Development, Characterization, and Evaluation Considerations of Particle Analysis to Support Generic Product Quality and Bioequivalence Determination

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Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.

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Drugs are no different.


FDA
Patients expect safe and effective medicine with every dose they take.

Pharmaceutical quality is

assuring *every* dose is safe and effective, free of contamination and defects.


It is what gives patients confidence in their *next* dose of medicine.

OPQ's Proactive Science and Research Approach

- The **science program** is designed to maintain preparedness
  - Consumer complaints
  - Public health issues

- The **research program** is "forward looking"
  - New and emerging technologies for analytics and manufacturing
  - Advanced analytics (instrument and modelling)
  - Forecasting generics for newly-approved new drug applications (NDAs)
  - Complex Drugs in new molecular entity (NME) and generic drugs

OTR's Role in Generic Drug Science

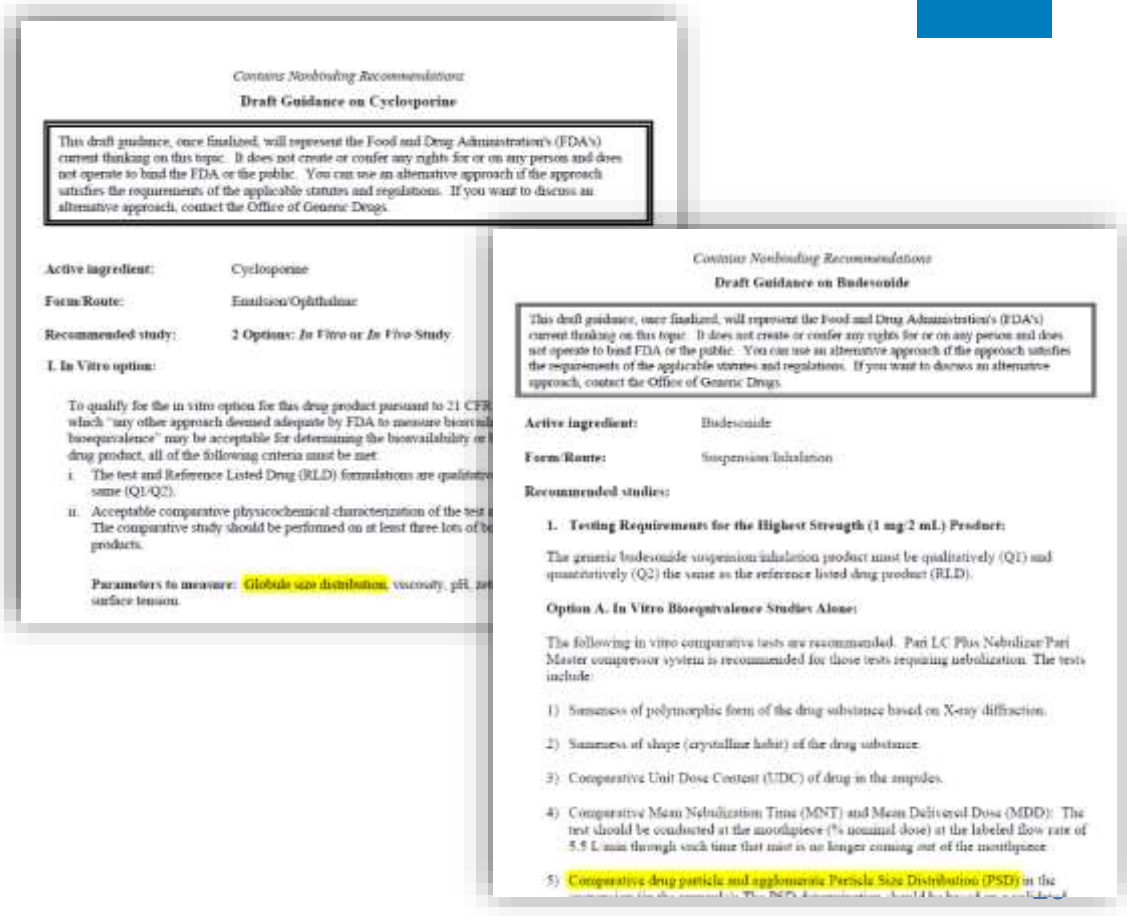
- Laboratory consults
  - Method evaluation (verification)
  - Product quality
  - Pharmaceutical equivalence and bioequivalence (in vitro approaches)
- Training
  - Provide training to quality assessment staff
- Guidance and Standard development
  - Provide scientific information to support product-specific guidances (PSG) and general guidances
  - Develop improved test methods for quality and equivalence standards

Outline

- The need and challenges associated with particle characterization
- Common techniques for particle size distribution (PSD)
- Common issues (during evaluation)
- Examples

Particle Characterization: the Need

- Particle size is an important product quality attribute for formulations in a dispersed state, e.g., emulsions, suspensions, liposomes, 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 Vivo* Study

I. In Vitro option:

To qualify for the in vitro option for this drug product pursuant to 21 CFR
which "any other approach deemed adequate by FDA to measure bioavail
bioequivalence" may be acceptable for determining the bioavailability or
drug product, all of the following criteria must be met:

i. The test and Reference Listed Drug (RLD) formulations are qualitative
same (Q1/Q2).

ii. Acceptable comparative physicochemical characterization of the test
The comparative study should be performed on at least three lots of b
products.

Parameters to measure: Globule size distribution, viscosity, pH,
surface tension.

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Draft Guidance on Budesonide

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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 each time that *must* is no longer coming out of the mouthpiece.

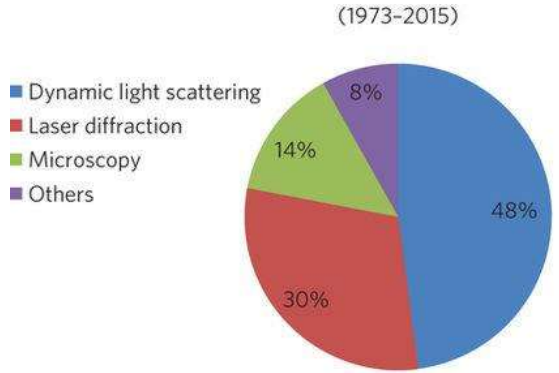
5) Comparative drug particle and agglomerate Particle Size Distribution (PSD) in the

Particle Characterization: the Challenges

- The analysis of the particle size is not an objective in itself, but is a means to an end
  - ❑ Physical stability
  - ❑ Dissolution/drug release
  - ❑ Bioavailability
  - ❑ Process capability
  - ❑ Bioequivalence
- Many factors could impact the PSD, in both method development and evaluation, e.g.,
  - ❑ Choice of the dispersion medium
  - ❑ Sample preparation procedure (e.g., dilution, sonication)
  - ❑ Instrument/software setting (e.g., laser power, measurement position, stirring speed, sonication, optical inputs, analysis algorithms)
  - ❑ Reporting (equivalent spheres, cumulant vs. distribution, meaning of means)
  - ❑ Trueness and precision (what to validate?)
  - ❑ Formulation specific considerations (e.g., **excipient interference**)

Common Techniques to Determine Particle Size Distribution

Technique	Size range	Shape	Resolution (1-5, relative scale)	Principle
Electron microscopy	0.1 nm to a few micron	Y	5	Electron density contrast
Homodyne/Heterodyne dynamic light scattering (DLS)	1 nm to 1 µm	N	2	Brownian motion + light scattering
Field flow fraction (FFF) + multi-angle light scattering (MALS) + DLS	1 nm to a few micron	N	5	Brownian motion + flow based separation + light scattering
Nanoparticle tracking analysis (NTA)	20 nm to 1 µm	N	4	Brownian motion + image analysis
Resonant mass measurement (RMM)	50 nm to 5 µm	N	4	Buoyant mass
Laser diffraction (LD)	30 nm to 3000 µm	N	3	Static light scattering (Mie or Fraunhofer)
Light obscuration	Subvisible particles (0.5 µm to 400 µm)	N	4	Single particle light blockage
Image analysis	1 µm to a few hundred micron	Y	4	Image analysis
Focused Beam Reflectance Measurement	1 µm to 1000 µm	N	4	Chord length



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

Points to consider:

- Sensitivity (e.g., formulation, process changes) and specificity (e.g., any interference?)
- Single technique (robustness) vs. complementary techniques (strength/limitation of each one, comparison)

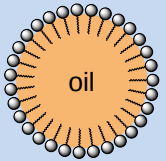
Common Deficiencies

- Incorrect choice of the instrument or technique, e.g., choosing laser diffraction (LD) for measuring particle size of colloidal iron product.
- Incorrect use of material/dispersant refractive index (RI), especially if reported based on distribution analysis (DLS).
- Not clear on which analysis was used, e.g., cumulant vs. distribution (DLS).
- Intensity- weighted distribution is always recommended; use volume- weighted distribution only if it is adequately justified; and avoid the use of number- weighted distribution (DLS).
- Validation performed incorrectly using only the reference standard (e.g., NIST standard); should use actual samples (RLD samples are also ok)
- Method precision (i.e., repeatability, reproducibility, robustness) is not demonstrated in the validation.
- Lack of method details, such as measurement position, attenuator settings, cuvettes (DLS).
- Sample preparation missing critical details or lack of justifications, e.g., if the dispersion medium has been saturated with the drug before measuring using LD, lack of justification for use of sonication.

Impact of the Dispersion Medium

- **Particle** exists in one of three forms: *gas*, *liquid*, or *solid*.
- Though not specifically mentioned, a particle should always be discussed in the context of its dispersion medium (either gas or liquid, solid is rare).

Particle State	Medium	Examples
Solid	Gas	Powders, granules, aerosols
Solid	Liquid	Suspensions, glass-lamella, other particulates
Liquid	Liquid	Emulsions (O/W or W/O), nano-emulsions, multiple-emulsions (e.g. W/O/W)
Liquid	Gas	Nasal spray (liquid)
Solid/liquid (soft-matter)	Liquid	Liposomes, micelles

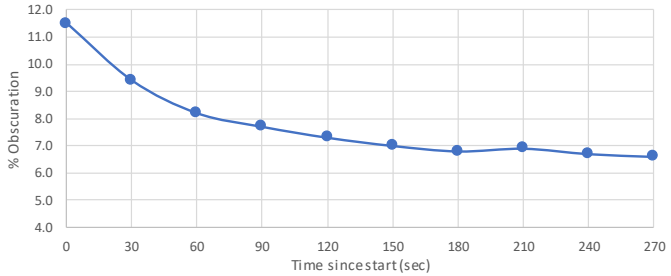


Aqueous (may contain electrolyte, buffers, polymers, etc.)

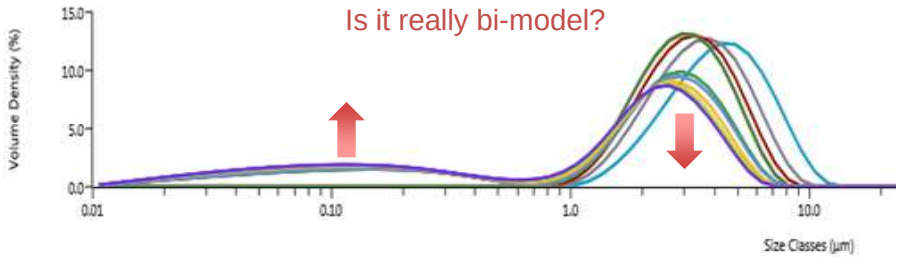
- Proper sample presentation is critical to ensure accurate and consistent particle size measurement. Most common dispersing medium is liquid. For solid samples, changing it's native dispersing medium may change particle stability (e.g., aggregation, dissolution), and hence the sample preparation procedures need to be carefully examined.

Impact of the Dispersion Medium (cont.)

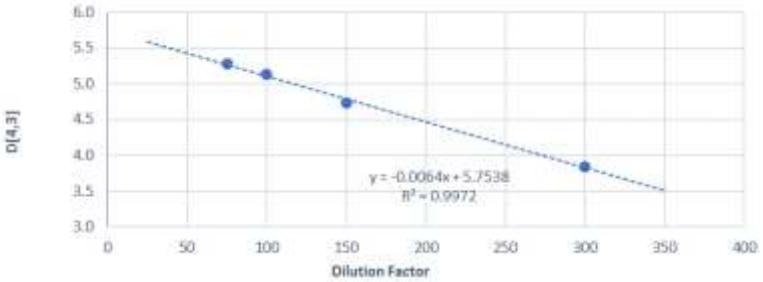
Possible signs that the dispersion medium is not ideal (examples):



Decreasing trend in %obscuration during the measurement (applicable to LD technique)



Appearance of a secondary smaller particle population, and accompanied by the reduction of the primary (larger) particle population



Decreasing trend of particle size upon dilution

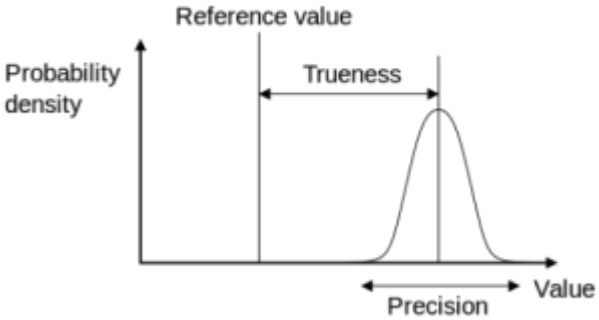
Possible causes:

- **Drug particles are dissolving during measurement**
- Drug particles are not stable (e.g., aggregation, sedimentation)

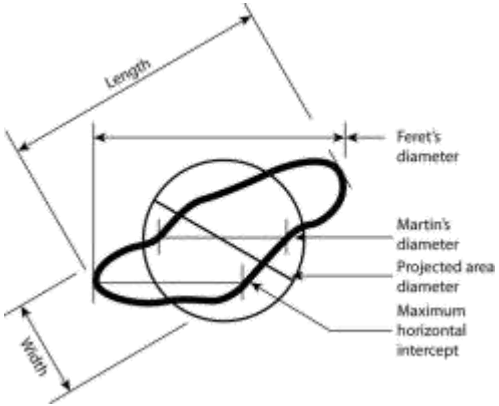
Solutions:

- **Pre-saturate the dispersion medium with the drug**
- Addition of wetting agents and/or electrolyte (to increase electrostatic repulsion)

Particle Size Evaluation: Trueness and Precision

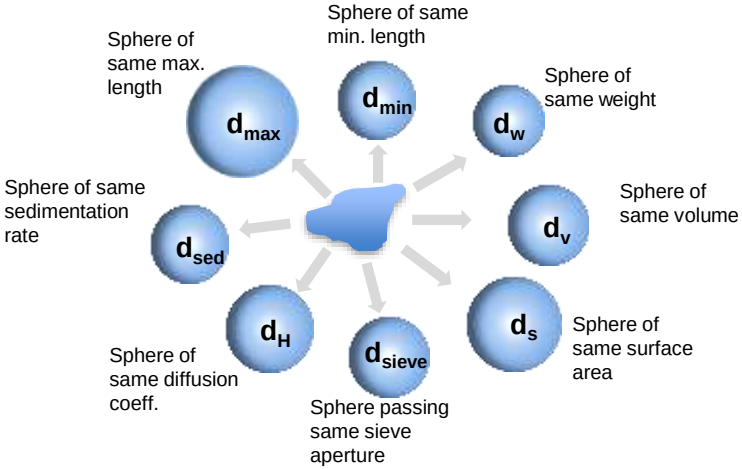


ISO 5725: “trueness” and “precision” to describe the accuracy of a measurement method.



USP <776> *Which one is true size?*

- ❑ Particles are 3-dimensional objects, and unless they are perfect spheres (e.g., oil globules, liposome vesicles, micelles, or air bubbles), they cannot be fully described by a single dimension such as a radius or diameter
- ❑ Concept of equivalent spheres (may not always be the most appropriate, e.g., for needle shaped particles)
- ❑ Different measurement technique assumes different equivalent sphere models, and hence may not necessarily give exactly the same results



Particle Size Evaluation: Precision (3R)

- It is important to demonstrate the method precision as part of the method validation, to show the procedure is suitable for its intended purpose.

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).

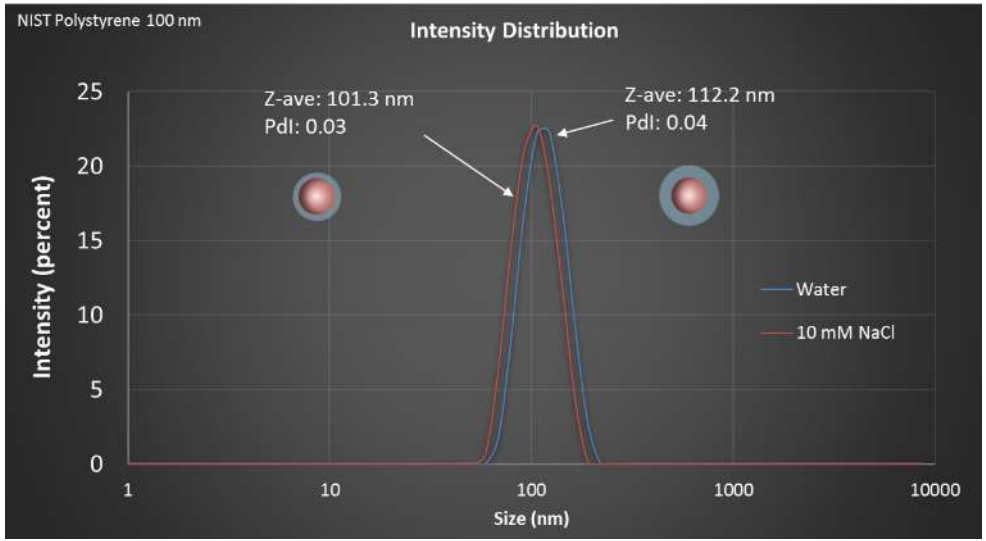
-Sampling procedure, dispersion, machine

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.

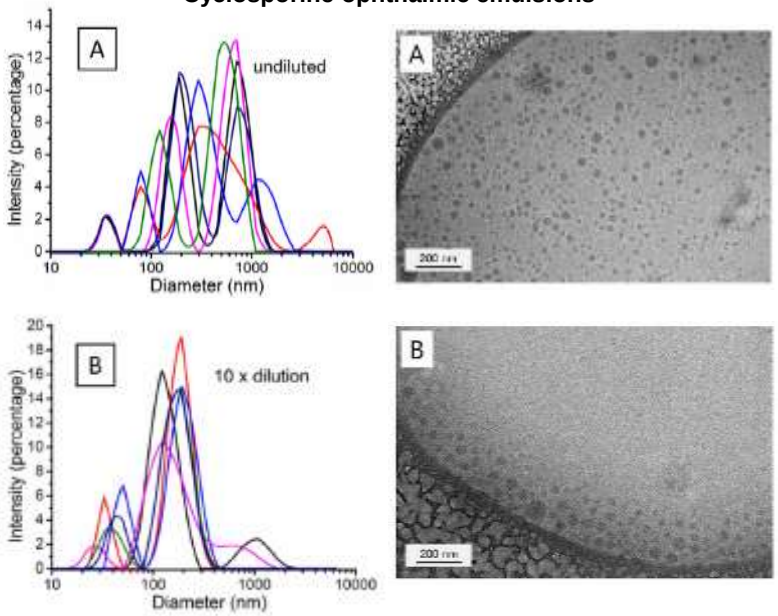
Impact of Dilution (Media Type and Dilution Factors)

Dilution could change the size measurement results in different ways, e.g.,

1) Change in structure/properties of the investigated particles
2) Change in the particle environment



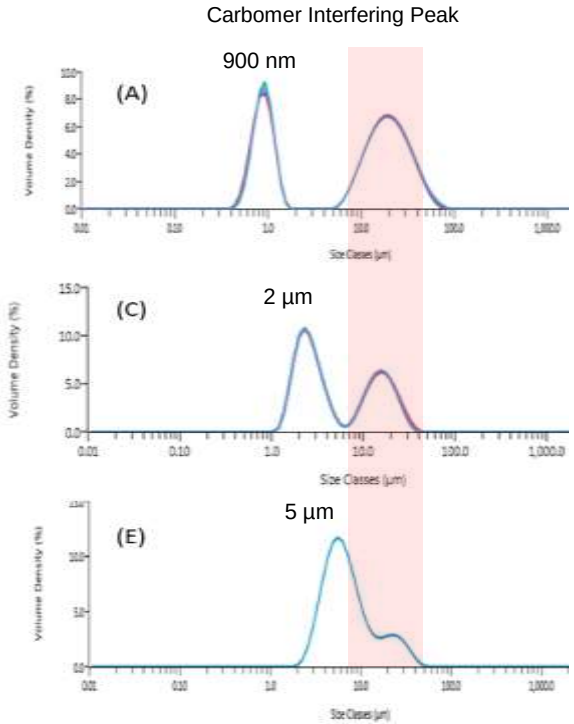
Cyclosporine ophthalmic emulsions



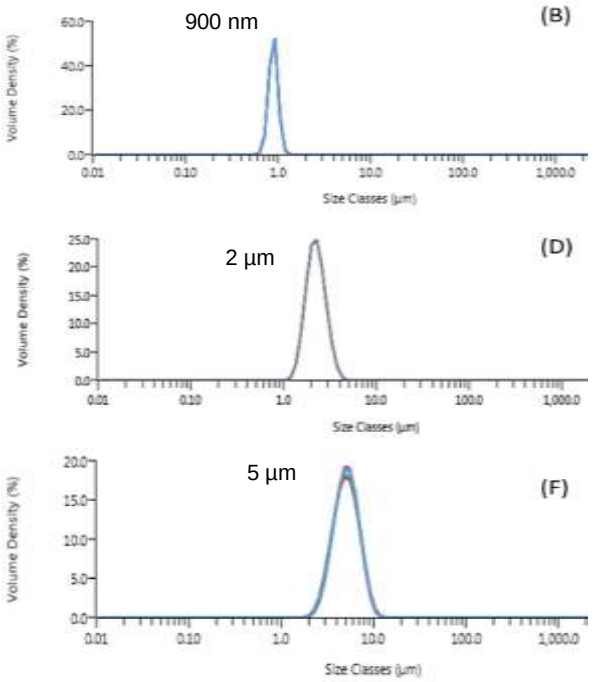
P. Petrochenko, et al. Analytical Considerations for Measuring the Globule Size Distribution of Cyclosporine Ophthalmic Emulsions. International Journal of Pharmaceutics (2018). 550(1-2), 229-239.

Method Interference (Excipients)

Formulation excipients (e.g., polymers, surfactants) may interfere with the size analysis, resulting high variability and erroneous results. For example, in an ophthalmic suspension formulation, presence of carbomer (a viscosity enhancer) was found to interfere with the laser diffraction measurement (demonstrated below using NIST standards with known sizes). The size of the API particle was close to 5 µm which overlapped with the excipient interfering peak.

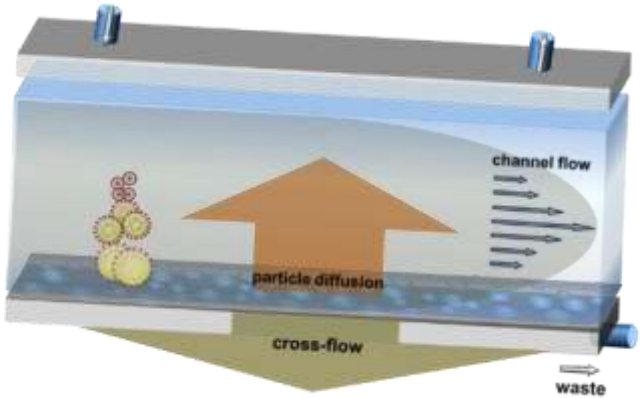


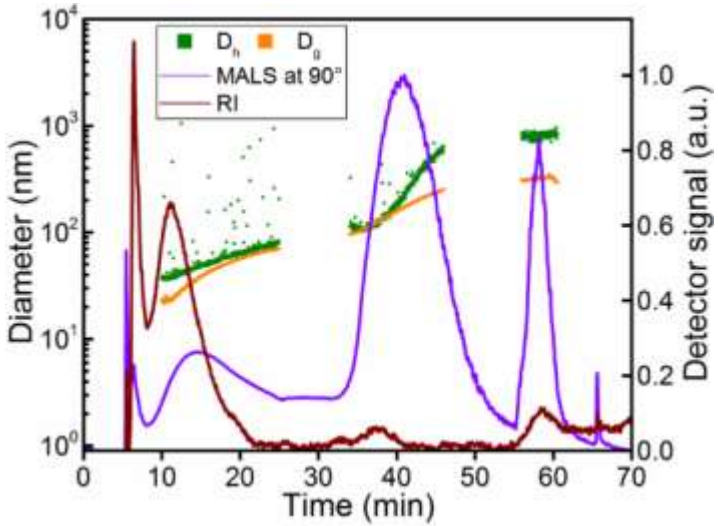
Interference eliminated by using the Placebo formulation as a blank



Other Particle Sizing Methods

Asymmetrical-Flow Field Flow Fractionation (AF4) to Analyze Emulsions





- Mild separation condition allowed delicate samples to be analyzed in their original state
- AF4: First globule population **30-80 nm**, second population 100 -600 nm
- For comparison, DLS shows Z-ave of **110 to 120 nm**, cryoTEM (**20-60 nm**), DOSY NMR (**70 nm**)

H. Qu, J. Wang, Y. Wu, J. Zheng, Y.S.R. Krishnaiah, M. Absar, S. Choi, M. Ashraf, C.N. Cruz and X. Xu. 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(1-2), 215-222.

Summary

- Particle size is one of the critical quality attributes that also affects the BE
- Do not underestimate the challenges of characterizing particle size
- Every particle sizing technique has its strengths and limitations (welcome new techniques which provide better understandings)
- It is important to ensure the method is properly developed and adequately validated
- Correct interpretation of the result relies on full and complete information of the method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