BASIC PRINCIPLES OF DETERMINING PARTICLE MORPHOLOGY

In static Image Analysis

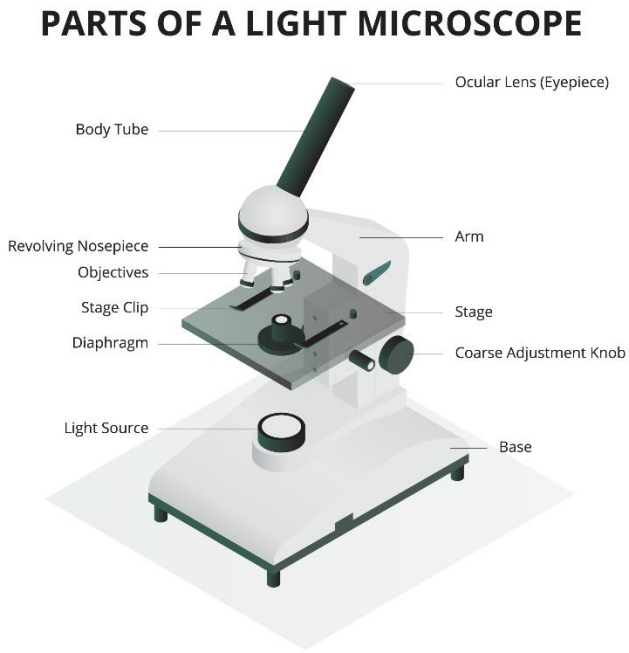
Particle morphology, generally defined and particle size and shape characteristics, are properties of a pharmaceutical drug product which can have a significant impact on the final product performance. Particle size can affect dissolution rate, activity, bioavailability. formulation stability, dosage. Particle shape can affect properties such as the processability, flowability, packing density as well as being a defining property of the crystal form of a product. With the introduction of technologies such as 3D printing into the pharmaceutical space, particle morphology is becoming increasingly important, where shape factors such as roundness, elongation and aspect ratio along with the surface texture of a particle can significantly impact the performance of the material within the printer. As manufacturing capabilities, such as continuous manufacturing, continue to advance, the basic science of particle morphology becomes increasingly important.

One of the primary techniques used to study the morphology of particles is static image analysis (SIA) using optical microscopy. In contrast to alternative technologies such as Scanning Electron Microscopy (SEM), Laser Diffraction (LD), or Dynamic Image Analysis (DIA), it is a readily accessible, cost effective, rapid and highly accurate technique for understanding the nature of particles. SIA can also be fully automated using an automated XYZ-stage, enabling thousands of particles in a sample to be analyzed in in minutes. The basic requirements for an optical microscopy particle image analysis workstation are a standard optical microscope suitable objectives and camera port, a digital camera, and analysis software such as CLAIRITY™ Particle Analysis Suite from ImageProVision, Inc.

This application note aims to discuss particle characterization and visualization using optical microscopy, and highlight the basic considerations for equipment, methods, and suitability to ensure both accurate and reliable size and morphological measurements.

1. **Components of an Optical Microscope**

   An optical microscope consists of a light source, diaphragm, sample stage, objectives and an ocular piece as illustrated below. Typically, a microscope used for particle analysis would consist of multiple objectives ranging from 4x to 100x magnification, where a 100x magnification would be an oil immersion objective. To perform particle analysis from digital images, a digital camera is attached to c-mount or camera port, to allow image acquisition. For automation, stationary stages can be replaced with an automated XYZ-stages, which allows the acquisition software to control the position and focal point of the sample on the stage to allow for large sections of a dispersed sample to be analyzed unattended.



2. Sample Preparation:

   Sample preparation is perhaps the most important step in particle analysis. Getting a sample onto a microscope slide evenly, well dispersed, and without agglomeration can be a considerable challenge. There are a variety of methods which can be used to obtain a well dispersed sample.

   a. Dry Dispersion: In some cases, it is possible to sprinkle the sample over the slide, and obtain good dispersion, especially if the powder is dry, and static free, but this is quite uncommon. Typically, a dry sample dispersion would be obtained using a sample dispersion system, where a sample is loaded into a dispersion chamber, and sprayed onto the slide using a burst of high-pressure air. Variation

of the air pressure in these systems can be used to control the breakdown of agglomerates for an aggregated sample.

b. Wet Dispersion: In wet dispersion, a sample is dispersed into a suitable solvent prior to placing on the stage. This process allows the use of ultrasonics and dispersants to help break down agglomerates in the sample. The sample is placed under a coverslip, which can be sealed in place using clear nail polish if the solvent is likely to evaporate prior to or during measurement. A drawer back of this technique is that the sample can undergo Brownian motion during analysis, making it difficult to analyze fine samples.
c. Evaporative Dispersion: In this technique, a sample is dispersed into a relatively volatile compatible solvent and placed onto the slide. The solvent is allowed to slowly evaporate, leaving the dispersed sample in place on the slide. The advantage of this technique is that ultrasonics and dispersants can be used to improve dispersion, without the challenges of a wet dispersion.

3. Measurement Techniques:

   There are generally two ways of obtaining particle size measurements from image analysis.

   a. Direct Measurement: In this technique, manual observation of particles using the ocular pieces is combined with a calibrated graticule to allow visual estimation of the size of observed particles. In this instance the morphology of a particle can only be performed as a visual observation.
   b. Image Analysis: Utilizing software such as the CLAIRITY™ Particle Analysis Suite, images of the dispersed particles are obtained directly from the microscope. Once collected, the images are processed and analyzed for particle morphology such as circularity, aspect ratio, elongation, and intensity can be accurately calculated. A benefit of this technique is that the image of each individual particle is saved and can be retrieved afterwards for further review or analysis.

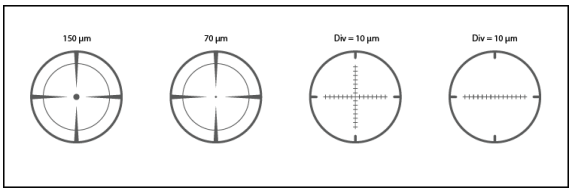
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5. Calibration and Standards:

   calibration of a microscope prior to sample analysis is a vital stage in particle size measurements. Accurate calibration ensures accurate, quality results, while validation standards can be used to verify the performance and limitations of a system.

   a. Calibration Graticule: If accurate size measurements are required, a microscope and image analysis software will require calibration. This is typically performed

using a calibration graticule as seen below, which is a microscope slide engraved length markings. They can be used in direct measurements for estimation of size, and in image analysis for calibration of pixel dimensions.



b. Reference Standards: Once calibrated, a system's performance can be verified using reference standards such as glass beads. These samples are generally polydisperse in nature and can be obtained from a variety of scientific instrument suppliers.

6. Considerations and Challenges:

Although considered a relatively simple to use technique, light microscopy does have some important considerations and challenges.

a. Resolution Limits: The typical resolution limit of light microscopy is considered to be about 0.5µm, which is a fundamental limit due to the physics of light. In an optical microscope, each objective will have its own limitation for particle resolution. In image analysis however, although particles can be detected down to very small sizes, for calculation of shape parameters it is recommended that a particle image contain no less than 100 pixels.
b. Sample Homogeneity: A fundamental challenge of optical microscopy is obtaining a evenly presented, homogeneous sample for analysis. Aggregated samples can misrepresent a sample and make it challenging to resolve particles into the individual components. A well-developed sample preparation method is essential to achieve quality, accurate and repeatable results.
c. Representative Sample: In microscopy, the amount of sample present on the slide is quite small, generally requiring it to be subsampled from a larger batch. It is important to ensure that you obtain a evenly dispersed, representative sample during subsampling, tools such as spinning rifflers can aid in this process.

7. Applications:
    a. Pharmaceuticals: In pharmaceutical development, measurement of particles are not only relevant to provide information about a products performance and specifications, but it is also mandated by regulatory authorities such as the USFDA. Regulations such USP <1776> provide guidance for qualitative or quantitative image analysis is the of two- digital images captured for pharmaceutical formulations and USP<787>, <788>, and <789> specify the use of optical microscopy to measure the particle morphology of ingredients as well as stipulate the physical limitations of the number and size of particulate matter contaminant particles within a final drug product.
    b. Materials Science: Within the world of material science, the measurement of particle size is a fundamental requirement to understand the nature of a material, from processing of ceramics to understanding the stability and emulsion, particle size influences a range of properties of a sample.
    c. Food and Beverages: For food and beverages, particle size dictates properties such as the mouth feel and flavor of chocolate, to the stability of products such as ketchup and mayonnaise. Particle size can even influence the color and opacity of beverages and flavoring agents.
    d. Geology: Particle size or grain size is a measurement commonly performed in geographical studies to understand the way rocks and minerals are forms, and to understand they way silts and sediments move about the environment.

Understanding the basic principles of particle sizing using microscopy is essential for obtaining reliable and meaningful results. Researchers and industrial professionals can leverage these insights to optimize processes, ensure product quality, and advance scientific understanding in various fields.

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