

Aptar Pharma

SEM-EDS and Raman Spectroscopy to
Accelerate Generic Drug Applications

Julie D. Suman, PhD

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Agenda

- Development and Regulatory Opportunities
- Application Overview
 - Raman Spectroscopy
 - Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS)
- Regulatory Expectations
- Case Studies
 - Nasal Sprays
 - Dry Powder Inhalers
 - Inhalation Suspensions



Questions Received from Generic Drug Developers

- Does the sonication or homogenization process produce the right particle size?
- Which particle engineering process produce in vitro similarity with the RLD?
- Can you quantify particles below 2 microns?
- What is the extent of agglomeration?
- How do I apply for a clinical biowaiver?



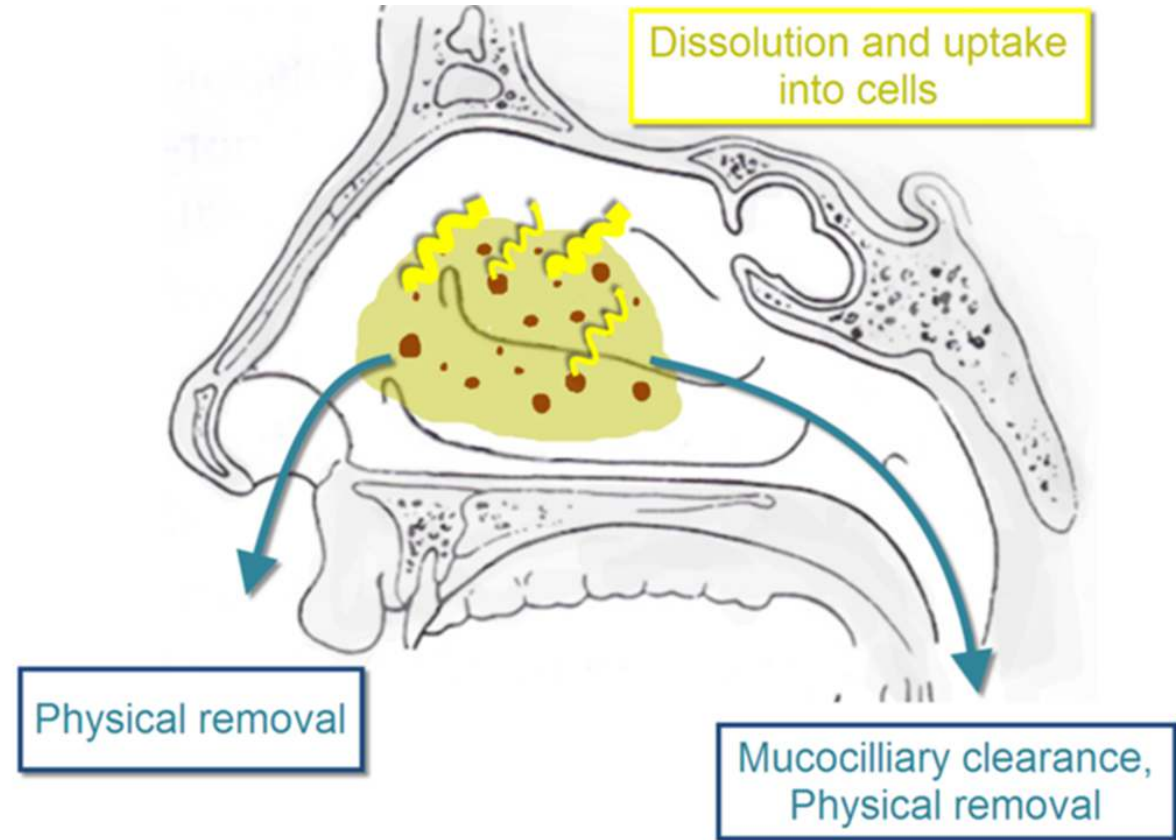
Biowaivers

- Typically considered a waiver of clinical bioequivalence studies
- Commonly used for oral solid dosage forms as classified by the BCS (biopharmaceutics classification system)
- In 2016, FDA accepted data using Raman spectroscopy in lieu of a clinical endpoint study for mometasone nasal spray



Linking Particle Size to Bioequivalence

- Rate of dissolution will potentially affect local activity and extent of absorption
- Impacts local effect
- Impacts bioequivalence
- Ingredient-specific particle size techniques objectively quantify API particle size and agglomerates

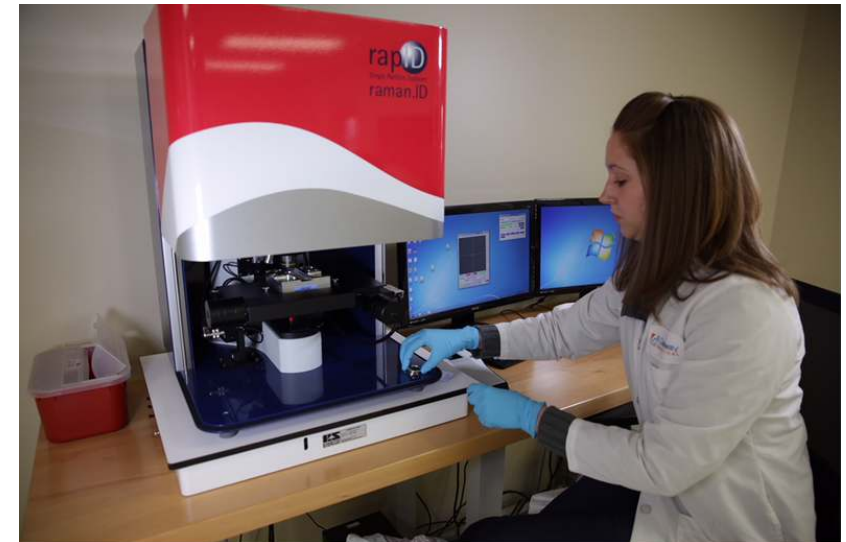


Microscopes on Steroids

- Automated
 - Rapidly analyze large populations of particles
- Identify a particle of interest by elemental or molecular fingerprint
 - Reduce subjectivity
- Quantify size and determine morphology



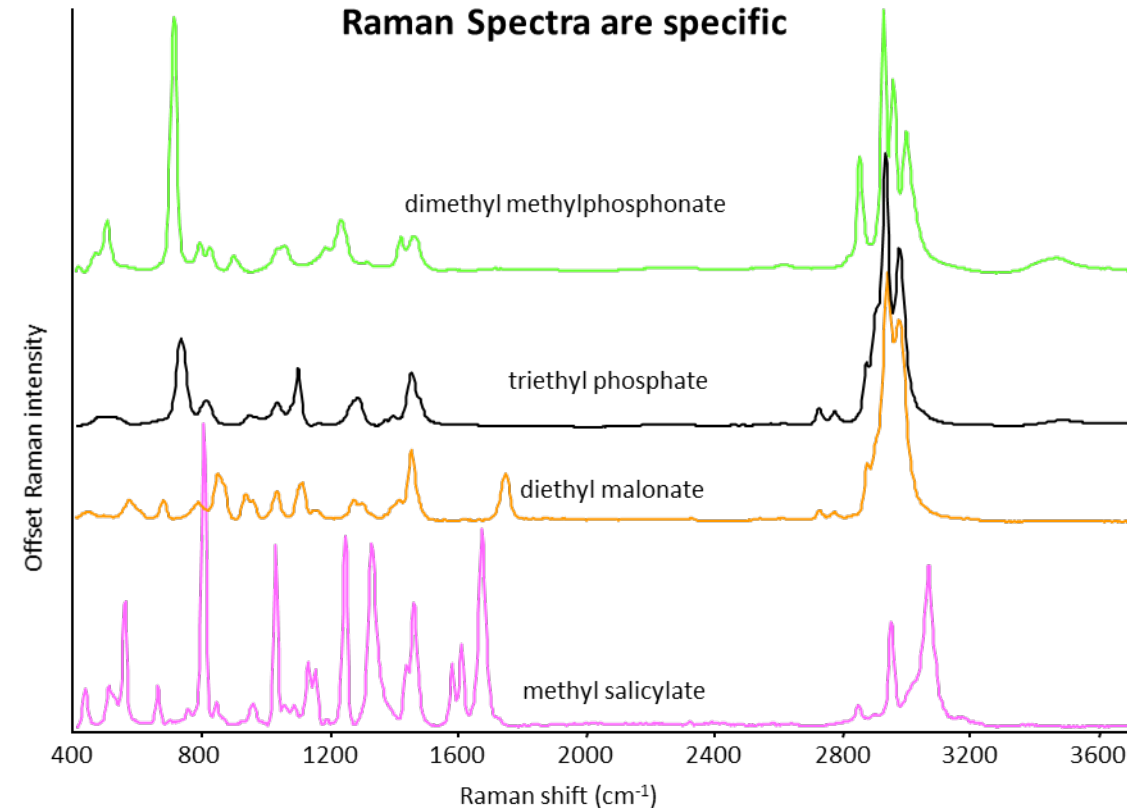
SEM-EDS Analysis at Gateway Analytical



Raman Spectroscopy **Aptar**
pharma

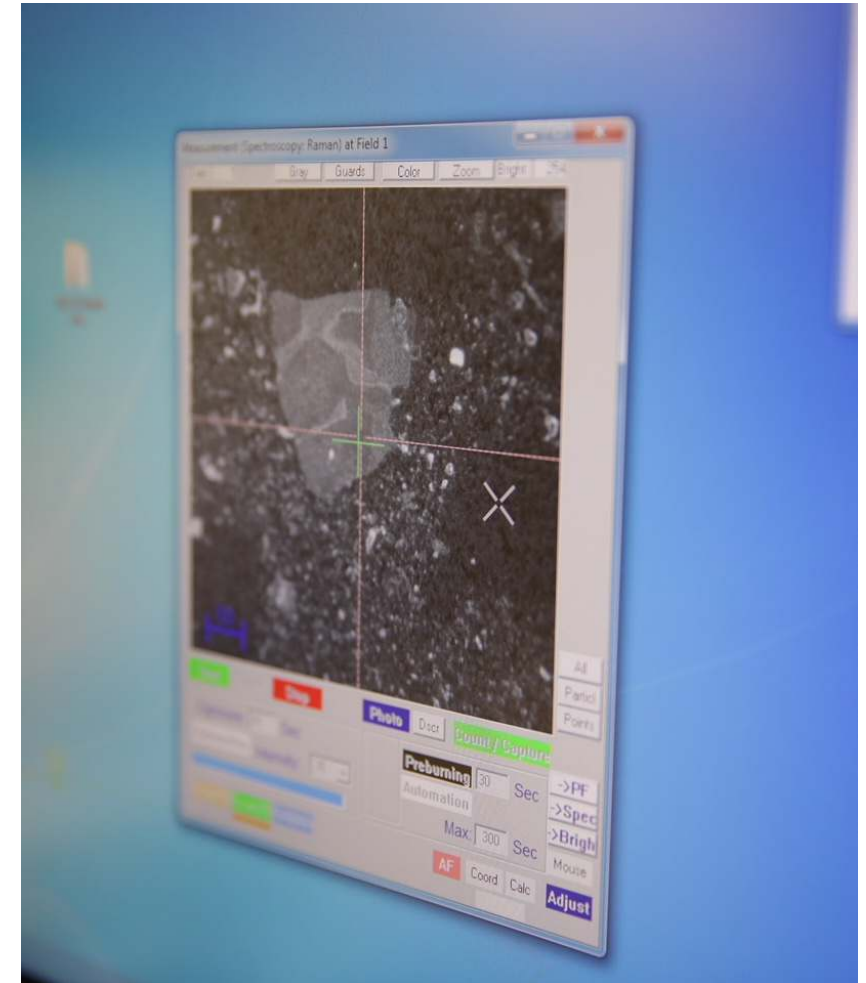
Raman Spectroscopy

- Based on scattering, rather than absorption, of light
- Laser based technique
- Probes energy of molecular vibrations
- Usually nondestructive and noninvasive
- Suitable for aqueous, gaseous and solid samples
- Inorganic and organic material analysis



Automated Raman Spectroscopy with Raman/LIBS

- The Rap.ID Single Particle Explorer (SPE)
- Combines optical microscopy with automated Raman analysis
- Allows for the counting, sizing and identification of an entire population of particles
- Types of analyses:
 - API-specific particle size distribution
 - Polymorphism studies
 - Agglomerates can be measured



API Specific Particle Size

Material	Size Distribution [μm]										
	Totals	≥ 2.0	≥ 3.0	≥ 4.0	≥ 5.0	≥ 6.0	≥ 7.0	≥ 8.0	≥ 9.0	≥ 10.0	≥ 25.0
API – Total Particles	61	14	10	5	9	11	5	1	2	4	0
API – Single Particles	38	14	6	4	4	3	5	1	1	0	0
Agglomerates	23	0	4	1	5	8	0	0	1	4	0
MCC (Avicel)	184	94	40	18	10	8	6	1	1	6	0

SEM-EDS

- A microscope that uses a beam of electrons instead of a beam of light
- The electrons interact with the sample prepped on a substrate such as a carbon-rich filter or aluminum coated slide
- Backscatter signal from API enables differentiation from background
- Allows a threshold to be set to detect the API particles
- Excipients can (generally) be ignored



SEM-EDS

- Measurement of particle size and morphology
- Separation of single particles and agglomerates limited (compared to Raman-SPE)
- Technique allows sub-micron detection

Material	Size Distribution [μm]										Total Particles
	0.2-1	1-2	2-3	3-4	4-5	5-10	10-25	25-50	50-100	≥ 100	
API	13	81	70	68	31	29	4	0	0	0	296

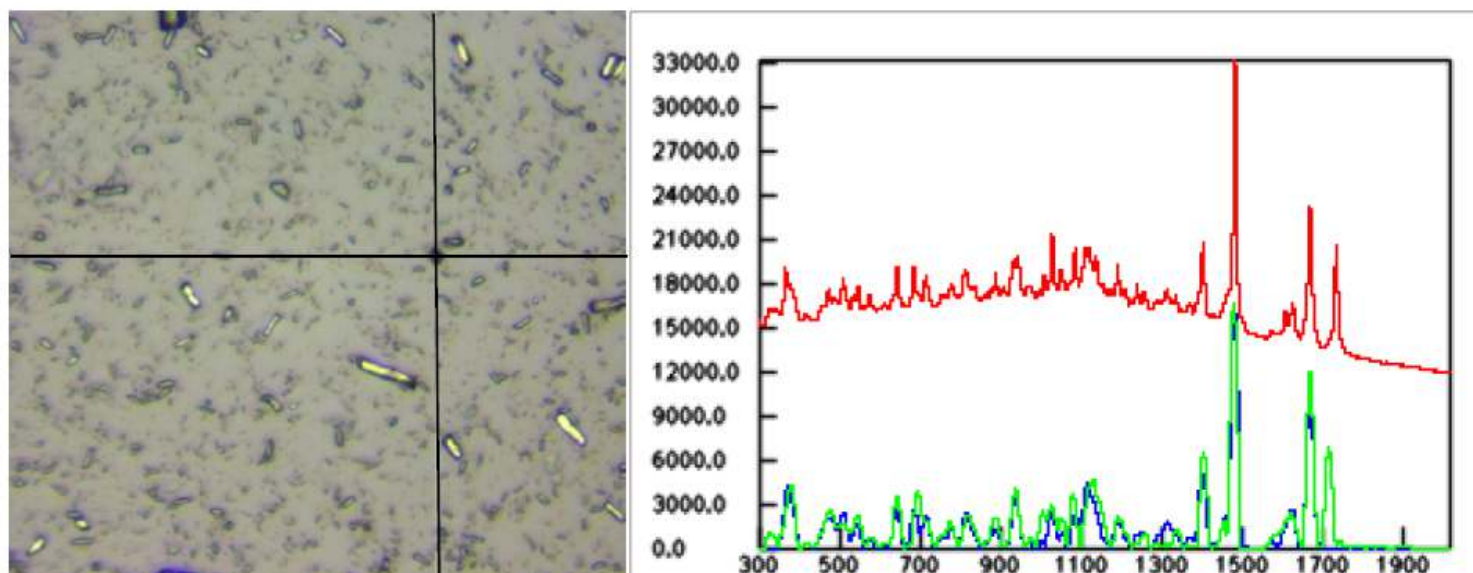
Regulatory Expectations

- Biowaiver applications
 - Request to report particle size results below 2 microns
 - SEM-EDS can offer a solution
- Orthogonal techniques
 - Method validation
 - Biowaiver applications
 - Raman and SEM-EDS supplement each other as well as optical microscopy



Nasal Spray Case Study

- Biowaiver Application-Raman-SPE & SEM-EDS
- Method feasibility
 - Build library of formulation components
 - Assess ability of instrumentation to identify and size API of interest
 - Determine appropriate number of particles to count

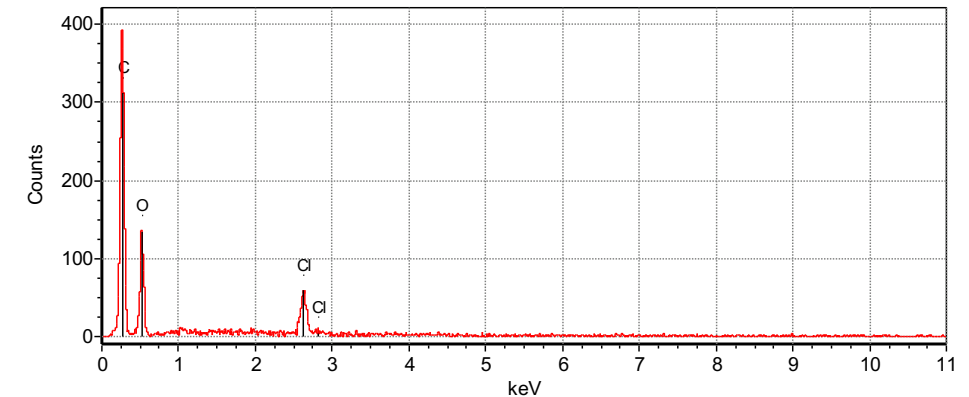
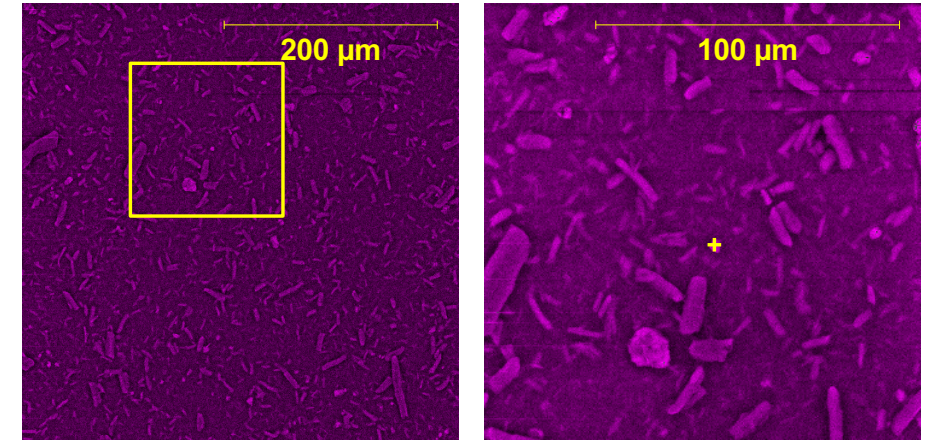


Representative 50x image (center of crosshair) and Raman spectrum collected

Particle Size Distribution

- SEM-EDS-1000 particles counted per sample

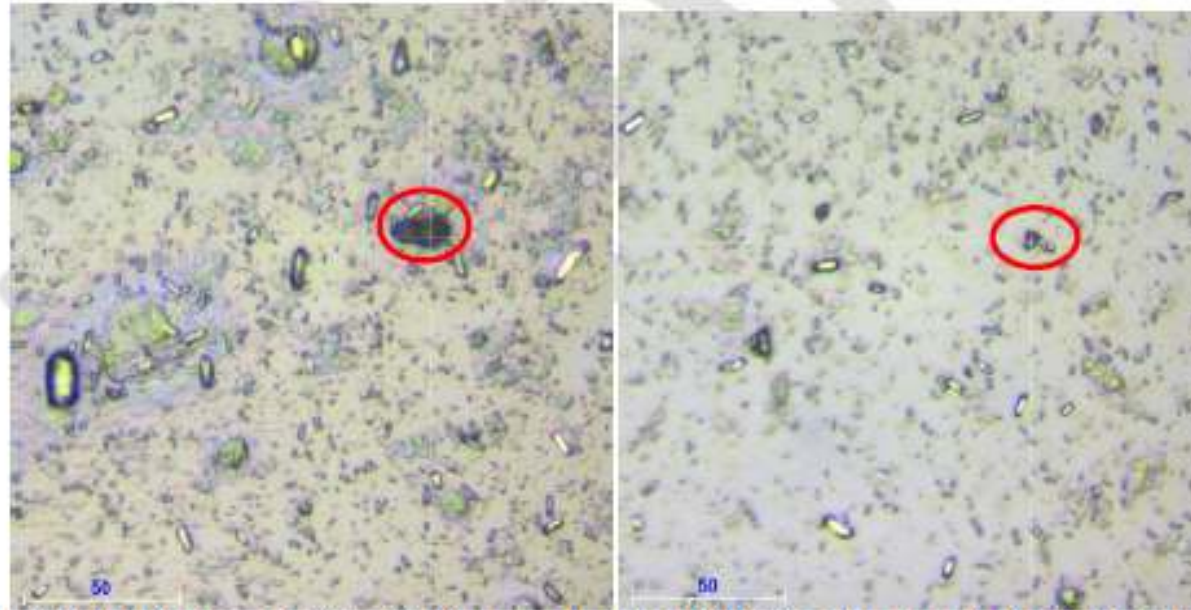
Sample	Test Product			RLD Product		
	D10	D50	D90	D10	D50	D90
1	0.90	1.60	3.00	0.90	1.50	2.60
2	0.90	1.50	2.60	0.90	1.50	2.60
3	0.90	1.40	2.50	0.90	1.50	2.50
Average	0.90	1.50	2.70	0.90	1.50	2.57
SD	0.00	0.10	0.26	0.00	0.00	0.06



Representative SEM-EDS spectrum of Sample. Point, API.

Agglomerates (Raman-SPE)

Material	Size Distribution [μm]										
	Totals	≥ 2.0	≥ 3.0	≥ 4.0	≥ 5.0	≥ 6.0	≥ 7.0	≥ 8.0	≥ 9.0	≥ 10.0	≥ 25.0
Agglomerates	27	0	3	9	6	1	2	2	3	1	0
Single Mometasone Furoate Monohydrate Particles	85	40	20	9	5	3	4	3	0	1	0
API + Agglomerates	112	40	23	18	11	4	6	5	3	2	0



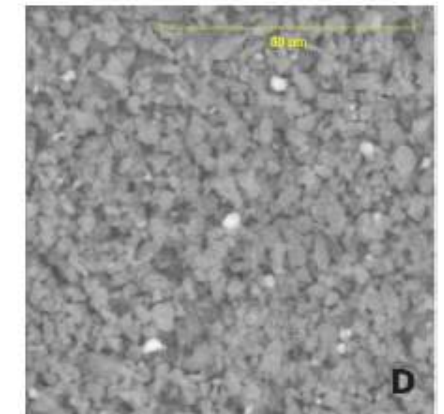
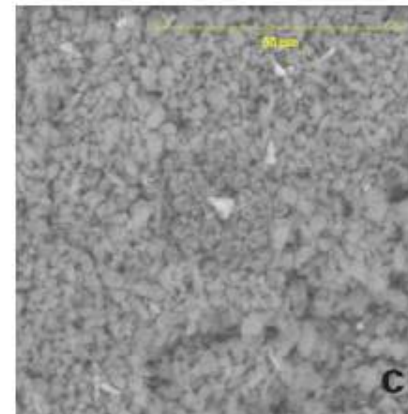
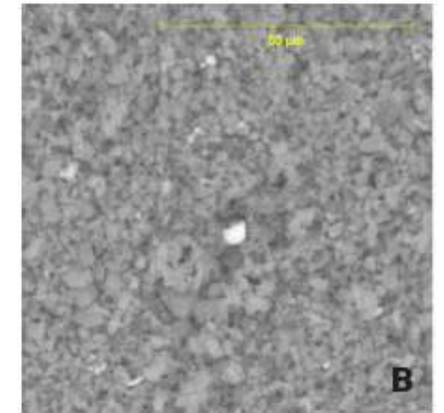
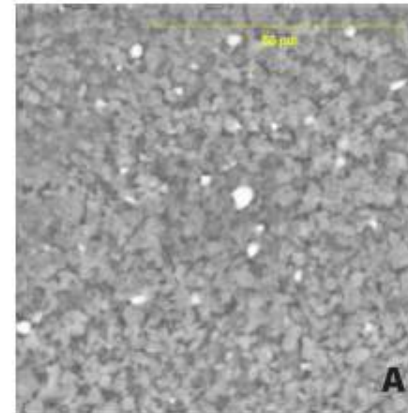
Representative 50x image (center of white crosshair) of agglomerated particles

Sub-Micron Particle Size Analysis (SEM-EDS)

Gateway Analytical Sample #	Material	Size Distribution [μm]- D_{ave}											
		0.2-0.5	0.5-1	1-1.5	1.5-2	2-3	3-4	4-5	5-10	10-25	25-50	50-100	≥ 100
CI-12056A	API	3	154	311	224	213	82	13	0	0	0	0	0
CI-12056B	API	3	145	338	244	233	34	3	0	0	0	0	0
CI-12056C	API	2	181	367	236	168	42	4	0	0	0	0	0

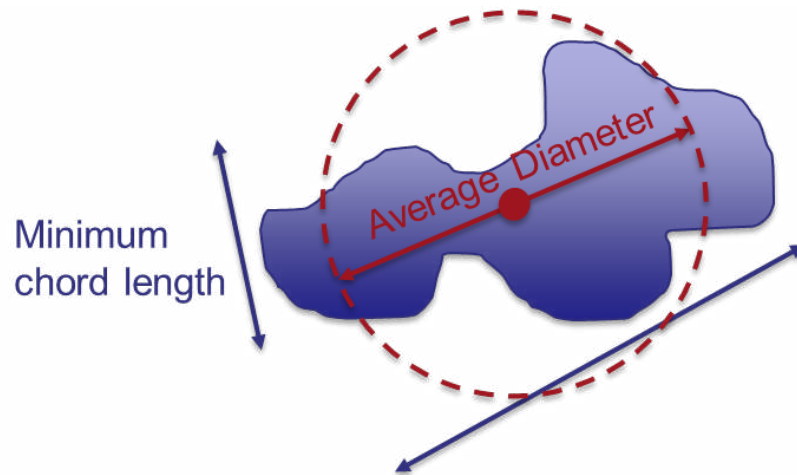
Dry Powder Inhalers

- Application to characterize in vitro similarity
 - RLD capsule based formulation and three test formulations
- Link particle size and morphology to aerodynamic performance
 - SEM-EDS analysis from Next Generation Impactor Stage 4



Morphology Aspects

Metric (µm)	Average Diameter				Aspect Ratio			
	RLD	Micronized	'MMAD'	'MMAD+'	RLD	Micronized	'MMAD'	'MMAD+'
Dv10	0.66	0.67	0.56	0.59	1.29	1.29	1.44	1.48
Dv50	1.01	1.03	0.91	1.01	1.86	1.87	2.78	2.61
Dv90	1.69	1.66	1.64	1.97	3.43	3.59	7.40	6.85



Aspect Ratio = max chord length / perpendicular chord length

Image courtesy of Circassia

Inhalation Suspensions

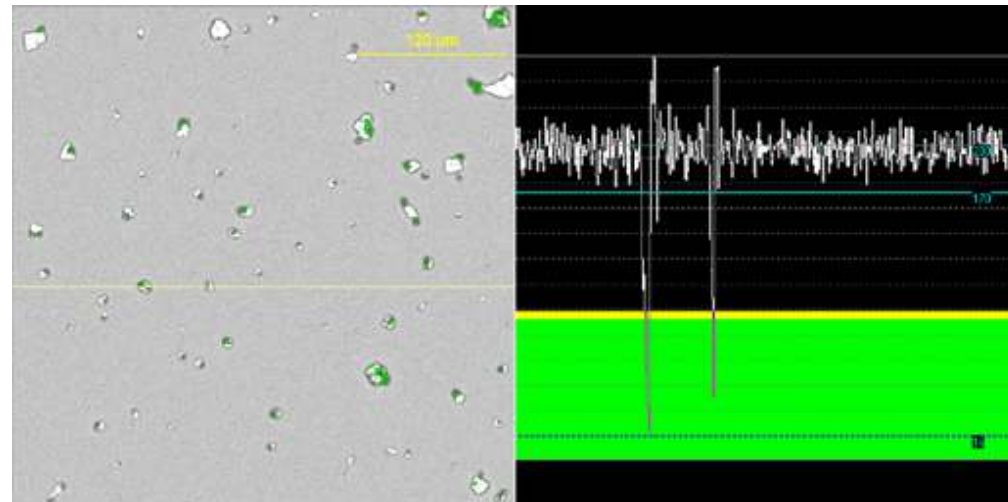
- FDA Draft Guidance on Budesonide
- In Vitro Options for Bioequivalence
 - Unit dose content
 - Mean nebulization time and mean delivered dose
 - Breath simulation per USP <1601>
 - Particle size distribution
 - **FDA emphasis on specificity, sensitivity and agglomerates**
 - Aerodynamic particle size distribution (APSD)
 - Droplet size by Laser Diffraction
- Raman spectroscopy and SEM-EDS offer combined approach to FDA expectations



Pari LC + and Pari Master Compressor

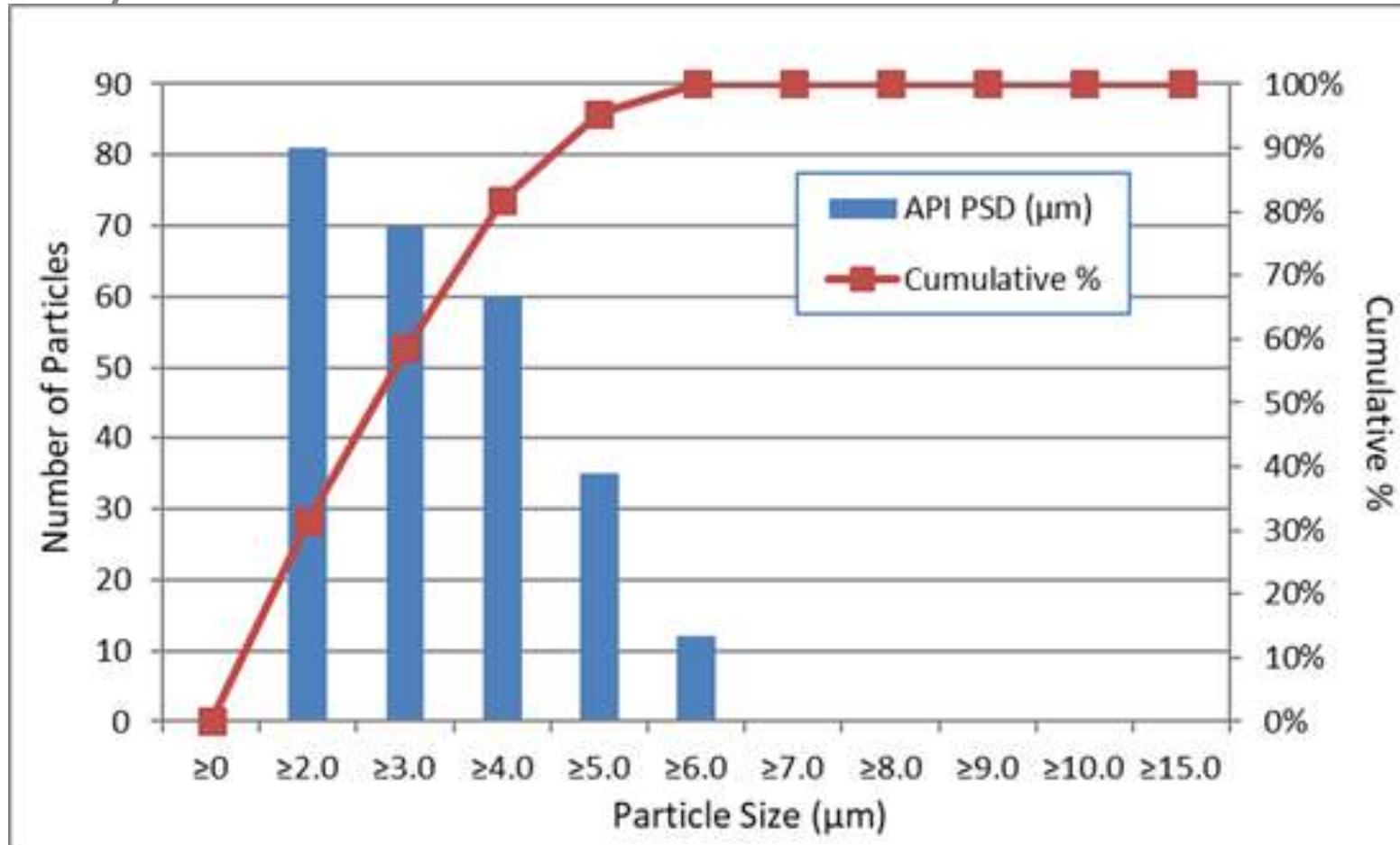
Methodology

- Micronized budesonide is the only solid component in the formulation
- Centrifuged formulation to separate API
- Collect API particles and re-suspend in ultra filtered DI water
- Method feasibility to confirm analysis of particles of interest
 - Raman spectra confirmed
 - SEM-EDS elemental determination (carbon and oxygen)



Setting thresholds for detection using SEM-EDS; black =background; yellow= detected by automated analysis, not measured; green= measured by automated analysis

Budesonide PSD by Raman SPE

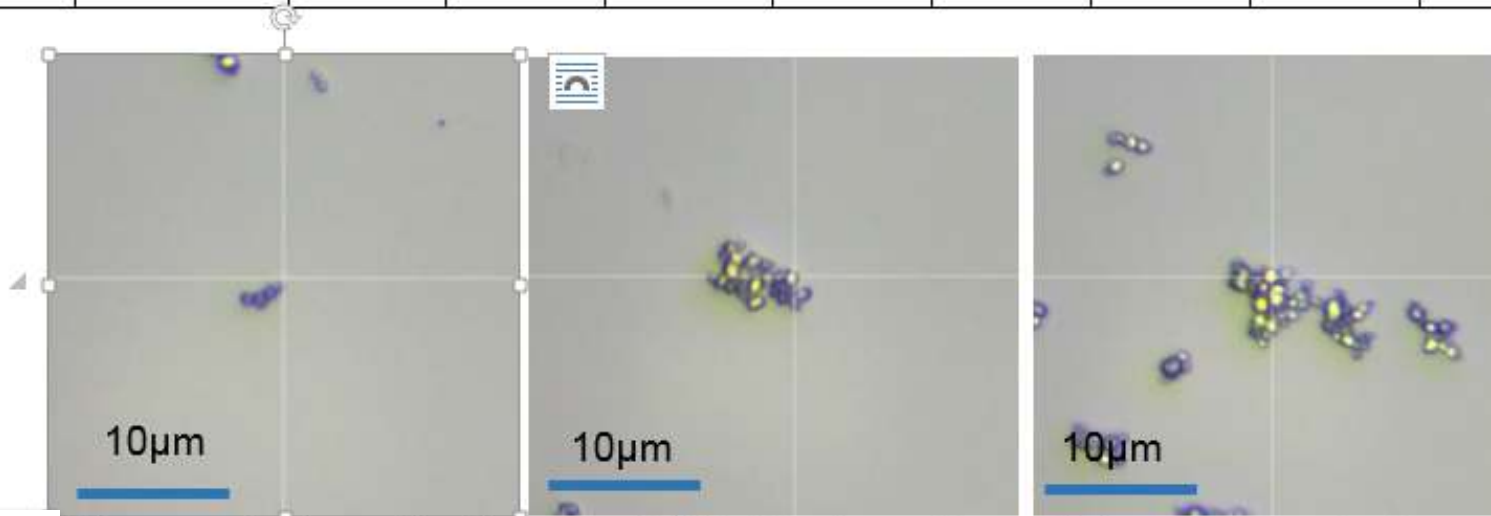


D10 (µm)	D50 (µm)	D90 (µm)
2.37	3.65	5.53

Agglomerates

Table 3. Particle Size Distribution of Particles in Suspension, based on length

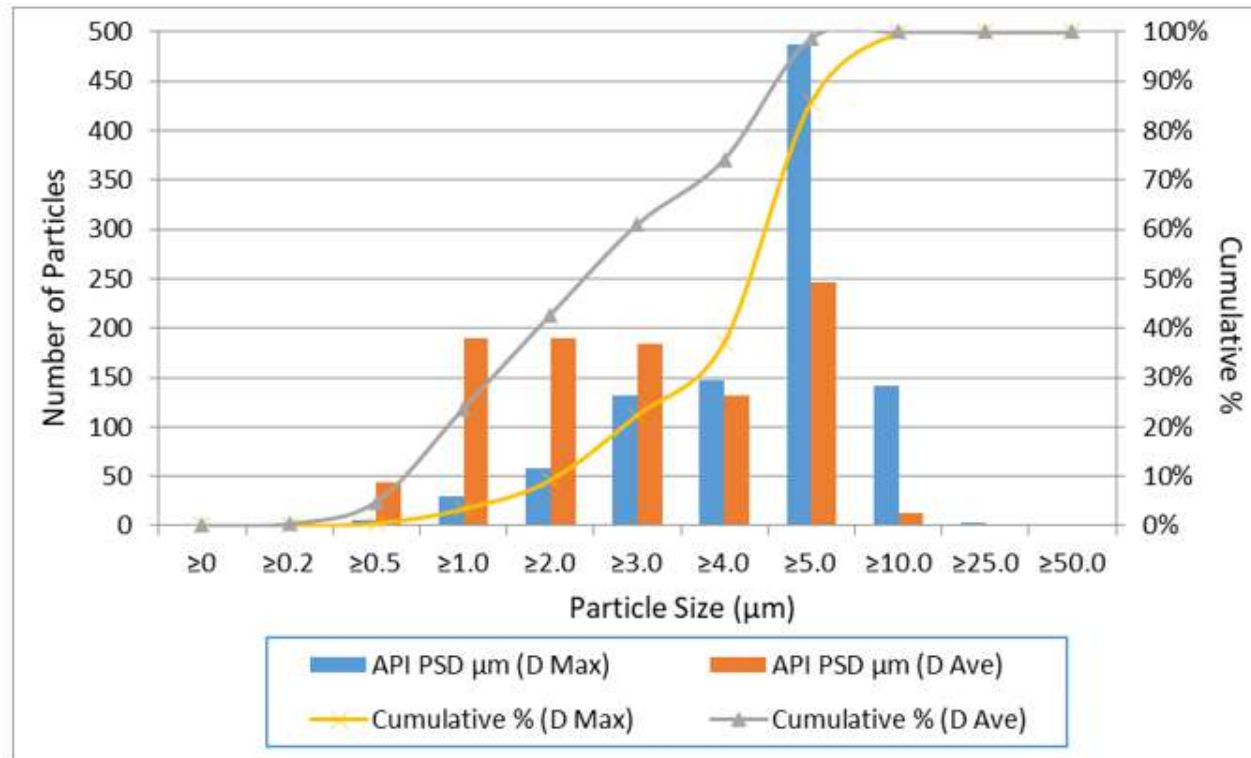
Length (μm) Budesonide API	Total Particles	≥ 2.0	≥ 3.0	≥ 4.0	≥ 5.0	≥ 6.0	≥ 7.0	≥ 8.0	≥ 9.0	≥ 10.0	≥ 15.0
All Particles	100	8	11	8	9	7	6	6	6	17	22
Single Particles	27	8	11	6	1	1	0	0	0	0	0
Agglomerates	73	0	0	2	8	6	6	6	6	17	22



Representative optical images (50x) of budesonide particle agglomerates in suspension.

Sub-Micron Particles

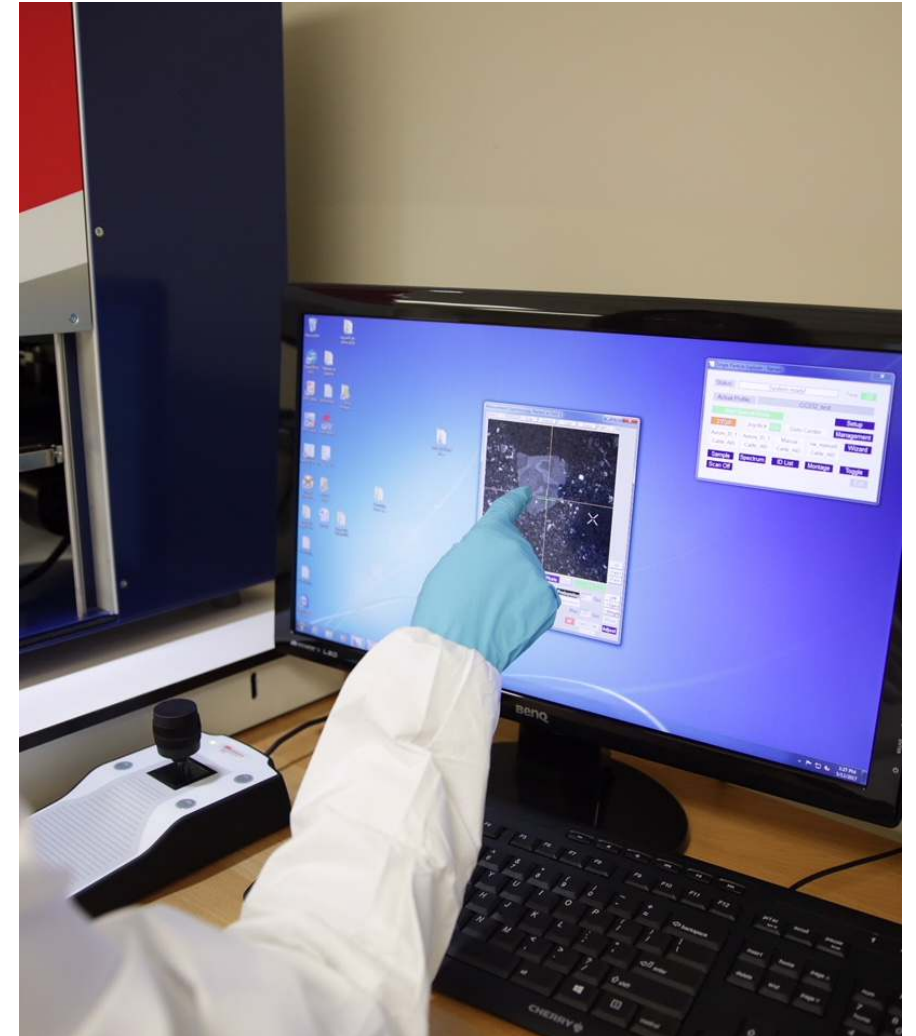
Budesonide API (μm)	Total Particles	≥ 0.20	≥ 0.50	≥ 1.0	≥ 2.0	≥ 3.0	≥ 4.0	≥ 5.0	≥ 10.0	≥ 25.0	≥ 50.0
D _{MAX}	1,002	0	5	29	58	132	147	487	141	3	0
D _{AVE}	1,002	4	44	189	190	184	132	246	13	0	0



Budesonide PSD histogram and cumulative percentage for both Maximum and Average Diameters.

Conclusions

- Raman Spectroscopy and SEM-EDS offer combined approaches
 - Particle ID
 - Particle size including submicron particles
 - Agglomerates
- Tools to Answer Questions
 - Formulation comparability
 - Manufacturing process similarity
 - Influence of morphology
 - Biowaiver applications



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