Particle Classroom Series VI: Method Development

November 12, 2019
Overview

• **Goal:** Reproducible method that tracks product performance
• Choose measurement approach (dry vs. suspension)
• Lock down RI
• Vary measurement settings that can influence result
  – Dry: measurement duration, concentration, air pressure
  – Wet: sampler selection, dispersion, duration, concentration, energy (mixing + ultrasound)
• Test method (reproducibility)
  – Meet ISO, USP or internal guidelines
  – Check COV at d10, d50, d90
Goals

- Reproducible method that tracks product performance
- You might have other goals
  - Accuracy: tricky subject, is it the “real” particle size?
  - Repeatability: liquid suspension re-circulating in sampler
  - Reproducibility: prepare, measure, empty, repeat
  - Resolution: optimize to find second populations
  - Match historic data (sieves), but quicker, easier technique
- Use structured approach for any decision/choice that may influence result
- Have data to support selections made
- Document process so colleagues understand your choices
Accuracy vs. Precision

(A) Low accuracy, low precision measurements form a diffuse, off-center cluster
(B) Low accuracy, high precision measurements form a tight off-center cluster
(C) High accuracy, low precision measurements form a cluster that is evenly distributed but distant from the center of the target
(D) High accuracy, high precision measurements are clustered in the center of the target.
Is it the "real particle size"?

- Comparison to referee technique
- Microscope (image analysis) is referee technique for particle characterization
- Two kinds of image analysis:
  - Dynamic image analysis; particles flowing
  - Static image analysis; particles sit on slide on automated stage
Image Analysis

**Dynamic:**
particles flow past camera

**Static:**
particles fixed on slide,
stage moves slide

Basic-Camera

Zoom-Camera
Guidelines

Drug substances used in pre-clinical studies → Document particle size and morphology using photomicroscopy

Development of instrumental methods may be initiated at this stage

Is the substance BCS Class I or III?

Document particle size and morphology using photomicroscopy

Initiate development of a particle-size distribution method using a quantitative technique (see table 1)

Compare with quantitative microscopy results

Evaluate the effect of any change in drug substance with respect to morphology or particle size from that initially studied.

If the drug is poorly soluble, you need to pay attention to particle analysis.

Compare with quantitative microscopy.

Decision tree outlining particle evaluation for Phase I clinical studies
Guidelines Phase III

1. **Develop PSD method**
2. **Compare with Quantitative Photomicroscopy results**
3. **Validate PSD method**
   - Evaluate need for particle size control in conjunction with other physical properties that may influence processing

   **Is the final dosage form chosen?**

   **Yes**
   - **Document particle size/morphology and evaluate impact on processing**
   - **Screen and develop suitable PSD method**
   - **Compare with quantitative photomicroscopy results**

   **No**
   - Go back to Develop PSD method

**Evaluate the effect of any change in drug substance with respect to morphology or particle size from that initially studied.**

**Compare**  **Validate**  **Document**
Types of Precision

Repeatability
Prepare sample, add to wet sampler, re-circulate, measure same multiple times (suspensions only)
Provides limited information (mostly a test of analyzer performance)

Reproducibility
Prepare sample, measure, drain, repeat (suspensions + dry)
Distinguishes great methods
Reproducibility

Yes, we emptied instrument between each analysis.
Reproducibility

Reproducibility: prepare, measure, empty, repeat

What would be good reproducibility?

Look at accepted standards

Measure 3 times, calculated COV at $d_{10}$, $d_{50}$, $d_{90}$

$$\text{COV (RSD)} = \frac{\text{st dev}}{\text{mean}} \times 100$$

ISO13320

- COV < 3% at median $d_{50}$
- COV < 5% at $d_{10}$ & $d_{90}$

USP<429>

- COV < 10% at median $d_{50}$
- COV < 15% at $d_{10}$ & $d_{90}$

Note: double all limits when $d_{10}$, $d_{50}$, or $d_{90}$ < 10 microns
Resolution

- Ability to measure small differences in particle size.
- Small differences between successive samples (different production lots) are most important. This is tied to reproducibility.
- Detection limit of small amount of material outside of main size distribution.
- Best defined by user’s real-world requirements.
• Resolve size difference between two materials of similar size
• 552nm and 600nm PSL
• Measured separately: high resolution
• Measure mixed together: peaks would blend and you cannot discern two populations.
Separate peaks

Resolution of multiple modes in a single sample
Next peak at least 2x of previous size

83nm, 204 nm, 503nm PSL
Separate Peaks

Next peak 2x of previous size

Resolution is independent of where you are on size scale

100µm, 200µm, 400µm glass beads
Identifying Trace Impurities

Ludox-50
Median Size: 0.031 μm

Geltech
Median Size: 1.65 μm

0.05wt% Geltech 1.5 in LUDOX
Wet Method Development
Workflow

First determine RI
Choose solvent (water, surfactants, hexane, etc.)
Sampler selection: sample volume
Pump & stirrer settings
Concentration
Measurement duration
Does the sample need ultrasound?
  - Document size-time plot
  - Disperse sample, but don’t break particles
  - Check for reproducibility
Refractive Index

Real component via literature or web search, Becke line, etc.
Measure sample, vary imaginary component to see if/how results change
Recalculate using different imaginary components, choose value that minimizes R parameter error calculation

More in the refractive index webinar at:
LA-960 Method Expert

Calculation Optimization

Imaginary Refractive Index Wizard - Result -

Graph Summary | Distributions | D90, D50, D10 | R Parameter

Distribution Graph

Step 6. Choose the best value
Please select the parameter listed below which provides the best particle size distribution.
If you would like assistance choosing the best value, please click the help symbol to the right.
Imaginary_RI: 1.58 ± 1.00i
Sample Handling

Larger, broad distributions require larger sample volume

Lower volume samplers for precious materials or solvents

<table>
<thead>
<tr>
<th>LA-960 Sample Handlers</th>
<th>Dispersing Volume (mL)</th>
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<tbody>
<tr>
<td>Aqua/SolvoFlow</td>
<td>180 - 330</td>
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<tr>
<td>MiniFlow</td>
<td>35 - 50</td>
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<tr>
<td>Fraction Cell</td>
<td>15</td>
</tr>
<tr>
<td>Small Volume Fraction Cell</td>
<td>10</td>
</tr>
</tbody>
</table>

- Median (D50): 114 µm
- Sample Amount: 1.29 mg
- Median (D50): 35 nm
- Sample Amount: 132 mg
- Median (D50): 9.33 µm
- Sample Amount: 0.165 mg

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Pump and Stir

- Must be high enough to suspend & circulate heavy particles-if too low speed, larger particles won’t be measured and apparent size decreases with time.
- Not so high that bubbles are introduced
- Adding energy – can disperse loose agglomerates
- Measure at several settings & select optimum (repeatability)
- Can be automated in software

<table>
<thead>
<tr>
<th>Exp #</th>
<th>Agitation</th>
<th>Circulation</th>
<th>D_{mean} (nm)</th>
<th>D_{10} (nm)</th>
<th>D_{90} (nm)</th>
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<td>187.03</td>
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<td>3</td>
<td>184.98</td>
<td>136.4</td>
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</tr>
</tbody>
</table>
Concentration

High enough for good S/N ratio
Low enough to avoid multiple scattering
Typically 95 – 80 %T
Measure at different T%, look at Chi Square calculation
Duration

- Long enough for reproducibility
- Typically 5 sec, up to several minutes
- Longer time for large, broad distributions
- Can be automated in software
- Could be used for robustness testing during method validation
Ultrasonic Dispersion

Adding energy to break up agglomerates – disperse to primary particles, without breaking particles

Similar to changing air pressure on dry powder feeder

Typically set to 100% energy, vary time (sec) on

Investigate tails of distribution

High end to see if agglomerates removed
Small end to see if new, smaller particles appear (breakage)

Test reproducibility, consider robustness

Note:

Do not use on emulsions
Can cause thermal mixing trouble w/solvents - wait
Use external probe if t> 2-5 minutes

More in the sampling and dispersion webinar at:
LA-960 Method Expert will systematically vary:

- Level (power)
- Time on
- Iterations
- Delay

Generate result graphs
Examples
Ultrasound
Plot summary data

Size ($\mu$m) vs. Ultrasound (sec) for D90, D50, and D10.
Reproducibility

- **ISO13320**
  - COV < 3% at median \(d_{50}\)
  - COV < 5% at \(d_{10} \& d_{90}\)

- **USP<429>**
  - COV < 10% at median \(d_{50}\)
  - COV < 15% at \(d_{10} \& d_{90}\)

<table>
<thead>
<tr>
<th>Data Name</th>
<th>Sample Name</th>
<th>D50</th>
<th>D10</th>
<th>D90</th>
<th>Comments</th>
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<td>15 sec US</td>
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</table>

Mean: 59.9667, 22.63733, 117.5578
Standard Deviation: 0.440191, 0.276966, 1.798666
COV (at d_{mean})*100: 0.74419, 1.230689, 1.631123
Reproducibility

58 methods

Image analysis for morphology

Laser diffraction for PSD

If RSD for d50 < 20%, then acceptable for QC environment

Note: RSD increases with decreasing size

Sampler Selection

Remove points from not acceptable region using Fraction Cell

Systematic analysis gives good hint about fraction cell use and importance of pumping for larger particles.
Dry Method Development
First get sampling right & determine RI
Measure at 3 different pressures (low, medium, high)
Determine optimum pressure based on good dispersion while not breaking particles
Can also compare dry vs. wet measurements
Adjust other settings to optimize sample concentration & duration
Ideally measure all of powder placed into the sampler
  Segregation can occur on vibrating tray
  Constant mass flow rate important for stable concentration during measurement
Once settings chosen, test reproducibility
Dispersion vs Breakage

Theoretical

Actual

Size

Stability

Increasing energy

Size

Increasing energy

Higher air pressure or longer ultrasound duration
Dispersion vs Breakage

- Dispersion and milling can be parallel rather than sequential processes

Theoretical

- Proper Air Pressure
  - Aggregated
  - Dispersed
  - Broken

- Air Pressure too High

Actual

- Proper Air Pressure
  - Aggregated
  - Dispersed
  - Broken

- Air Pressure too High
Pressure Titration
Mg Stearate

Graph Type | Remarks 1 | Median Size | Air
---|---|---|---
small nozzle | | 7.50244 (μm) | High
small nozzle | | 8.14897 (μm) | Mid
small nozzle | | 8.62565 (μm) | Low

High = 3 bar
Mid = 2 bar
Low = 1 bar
Mg Stearate

![Graph showing D90, D50, and D10 values for Mg Stearate]

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>D90</td>
<td>15.258</td>
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<td>D50</td>
<td>8.626</td>
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<td>D10</td>
<td>4.862</td>
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Reproducibility at 3 Bar

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<th>Remarks</th>
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<td>Small nozzle</td>
<td>7.43656</td>
<td>High</td>
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<table>
<thead>
<tr>
<th>Code</th>
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<tr>
<td>200707180862099.NGB</td>
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<td>2007071808654102.NGB</td>
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</table>

Mean: 7.525, 4.232, 12.919
Standard Deviation: 0.110, 0.040, 0.285
COV (2d/memean)*100: 1.456, 0.956, 2.209
Reproducibility at 2 Bar

<table>
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<tr>
<th>Date</th>
<th>Type</th>
<th>Median Size (μm)</th>
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<th>D10</th>
<th>D90</th>
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<tr>
<td>2007/07/180856106 NGB</td>
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<td>8.236</td>
<td>4.595</td>
<td>14.722</td>
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</tbody>
</table>

Mean: 8.226, 4.581, 14.720
Standard Deviation: 0.024, 0.011, 0.132
COV (st dev/mean)*100: 0.288, 0.242, 0.896
Reproducibility at 1 Bar

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample</th>
<th>Median Size</th>
<th>D50</th>
<th>D10</th>
<th>D90</th>
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<tr>
<td>200707190903111. NGB</td>
<td>Mg Stearate</td>
<td>8.625</td>
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<td>4.051</td>
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</tbody>
</table>

Mean: 8.616
Standard Deviation: 0.053
CCV (st dev/mean)*100: 0.612
Summarize Reproducibility

<table>
<thead>
<tr>
<th>Pressure</th>
<th>D10 COV</th>
<th>D50 COV</th>
<th>D90 COV</th>
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</thead>
<tbody>
<tr>
<td>1 Bar</td>
<td>0.659</td>
<td>0.612</td>
<td>1.007</td>
</tr>
<tr>
<td>2 Bar</td>
<td>0.242</td>
<td>0.288</td>
<td>0.896</td>
</tr>
<tr>
<td>3 Bar</td>
<td>0.956</td>
<td>1.456</td>
<td>2.209</td>
</tr>
</tbody>
</table>

This is reproducibility; Sampling is important!

Must have representative sample
Summary

Must have representative sample
Powders: select air pressure
Suspensions: wet, disperse
Check accuracy w/microscope or DLS
Investigate system settings: concentration, agitation, ultrasound
Design for maximum precision
Follow guidelines in standards
Thank you
Thank you

감사합니다

Cảm ơn

Большое спасибо

Obrigado

Σας ευχαριστούμε

Gracias

Danke