Developing and Validating Advanced Microscopy Methods for Supporting Complex Product Equivalence

SBIA 2020: Advancing Innovative Science in Generic Drug Development Workshop

Session 1: Method Development / Validations for Non-traditional Analytical Methods

Topic 2: Advanced Analytical and Statistical Methods for Assessing Particle Size Distributions

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Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.

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A quality product of any kind consistently meets the expectations of the user.




Drugs are no different.


FDA
Patients expect safe and effective medicine with every dose they take.

Pharmaceutical quality is

assuring *every* dose is safe and effective, free of contamination and defects.


It is what gives patients confidence in their *next* dose of medicine.

Learning Objectives

- Background: Current Challenges in Characterizing Particle Size Distribution for Complex Drug Products.
- Overview of Advance Raman Microscopy Methods
  - Cryo Scanning Electron Microscopy (CryoSEM) – Raman Spectroscopy
  - Morphologically-Directed Raman Spectroscopy (MDRS)
- Case Study: MDRS application on Nasal Spray Suspensions
- Summary: General Considerations for Advance Microscopy Method Development and Validation.

Background

Particle size distribution (PSD) of active pharmaceutical ingredient (API) **in the drug product** is a critical attribute in evaluating complex drug products

- Quality
- Effectiveness
- Bioequivalence (BE) (for evaluating generic drugs)

Challenges:

- **API and excipient particles coexist in the formulation**
- **More than one API in the formulation**
- **API may have more than one polymorphic form**

Traditional particle sizing techniques, such as sieving, laser diffraction, and microscopy, cannot distinguish particles with different chemical identities.

Advanced Microscope Methods

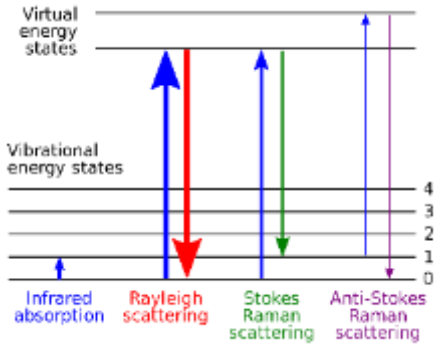
Microscopy – Raman Spectroscopy Combination Technology

Raman spectroscopy provides information on molecular vibrations and crystal structures.

Raman Spectra - “fingerprints” of molecules

--- Each molecule has its own unique spectrum.

--- API / excipient particles show significantly different Raman profiles.

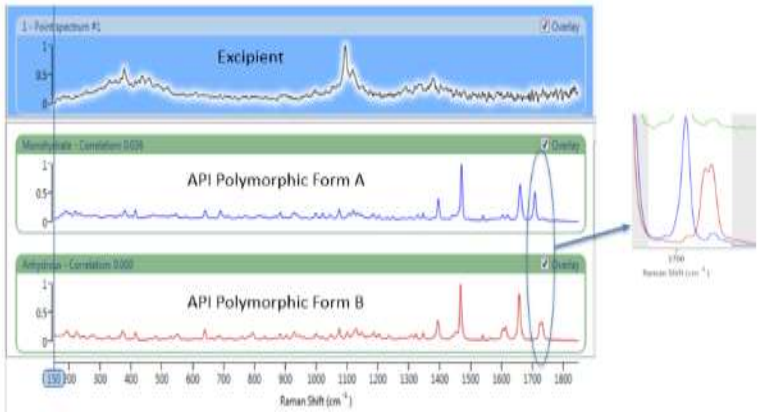


Raman Advantages:

- ✓ Chemical identification for each individual particle.
- ✓ Identify different polymorphic forms of an API.

Raman Disadvantage:

- ❖ **Relatively slow measurement, time-consuming.**

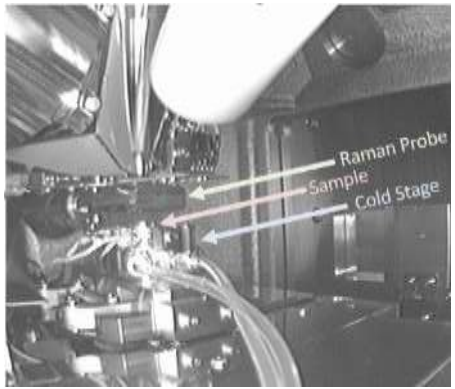


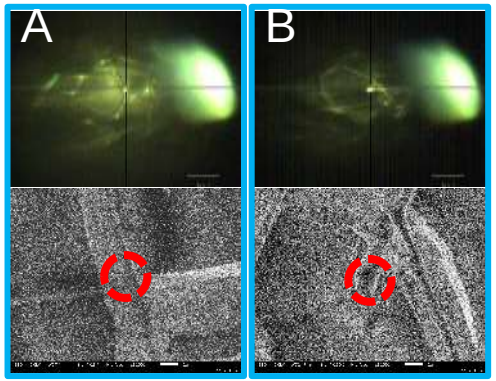
Advanced Raman Microscope Methods

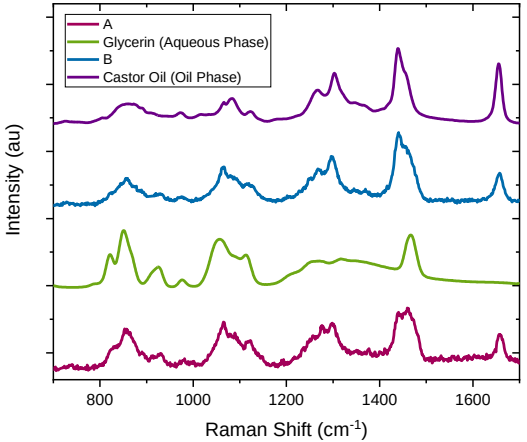
Example 1 (advanced sampling technique):

Cryo Scanning Electron Microscopy (CryoSEM) – Raman Spectroscopy

- Cryofixation: a rapid freezing technique able to preserve samples in their native states.
- SEM can measure particles in nanoscale size range.







Advanced Raman Microscope Methods

Example 2 (advanced analytical methodology):

Morphologically-Directed Raman Spectroscopy (MDRS) - Apply morphology screening procedure to significantly reduce total measurement time.

- Has been used as an alternative approach to the comparative clinical endpoint BE study
  - First used as supporting evidence for the approval of the first generic Mometasone Furoate Nasal Suspension Spray in 2016
  - Recommended in several FDA Product-Specific Guidances (PSGs)

Malvern Panalytical: Morphologi

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Morphologically-Directed Raman Spectroscopy (MDRS)

MDRS application on Nasal Spray Suspensions is used as an example for developing and validating advanced microscopy methods for complex drug products.

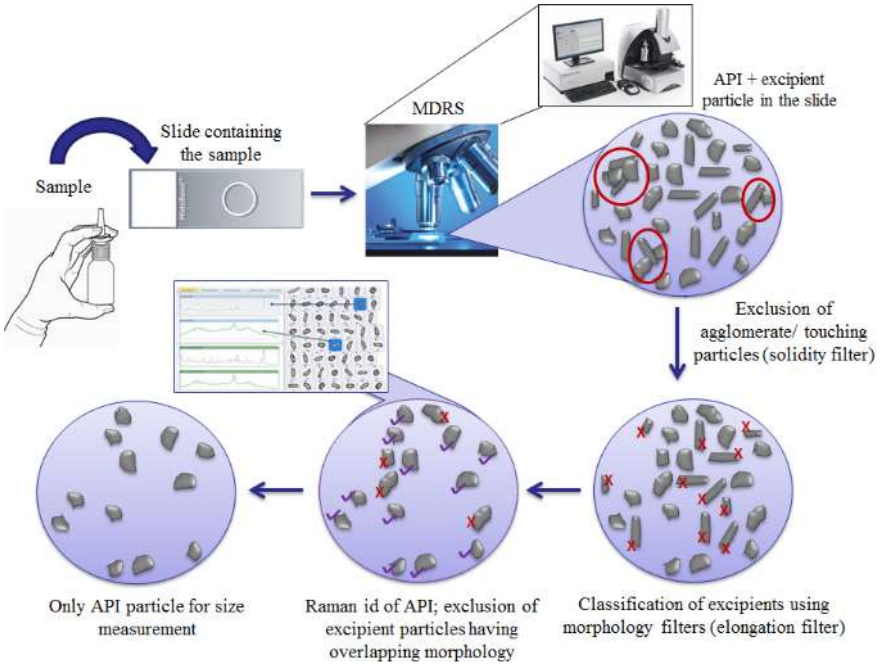
MDRS strategy

Morphology Screening

- Capture particle image using digital microscope.
- Apply morphology filters to exclude as many excipient particles as possible

Raman Confirmation

- Perform Raman measurements on selected particles for chemical identification.



Morphology Measurement

Particle Morphology

Size

- Circular equivalent (CE) diameter

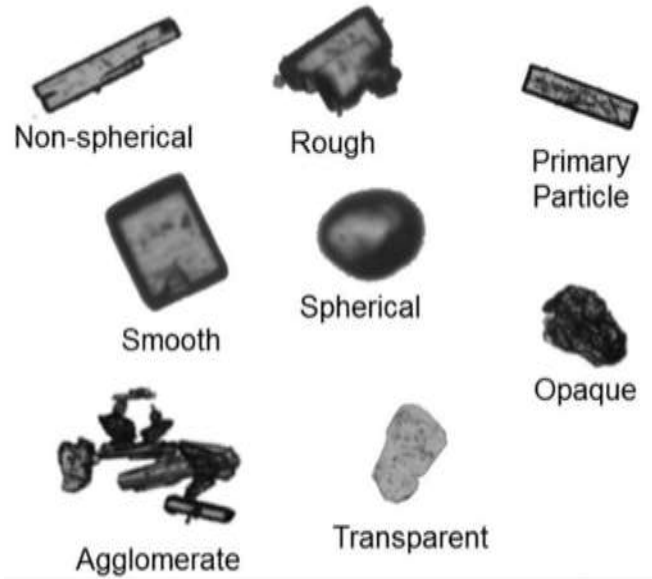
Shape

- Aspect Ratio
- Elongation
- Convexity
- Solidity
- Circularity

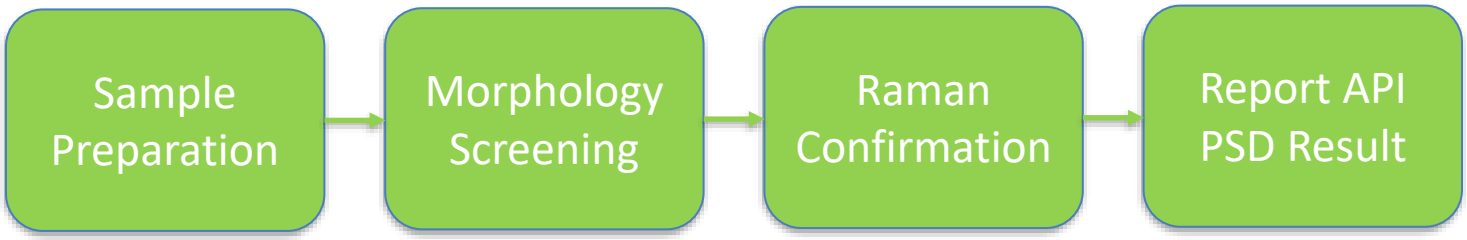
Other physical feature

- Intensity Mean

--- Measurements of light transmission or light reflection of the particles.



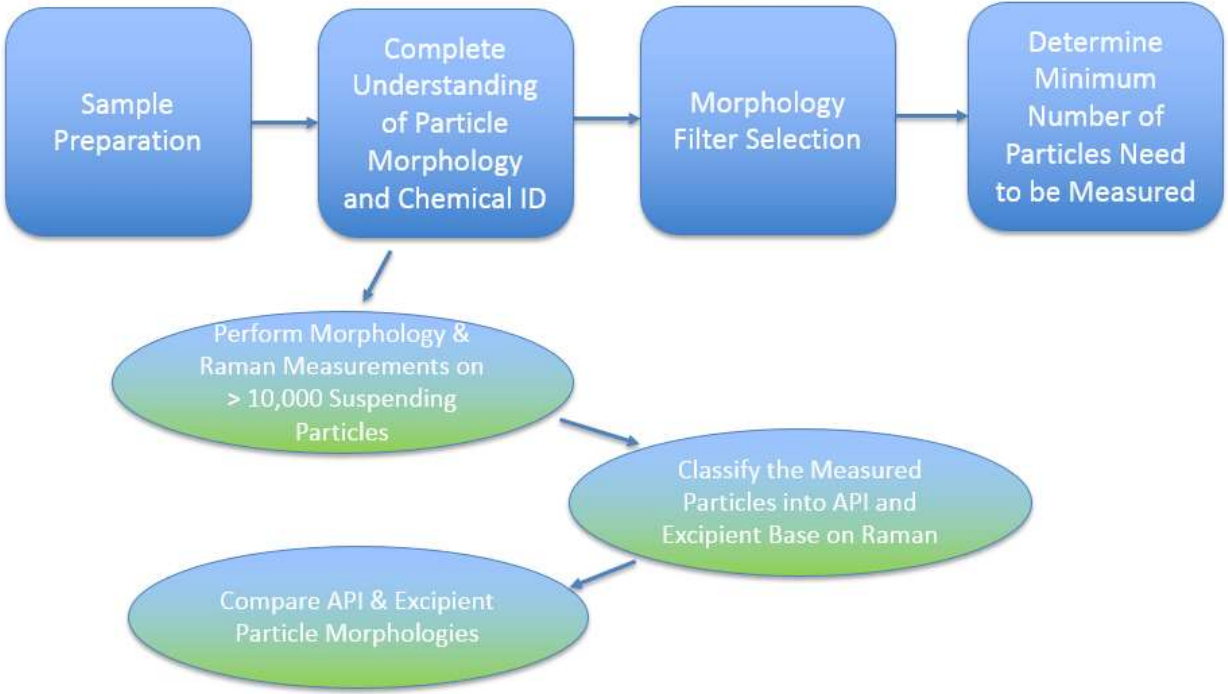
Typical MDRS Protocol



- Since the majority of particles in a formulation are excipients, it is important to apply **appropriate** morphology filters to exclude as many excipient particles as possible before performing time-consuming Raman measurements.
  - Be alert: morphology filters will also discard API particles with overlapping morphologies. A loose morphology filter selection will cause biased API PSD results.

Method Development is the key to success.

MDRS Method Development and Validation



Sample Preparation

Proper sample presentation is critical to ensure accurate and consistent PSD measurement.

Goal: keep the sample in its native state with minimum sample preparation involved.

Consideration: if the sample needs to be prepared in a diluted or dried condition

- The process of dispersion, ultrasonication, evaporation, or freezing may cause change in particle stability.
- Validation is needed to ensure particles are measured in their native state.

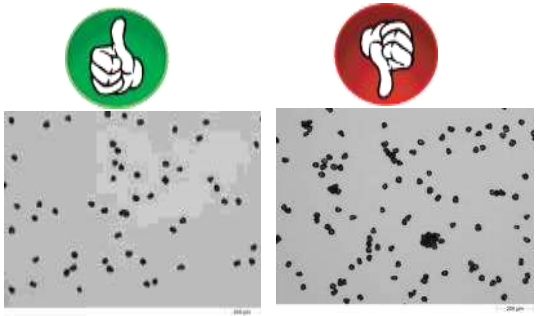
Preferred Sample Preparation Example for Nasal Spray Suspension (Wet Method / post-spray):

- Gently shake nasal spray bottle and prime the pump.
- Deliver next two actuations into a small glass vial (post-spray).
- Transfer 5 µL of the post-spray sample to a quartz microscope slide.
- Cover sample with a quartz coverslip and apply petroleum jelly to the coverslip edges to prevent sample evaporation.
- Allow time for particles to settle.

Sample Preparation

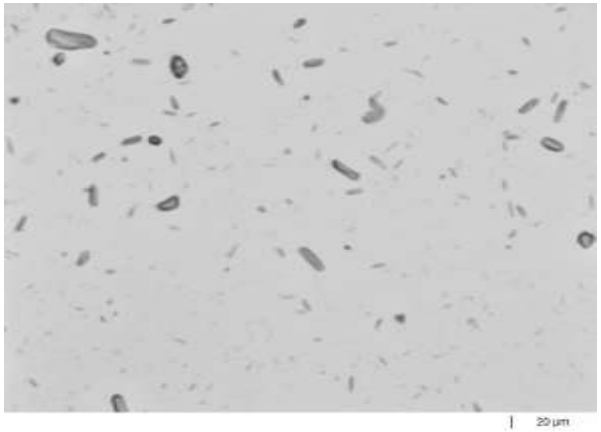
Considerations:

- Volume of sample should be minimized and optimized for a reproducible thin layer
  - Avoid possible overlapping particles (touching particles)
  - Provide better focus on all particles in the field of view
- Time of settlement: particles should not move after settling
  - High viscous formulation: 30 mins to 2 hrs
  - Low viscous formulation: 12 hrs (overnight)



Understanding the Sample

Perform morphology & Raman measurements on a training set > 10,000 Particles



Particle #	1	2	3	4	5	6
CE Diameter	2.28 µm	4.69 µm	2.53 µm	8.91 µm	7.36 µm	6.85 µm
Aspect Ratio	0.74	0.46	0.822	0.418	0.709	0.585
Circularity	0.965	0.86	0.974	0.853	0.937	0.795
Intensity Mean	124	155	115	154	107	108
Convexity	0.994	0.974	0.994	0.986	0.988	0.923
Solidity	0.997	0.986	0.998	0.994	0.99	0.886

Touching particles or agglomerates / aggregates: Relatively low convexity / solidity values

- If more than 5% of particles are touching particles
  - Poor sample preparation (particle concentration too high)
- If more than 5% of particles are agglomerates / aggregates
  - May indicate a potential stability issue
  - Need to be investigated as a separate class

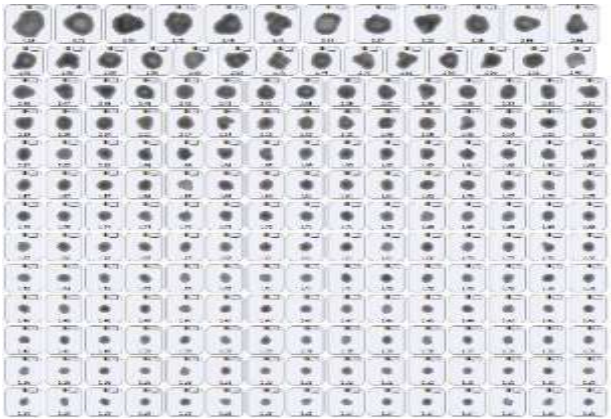
Understanding the Sample

Classify the Measured Particles into API and Excipient Base on Raman

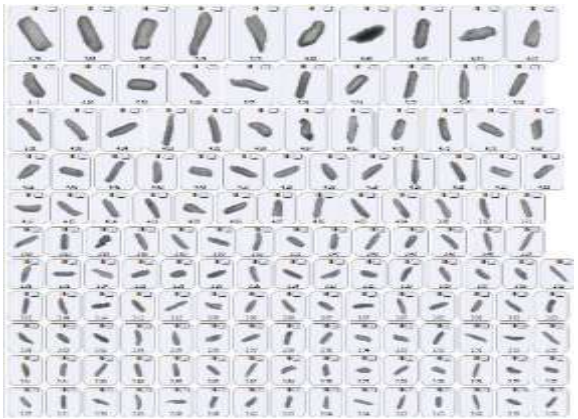
Limit of detection for Raman chemical identification

- API / excipient could be weak Raman scatterers (longer exposure time needed)
- Lower size limitation of the microscope (Light microscope: ~ 1 µm; SEM: nm)
- Laser spot size limitation (exposure area and depth of field)

❖ Limit of detection for MDRS: 1 µm



API

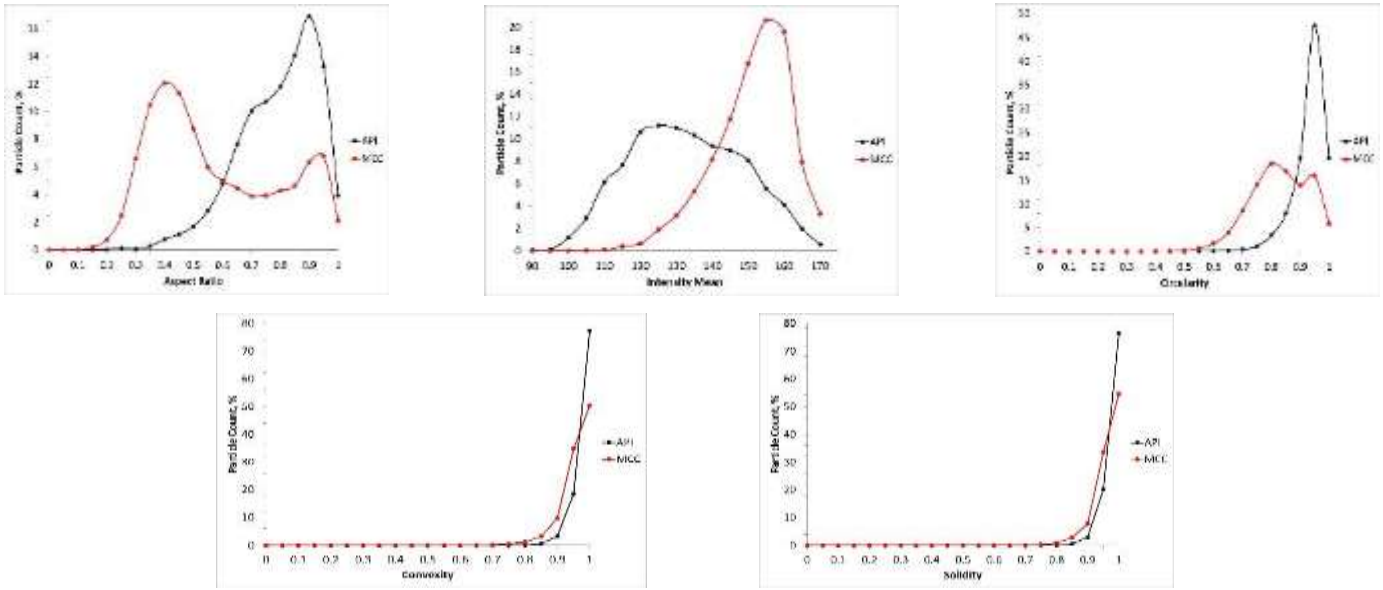


Excipient

Understanding the Sample

Compare API and Excipient Particle Morphologies

API vs. Excipient Morphology Distribution (Normalized Plots)



None of the morphology parameters or morphology parameter combinations can provide a complete separation between API and excipient.

Morphology Filter Selection

Goal: Select one or more morphology filter(s) with proper cutoff value(s) to

- Exclude as many excipient particles as possible
- At the same time, keep as many API particles as possible
  - It is inevitable to discard some API particles due to overlapping morphologies
  - After applying morphology filters, keep at least 85% API particles

❖ Applying morphology filter(s) will not change the PSD results

Validate the morphology filter selection

- Re-analyze the training set by applying morphology filters with selected cutoff values
- Evaluate the filter cutoff values by comparing API PSD results before and after morphology filter application
  - Criteria: < 5% difference in D10, D50, and D90
- If the criteria cannot be met, need to tighten filter selection to include more API particles

Determine Minimum Number of Particles

Goal: The API PSD results should be statistically meaningful and representative of the actual product.

The minimum number of particles to be measured should be determined by evaluating the accuracy and repeatability of API PSD measurements.

Selection Rule:

1. Measuring more particles will not result in statistically significant changes in PSD results.
2. The PSD measurement results should be repeatable.

Method:

Compare PSD results from counting different number (100, 200, 300 …) of API particles.

Proposed criteria for determining minimum number of particles:

- Accuracy: < 5% difference in D10, D50, and D90 if more particles were counted.
- Repeatability: RSDs of D10, D50, and D90 from 5 replicates are < 5%.

Consideration: influence from particle size distribution profile

- Single distribution: narrow vs. wide (span)
- Single distribution vs. multimodal distribution

Summary

Advanced Raman microscopy methods are capable to perform ingredient-specific particle sizing for characterizing complex drug products.

Advantages:

- Automated imaging
- High-resolution particle morphology analysis
- Integration of Raman spectroscopy
  - Chemical identification of individual particles
  - Identification of different polymorphic forms of drug substance in a formulation
  - In-depth investigation of API-excipient interaction in complex drug structures (liposomes, emulsion)

Considerations: complex analytical methods, emerging technology

- Extra effort needed in order to develop robust methods
- More room to improve in both hardware and analytical methodology

Summary

MDRS method development and validation considerations for pharmaceutical products:

- Sample Preparation
  - Prepare sample in its native state
  - Prepare sample as a thin layer for better focus and dispersion
- Particle morphology analysis
  - Watch for possible agglomerates / aggregates
- Limit of detection for Raman Microscope
  - Lower size limit from the microscope
  - Raman sensitivity of the ingredients
- Morphology filter selection
  - Avoid biased filter selection
  - PSD results should not change before and after filter application
- Minimum number of particles to be measured
  - Statistically meaningful and representative of the actual product

Challenge Question #1

Which of the following morphology parameters is (are) used to describe particle shape?

A. Aspect Ratio

B. Solidity

C. Convexity

D. All of the above

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