HORIBA Scientific

Application Note

Size and Shape Characterization of Pharmaceutical Active Ingredients

AN182

Automated image analysis is rapidly replacing manual microscopy in laboratories wanting the benefits of accuracy, resolution, and shape information coupled with the statistical confidence coming from inspecting thousands of particles. The most common application for static image analysis systems is currently the characterization of active pharmaceutical ingredients (APIs). Pharmaceutical laboratories around the world are investing in image analysis systems as a valuable tool for complete particle size and shape characterization of dosage forms including solid oral, aerosol, and transdermal.

Introduction

The static image analysis technique uses an optical microscope to characterize particles dispersed on a slide that is moved by an automated stage. The slide moves to reveal a new section (think of a grid), an image is captured by a digital camera, and a software routine then performs various tasks to distinguish the particles from the background, to separate touching particles, and assign size and shape parameters. Image analysis provides unique particle size and shape information for pharmaceutical scientists working with APIs. Some of the advantages of using image analysis for particle characterization are summarized in the paragraphs below.

Accuracy

Microscopy is the referee technique for particle size analysis since it is the most direct measurement. While light scattering techniques such as laser diffraction are more popular for reasons including ease of use and dynamic range, the user is typically uncertain of the accuracy of the measurement. A validated image analysis system provides the “view of reality” often wished for when interpreting results from other techniques.

Quantitative microscopic analysis

Manual microscopy can provide a feel for particle size and shape, but should be considered qualitative unless a statistically significant number of particles are inspected (1). Automated image analysis systems can quickly measure thousands (or tens or hundreds of thousands) of particles, delivering truly quantitative results.

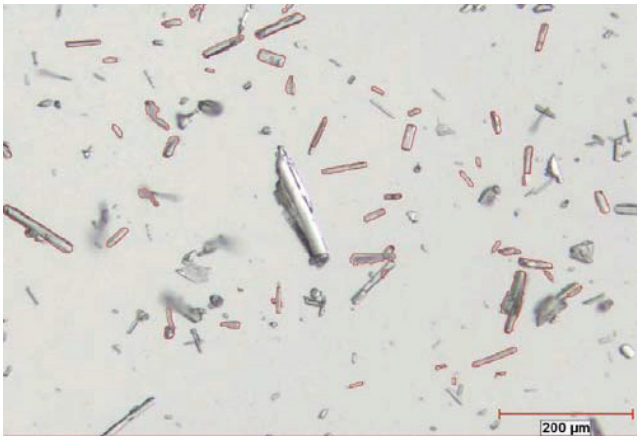


Figure 1: API image taken by the PSA300

Shape characterization

Along with high resolution particle size information, image analysis also provides shape information on the particles. Particle shape can be expressed using many different parameters (2, 3). Particle shape affects important particle behavior including powder flow (4), compaction, and other bulk properties that affect the manufacturing processes used to create solid oral dosage forms.

Finding outlier populations

All techniques that inspect particles one at a time are inherently higher resolution than ensemble light scattering methods. Since image analysis is a high resolution counting technique, it can effectively detect outlier populations (both larger and smaller than the main population). For active pharmaceutical ingredients, this could be a critical advantage for finding small amounts of large particles that could negatively impact dose uniformity.

Size analysis of high aspect ratio particles

All light scattering techniques, optical counters, and electric sensing zone counters are based on equivalent spherical diameter models. Microscopy/image analysis is the only technique that provides accurate size and shape distribution information for particles of any shape. Several industry-leading pharmaceutical companies now regularly switch to image analysis for particles above a defined aspect ratio.

Support method development and validation

Even when laser diffraction will be the release test for an API, image analysis can play a critical role in both method development and validation. Since microscopy/image analysis is the referee technique in particle size analysis, image analysis could be used as an *accuracy* test during method validation. The FDA guidance document for method validation (5) states in section 4.11.b:

"Several methods of determining accuracy are available."… "Comparison of the results of the proposed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and/or defined."

A validated automated image analysis system such as the HORIBA PSA300 (see Figure 2) certainly qualifies as a "second well-characterized procedure".

An often referenced publication on particle sizing method validation (6) suggests that microscopic analysis be used when investigating the *range* component of the method validation process:

Range

Ideally, the range of the specific technique chosen should cover the potential particle size ranges of the samples to be analyzed. This … In addition, the method should be checked directly using, for example, microscopy."

This idea of using microscopy/image analysis to support laser diffraction method development and validation is becoming common in the pharmaceutical industry.

Figure 2: The PSA300 image analysis system

The same publication (6) suggests that the method validation report should include a material description section describing the substance using USP definitions for particle shape (7), along with a photomicrograph of the material. With the capability of modern image analysis systems, a full description of particle shape is possible rather than merely stating, for example, that the particles were acicular. It is now possible to measure and report parameters such as length and width distributions, aspect ratio, roundness, etc. Rather than a single micrograph, all images of particles examined can be stored and viewed at any time.

PQRI Recommendations

The Product Quality Research Institute (PQRI) is a collaborative process involving the Center for Drug Evaluation and Research (CDER) in the Federal Drug Administration (FDA), industry, and academia. The mission of PQRI is to conduct research to generate scientific information to support regulatory policy. The PQRI formed a work group to study and report on particle size analysis techniques and practices. The group published their report in 2006 (8). This report suggests that microscopy/image analysis should be used throughout the drug development process. Pertinent comments found in this publication include:

"A preliminary microscopic evaluation of a representative lot of drug substance using manual optical microscopy or image analysis should be conducted prior to selecting the technique."

"Once the preliminary microscopic examination of size and morphology has been conducted, select a technique that is appropriate for the material being analyzed and provides particle size distribution results that are consistent with the information obtained by microscopic examination."

Figure 3 below shows a proposed decision tree outlining particle evaluation for Phase II clinical studies as described in the PQRI report. This decision tree suggests that when screening and developing a suitable particle size distribution method, the scientist(s) should compare results from techniques such as laser diffraction with quantitative microscopy results (image analysis).

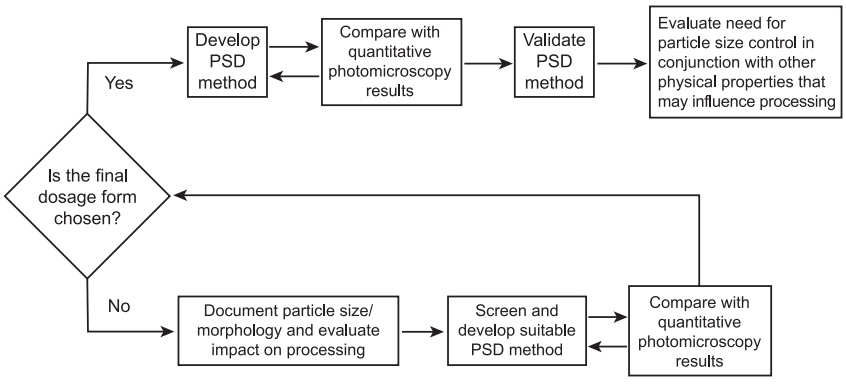


Figure 3: Decision tree for Phase II

Example: acetaminophen

Acetaminophen (or paracetamol) is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). Like many other powder APIs, the particle size and shape of acetaminophen will influence powder flow and compaction behavior that is critical when manufacturing solid oral dosage forms (9).

Acetaminophen (Sigma Aldrich A7085) was analyzed using the HORIBA PSA300 static image analyzer in its natural state as a dry powder. The sample was prepared on a slide using the Powder Disperser (see Figure 5) at 200 Torr, normal settings. Preparations were investigated at vacuum settings at 500, 350, 200, and 100 Torr before determining that 200 provided the best powder dispersion without breaking and particles.

Figure 5: Powder disperser for the PSA300

After sample preparation, the slide was analyzed on the PSA300 using the 200x objective. A total of 400 fields were viewed during the analysis. The software routine was optimized for this sample using several functions unique to the PSA300 software (10) including bridge removal to separate touching particles and split long objects to separate crossing fibers without breaking them into four individual particles. One of the images captured during the analysis is shown in Figure 6 below.

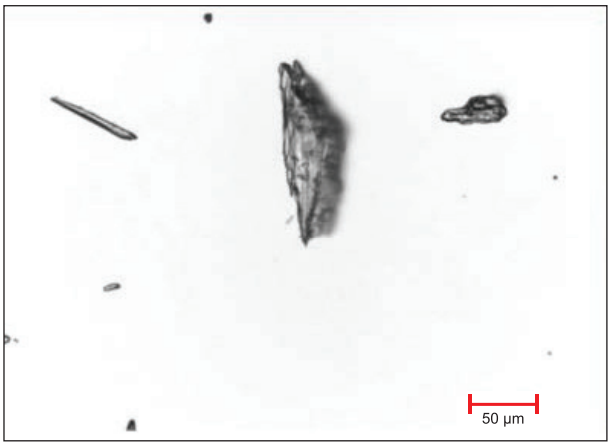
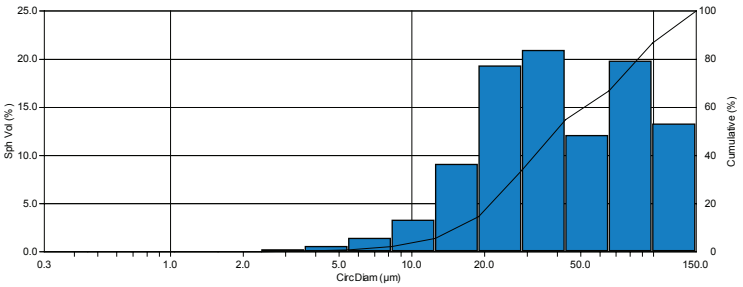


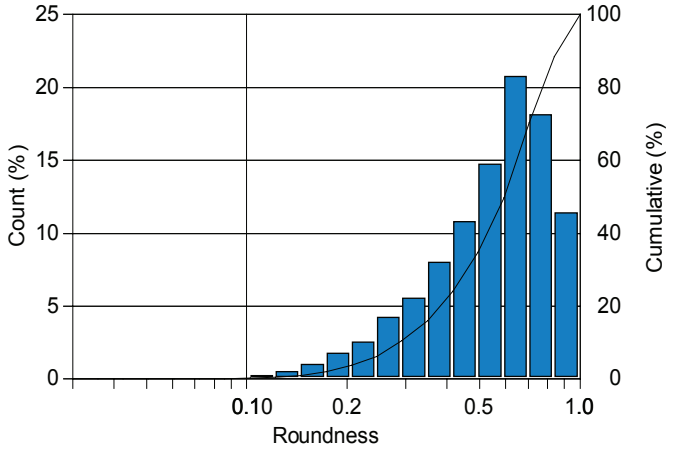
Figure 6: Acetaminophen at 200X

The particle size and shape distributions from this analysis are shown in Figures 7-9 below.



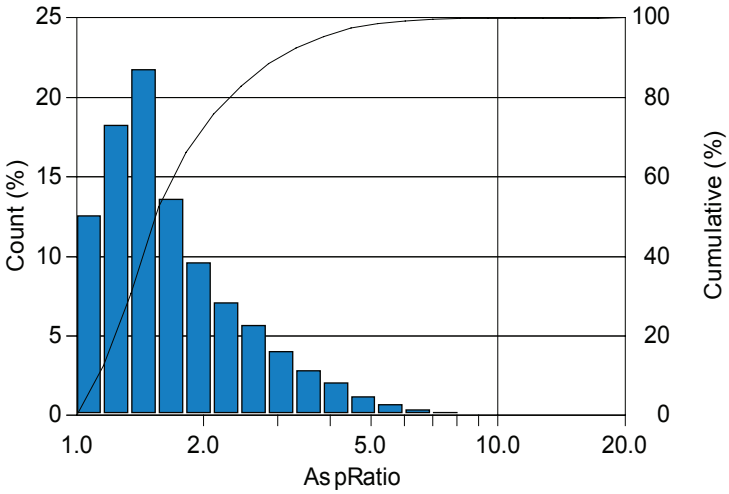
Statistics	
Minimum:	0.3 µm
Maximum:	117.7 µm
D[4.3]:	52.3 µm
Std Dev.:	34.6 µm
Sum:	553229657.7 µm
Count:	11208
Under:	0
Over:	0
Accepted:	100.0 %
Field Count:	400
Field Area:	183702.5 µm²
Total Area:	73480997.2 µm²
D10:	15.9 µm
D50:	40.1 µm
D90:	100.0 µm

Figure 7: Particle size distribution



Mean:	0.6
Std Dev:	0.2
Count:	11208
Field Count:	400

Figure 8: Roundness distribution



Mean	1.9
Std Dev	1.0
Min	1.0
Max	17.6
Count	11208
Field Count	400

Figure 9: Aspect ratio distribution

Conclusions

This application note summarizes the reasons why static image analysis is becoming a popular technique for characterizing APIs. The technique can be used on its own or as a support tool for verifying laser diffraction method development and validation. This adds a vital capability to any pharmaceutical laboratory serious about particle characterization. The HORIBA PSA300 combines the most advanced image analysis hardware and software currently available and packages them into a single turn-key system.

References

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2. ISO 9276-6:2008 Representation of results of particle size analysis - Part 6: Descriptive and quantitative representation of particle shape and morphology, available at: www.ansi.org

3. TN150 Size and Shape Parameters Defined in the PSA300, available at: www.horibalab.com

4. Bumiller M, Carson J, Prescott J. A preliminary investigation concerning the effect of particle shape on a powder's flow properties. World Congress on Particle Technology IV. July 22–25, Sidney, AU.

5. Guidance for Industry, Q2B, Validation of Analytical Procedures, Methodology, available at: www.fda.gov/cder/guidance/index.htm

6. Bell et. al, Position Paper on Particle Sizing: Sample Preparation, Method Validation and Data Presentation, Pharmaceutical Technology Europe, November 1999

7. USP<776> Optical Microscopy

8. Snorek, et.al, PQRI Recommendations on Particle-Size Analysis of Drug Substances Used in Oral Dosage Forms, Journal of Pharmaceutical Sciences, vol. 96, no.6, June 2007

9. Kaerger, J., Edge, S., Price, R., Influence of particle size and shape on flowability and compactibility of binary mixtures of paracetamol and microcrystalline cellulose, European Journal of Pharmaceutical Sciences 22 (2004) 173–179

10. TN152 Unique PSA300 Image Analysis Software Features, available at: www.horibalab.com