.01	0.89 ± 0.00	1.19 ± 0.01	0.601 ± 0.024	0.91 ± 0.00
	F7	0.64 ± 0.02	0.89 ± 0.01	1.21 ± 0.01	0.646 ± 0.034	0.91 ± 0.01
	F8	0.77 ± 0.02	0.90 ± 0.00	1.06 ± 0.02	0.330 ± 0.045	0.91 ± 0.00
	F9	0.63 ± 0.02	0.88 ± 0.01	1.21 ± 0.01	0.652 ± 0.029	0.90 ± 0.00
Population 2 (Excipients)	F1	8.69 ± 0.01	14.70 ± 0.00	24.20 ± 0.10	1.057 ± 0.003	15.72 ± 0.05
	F2	9.22 ± 0.02	15.44 ± 0.06	24.88 ± 0.13	1.014 ± 0.004	16.34 ± 0.05
	F3	8.89 ± 0.00	15.34 ± 0.06	25.68 ± 0.11	1.095 ± 0.005	16.44 ± 0.06
	F4	9.52 ± 0.02	16.60 ± 0.16	27.48 ± 0.37	1.082 ± 0.013	17.70 ± 0.16
	F5	8.04 ± 0.01	13.54 ± 0.06	22.50 ± 0.14	1.066 ± 0.006	14.52 ± 0.05
	F6	10.42 ± 0.04	22.86 ± 1.58	77.20 ± 34.14	2.864 ± 1.230	35.46 ± 7.74
	F7	8.65 ± 0.05	14.80 ± 0.07	24.72 ± 0.08	1.085 ± 0.011	15.86 ± 0.06
	F8	-	-	-	-	-
	F9	9.99 ± 0.02	19.52 ± 0.18	38.98 ± 0.86	1.484 ± 0.034	22.42 ± 0.34

Impact of Sodium Chloride and Sample Processing on PSD Analysis of Placebos

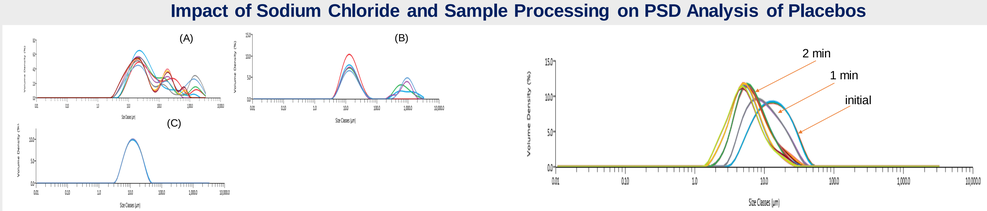

Figure 2. PSD histogram of placebo treated with various concentrations of NaCl solution (A) 0%, (B) 1%, (C) 2%,

Figure 3. Overlay PSD histogram of placebo treated NaCl and sonicated for 0, 1, 2 min

- *At below 2% (w/w), sodium chloride exhibited concentration dependent reduction on the polycarbophil interference.*
- *Sonication in the presence of sodium chloride could further narrow PSD of polycarbophil, and its effect was time dependent.*
- *However, sodium chloride alone could not eliminate the interference of polycarbophil.*

Approaches to Eliminate the Excipients Interference on PSD Using Size Standards

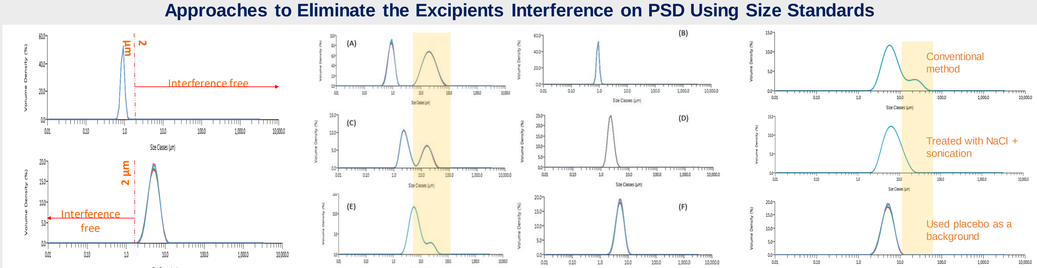

Figure 4. PSD histogram of placebo spiked with (A) 900 nm and (B) 5µm STD using placebo dispersion background.

Figure 5. Comparison of PSD histogram of placebo + 0.9 µm, 2.0 µm, or 5.0 µm STD without (A, C, E) and with (B, D, F) background subtraction, respectively.

Figure 6. PSD histogram of placebo spiked with 5 µm STD measured using various approaches

- *False interpretation of the PSD could arise, if the LD peak generated by the excipients overlapped with the peak from the particles of interest.*
- *Using placebo dispersion as a background could eliminate the interference of excipients.*
- *Pretreating samples using sodium chloride could reduce (disguise) the interference, rather than eliminating it.*

Measurement PSD of Commercial Loteprednol Etabonate Gel 0.5%

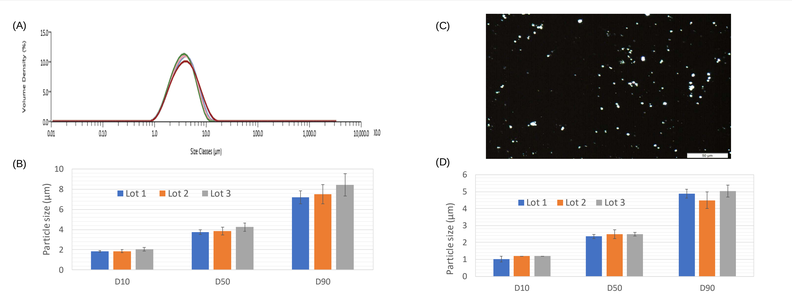

Figure 7. PSD measurement results of Lotemax® using LD technique with total background subtraction (A, B) (volume based) and polarized microscope (C, D)(number based)

- *The excipient interference was eliminated, results were consistent in three commercial lots.*
- *The PSD measurement results were comparable between two techniques confirming the suitability of the newly developed method.*

Measurement PSD of Commercial Brinzolamide (BRZ) Suspension 1%

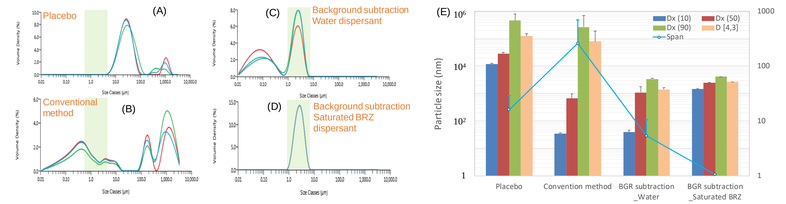

Figure 8. Representative PSD histogram of Azopt® (results from one lot showing) and its placebo, measured using different approaches (A, B, C, D) and the corresponding PSD measurement results (E).

- *Using placebo background subtraction could eliminate the interference of carbomer.*
- *Dissolution of brinzolamide during measurement led to underestimation of PSD as well as appearance of a bimodal distribution.*
- *Combination of placebo background subtraction and saturated BRZ dispersant allowed to measure PSD of BRZ in the product reliably.*

CONCLUSIONS

The newly developed LD method successfully eliminated the interference of excipients, and thus allowed more reliable measurement of the PSD in ophthalmic suspensions. A similar strategy can also be applied to other heterogeneous dispersed systems where the excipients interferences could be of concern.

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