One Size Does Not Fit All:

Challenges of Particle Size Measurement in Pharmaceutical Applications

Xiaoming Xu, Ph.D.
Director, Division of Product Quality Research
Office of Testing and Research, Office of Pharmaceutical Quality
CDER | U.S. FDA

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This presentation reflects the views of the presenter and should not be construed to represent FDA's views or policies.

Examples of Particles in Pharmaceutical Applications

- Drug substance powder (micron-, nano-)
- Excipient powder
- Granules (wet- or dry- process)
- Suspension, for i.v. injection (really small), or more commonly ophthalmic and oral routes
- Emulsions (oil globules) for i.v., ophthalmic, oral
- Aerosols, nasal spray, metered dose inhalers (MDI), dry powder inhalers (DPI)
- Liposomes, micelles, protein-drug complex, lipid nanoparticles (covid-vaccine)
- Iron colloids
- Particulates (both visible and sub-visible)
- Glass fragments (a unique undesired particulate)
- Protein or peptide aggregates (undesired)

Particle sizing is a poorly posed problem.

The apparent simplicity of particle size analysis is deceptive.

Burgess, D.J., Duffy, E., Etzler, F. et al. AAPS J (2004) 6: 23. https://doi.org/10.1208/aapsj060320

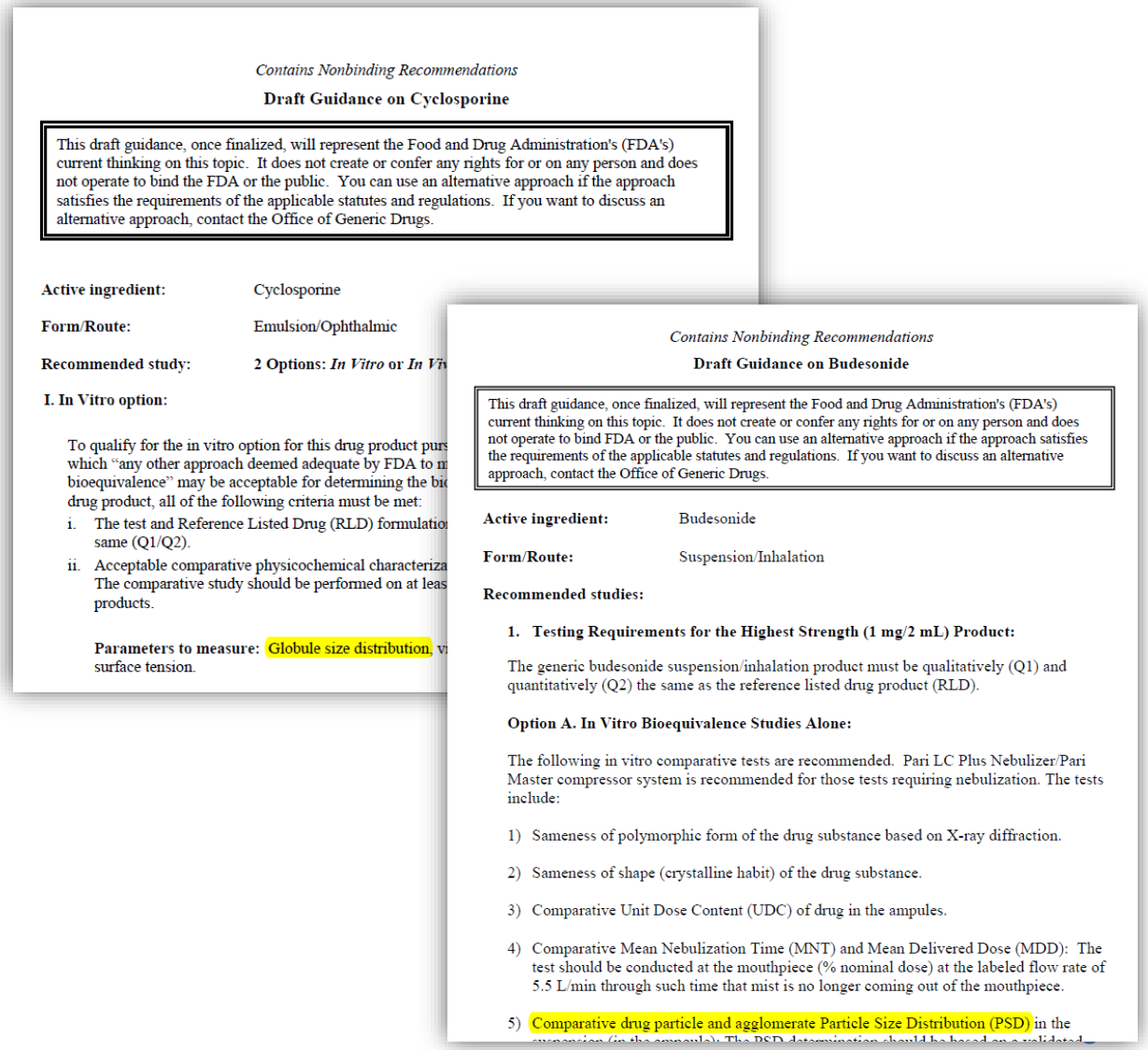
- What we are truly interested is not the “size”
- What we are actually measuring may not be the “size”

Particle Sizing: the Why

- Particle size is an important product quality attribute for pharmaceutical formulations in a dispersed state, e.g., emulsions, suspensions, liposomes, aerosols, colloidal irons

- Also, a critical physicochemical property in supporting the bioequivalence (BE) determination (in vitro option), e.g., budesonide suspension, cyclosporine emulsion

- Concerns with products of a wide range of sizes (e.g., 10 nm to 100 µm) and with different distributions (e.g., unimodal or multi-modal)



Contains Nonbinding Recommendations

Draft Guidance on Cyclosporine

This draft guidance, once finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind the FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the Office of Generic Drugs.

Active ingredient: Cyclosporine

Form/Route: Emulsion/Ophthalmic

Recommended study: **2 Options: *In Vitro* or *In Vi***

I. In Vitro option:

To qualify for the in vitro option for this drug product purs
which "any other approach deemed adequate by FDA to m
bioequivalence" may be acceptable for determining the bi
drug product, all of the following criteria must be met:

i. The test and Reference Listed Drug (RLD) formulatio
same (Q1/Q2).

ii. Acceptable comparative physicochemical characteriza
The comparative study should be performed on at leas
products.

Parameters to measure: Globule size distribution, v
surface tension.

Contains Nonbinding Recommendations

Draft Guidance on Budesonide

This draft guidance, once finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the Office of Generic Drugs.

Active ingredient: Budesonide

Form/Route: Suspension/Inhalation

Recommended studies:

1. Testing Requirements for the Highest Strength (1 mg/2 mL) Product:

The generic budesonide suspension/inhalation product must be qualitatively (Q1) and quantitatively (Q2) the same as the reference listed drug product (RLD).

Option A. In Vitro Bioequivalence Studies Alone:

The following in vitro comparative tests are recommended. Pari LC Plus Nebulizer/Pari Master compressor system is recommended for those tests requiring nebulization. The tests include:

1) Sameness of polymorphic form of the drug substance based on X-ray diffraction.

2) Sameness of shape (crystalline habit) of the drug substance.

3) Comparative Unit Dose Content (UDC) of drug in the ampules.

4) Comparative Mean Nebulization Time (MNT) and Mean Delivered Dose (MDD): The test should be conducted at the mouthpiece (% nominal dose) at the labeled flow rate of 5.5 L/min through such time that mist is no longer coming out of the mouthpiece.

5) Comparative drug particle and agglomerate Particle Size Distribution (PSD) in the

But, what we are interested usually is not “size”

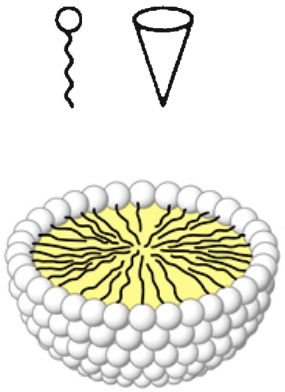
The analysis of the particle size is not an objective in itself but is a means to an end.

- Physical stability (e.g., dispersion sedimentation, agglomeration)
- Dissolution/drug release (e.g., due to surface area differences)
- Bioavailability
- Process capability (e.g., power flow, packing density)
- Bioequivalence (e.g., sameness or difference)
- Safety (e.g., particulate matter)

Depending on the purpose of the size measurement, the expectations on analysis outcome (i.e., numbers) may vary, e.g., Product A is 10 nm smaller than Product B ($p<0.05$). Are they “different”?

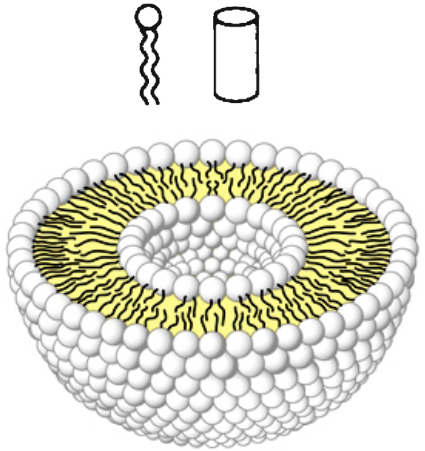
Particle Size Concepts: Dispersed Particles

Pharmaceutical dosage form examples:



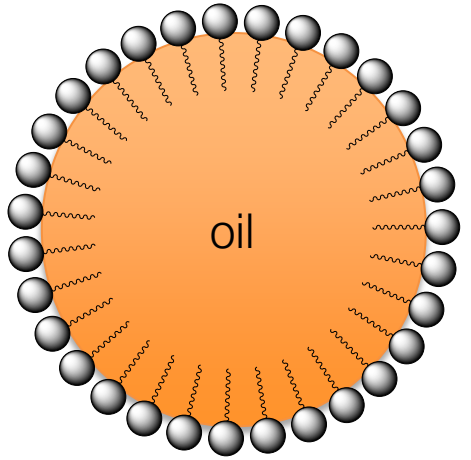
Micelles

- <30 nm
- Thermodynamically stable
- Change upon dilution (relative to critical micelle concentration, CMC)



Liposomes

- 70-300 nm
- Kinetically stabilized system
- Dilution generally has no impact on liposome integrity and size (individual lipid components are kinetically trapped)



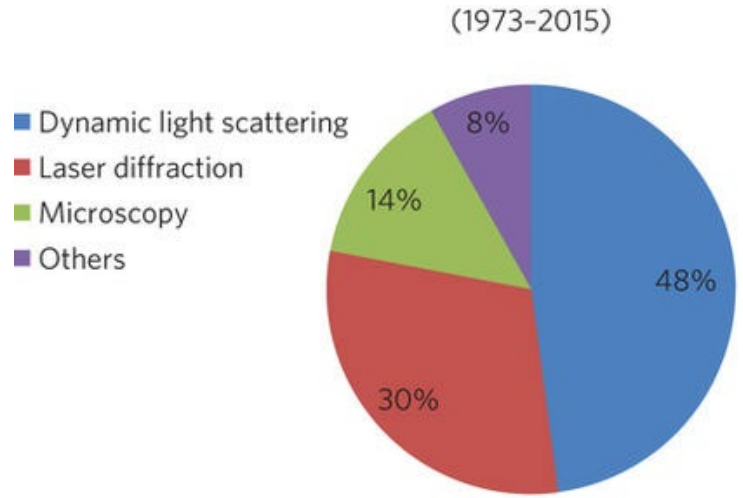
O/W emulsions

- 20 nm to few hundred nanometer (nanoemulsions), or larger than 1 micron (coarse emulsions)
- Thermodynamically unstable (agglomeration, coalescence, Ostwald-ripening, phase-separation, creaming, etc.)
- Dilution may impact globule size analysis (examples later)

Note: microemulsions (or swollen micelle) is a thermodynamically stabilized system, its size generally overlaps with the nanoemulsions (but the formulation composition and process conditions are completely different)

Techniques to Determine Particle Size Distribution

Technique	Size range	Principle
Dynamic light scattering (DLS)	1 nm to 1 µm	Brownian motion + light scattering
Laser diffraction (LD)	30 nm to 3000 µm	Static light scattering (Mie or Fraunhofer)
Electron microscopy (EM)	0.1 nm to a few micron	Electron density contrast
Image analysis	1 µm to a few hundred micron	Image analysis
Light obscuration	Subvisible particles (0.5 µm to 400 µm)	Single particle light blockage
Nanoparticle tracking analysis (NTA)	20 nm to 1 µm	Brownian motion + image analysis
Field flow fraction (FFF) + multi-angle light scattering (MALS) + DLS	1 nm to a few micron	Brownian motion + flow based separation + light scattering
Resonant mass measurement (RMM)	50 nm to 5 µm	Buoyant mass
Focused Beam Reflectance Measurement	1 µm to 1000 µm	Chord length

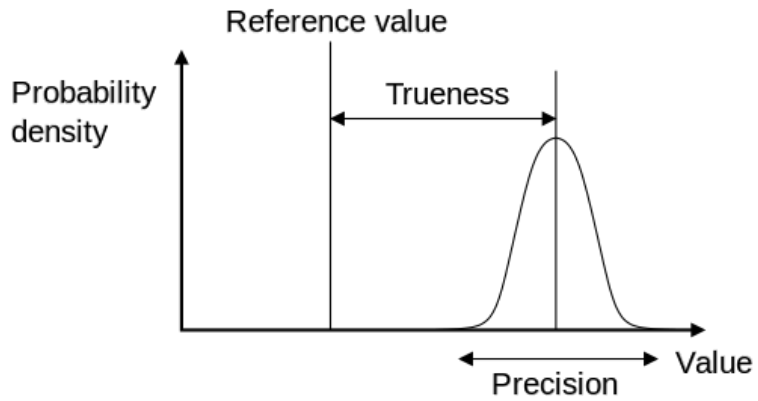


S. D'Mello, et al. *Nature Nanotechnology*, 2017, 12, p.523-529

PSD Method Validation: Which Ones Are Critical?

PSD method needs to be properly validated to demonstrate it is suitable for its intended purpose. However, validation of particle sizing methods is not the same as validation of other analytical methods described in ICH Q2 guideline.

Characteristics recommended in ICHQ2	Relevant for Size?	Comment
Specificity	No	Almost all PSD methods are non-specific to the particles being measured
Linearity	No	Most of the size measurement do not rely on calibration, therefore no need to establish linearity
Range	No	Range of the method is pre-defined by the choice of the technique, e.g., DLS or cryo-TEM, and not the method itself
Accuracy	Maybe	For system qualification, it is useful to use size standards to ensure the system operates correctly. But accuracy of the method cannot be determined using the size standards. It is more important to demonstrate that the method is reliable in measuring test and reference samples
Detection limit	No	Not relevant
Quantitation limit	No	Not relevant
Precision	Yes	Very important (3Rs, see next slide)
Robustness	Yes	Very important



Validation: Repeatability, Reproducibility, Robustness

Repeatability: closeness of agreement between multiple measurement results of a given property in the same dispersed sample aliquot, executed by the same operator in the same instrument under identical conditions within a short period of time (e.g., 6 measurements for the same sample).

-Machine, Testing method, Sample stability

Reproducibility: closeness of agreement between multiple measurement results of a given property in different aliquots of a sample, prepared and executed by same or different operators in similar instruments according to the same method (e.g., 6 samples prepared by the same operator).

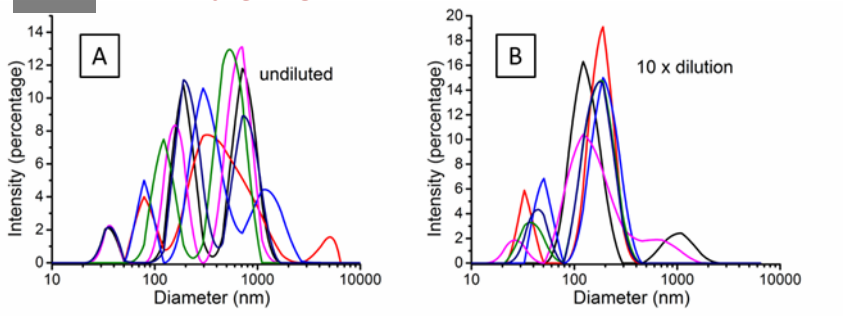
-Sampling procedure, dispersion, machine

Robustness: reliability of an analysis with respect to deliberate variations in method parameters, i.e., it should be both sensitive (able to detect significant changes in the underlying measured parameter) and precise (repeatable with a high signal to noise ratio). For example, change in sonication power, sonication duration, flow rate, particle concentration (i.e., obscuration%), temperature, analysis algorithm.

Example 1: Though All were in the Wrong, Each was Partly in the Right

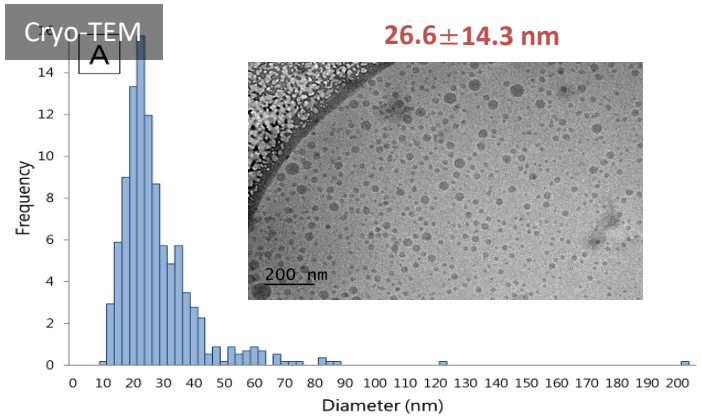
DLS

Zave=118 nm

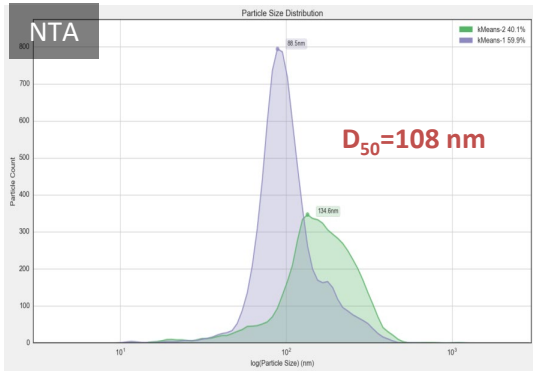


Cryo-TEM

26.6±14.3 nm

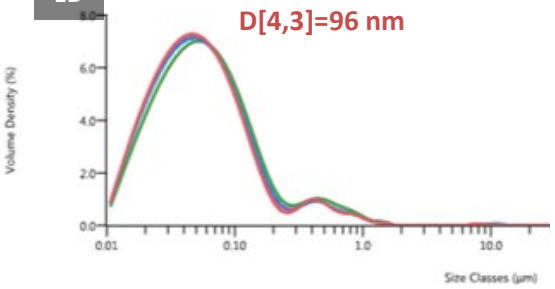


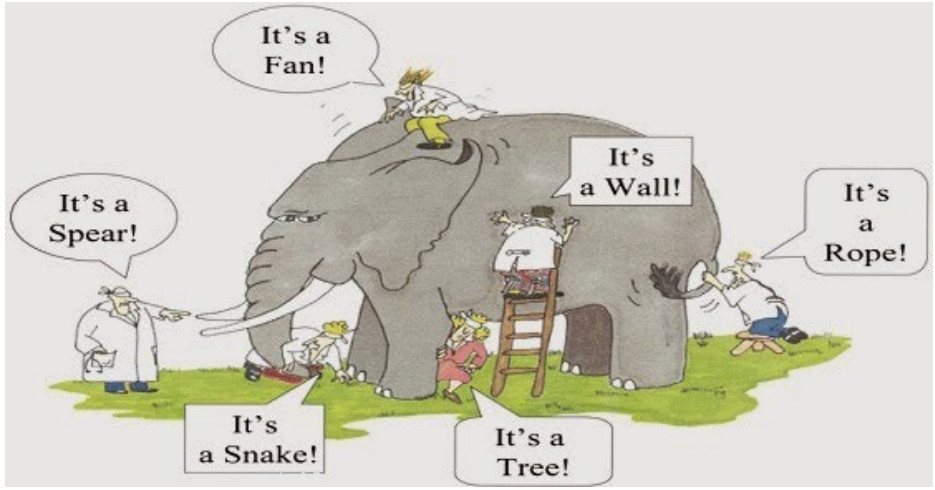
NTA



LD

D[4,3]=96 nm

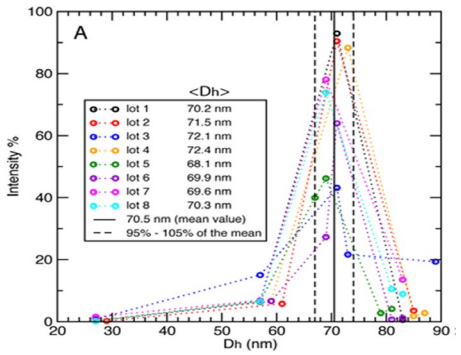




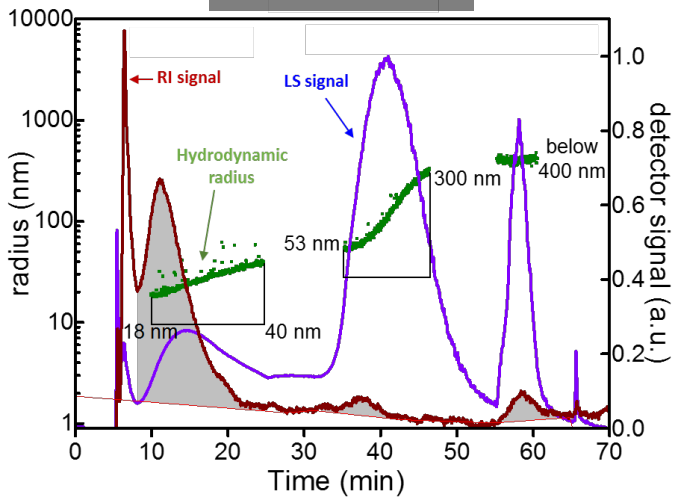
https://medium.com/betterism/the-blind-men-and-the-elephant-596ec8a72a7d

DOSY NMR

mean=70.5 nm



AF4+MALS+DLS+RI

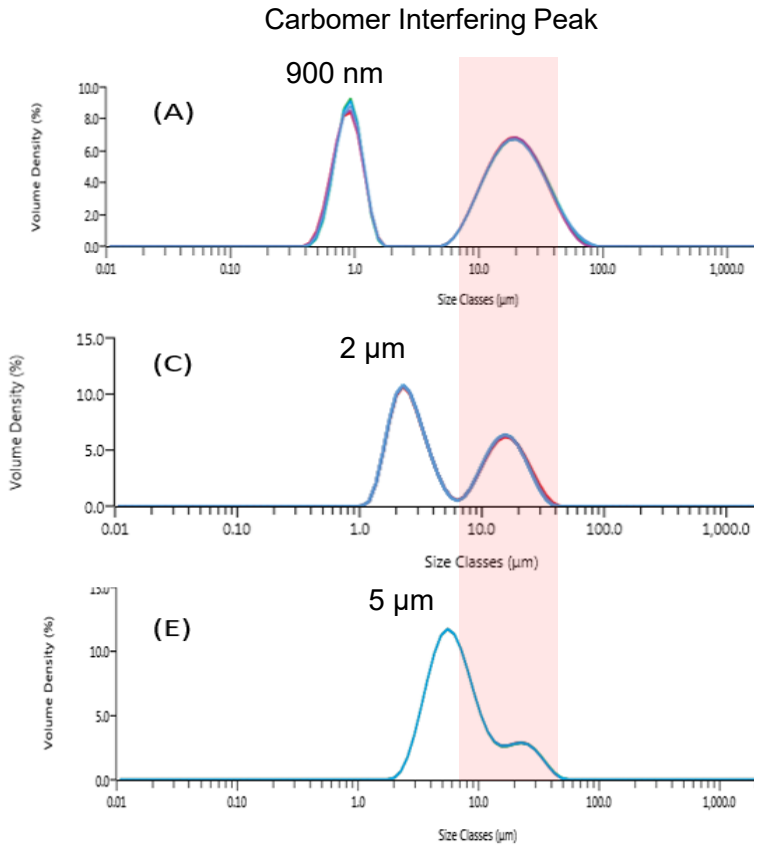


1st: 30 - 80 nm (87.8%); 2nd: 100 – 600 nm (5.3%)

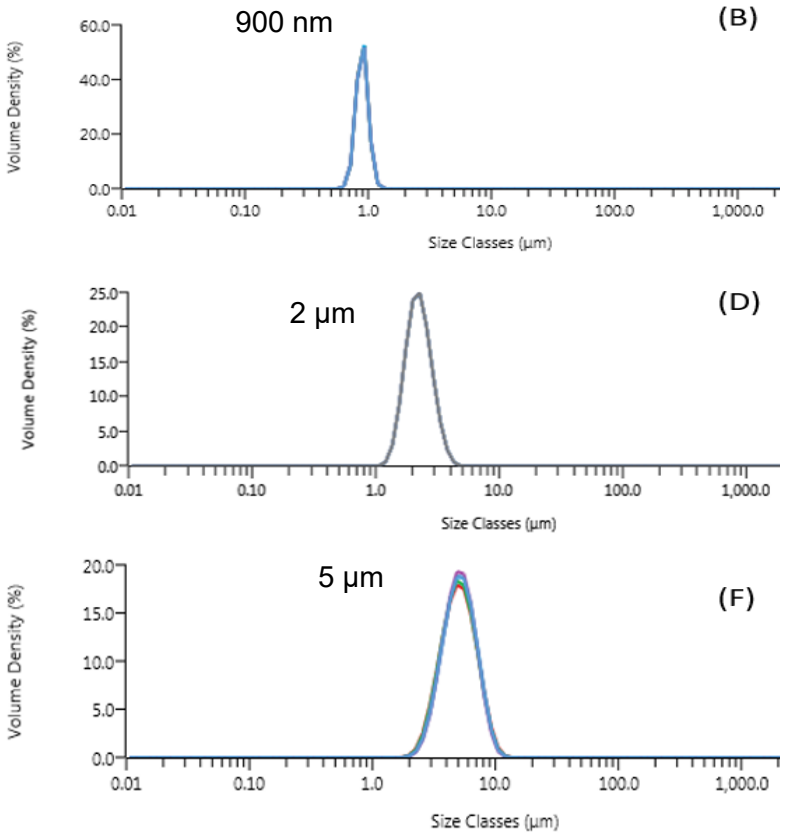
- P. Petrochenko, et al. Analytical Considerations for Measuring the Globule Size Distribution of Cyclosporine Ophthalmic Emulsions. International Journal of Pharmaceutics (2018). 550(1-2), 229-239.
- H. Qu, et al. Asymmetric flow field flow fractionation for the characterization of globule size distribution in complex formulations: A cyclosporine ophthalmic emulsion case. International Journal of Pharmaceutics , 2018, 538, p.215-222

Example 2: Identifying and Removing Excipient Interferences

For suspension drug products, presence of polymers (e.g., for stabilization) can often interfere with the laser diffraction measurement. Many of such interferences go unreported. However, the excipient interferences can impact the quality and BE assessment of the drug product. Shown below is an example of Lotemax (loteprednol ophthalmic suspensions), where the size of the API particle was close to 5 µm which overlapped with the excipient interfering peak.

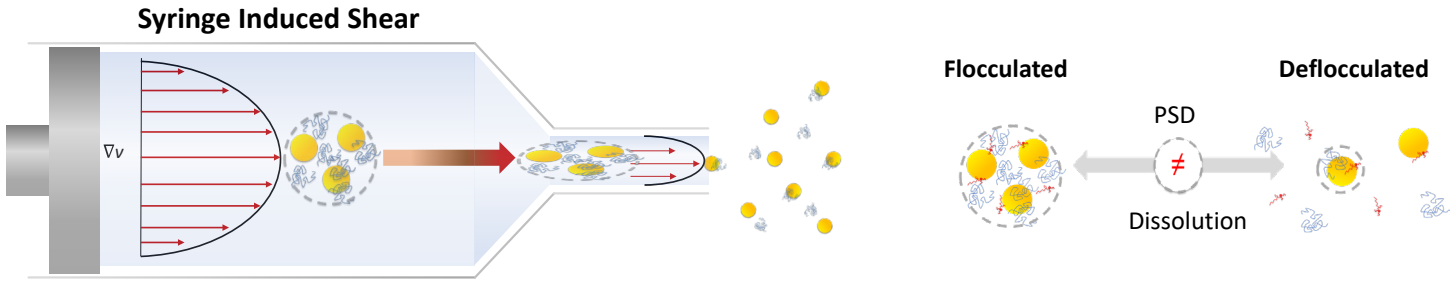


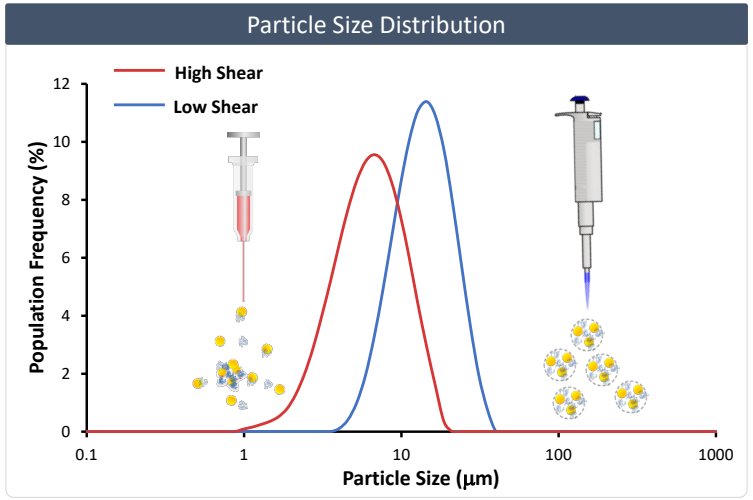
Interference eliminated by using the Placebo formulation as a blank

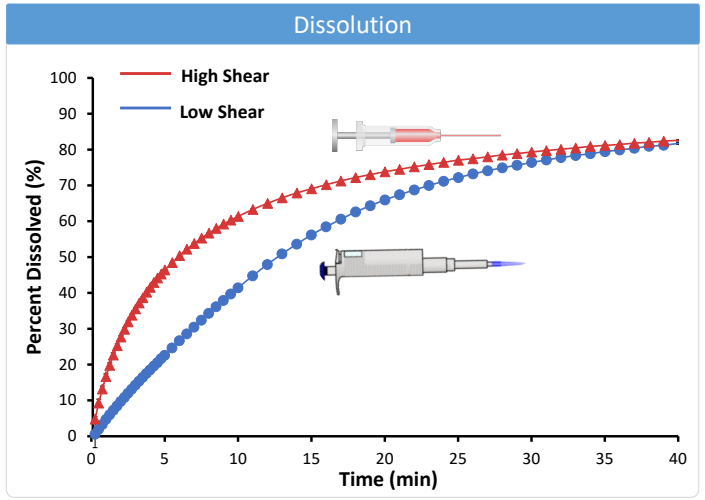


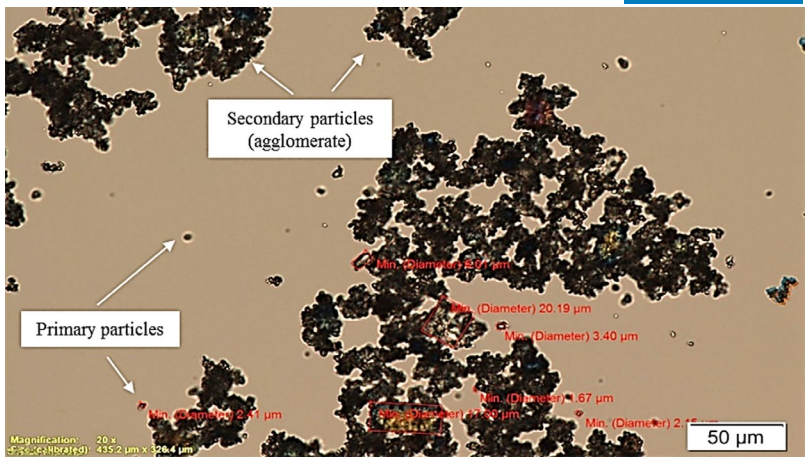
A. Vo, X. Feng, W. Smith, D. Zhu, M. Patel, D. Kozak, Y. Wang, J. Zheng, M. Ashraf, X. Xu. *Analyzing ophthalmic suspension particle size distributions using laser diffraction: Placebo background subtraction method*. International Journal of Pharmaceutics (2021), 598, 120401.

Example 3: Assessing Impact of Flocculation on Particle Size Distribution and Dissolution









- Flocculated particles increase difficulty in measuring the size (shear dependent and method dependent!)
- Impact of flocculation on drug dissolution and bioavailability is less understood (very critical)

- Crystalline suspensions are often formulated with specific excipients and wetting agents to induce flocculation for increased shelf stability.
- Flocculation inherently impacts particle size distribution (PSD) and conversion between primary and secondary particles is highly shear dependent.
- Syringe induced stress shifts particle size distribution from large secondary particles (flocculates/agglomerates) to a mixture of primary and secondary particles, exhibiting significantly higher initial dissolution rates than the low shear introduction method.

Smith, W., Bae, J., Zhang, Y., Qin, B., Wang, Y., Kozak, D., Ashraf, M. and Xu, X., 2021. Impact of Particle Flocculation on the Dissolution and Bioavailability of Injectable Suspensions. International Journal of Pharmaceutics, p.120767. DOI: 10.1016/j.ijpharm.2021.120767

Closing Thoughts

- Appropriate selection of the technique for measuring particle size is essential
- Clear description and understanding of use of algorithm is critical (e.g., CONTI, Cumulant, Mie).
- Need clear expectation on what parameters are important to validate, and what types of materials to use for validation.
- Bi-modality needs careful examination (but the goal should be to understand the cause rather than to eliminate it).
- Often seeing in submissions missing critical method details and justification, such as: measurement position, attenuator settings, cuvette type (DLS), if the dispersion medium has been saturated with the drug before measuring using LD, lack of justification for use of sonication.
- Need to focus more on the purpose of particle size measurement.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