U.S. FOOD & DRUG ADMINISTRATION

# Regulatory Perspectives: Method Validation and Common Deficiencies

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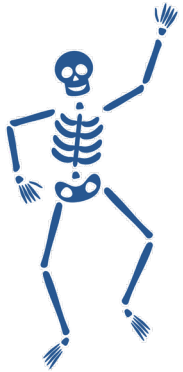
09/23/2025



# Skeleton of the Next 15-20min

- ❑ Considerations for Particle Size Measurement and Method Development
- ❑ What does Method Validation mean for Particle Size Measurement
- ❑ Common Deficiencies in DLS and LD Method and Data Reporting
- ❑ What's Next…

# Particle Sizing: Development Considerations

*…not an exhaustive list…*

- ❑ What is the size range?
  - <100 nm: dynamic light scattering (DLS)
  - >1 µm: laser diffraction (LD)
  - Highly poly-dispersed mixture: separation technique (e.g., AF4, SEC)
- ❑ Interested in shape or if the shape is important: microscopy, e.g., Cryo-TEM
- ❑ Is the sample colored (e.g., may absorb light and thus reduce the signal quality in DLS or LD)?
- ❑ Does sample disperse well or remain stable in the medium (e.g., in air or in water)?
- ❑ Does sample preparation impact the stability of the particles (e.g., sonication, dilution, filtration)?
- ❑ Is the sample sensitive to dilution (e.g., suspension particles may dissolve)?
- ❑ Does the formulation component (e.g., polymeric excipient) interfere with the analysis?

# Particle Sizing: Development Considerations

*… a non-exhaustive list continued…*

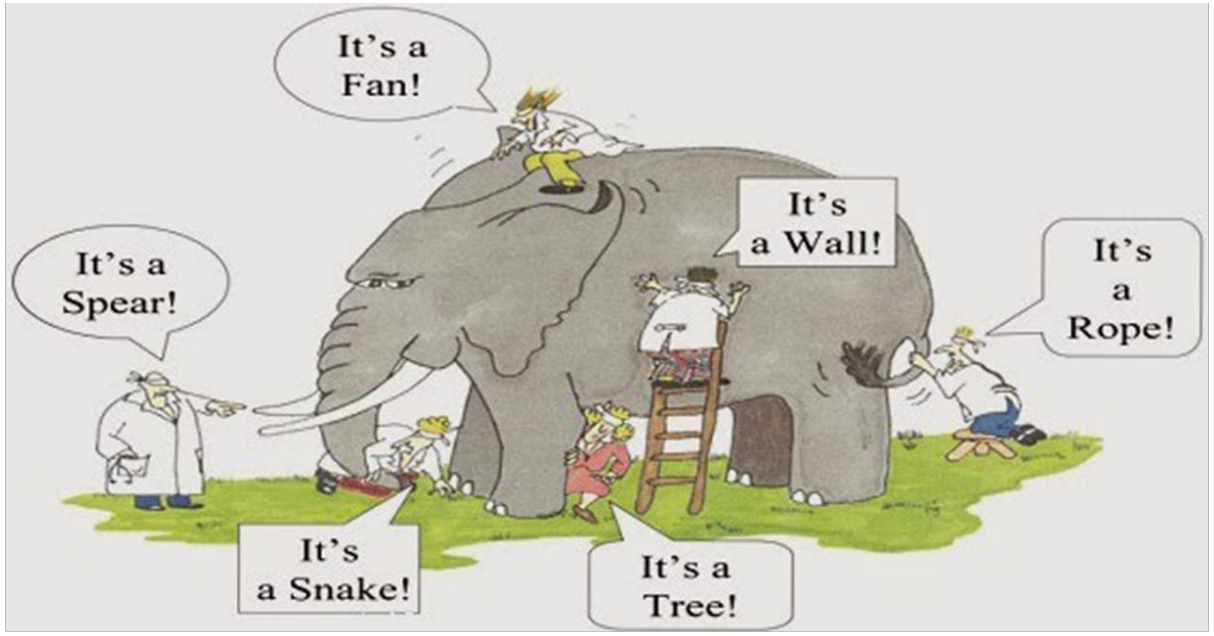
- ❑ What are the appropriate measurement setting (instrument/software dependent)?
  - Laser power (DLS)
  - Measurement position (DLS)
  - Optical inputs (DLS and LD)
  - Stirring speed (LD)
  - Sonication (LD)
  - Analysis algorithms (e.g., cumulant vs. distribution, Mie vs. Fraunhofer)

- ❑ What should be reported?
  - Mean or median (e.g., D[4,3], D[3,2], D[1,0], z-average, $D_{50}$, $D_{10}$, $D_{90}$, number, volume, weight, etc.)
  - Distribution (e.g., Polydispersity index, SPAN)
  - Any data processing or transformation… Cumulant vs. distribution (for DLS)
  - Everything has to do with how the data is generated (e.g., settings, sample preparation)

# Particle Sizing: Development Considerations

*… yet more considerations…*

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




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

# PSD Method Validation

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).

-Instrument, 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, instrument

**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.

# DLS: Choosing and Reporting the Measurement Settings

Example 1

You used a dispersant viscosity value of 0.97 cP in your globule size distribution (GSD) method (3.2.P.5.2). Your value of viscosity for water differs from the value reported in the literature at 25° C (0.89 cP). Considering that the viscosity is extremely important in calculating the correct hydrodynamic size of globule, please provide justification for this viscosity value given the extent of dilution or consider updating your current GSD method to use the appropriate viscosity for the dispersant. Additionally, using the correct viscosity, please recalculate all the results for the reference and test products. Please also include representative sample reports (generated on the instrument) in your response.

Related topic:

1) How does the viscosity and measurement temperature impact the DLS analysis

# DLS: Fixing Measurement Conditions

Example 2

Please provide the “measurement position” and the “attenuator setting” used in the PSD method in section 3.2.P.5.2. Please note that the identical settings should be used for both the reference and test products. Based on your response, please update the method in section 3.2.P.5.2 accordingly.

Related topic:

1) How to compare two products or batches – BE and QC
2) What is the impact of inter-measurement changes in measurement position and/or attenuator setting

# DLS: Setting Specifications

Example 3

a. We acknowledge that you have set acceptance limit for GSD based on Z-ave and PdI in 3.2.P.5.1. Please provide justification for the selected range of 50-130 nm for Z-ave.

b. We acknowledge that ….However, the acceptance limit should be a range (both the upper and lower limit). Furthermore, the increase in the acceptance limit from NMT150 nm (product release) to NMT170 nm (stability test) is not justified based on the 9-months stability testing results. Please revise the acceptance limit for globule size to a range and use the same acceptance limit for both product release and stability test.

c. We acknowledge that .... However, the variability in the provided results appears much narrower than the proposed range of 75-200 nm. Please tighten the acceptance limit for globule size, either based on the variation of the reference listed drug (e.g., $\pm$ 3$\sigma$) or a range that you can justify. In addition, please establish appropriate acceptance limit for PdI, and provide justification accordingly.

Related topic:

1) How to set the specification and acceptance limit
2) Which parameter to set the specification

# DLS: Impacts of Sample Preparation

Example 4

a. We acknowledge that you have validated the method for particle size for precision. However, the robustness of the method against dilutions has not been determined using [*Specific Instrument*]. Please validate the method robustness in terms of serial dilutions (e.g., 50, 100, and 200 times with dispersant - water) using [*Specific Instrument*]. Additionally, please include polydispersity index (PdI) in the validation result (precision and robustness).

b. We acknowledge that you have performed method validation for particle size. However, the robustness of the method against dilutions has not been determined. You have diluted the product to 1250x times prior to the size measurement. The impact of serial dilutions on size results should be determined. Accordingly, please validate the method robustness in terms of serial dilutions (e.g., 10, 100, 1000 times using water). Additionally, please include polydispersity index (PdI) in the validation result (precision and robustness).

Related topic:

1) What should (not) be validated
2) 3Rs (repeatability, reproducibility, and robustness)

# DLS: Sample Preparation and Interference

Example 5

In your method to determine the globule size distribution of the emulsions in 3.2.P.5.2, samples were analyzed directly without dilution. This has resulted in high variability and inconsistency in distribution results (page 11 of the validation report, 3.2.p.5.3), which was also evident from the high reported polydispersity values (>0.4 in most cases). Considering the potential interference(s) of the polymers at higher concentrations, please select an appropriate dilution media and condition to minimize the effect. Please revise the analytical procedure for globule size distribution, reanalyze the samples, and revise the acceptance limit for globule size distribution based on new data. In addition, please establish appropriate acceptance limit for PdI, and provide justification accordingly.

Related topic:

1) How to identify the problem (variability)
2) Interferences

# DLS: Data Processing and Reporting

Example 6a

We acknowledge that you have set acceptance limit for GSD based on distribution analysis in 3.2.P.5.3. Please clarify if the results (i.e., d10, d50, and d90) were "intensity-" or "volume-" weighted and if the mode of the analysis was "General purpose" or "Multi narrow". Based on your response, please update the method in section 3.2.P.5 accordingly.

Related to:

1) How data are reported (i.e., cumulant or distribution)
2) What is the analysis mode (data processing)

# DLS: Optical Properties and Data Processing

Example 6b

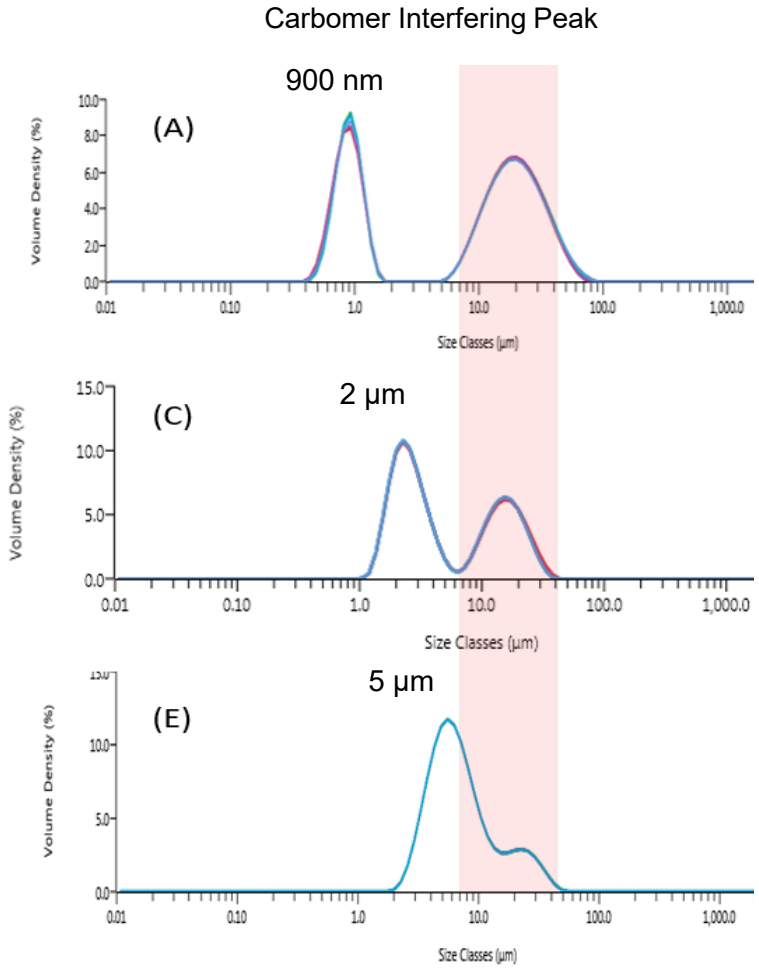
You used a Refractive Index (RI) value of 1.52 for castor oil in your globule size distribution (GSD) method (3.2.P.5.3 and 5.3.1.2). This differs from the literature reported value for castor oil . Considering that the RI may have an impact on the distribution analysis results (i.e., d10, d50, and d90) please update your current method to use the correct RI value for castor oil.

Related to:

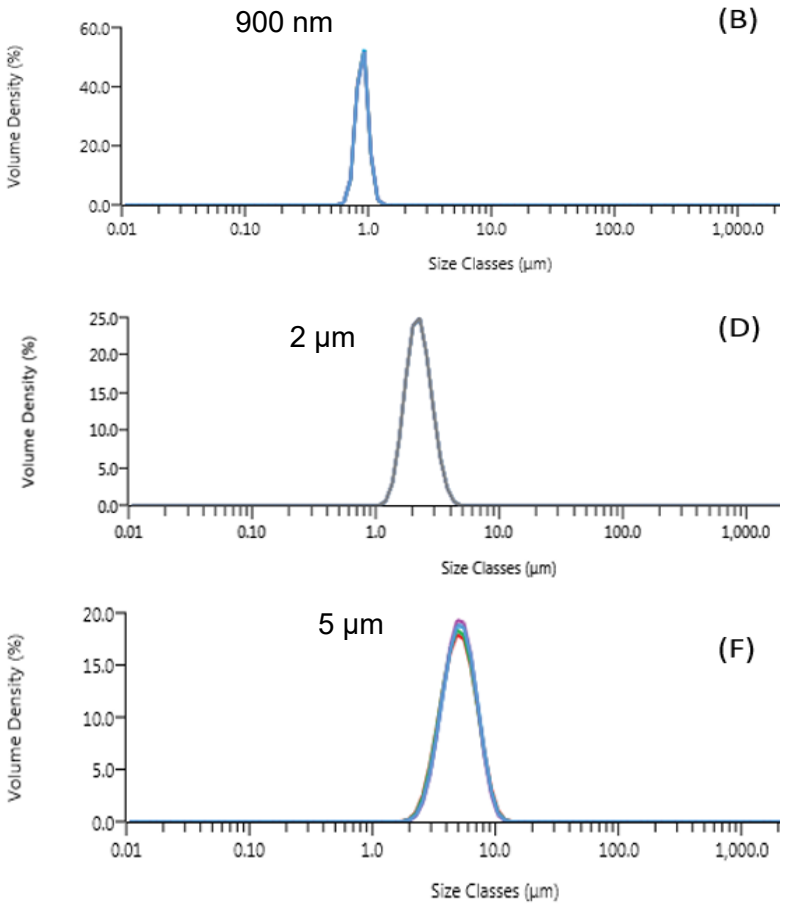
1) How RI values are used for DLS analysis
2) How data are reported (i.e. cumulant or distribution)

# LD: Method Interference (Excipients)

Formulation excipients (e.g., polymers, surfactants) may interfere with the size analysis, leading to 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.



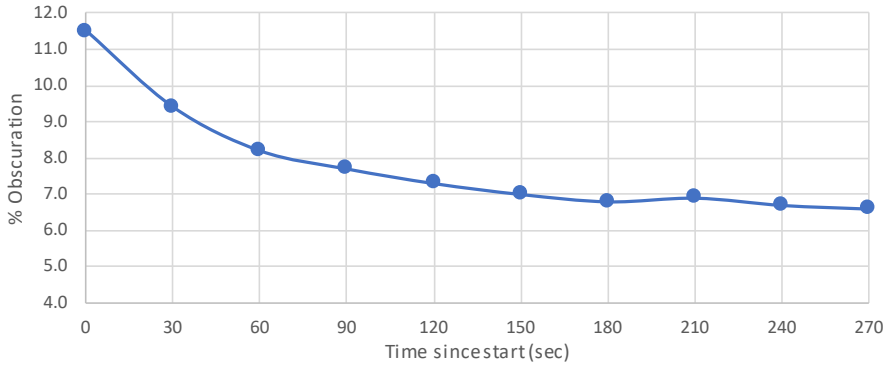

Interference eliminated by using the Placebo formulation as a blank



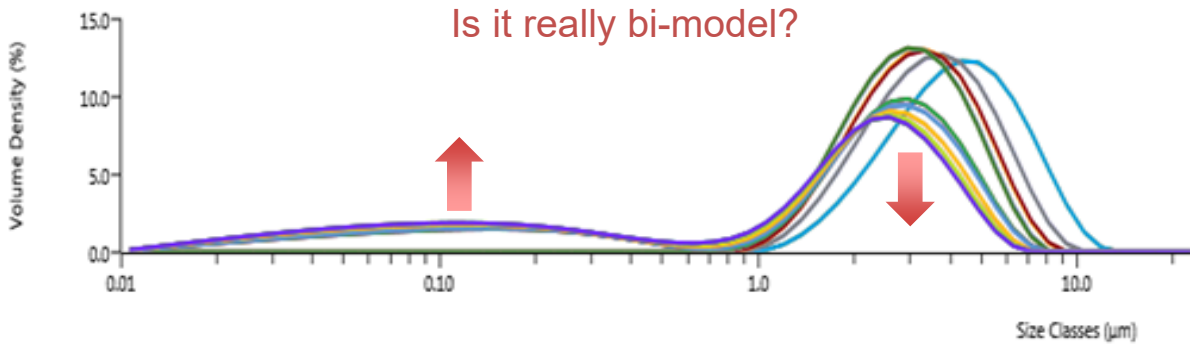

Anh Vo, et al. Analyzing ophthalmic suspension particle size distributions using laser diffraction: Placebo background subtraction method. Int J Pharmaceutics (2021) 598. 12041.

# LD: Impact of the Dispersion Medium

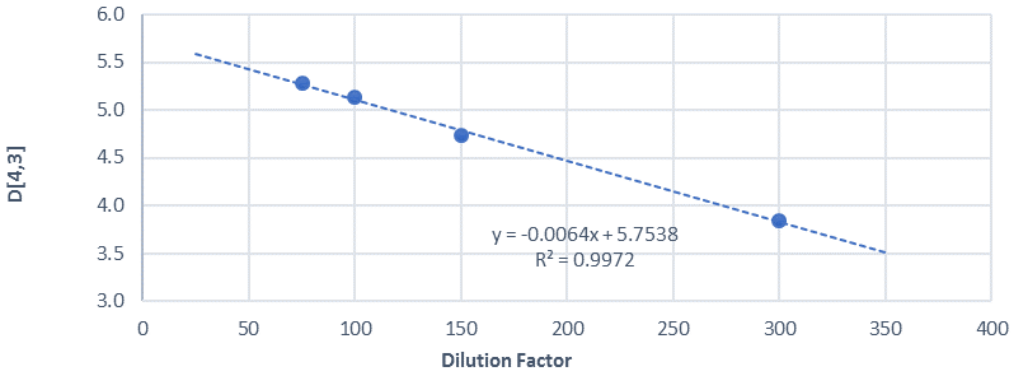
Possible signs that the dispersion medium is not ideal (examples):




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)

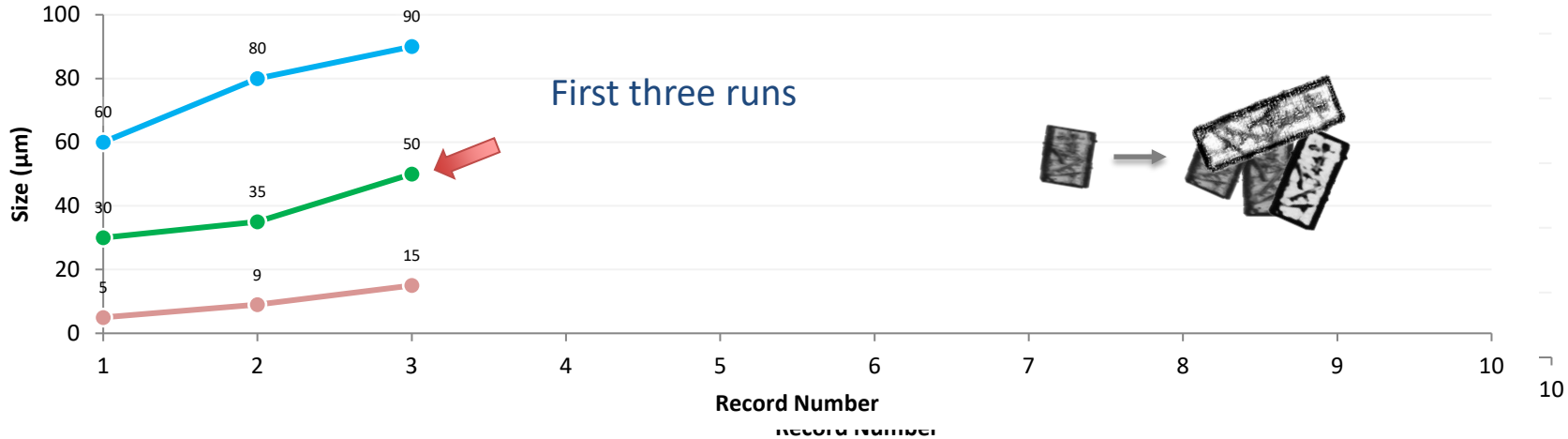
# LD: What's wrong … Measurement Conditions

**Information about the sample (obtained via microscope):**
Particles with rectangular shape, size approx. 1-9 µm (appear poly-dispersed). Upon dispersing in water, particles appear forming agglomerates.

**Method details:**
Particle type: spherical
Particle RI: 1.67
Sample volume: 10 mL (20% w/w)
Dispersant: Millipore Water (RI=1.33)
Dispersant volume: 400 mL
Sample measurement duration: 5 sec
Number of measurement: 3
Sonication: None

**Revised Method:**
Particle type: Non-spherical
Particle RI: 1.67
Sample volume: 2.5 mL (5% w/w)
Dispersant: Millipore Water (RI=1.33)
Dispersant volume: 400 mL
Sample measurement duration: 10 sec
Number of measurement: 6
Sonication: continuous vs prior

***Consider Variability in Time***:
- Obscuration
- Size Values



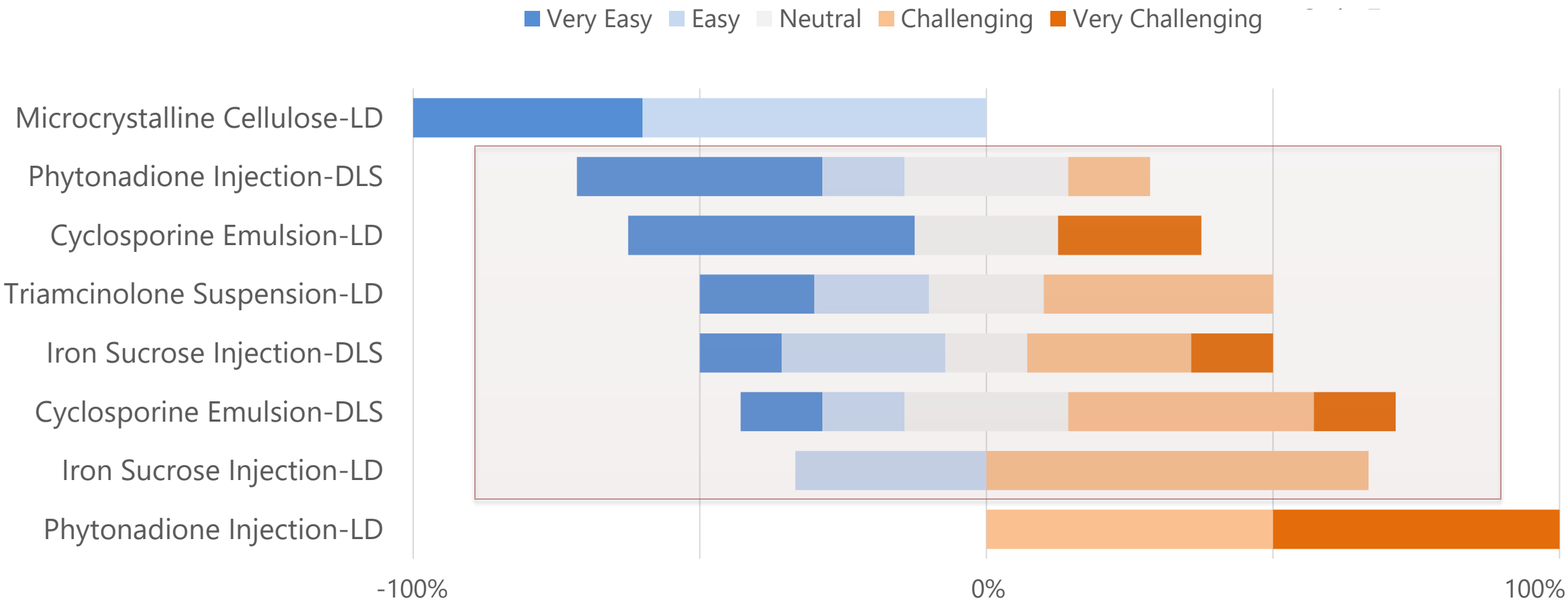
# Other Common Deficiencies

- Incorrect choice of the instrument or technique, e.g., choosing laser diffraction (LD) for measuring particle size of colloidal iron product.
- Lack of method details, such as measurement position, attenuator settings, cuvettes (DLS); Stir rate, sonication - mode/power (LD).
- 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 sonication – to use or not to use.
- Not clear on what data processing or 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).
- Incorrect use of material/dispersant refractive index (RI) or optical properties, influence on reported on distribution analysis (DLS - LD).
- Validation performed incorrectly using only the reference standard (e.g., NIST standard); should *use actual samples* (reference list drug (RLD) samples are also suitable)

# Summary

- Particle size is one of the critical quality attributes that affects the *BE*
- 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 – 3Rs : *Repeatability, Reproducibility, Robustness*
- Correct interpretation of the result relies on *full and complete information* of the method (documentation and reporting)

# What's Next - Why Were These Samples Chosen?




* Pre-workshop Sample Measurement Survey Results: seven vendors participated, two techniques (DLS, LD), five materials, 19 survey responses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