Method Development and Validation for Particle Size and Shape Measurements

Ulf Willén
Divisional Product Manager
Analytical Imaging Systems
Malvern Instruments Ltd, Malvern, UK.
3.3.1 New Drug Substances

(b) Particle size: For some new drug substances intended for use in solid or suspension drug products, particle size can have a significant effect on dissolution rates, bioavailability, and/or stability. In such instances, testing for particle size distribution should be carried out using an appropriate procedure, and acceptance criteria should be provided.

Decision Tree #3 provides additional guidance on when particle size testing should be considered.

*Federal Register/Vol. 65, No.251, Friday December 29, 2000/ Notices p. 83041-83054
FDA guidance: when should particle size be measured?

Solid dosage form or liquid with undissolved drug?

Yes

Critical to dissolution, solubility, bioavailability?

Critical to drug product processability?

Critical to product stability?

Critical to product content uniformity?

Yes to any?

Set acceptance criterion

No to all?

No acceptance criterion necessary

No drug substance particle size criterion required for solution dosage forms

No
Comparing techniques: assessing different technologies

Figure 1. Criticalities in the PSD analysis of nano- and submicron liquid dispersions

A.P. Tinke, Ph.D., R. Govoreanu, Ph.D., and K. Vanhoutte, Ph.D.
Johnson & Johnson Pharmaceutical Research and Development

American Pharmaceutical Review
Comparing techniques: assessing different technologies

<table>
<thead>
<tr>
<th>Technology</th>
<th>nm</th>
<th>μm</th>
<th>Shape</th>
<th>PSD</th>
<th>Homogeneity</th>
<th>Interactive State</th>
<th>Dynamics</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser Diffraction (traditional)</td>
<td></td>
<td></td>
<td>🍓</td>
<td>🧵</td>
<td>🎨</td>
<td>🌈 🌈 🌈 🌈</td>
<td>🌈 🌈 🌈</td>
<td>🌈 🌈 🌈 🌈</td>
</tr>
<tr>
<td>Laser Diffraction (extended range)</td>
<td></td>
<td></td>
<td>🍓</td>
<td>🧵</td>
<td>🎨</td>
<td>🌈 🌈 🌈 🌈</td>
<td>🌈 🌈 🌈</td>
<td>🌈 🌈 🌈 🌈</td>
</tr>
<tr>
<td>Dynamic Light Scattering</td>
<td></td>
<td></td>
<td>🍓</td>
<td>🧵</td>
<td>🎨</td>
<td>🌈 🌈 🌈 🌈</td>
<td>🌈 🌈 🌈</td>
<td>🌈 🌈 🌈 🌈</td>
</tr>
<tr>
<td>Back Scattering</td>
<td></td>
<td></td>
<td>🍓</td>
<td>🧵</td>
<td>🎨</td>
<td>🌈 🌈 🌈 🌈</td>
<td>🌈 🌈 🌈</td>
<td>🌈 🌈 🌈 🌈</td>
</tr>
<tr>
<td>Centrifugal Sedimentation</td>
<td></td>
<td></td>
<td>🍓</td>
<td>🧵</td>
<td>🎨</td>
<td>🌈 🌈 🌈 🌈</td>
<td>🌈 🌈 🌈</td>
<td>🌈 🌈 🌈 🌈</td>
</tr>
<tr>
<td>Hydrodynamic Chromatography</td>
<td></td>
<td></td>
<td>🍓</td>
<td>🧵</td>
<td>🎨</td>
<td>🌈 🌈 🌈 🌈</td>
<td>🌈 🌈 🌈</td>
<td>🌈 🌈 🌈 🌈</td>
</tr>
<tr>
<td>Scanning Electron Microscopy</td>
<td></td>
<td></td>
<td>🍓</td>
<td>🧵</td>
<td>🎨</td>
<td>🌈 🌈 🌈 🌈</td>
<td>🌈 🌈 🌈</td>
<td>🌈 🌈 🌈 🌈</td>
</tr>
<tr>
<td>Optical Microscopy (Static)</td>
<td></td>
<td></td>
<td>🍓</td>
<td>🧵</td>
<td>🎨</td>
<td>🌈 🌈 🌈 🌈</td>
<td>🌈 🌈 🌈</td>
<td>🌈 🌈 🌈 🌈</td>
</tr>
<tr>
<td>Acoustic Spectroscopy</td>
<td></td>
<td></td>
<td>🍓</td>
<td>🧵</td>
<td>🎨</td>
<td>🌈 🌈 🌈 🌈</td>
<td>🌈 🌈 🌈</td>
<td>🌈 🌈 🌈 🌈</td>
</tr>
<tr>
<td>Near Infrared Spectroscopy</td>
<td></td>
<td></td>
<td>🍓</td>
<td>🧵</td>
<td>🎨</td>
<td>🌈 🌈 🌈 🌈</td>
<td>🌈 🌈 🌈</td>
<td>🌈 🌈 🌈 🌈</td>
</tr>
</tbody>
</table>
Better Characterisation - Size and Shape

Size Only

Size and Shape

- Large Primary Particles
- Aggregates (Low circularity)
Convexity - Dissolution behaviour

- Lower convexity – higher surface area
- Increased surface area – faster dissolution
Laser Diffraction & Optical Imaging

Optical Microscopy
(USP <776>, ISO 13322-1)

Laser Diffraction
(USP <429>, EP 2.9.31, ISO13320-1)
What information should be included in the particle size specification?

**ICH Q6A Guidance**

- Analytical Procedure
  (system suitability, sampling, dispersion, etc.)
- Method Validation
  (precision, ruggedness, dispersion stability, robustness, etc.)
- Acceptance Criteria
  (upper and lower limits)
The normal concepts of validation may differ for particle size methodologies as compared to other analytical methodologies such as HPLC.

The system should be calibrated according to the manufacturers and/or the laboratory’s specification, as appropriate.

The methods validation usually involves evaluation of intermediate precision and robustness.

Assurance should be provided that the data generated are reproducible and control the product’s quality.

*Section F: Methodologies relating to particle size analysis;
“However, it must be realised that particle size analysis is not an objective in itself but is a means to an end, the end being the correlation of powder properties with some process of manufacture, usage or preparation”

FDA guidance: why should accuracy not be assessed?

- Accuracy can be difficult to define for size analysis
  - Easy for spherical particles
  - For non-spherical particles all sizing techniques give different answers.

- For laser diffraction:
  - You do need to verify the system
    - See ISO13320 / USP<429> / EP 2.9.31 for details
  - Microscopy is the most important referee method
Method development and validation: PASG
definition of sample preparation*

“The pre-treatment and the presentation of the sample to the measuring technique in a meaningful manner.”

Need to consider

- How the primary sample is obtained
- How the material is dispersed
  - Wet or dry dispersion?
  - Dispersed or agglomerated state?

Sample presentation: what do we need to consider?

“Novices in the size measurement field must understand that most errors in size measurement arise through poor sampling and dispersion and not through instrument inadequacies.”

Sampling: particle segregation

Classification of particles in transit

The large particles in a bag of powder migrate to the top in transit.

As the bag vibrates in transit

Large particles part to allow fines to fall through

...and then close together over the fines - being lifted in the process
# Sampling: typical errors associated with different techniques

<table>
<thead>
<tr>
<th>Method</th>
<th>Estimated max error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cone &amp; Quartering</td>
<td>22.7</td>
</tr>
<tr>
<td>Scoop Sampling</td>
<td>17.1</td>
</tr>
<tr>
<td>Table Sampling</td>
<td>7.0</td>
</tr>
<tr>
<td>Chute Riffler</td>
<td>3.4</td>
</tr>
<tr>
<td>Spinning Riffler</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Sampling: using a spinning riffler

- Sample stream from a vibrating hopper
- Sample pots on a revolving tray
Sampling: riffled sample measurements

- **Dv10**: 2.8%
- **Dv50**: 2.9%
- **Dv90**: 1.4%
Sampling: obtaining unbiased samples from slurry systems

- Stirrer
- Isokinetic sampling probe
- Flow baffles

Speed of sample rise matches sample extraction velocity
Sample preparation: product form
Sample presentation: force of adhesion / cohesion between particles

Sample presentation: affect of sonication on the particle size reported by laser diffraction

![Graph showing the effect of sonication on particle size](image-url)
Using image analysis as a referee method

After inappropriate level of sonication – broken particles

With reduced level of sonication – no broken particles
Using image analysis as a referee method

- Pharmaceutical dispersed in cyclohexane
- With no surfactant a high level of agglomeration is observed

Wide size and shape distributions

Images show high degree of agglomeration
Using image analysis as a referee method

- Narrower and smoother size and shape distributions
- Images confirm individual particles and little agglomeration
- Same sample but with addition of lecithin
Sample presentation: changes in size as a function of pressure
Sample presentation: changes in size as a function of pressure – microscopy as a referee technique.

High Pressure – Increased proportion of fines less large material

Low Pressure – More large material fewer fines
Sample presentation: changes in size as a function of pressure – microscopy as a referee technique

Pressure titration against Particle Size and Aspect ratio

- Increased pressure = reduced Size
- Increased pressure = Increased Aspect Ratio
Sample presentation: changes in size as a function of pressure – microscopy as a referee technique

Low pressure dispersion – example images of largest particles – long, low aspect ratio (needle like)

High pressure dispersion – example images of largest particles - short, high aspect ratio.
Using image analysis as a referee method

<table>
<thead>
<tr>
<th></th>
<th>Dv10 / Microns</th>
<th>Dv50 / Microns</th>
<th>Dv90 / Microns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mastersizer 2000</td>
<td>1.03</td>
<td>2.31</td>
<td>5.00</td>
</tr>
<tr>
<td>Morphologi G3</td>
<td>1.44</td>
<td>2.66</td>
<td>5.30</td>
</tr>
</tbody>
</table>
Sample presentation: using image analysis as a referee method

![Graph showing particle size distribution](image-url)
Sample presentation: using image analysis as a referee method

- **USP<792>:** ‘For irregularly shaped particles, characterisation of particle size must include information on particle shape.'
Using image analysis as a referee method: verifying optical properties
Comparing imaging and diffraction: verifying optical properties
Method development: available guidance for laser diffraction measurements

- **ISO13320-1: Section 6.4**
  - Dv50 - 5 different readings: COV < 3%
  - Dv10 and Dv90: COV < 5%
  - “Below 10μm, these maximum values should be doubled.”

- **USP <429>**
  - Provides reproducibility ranges
  - Dv50 or any central value: <10%
  - Dv10, Dv90 or any non-central value: <15%
  - “Below 10μm, these maximum values should be doubled.”

- **EP 2.9.31** provides similar advice to USP<429>
Method validation: precision for excipient measurements using laser diffraction

- Measurements of multiple samples (n≥6) by a single operator
- RSD within USP <429> and ISO13320 limits for laser diffraction
  - variation in Dv10 due to dispersion
  - variation in Dv90 due to sampling
    - Scoop sampling used in this case….
Method validation: intermediate precision for excipient measurements using laser diffraction

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Dv10 / μm</th>
<th>Dv50 / μm</th>
<th>Dv90 / μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.06</td>
<td>22.92</td>
<td>61.01</td>
</tr>
<tr>
<td>2</td>
<td>1.08</td>
<td>22.08</td>
<td>56.54</td>
</tr>
<tr>
<td>3</td>
<td>1.04</td>
<td>21.66</td>
<td>62.17</td>
</tr>
<tr>
<td>4</td>
<td>0.97</td>
<td>22.55</td>
<td>60.23</td>
</tr>
<tr>
<td>5</td>
<td>1.04</td>
<td>22.74</td>
<td>57.98</td>
</tr>
<tr>
<td>6</td>
<td>0.99</td>
<td>23.58</td>
<td>59.86</td>
</tr>
<tr>
<td>7</td>
<td>0.95</td>
<td>22.11</td>
<td>62.78</td>
</tr>
<tr>
<td>Mean</td>
<td>1.02</td>
<td>22.52</td>
<td>60.08</td>
</tr>
<tr>
<td>COV (%)</td>
<td>4.79</td>
<td>2.83</td>
<td>3.69</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pooled Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Dv50 / μm</td>
<td>22.85</td>
</tr>
<tr>
<td>Standard Deviation COV (%)</td>
<td>0.68</td>
</tr>
<tr>
<td>COV (%)</td>
<td>2.98</td>
</tr>
</tbody>
</table>

Pooled RSD for both analysts is within the USP<429> and ISO13320 limits.
Method validation: reproducibility

- Should now go on to test reproducibility
- Defined as the precision between laboratories*

- Modern sizing systems can store and replay measurement procedures
  - method files can then be emailed to other sites
- Main challenge is related to the control of the laboratory conditions and dispersant quality

Microscopy - how many particles do I need to measure?

- **ISO 13322-1** Particle size analysis – Image analysis methods – Part1: Static image analysis methods - proposes a method to evaluate minimum number of particles to achieve certain confidence of mass median diameter (Dv50) being within a certain statistical error.

Table A.2(a)- Number of particles required $n^*$, $\delta = 0.05, p = 0.95, (u = 1.96)$

<table>
<thead>
<tr>
<th>$\delta$</th>
<th>GSD</th>
<th>$n^*$ (MMD)</th>
<th>$n^*$ (Sauter)</th>
<th>$n^*$ (MVD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>1.10</td>
<td>585</td>
<td>389</td>
<td>131</td>
</tr>
<tr>
<td>1.15</td>
<td>1</td>
<td>1 460</td>
<td>934</td>
<td>294</td>
</tr>
<tr>
<td>1.20</td>
<td>1</td>
<td>2 939</td>
<td>1 808</td>
<td>528</td>
</tr>
<tr>
<td>1.25</td>
<td>1</td>
<td>5 223</td>
<td>3 103</td>
<td>843</td>
</tr>
<tr>
<td>1.30</td>
<td>1</td>
<td>8 526</td>
<td>4 920</td>
<td>1 247</td>
</tr>
<tr>
<td>1.35</td>
<td>1</td>
<td>13 059</td>
<td>7 355</td>
<td>1 750</td>
</tr>
<tr>
<td>1.40</td>
<td>1</td>
<td>19 026</td>
<td>10 504</td>
<td>2 363</td>
</tr>
<tr>
<td>1.45</td>
<td>1</td>
<td>26 617</td>
<td>14 457</td>
<td>3 096</td>
</tr>
<tr>
<td>1.50</td>
<td>1</td>
<td>36 007</td>
<td>19 295</td>
<td>3 956</td>
</tr>
<tr>
<td>1.55</td>
<td>1</td>
<td>47 358</td>
<td>25 093</td>
<td>4 952</td>
</tr>
<tr>
<td>1.60</td>
<td>1</td>
<td>60 811</td>
<td>31 919</td>
<td>6 092</td>
</tr>
</tbody>
</table>

- Maths can be reduced to one input: standard deviation
- Example: Sample with GSD of 1.6 needs 61,000 particles to achieve mass median diameter within 5% error with 95% probability.
Using image analysis as a referee method
Morphologi G3 – Automated Particle Image Analyser
Mixture of API and Starch

Volume transformation: CE Diameter (µm) smoothed over 50 points

Record 2: API Starch 1 classed
Mixture of API and Starch

- Starch - only
  - High circularity
  - Low elongation
Mixture of API and Starch

- API - only
  - High elongation
  - Low circularity
Mixture of API and Starch

CE Diam. (vol) for Mixture, API(39%) and Starch (61%)
Conclusions

- Need to consider the Precision, Intermediate Precision and Robustness of measurements during method development and validation.

- Requires an understanding of how the sampling and dispersion is achieved.

- Need to ensure that the sample preparation method is reasonable in terms of predicting the properties of the product being tested.

- Remember to look for specific guidance relating to the expected precision of the measurements.
Where can I find out more?

- www.malvern.com
  - Product information
  - Application Notes
  - Live Webinars
  - On-demand Presentations
  - Useful Links