AUTOMATED IMAGE ANALYSIS PROCEDURES:
An effective tool for drug substance and drug product characterization

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INTRODUCTION

Particle size and particle shape properties have been recognized as key factors for drug substance and drug product quality; they can have a tremendous impact on reactivity, physic-chemical stability and workability of drug substance; they can dramatically affect the drug product biopharmaceutical properties, "in vivo" performance and bioavailability.
Today, increasing emphasis has been covered by particle size and particle shape measurements, identifying those among the critical quality attributes (CQAs) of drug substance and drug product (ICHQ8, ICHQ11). Process Analytical Technology (PAT) initiative and Quality by Design (QbD) approach have focused interest on these physical properties as means to better understanding and control of manufacturing processes and then generate specifications.
Many technologies have been successfully determined for quickly and easily characterization of particle size and shape: optical Microscopy, Sieving, Sedimentation, Optical and Electrical Sensing Zone method, Laser light scattering techniques, including Laser Diffraction Particle Size Analysis and Photon Correlation Spectroscopy, Surface area measurement.
Recently the availability of innovative image analysis analyzers, such the Morphologi GS3 (Malvern, UK) has given a renewed impulse and new prospective. The reason is firstly the ability of measuring shape as well as size; then Morphologi GS3 analysis provides a statistically significant number of detected particles and each single measured particle is recorded for obtaining qualitative and quantitative data.

MORPHOLOGI GS3

MORPHOLOGI GS3 - KEY FEATURES
(***Picture 1 – Picture 2***)

- Particle properties measured: shape, count, size, trasparency, location.
- Particle size measurement range : 0.5-1000 µm
- Particle size properties: Circle equivalent (CE) diameter, length, width, area.
- Particle shape parameters: aspect ratio, circularity, convexity, elongation, solidity fiber elongation
- Sample dispersion Unit: dry and wet method; control of pressure dispersion and injection time.

Picture 1: Sample Unit Dry powder Dispersion

Picture 2 – Lens 2,5X, 5X, 10X, 20X,100X

CASE STUDIES

In our CQ Laboratory, we have developed and implemented several analytical procedures by means of Morphologi GS3 for supporting both drug development program and final product QC release.

The poster illustrates actual data and case studies of most significant applications.

- Case Study 1: API inter-batches Comparison.
- Case Study 2: Formulation screening Application.
- Case Study 3: Particle size Method Validation and CQ Release

CASE STUDY 1 – API INTER LOTS COMPARISON

We analyzed batches of an API, produced by two different synthetic path ways (A and B) by means of Morphologi G3 for both size and shape: particle size and particle shape characterization were needed for understanding potential incoming differences between batches.

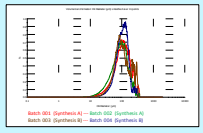
METHODS

- Sample Dispersion Unit: dry cell
- Dispersion Pressure Range: 2.5-3.0 bar
- Sample Amount: 3 mm 3
- Injection Time: 10 msec
- Sample Settling Time: 60 sec
- Optical Lens: 5X (6.5-420 µm)
- Particle size parameters: length, area, Circle equivalent (CE) diameter
- Particle shape descriptors: elongation, as an indication of length/width ratio of the particles

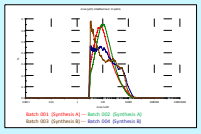
RESULTS

The collected results show all the analyzed batches had similar size distributions in terms of volume, by the Circular Equivalent Diameter (µm) distribution ***(Graph 1)***. Significant inter-batches differences were highlighted by the particles Area (μm^2) and the length (µm) distribution ***(Graph 2 and 3);*** the use of a shape descriptor, such as ***ELONGATION,*** confirming different crystals shape properties can be appropriately detected by a high sensitive image analyzer technique. ***(Photo 1 - Photo 2).***

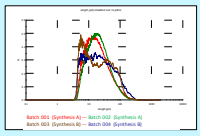
RESULTS



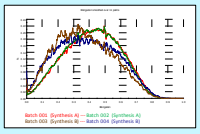
Graph 1 – CE Diameter distribution



Graph 2 – Area distribution



Graph 3 – Length distribution



Graph 4 – Elongation (Length/Wight ratio)

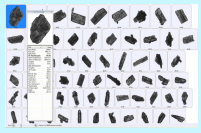


Photo 1 – Batch 001 (Synthesis A)

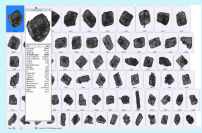


Photo 2 – Batch 003 (Synthesis B)

CASE STUDY 2 – FORMULATION SCREENING APPLICATIONS

In the Study Case 2 an example of an automated image analysis on cream formulation is presented; two batches were analyzed both by means of Morphologi GS3 and Beckman Coulter laser Granulometer; a comparison between the obtained results by Morphologi GS3 and the ones by a conventional laser diffraction is also proposed.

METHODS

- Sample Dispersion Unit: wet cell
- Disperdant Vehicle: Silicon Oil
- External Sonication: 5 sec
- Apparatus: Morphologi GS3 Optical equipped with 20X Len; Beckman Coulter Laser Granulometer model LS100Q.
- Particle Size Descriptor: CIRCULARITY

RESULTS

The automated image analysis allowed to well characterize the two selected batches: Length distribution, CE diameter vs number distribution, CE diameter vs volume distribution are reported respectively in ***Table 1, 2, and 3.***
Good values in terms of circularity were reported for both analyzed batches: 0.962 and 0.888. The particle size distribution results, obtained by laser diffraction technique, are reported in ***Table 4.***
The two applied techniques provided comparable results in terms of particle size distribution and no significant difference were highlighted in terms of shape and particle size distribution.

RESULTS

Batch	d10 (µm)	d50 (µm)	d90 (µm)
350129	3.3	7.2	17.3
301025	4.1	8.7	17.8

Table 1: Lenght Distribution

Batch	d10 (µm)	d50 (µm)	d90 (µm)
350129	2.7	6.3	14.4
301025	3.1	7.6	15.1

Table 2: CE diameter vs Number Distribution

Batch	d10 (µm)	d50 (µm)	d90 (µm)
350129	8.3	15.34	21.7
301025	8.6	15.1	21.8

Table 3: CE diameter vs Volume Distribution

Batch	d10 (µm)	d50 (µm)	d90 (µm)
350129	5.35	11.7	24.8
301025	4.42	11.2	29.8

Table 4: Particle Size Distribution

CASE STUDY 3 – VALIDATION AND CQ RELEASE

Method set-up, development and validation were performed by means of Morphologi GS3, in alternative to a manual microscopy procedure for QC release of a micronized product.

SAMPLE PREPARATION

Sample dispersion procedure was selected according to the solubility properties of the compound: insoluble in water, soluble in Alcohols and Acetone. Suitable dispersion solvents were the following: Cyclohexane, Hexane and Tegiloxan 3. Wet cell dispersion method in Cyclohexane was identified as better sample dispersion procedure; dry sample dispersion did not guarantee appropriate crystals dispersion: large aggregates were detected applying pressure from 3.5 to 5.0 bar. ***(Photo 3 and 4)***.

METHODS

- Sample Dispersion Unit: wet cell with CycloHesane (10 mL).
- Sample amount: 2 mm^3
- External sonication: 5 min
- Optical Lens: 20X (1,75-100 µm)

VALIDATION

The following validation parameters were evaluated: Precision, Intermediate Precision, Acccuracy and Robustness. All the acquired results satisfaied the fixed acceptance criteria. (***Table 5-6-7***).
The developed paritcle size analysis method by means of Morphologi GS3 was appropriately applied for the CQ release of micronized compound.

RESULTS

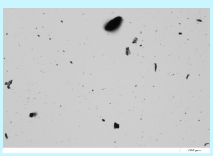


Photo 3: Dry Sample Dry Dispersion – 3.5 bar

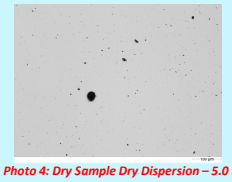


Photo 4: Dry Sample Dry Dispersion – 5.0 bar

PRECISION						
PARAMETER	No. Analysis	No. Particles	RESULTS d10(µm)	d50(µm)	d90(µm)	
Analyst 1, Day 1	6	20422	0.875	1.42	3.13	
			2.01	3.14	5.14	CV%
			30%	20%	30%	Limits
			YES	YES	YES	Compliance
PARAMETER	No. Analysis	No. Particles	RESULTS d10(µm)	d50(µm)	d90(µm)	
Analyst 1, Day 2	6	20565	0.970	1.76	3.46	
			6.42	12.27	8.26	CV%
			30%	20%	30%	Limits
			YES	YES	YES	Compliance

Table 5: Precision Results

UPDATE/MEDIATE PRECISION						
PARAMETER	No. Analysis	No. Particles	RESULTS d10(µm)	d50(µm)	d90(µm)	
Analyst 1, Day 1, Analyst 2, Day 2	6	20422	0.879	1.45	3.29	
			3.23	6.49	13.96	CV%
			30%	20%	30%	Limits
			YES	YES	YES	Compliance

Table 6: Intermediate Precision Results

PARAMETER	No. Analysis	No. Particles	RESULTS d10(µm)	d50(µm)	d90(µm)	
SONICATION TIME	6	20269	0.882	1.39	2.68	
			6.678	9.78	6.85	CV%
			30%	20%	30%	Limits
			YES	YES	YES	Compliance
PARAMETER	No. Analysis	No. Particles	RESULTS d10(µm)	d50(µm)	d90(µm)	
WEIGHT VARIATION	6	20565	0.847	1.27	2.54	
			6.670	9.78	6.85	CV%
			30%	20%	30%	Limits
			YES	YES	YES	Compliance

Table 7: Robustness Results

CONCLUSIONS

In conclusion, all the reported case studies demonstrated the promising role of image analyzer technology as suited and valid tool for measuring both size and shape of drug substance and drug product allowing better solid-state characterization: shape descriptors, such as elongation, circularity and convexity are suitable parameters for better particle characterization suitable for several and wide pharmaceuticals applications. product quality in accordance with the regulatory compliance (USP Monograph <776>: "Optical Microscopy").