Image analysis for particle characterization

Characterizing particle size and shape in MDI and DPI formulations using automated image analysis

Mark Bumiller
Horiba Instruments

Many scientists working with metered dose inhaler (MDI) and dry powder inhaler (DPI) formulations associate particle characterization only with cascade impaction to determine aerodynamic particle size distribution (PSD). However, a number of factors in addition to the PSD that are related to the size and shape of the active ingredients can affect the ability of an inhalation product to deliver the expected dose to the patient. For example, the crystalline form of the drug substance can affect the bioavailability, performance, stability, or other properties of the drug product, as can agglomerations.

As a result, regulatory agencies recognize the benefits of visual analysis for the evaluation of inhalation formulations. US Pharmacopoeia chapter <601> approves of microscopy for determination of the number of large particles, agglomerates, and foreign particulates in MDI emissions. In addition, an FDA draft guidance document suggests that the use of the microscope "has certain merits and, therefore, should be retained for release and stability purposes," particularly control and monitoring of morphic form changes on stability. Microscopy also provides useful information for early characterization of drug substance and the evaluation of crystal growth and agglomeration during temperature cycling studies.

Recent advances in automated image analysis have resulted in a preference for automation over manual microscopy, which has been used for particle characterization in the pharmaceutical industry for many years. The first attempts by microscope vendors and large pharmaceutical laboratories to create computer-controlled image analysis systems began in the mid-1980s. Complete turnkey systems became commercially available in the mid-1990s as computer and digital camera technologies became more advanced, and equipment and software continue to improve today. Since few contract laboratories offer specific expertise in automated visual analysis, pharmaceutical companies often bring this technology in house and develop internal capabilities.

The benefits of automation

The primary benefit of automation is that it allows for a significant increase in the number of particles analyzed, typically collecting thousands of particle images compared to a manual inspection that would usually look at no more than 60-100 particles. In addition, modern algorithms can automatically determine separate characteristics for particles that are touching each other on the slide, facilitating data collection and providing information on single particle characteristics.

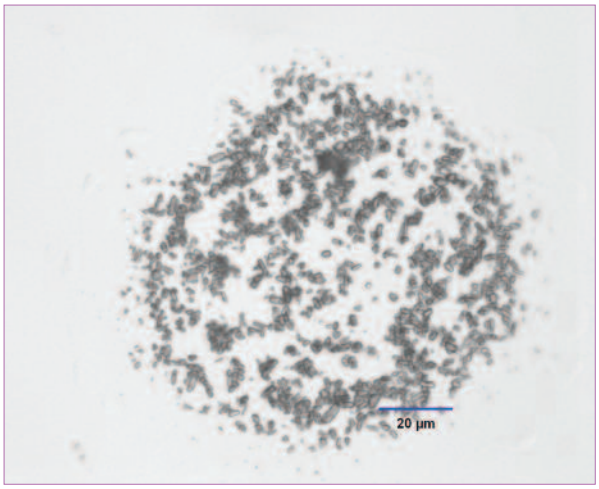
Only automated microscopy can efficiently inspect the large number of particles required to produce a high statistical confidence in the quantification of particle size and shape distributions as defined in ISO standard 13322-1, "Particle size analysis—Image analysis methods—Part 1: Static image analysis methods." The ISO standard includes tables that define the number of particles, numbering in the thousands, requiring inspection in order to achieve desired confidence limits depending on the breadth of the particle size distribution. Commercially available systems also include validation support such as IQ/OQ procedures and 21 CFR Part 11 compliant software.

Preparing slides for MDI and DPI formulations

Image analysis requires the distribution of particles on a slide with the particles as separate as possible, a procedure that can be tricky when the slide preparation involves dispersion of particles from an MDI. Traditional industry guidelines suggest actuation of the MDI onto a clean, dry slide held 5 cm from the device, perpendicular to the direction of the spray; however, actuation at this distance can result in an overly concentrated area of particles on the slide (Fig. 1), resulting in both a high number of particles within one field and a prevalence of overlapping particles.

Figure 1

Particles on slide held 5 cm from an MDI

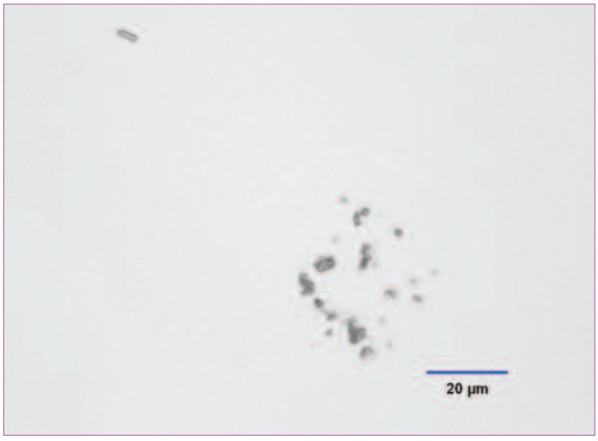


Although modern image analysis algorithms can automatically separate touching particles, the system will more likely analyze overlapping particles as agglomerates rather than individual particles, skewing the results. Studies have shown that placing the MDI at approximately 15 cm from the slide and actuating twice provides the optimum particle placement on the slide (Fig. 2).

DPI powders require an additional sample preparation step in order to create an even dispersion of individual particles onto a microscope slide, a process that involves a specialized piece of equipment. This step involves collection of the powder dose after actuation of the DPI device and transfer of the powder into the nozzle of a disperser unit. With the nozzle locked into place inside a chamber containing microscope slides, the unit pulls a vacuum on the chamber and then releases it, providing enough energy to disperse the particles, which then fall onto the microscope slides.

Figure 2

Particles on slide held 15 cm from an MDI



Choosing optimum settings

Automatic image analysis systems allow operators to create routines that control how the microscope views and analyzes each slide. An automated stage moves the slide over the desired number of fields, following a pattern contained in the routine. Each time the slide moves to a new field, a digital camera captures an image of the particles within the field of view and performs the image analysis steps chosen by the operator. Once the routine has been programmed, the image analyzer provides the complete particle size and shape distribution characterization of a sample collected from an MDI and DPI in a matter of minutes with the push of a button.

Important aspects of the measurement controlled by the routine include:

- magnification (500x or 100x)
- light intensity
- portion of the slide inspected
- threshold setting
- particle separation approach and sensitivity
- parameters assigned to each particle.

Magnification choice depends in part on the size of the particles that the operator wants to know the most about. The 500x objective maximizes sensitivity to small particles, while the 100x objective can zoom out and scan the slide for the presence of large particles or foreign material. The particle size and shape information at 100x does not include the smaller particles due to the loss in sensitivity, but switching to the 100x objective greatly decreases the analysis time for inspecting a larger area.

Although the 500x results represent the most accurate information for both the particle size and shape distributions of the sample, the 100x objective allows operators to locate individual oversize particles in samples containing thousands of particles. Operators can then manually inspect those oversize particles to determine whether they consist of agglomerations, single large crystals, or foreign materials. A 100x inspection of an MDI sample with two active ingredients, for example, looked at more than 16,000 particles, and a 100x inspection of a DPI sample included analysis of 7,200 particles.

A data browser that sorts all inspected particles on any chosen criteria—for example, particle size in descending order—facilitates visual inspection of outlier particles. The operator clicks on any particle in the browser, and the stage moves the slide to that

particle for inspection, rejection, or saving of the individual image. Large foreign particles may be collected for further analysis, possibly for identification as active ingredient or foreign material using techniques such as Raman spectroscopy. In the 16,000-particle MDI sample, the analyzer captured an image of a 20 µm particle that shows it to be a single particle, not an agglomerate (Fig. 3). In the DPI study, the image analyzer located a 143 mm particle (Fig. 4).

Figure 3

Large particle from an MDI analysis

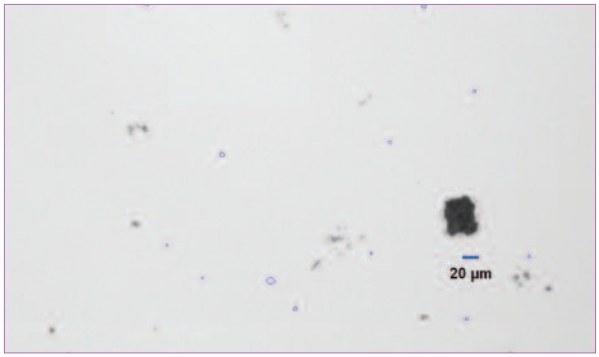
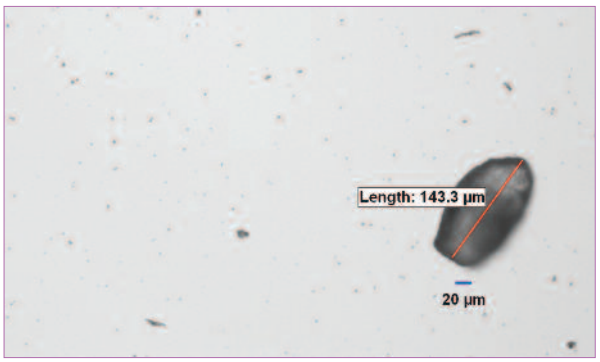


Figure 4

Large particle from a DPI analysis



Interpretation of results

PSD results from image analysis typically include a table reporting size as circle diameter vs. spherical volume in µm, with both roundness and aspect ratio reported on a count basis (Figs. 5 and 6). Although the practice of converting PSD from a count to volume basis while reporting shape parameters on a count basis can lead to confusion, the industry has so far not come up with a more consistent method of reporting.

Since image analysis and other light scattering techniques like laser diffraction do not differentiate between chemical species, users may find subsequent Raman mapping useful to determine the chemical species of large particles that have been isolated to distinguish between API, excipients, and foreign materials. The pharmaceutical industry has increasingly accepted Raman mapping for such applications. Analysis of a large particle found on a section of a slide prepared from a DPI formulation, for example, can reveal whether API remains attached to larger carrier particles, making it unlikely to deposit in the lower lung and therefore reducing the delivered dose.

Figure 5

MDI at 500X

	Size	Roundness	Aspect ratio
D10:	2.3	0.43	1.19
D50:	3.9	0.6	1.51
D90:	6.4	0.78	2.05
Minimum:	0.6	0.27	1.07
Maximum:	8	0.93	3.27
Mean:	4.21	0.61	1.58

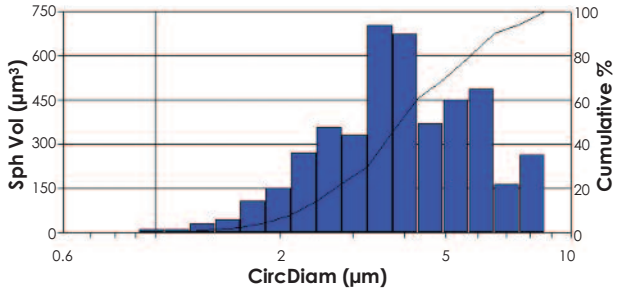
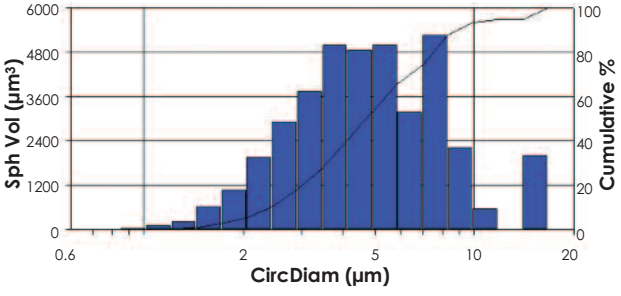


Figure 6

DPI at 500x

	Size	Roundness	Aspect ratio
D10:	2.4	0.48	1.15
D50:	4.8	0.68	1.37
D90:	8.6	0.83	1.86
Minimum:	0.6	0.24	1.05
Maximum:	15.7	0.93	3.68
Mean:	5.49	0.667	1.46



Mark Bumiller is Vice President of Particle Products, Horiba Instruments, 18 Lyman Street, Suite 203, Westborough, MA 01581. Tel. +1 508 366-5664. mark.bumiller@horiba.com