Particle Size Analysis in the Pharmaceutical Industry

Mark Bumiller
Outline

- Why is particle size important?
  - Relationship between particle size and dissolution, absorption and content uniformity
- Do I need to set a specification?
- Recent recommendations from PQRI
- Particle sizing techniques
  - Microscopy/image analysis, dynamic light scattering, laser diffraction, acoustic attenuation
    - ISO standards & USP <429>
Monitor Particle Processes

- Milling/size reduction
- Mixing/blending
- Separation
- Filtration
- Granulation
- Homogenization
- Crystallization

Narrow particle size distributions minimize segregation problems during mixing – more homogeneous distribution of components in final product.
Particle Size and Dissolution

Equation 1.

$$\frac{dX_S}{dt} = -\frac{3DX_0^{1/3}X_S^{2/3}}{\rho hr_0} \left( C_S - \frac{X_d}{V} \right)$$

XS is the mass of solid drug (mg),
t is time (minutes),
D is the drug diffusivity (cm²/min),
X₀ is the initial drug mass (mg),
r is the drug density (mg/mL),
h is the diffusion layer thickness (cm),
\( r_0 \) is the initial particle radius (cm),
CS is the drug solubility (mg/mL),
Xd is the mass of dissolved drug (mg),
V is the volume of dissolution media (mL).

David R. Friend, PhD; Gregory E. Parry, PhD; T. Francis, PhD; Gary Kupperblatt, PhD; Suggy S. Chrai, PhD; and Gerald Slack,
Mathematical Modeling of a Novel Controlled-Release Dosage Form
Drug Delivery Technology,
Content Uniformity

good  good  good  bad
<table>
<thead>
<tr>
<th>Tablets</th>
<th>Suspensions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of active ingredient effects</td>
<td>Dissolution and absorption</td>
</tr>
<tr>
<td>content uniformity</td>
<td>Content uniformity</td>
</tr>
<tr>
<td>Size influences</td>
<td>Ability to stay in suspension</td>
</tr>
<tr>
<td>tablet hardness</td>
<td>Feel in mouth</td>
</tr>
<tr>
<td>Size and shape effects packing</td>
<td></td>
</tr>
<tr>
<td>Size and shape effect powder flow</td>
<td></td>
</tr>
</tbody>
</table>
Excipients

- Particle size and physical characteristics critical in selection and performance

<table>
<thead>
<tr>
<th>HPC Grade</th>
<th>SSL</th>
<th>SL</th>
<th>L</th>
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</thead>
<tbody>
<tr>
<td>Material</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose (g)</td>
<td>700</td>
<td>700</td>
<td>700</td>
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<tr>
<td>Corn starch (g)</td>
<td>300</td>
<td>300</td>
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<tr>
<td>8% HPC aqueous solution (g)</td>
<td>375</td>
<td>375</td>
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<tr>
<td>Property of granule</td>
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</tr>
<tr>
<td>Particle size distribution (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1400μ on</td>
<td>0.2</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>500μ</td>
<td>0.2</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>355μ</td>
<td>0.4</td>
<td>0.6</td>
<td>1.7</td>
</tr>
<tr>
<td>250μ</td>
<td>1.3</td>
<td>2.1</td>
<td>8.4</td>
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<tr>
<td>180μ</td>
<td>4.0</td>
<td>6.0</td>
<td>14.4</td>
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<tr>
<td>150μ</td>
<td>8.0</td>
<td>9.6</td>
<td>15.6</td>
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<tr>
<td>106μ</td>
<td>22.1</td>
<td>22.1</td>
<td>24.7</td>
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<tr>
<td>75μ</td>
<td>30.3</td>
<td>26.9</td>
<td>19.6</td>
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<tr>
<td>75μ under</td>
<td>33.6</td>
<td>32.3</td>
<td>15.0</td>
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<tr>
<td>Bulky density (kg/cm³)</td>
<td></td>
<td></td>
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<tr>
<td>Loose packed</td>
<td>90</td>
<td>95</td>
<td>130</td>
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<tr>
<td>Packed</td>
<td>0.5</td>
<td>0.47</td>
<td>0.46</td>
</tr>
<tr>
<td>Property of tablet</td>
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<td></td>
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</tr>
<tr>
<td>Hardness (kg)</td>
<td>14</td>
<td>14</td>
<td>12</td>
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<tr>
<td>Friction loss (%)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Disintegration time (min)</td>
<td>6</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>
Included in the January FDA-483 report were observations alleging that the product quality review methods for *unnamed drug* delivery and particle size "were inadequate in that the methods exhibit various unidentified extraneous peaks."

**PRODUCT**
*Unnamed Drug* Inhalation Aerosol with mouthpiece, Net Contents 14g (10 ml), Metered Dose Inhaler, 200 metered doses. Recall # D-840-2007

**CODE**
Lot Numbers: 050912W, expiration date Dec 07 and lot # 060359W, expiration date Jun 08.

**RECALLING FIRM/MANUFACTURER**

**REASON**
*Product failed particle size distribution analysis during stability.*

**VOLUME OF PRODUCT IN COMMERCE**
137,491 canisters
Need a Specification?  Decision Tree 3

- Is the drug product a solid dosage form or liquid containing undissolved drug substance?
  - NO: No drug substance particle size acceptance criterion needed for solution dosage forms.
  - YES:
    1. Is the particle size critical to dissolution, solubility, or bioavailability?
    2. Is the particle size critical to drug product processability?
    3. Is the particle size critical to drug product stability?
    4. Is the particle size critical to drug product content uniformity?
    5. Is particle size critical for maintaining product appearance?
      - NO to all: No Acceptance Criterion Needed
      - YES to any:
        - Set Acceptance Criterion
Preclinical and Phase I*

Scheme for outlining particle evaluation for preclinical studies.

Decision tree outlining particle evaluation for Phase I clinical studies

*PQRI Recommendations on Particle-Size Analysis of Drug Substances Used in Oral Dosage Forms
JOURNAL OF PHARMACEUTICAL SCIENCES, VOL. 96, NO. 6, JUNE 2007
Phase II

Scheme for outlining particle evaluation for Phase III clinical studies
### PQRI Recommendations: Techniques

#### Table 1. Nominal Particle-Size Ranges Measured by Laboratory Sizing Methods

<table>
<thead>
<tr>
<th>Technology</th>
<th>Optimal Particle Shape</th>
<th>Size Range (µm)</th>
<th>Distribution</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acoustic spectroscopy</td>
<td>□</td>
<td>0.01 - 10</td>
<td></td>
<td>Wet</td>
</tr>
<tr>
<td>Chord-length measurement</td>
<td>□</td>
<td>1 - 10000</td>
<td></td>
<td>Wet</td>
</tr>
<tr>
<td>Disc centrifuge</td>
<td>□</td>
<td>0.05 - 100</td>
<td></td>
<td>Wet</td>
</tr>
<tr>
<td>Dynamic image analysis</td>
<td>□</td>
<td>0.05 - 3500</td>
<td></td>
<td>Wet</td>
</tr>
<tr>
<td>Elliptically polarized light scattering</td>
<td>□</td>
<td>0.05 - 100</td>
<td></td>
<td>Wet</td>
</tr>
<tr>
<td>Electrical sensing zone</td>
<td>□</td>
<td>0.4 - 1600</td>
<td></td>
<td>Wet</td>
</tr>
<tr>
<td>Hydrodynamic chromatography</td>
<td>□</td>
<td>0.01 - 50</td>
<td></td>
<td>Wet</td>
</tr>
<tr>
<td>Laser diffraction</td>
<td>□</td>
<td>0.01 - &gt;5000</td>
<td></td>
<td>Wet &amp; dry</td>
</tr>
<tr>
<td>Light obscuration</td>
<td>□</td>
<td>0.5 - 5000</td>
<td></td>
<td>Wet</td>
</tr>
<tr>
<td>Photon correlation spectroscopy</td>
<td>□</td>
<td>0.003 - 3</td>
<td></td>
<td>Wet</td>
</tr>
<tr>
<td>Polarization intensity differential scattering</td>
<td>□</td>
<td>0.04 - 0.4</td>
<td></td>
<td>Wet</td>
</tr>
<tr>
<td>Sieve analysis</td>
<td>□</td>
<td>5 - 10000</td>
<td></td>
<td>Wet &amp; dry</td>
</tr>
<tr>
<td>Scanning electron microscopy</td>
<td>□</td>
<td>0.001 - 5</td>
<td></td>
<td>Wet &amp; dry</td>
</tr>
<tr>
<td>Static image analysis (optical microscopy)</td>
<td>□</td>
<td>0.3 - 500</td>
<td></td>
<td>Wet &amp; dry</td>
</tr>
</tbody>
</table>

□, spherical; □, blocky; □, acicular; □, flat, tabular, bladed; □, fibrous.
Microscopy in the Pharm Industry

- Microscopy used for many years
  - USP <776> “Optical Microscopy”
- USP <788> for parenterals
  - Light obscuration or microscope
- Home made, components since 1985
- Automated image analysis since ~1995
- New generations vastly superior
  - Computer speed, cameras, software
USP <776> Terms

**Acicular**—Slender, needle-like particle of similar width and thickness.

**Columnar**—Long, thin particle with a width and thickness that are greater than those of an acicular particle.

**Flake**—Thin, flat particle of similar length and width.

**Plate**—Flat particles of similar length and width but with greater thickness than flakes.

**Lath**—Long, thin, and blade-like particle.

**Equant**—Particles of similar length, width, and thickness; both cubical and spherical particles are included.
Don’t use words to describe particle shape
Quantify morphology through image analysis

Two Approaches

Dynamic:
particles flow past camera

Static:
particles fixed on slide, stage moves slide
Multi-particulates, Dynamic IA

- Inert Core
- Drug + Binder
- Polymer Coating
- Drug release

Heinicke et al., *Pharm Dev Tech*, 10, 85, 2005
Drug Layering*

13% Weight gain

Sphericity = 0.96

15% Weight gain

*Heinicke et al., Pharm Dev Tech, 10, 85, 2005
Drug Layering*

Heinicke et al., Pharm Dev Tech, 10, 85, 2005
Pharmaceutical Applications

Sugar Spheres

Coating thickness

Globules

20% < 0.98 Roundness
1% < 0.98 Roundness
Static Image Analysis: PSA300

- Release vacuum
- No point of impact
- Software controlled
- Break up agglomerates
- Don’t break fragile particles
- Even distribution on slide

Undispersed vs. dispersed samples
Image Processing

- Erosion: remove smaller valleys
- Dilation: add
- Closing: both smaller valleys filled

Delineation: increase contrast at edges

Tool box to improve images before assigning size & shape values
Image Processing

Multi-layer grab

Contrast Thresholding

Convex hull
Fiber Separation

Acicular Separation
Each fiber has its own bitplane
Actually measure longest diameter
Screen Excipients: Magnesium Stearate

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Diameter (mic.)</th>
<th>Main Length (mic.)</th>
<th>Sphericity</th>
<th>Roundness</th>
<th>Aspect Ratio</th>
<th>Spherical Volume (mic.(^3))</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pattern 1</td>
<td>3.49</td>
<td>4.33</td>
<td>0.92</td>
<td>0.61</td>
<td>1.55</td>
<td>49.09</td>
<td>18435</td>
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<tr>
<td>Pattern 2</td>
<td>3.75</td>
<td>4.65</td>
<td>0.91</td>
<td>0.61</td>
<td>1.55</td>
<td>63.53</td>
<td>21669</td>
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<tr>
<td>Pattern 3</td>
<td>3.65</td>
<td>4.54</td>
<td>0.91</td>
<td>0.60</td>
<td>1.55</td>
<td>54.37</td>
<td>30720</td>
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<tr>
<td>Pattern 4</td>
<td>3.64</td>
<td>4.51</td>
<td>0.91</td>
<td>0.61</td>
<td>1.55</td>
<td>58.67</td>
<td>21809</td>
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<tr>
<td>Pattern 5</td>
<td>3.08</td>
<td>3.61</td>
<td>0.93</td>
<td>0.60</td>
<td>1.58</td>
<td>29.61</td>
<td>27605</td>
</tr>
</tbody>
</table>

| Std Dev: | 0.26           | 0.33               | 0.01       | 0.00      | 0.01        | 13.07                         |
| Mean:    | 3.52           | 4.37               | 0.91       | 0.60      | 1.55        | 51.13                         | 24048  |
| %RSD:    | 7.50           | 7.59               | 1.00       | 0.30      | 0.79        | 25.55                         |
Active Ingredients

Statistics
Minimum: 0.3 µm
Maximum: 117.7 µm
D[4,3]: 52.3 µm
Std Dev.: 34.6 µm
Sum: 55329657.7 µm
Count: 11208
Under: 0
Over: 0
Accepted: 100.0%
Field Count: 400
Field Area: 183702.5 µm²
Total Area: 73480997.2 µm²
D10: 15.9 µm
D50: 40.1 µm
D90: 100.0 µm
Recommendations
Of the possible validation headings discussed above, the following items are considered significant and should be included in a particle sizing validation report:
• the procedure (or reference to it)
• precision
• range (suitability assessment including microscopic comparison)
• robustness.

*Bell, Dennis, Hendriksen, North, Sherwood, Position Paper on Particle Sizing: Sample Preparation, Method Validation and Data Presentation, Pharmaceutical Technology Europe, November 1999
Contamination: Image Analysis

Effective filtration diameter of membrane filter: 47mm
Area of single field: 3584998 \( \mu \text{m}^2 \)
Number of fields analyzed: 314

<table>
<thead>
<tr>
<th>Method of analysis</th>
<th>Tiling</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0-15</td>
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<tr>
<td>Dark particles</td>
<td>705</td>
</tr>
<tr>
<td>Rust particles</td>
<td>951</td>
</tr>
<tr>
<td>Total count</td>
<td>1,656</td>
</tr>
<tr>
<td>Total count, all</td>
<td>12,713</td>
</tr>
</tbody>
</table>
Particles in suspension undergo Brownian motion due to bombardment by solvent molecules in random thermal motion.

Nanoparticles attached to red blood cells
Dynamic Light Scattering: 1 nm – 1 µm

Particles in suspension undergo **Brownian motion** due to solvent molecule bombardment in random thermal motion.

- **Brownian Motion**
  - Random
  - Related to Size
  - Related to viscosity
  - Related to temperature
**Diffusion**

- Particle is randomly diffusing
  - Larger particles will diffuse more slowly
  - Larger particles have more Inertia
- Scatter light off this diffusing particle
- Measure the Frequency Shift of the signal

![Diagram of laser beam and detector with frequency shifted signal graph]
Dynamic Light Scattering

- Measured frequency-intensity distribution (power spectrum)

- Power spectrum takes form of Lorentz distribution, whose half-value width can be expressed as $2Dq^2$

- All parameters in the half-width are known or measured

- The Diffusion Coefficient D is related to the Particle Size

Stokes-Einstein

$$R_H = \frac{kT}{6\pi \eta D}$$
Liposomes to target tumor growth

- Encapsulates protein
- Functions within body
- Remains stable over time
- Delivers the protein
2mg/mL filtered BSA

- BSA- well characterized protein
- DLS – Can be used to determine the aggregation state of the protein
Protein Aggregation Time Study*

- Unstabilized 10mg/ml lysozyme at pH 2

*Lisa Cole and Ben Burnett at the Florida Institute of Technology
Time Evolution

Stability is influenced by:
- Temperature
- Protein concentration
- pH
- Ionic strength

Aggregation is influenced by:
- Freezing
- Exposure to air
- Interactions with metal surfaces
Acoustic Spectroscopy

- Pulsed electric field applied to sample
- Particles vibrate
- Sound waves converted to size

- ADVANTAGES:
  - Can accommodate high sample concentrations
  - No need to consider complex optical properties

- Applications
  - Emulsions & suspensions
  - Dispersion stability with zeta potential
Dispersion Stability

stable

stable

85nm suspension aggregates at IEP
Laser Diffraction

Particle size 0.01 – 3000 µm

• Converts scattered light to particle size distribution
• Quick, repeatable
• Most common technique
• Suspensions or powders
Low End Sensitivity

30, 40, 50, 70 nm latex standards
Low End Sensitivity

- Sensitivity: small particle detection

30 nm silica
- S.P.Area: 2.0183E+6 (cm$^2$/cm$^3$)
- Mean Size: 0.02990 (µm)
- Variance: 5.0313E-6 (µm$^2$)
- Median Size: 0.03013 (µm)
- Mode Size: 0.0302 (µm)
- Skewness: -0.2901

40 nm latex
- S.P.Area: 1.4253E+6 (cm$^2$/cm$^3$)
- Mean Size: 0.04241 (µm)
- Variance: 1.2759E-5 (µm$^2$)
- Median Size: 0.04214 (µm)
- Mode Size: 0.0422 (µm)
- Skewness: -0.1514
Low End Sensitivity: Cosmetics

- Some (unfounded?) concerns with particles <100nm
- LA-950 good at determining sub 100nm particles
- Software set to display % under any given size
- Data shown left is for skin cream and TiO2 suspension
- Other systems capable of these measurements:
  - DT-1201 acoustic spectrometer
  - LB-550 DLS system
Small Sample Volume (MiniFlow)

Colloidal Silica (weak scatterer)
Median (D50): 35 nm
Sample Amount: 132 mg

Magnesium Stearate
Median (D50): 9.33 μm
Sample Amount: 0.165 mg

Bio-degradable Polymer
Median (D50): 114 μm
Sample Amount: 1.29 mg
Reproducibility: Dry Powder Feeder

Direct flow of powder down to cell rather than turn 90°, then around plastic tube.
Reproducibility: Dry Powder Feeder

- Automatic control of sample feed rate
  - LA-950 monitors amount of sample supplied by the vibratory feeder. Automatic feed back control keeps constant mass flow rate of powder during measurement
  - This is CRITICAL
    - More reproducible, robust
    - No ghost peaks
    - No cutting off results
USP<429> Software

- EP 2.9.31 “Laser Diffraction Measurement of Particle Size”; Lead for this monograph
- Appearance in Pharmacopeial Forum 28, Number 3 2002
- Now in USP 28, NF25
  – in Stage 4 of the harmonization process with the EP and the JP

<table>
<thead>
<tr>
<th>Chapter Title</th>
<th>Summary Status</th>
<th>Most Recent/Proposed PF</th>
<th>Official/Proposed Official Date</th>
<th>Expert Committee</th>
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</thead>
<tbody>
<tr>
<td>&lt;429&gt; LIGHT DIFFRACTION MEASUREMENT OF PARTICLE SIZE</td>
<td>Official in PF</td>
<td>31(4)</td>
<td>USP 27 1S</td>
<td>GC</td>
</tr>
</tbody>
</table>
USP<429> Software

- USP <429>: Replicates
  - May call this reproducibility

- At least three different representative samples from the same batch
  - CV < 10% at median d 50
  - CV < 15% at d10 & d 90

- Can double when below 10 µm

---

<table>
<thead>
<tr>
<th>ID</th>
<th>Material</th>
<th>D50</th>
<th>D10</th>
<th>D90</th>
</tr>
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<tbody>
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<td>14.698</td>
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<td>200707180911077.GBE</td>
<td>Mg Stearate</td>
<td>8.265</td>
<td>4.698</td>
<td>14.678</td>
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<td>Mg Stearate</td>
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<td>4.679</td>
<td>14.563</td>
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<tr>
<td>200707180911109.GBE</td>
<td>Mg Stearate</td>
<td>8.266</td>
<td>4.695</td>
<td>14.722</td>
</tr>
</tbody>
</table>


Standard Deviation: 0.004, 0.011, 0.132

CV (at d10 mean)*100: 0.288, 0.242, 0.896
COV Calculations
Qualification: Accuracy and Repeatability

- Three independent measurements, calculate mean
- X50 <3% “certified range of values”
- X10 & X90 < 5% “certified range of values”
- Also check repeatability
- COV X50 < 3%
- COV X10 & X90 < 5%
Accuracy Calculations

[Image of a software interface showing data on mean size, variance, median size, mode size, standard deviation, chi square, R parameter, distribution graph, cumulative % on diameter, and verification details, including data names, graph type, transmittance, median size, and R parameter values.]
ISO 13320

ISO 13320 has same requirements

Just different specs

EVERY user should
Use these features
Conclusions

- Particle size and shape are critical physical characteristics for pharmaceutical powders & suspensions.

- Laser diffraction most popular technique
  - USP<429> may drive method development

- Image analysis becoming more popular
  - PQRI recommendations suggest use

- Acoustic spectroscopy w/zeta potential useful in formulation