⟨776⟩ OPTICAL MICROSCOPY

Optical microscopy for particle characterization can generally be applied to particles 1 µm and greater. The lower limit is imposed by the resolving power of the microscope. The upper limit is less definite and is determined by the increased difficulty associated with the characterization of larger particles. Various alternative techniques are available for particle characterization outside the applicable range of optical microscopy. Optical microscopy is particularly useful for characterizing particles that are not spherical. This method may also serve as a base for the calibration of faster and more routine methods that may be developed.

APPARATUS

Use a microscope that is stable and protected from vibration. The microscope magnification (product of the objective magnification, ocular magnification, and additional magnifying components) must be sufficient to allow adequate characterization of the smallest particles to be classified in the test specimen. The greatest numerical aperture of the objective should be sought for each magnification range. Polarizing filters may be used in conjunction with suitable analyzers and retardation plates. Color filters of relatively narrow spectral transmission should be used with achromatic objectives, are preferable with apochromats, and are required for appropriate color rendition in photomicrography. Condensers corrected at least for spherical aberration should be used in the microscope substage and with the lamp. The numerical aperture of the substage condenser should match that of the objective under the conditions of use and is affected by the actual aperture of the condenser diaphragm and by the presence of immersion oils.

ADJUSTMENT

The precise alignment of all elements of the optical system and proper focusing are essential. The focusing of the elements should be done in accordance with the recommendations of the microscope manufacturer. Critical axial alignment is recommended.

ILLUMINATION

A requirement for good illumination is a uniform and adjustable intensity of light over the entire field of view; Kohler illumination is preferred. With colored particles, choose the color of the filters used so as to control the contrast and detail of the image.

VISUAL CHARACTERIZATION

The magnification and numerical aperture should be sufficiently high to allow adequate resolution of the images of the particles to be characterized. Determine the actual magnification using a calibrated stage micrometer to calibrate an ocular micrometer. Errors can be minimized if the magnification is sufficient that the image of the particle is at least 10 ocular divisions. Each objective must be calibrated separately. To calibrate the ocular scale, the stage micrometer scale and the ocular scale should be aligned. In this way, a precise determination of the distance between ocular stage divisions can be made. Several different magnifications may be necessary to characterize materials having a wide particle size distribution.

PHOTOGRAPHIC CHARACTERIZATION

If particle size is to be determined by photographic methods, take care to ensure that the object is sharply focused at the plane of the photographic emulsion. Determine the actual magnification by photographing a calibrated stage micrometer, using photographic film of sufficient speed, resolving power, and contrast. Exposure and processing should be identical for photographs of both the test specimen and the determination of magnification. The apparent size of a photographic image is influenced by the exposure, development, and printing processes as well as by the resolving power of the microscope.

PREPARATION OF THE MOUNT

The mounting medium will vary according to the physical properties of the test specimen. Sufficient but not excessive contrast between the specimen and the mounting medium is required to ensure adequate detail of the specimen edge. The particles should rest in one plane and be adequately dispersed to distinguish individual particles of interest. Furthermore, the particles must be representative of the distribution of sizes in the material and must not be altered during preparation of the mount. Care should be taken to ensure that this important requirement is met. Selection of the mounting medium must include a consideration of the analyte solubility.

CRYSTALLINITY CHARACTERIZATION

The crystallinity of a material may be characterized to determine compliance with the crystallinity requirement where stated in the individual monograph of a drug substance. Unless otherwise specified in the individual monograph, mount a few particles of the specimen in mineral oil on a clean glass slide. Examine the mixture using a polarizing microscope: the particles show birefringence (interference colors) and extinction positions when the microscope stage is revolved.

LIMIT TEST OF PARTICLE SIZE BY MICROSCOPY

Weigh a suitable quantity of the powder to be examined (for example, 10 to 100 mg), and suspend it in 10 mL of a suitable medium in which the powder does not dissolve, adding, if necessary, a wetting agent. A homogeneous suspension of particles can be maintained by suspending the particles in a medium of similar or matching density and by providing adequate agitation. Introduce a portion of the homogeneous suspension into a suitable counting cell, and scan under a microscope an area corresponding to not less than 10 µg of the powder to be examined. Count all the particles having a maximum dimension greater than the prescribed size limit. The size limit and the permitted number of particles exceeding the limit are defined for each substance.

PARTICLE SIZE CHARACTERIZATION

The measurement of particle size varies in complexity depending on the shape of the particle, and the number of particles characterized must be sufficient to ensure an acceptable level of uncertainty in the measured parameters. Additional information on particle size measurement, sample size, and data analysis is available, for example, in ISO 9276. For spherical particles, size is defined by the diameter. For irregular particles, a variety of definitions of particle size exist. In general, for irregularly shaped particles, characterization of particle size must also include information on the type of diameter measured as well as information on particle shape. Several commonly used measurements of particle size are defined below (see *Figure 1*):

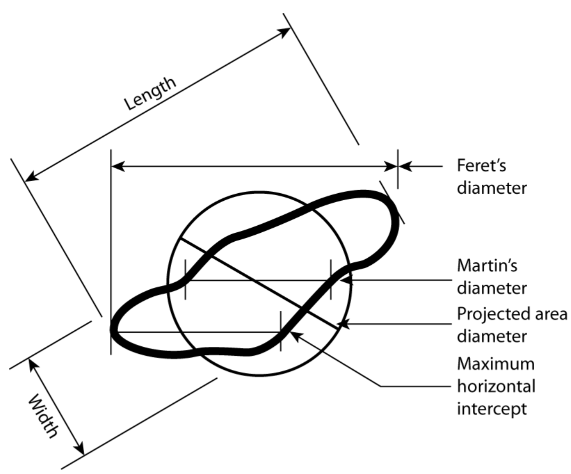

Figure 1. Commonly used measurements of particle size.

Feret's Diameter—The distance between imaginary parallel lines tangent to a randomly oriented particle and perpendicular to the ocular scale.
Martin's Diameter—The diameter of the particle at the point that divides a randomly oriented particle into two equal projected areas.
Projected Area Diameter—The diameter of a circle that has the same projected area as the particle.
Length—The longest dimension from edge to edge of a particle oriented parallel to the ocular scale.
Width—The longest dimension of the particle measured at right angles to the length.

PARTICLE SHAPE CHARACTERIZATION

For irregularly shaped particles, characterization of particle size must also include information on particle shape. The homogeneity of the powder should be checked using appropriate magnification. The following defines some commonly used descriptors of particle shape (see *Figure 2*):

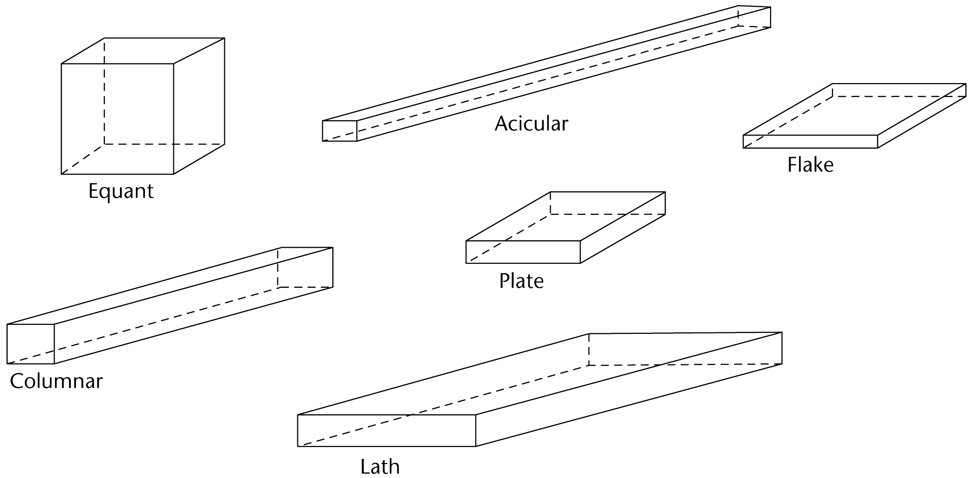

Figure 2. Commonly used descriptions of particle shape.

Acicular—Slender, needle-like particle of similar width and thickness.
Columnar—Long, thin particle with a width and thickness that are greater than those of an acicular particle.
Flake—Thin, flat particle of similar length and width.
Plate—Flat particles of similar length and width but with greater thickness than flakes.
Lath—Long, thin, and blade-like particle.
Equant—Particles of similar length, width, and thickness; both cubical and spherical particles are included.

GENERAL OBSERVATIONS

A particle is generally considered to be the smallest discrete unit. A particle may be a liquid or semisolid droplet; a single crystal or polycrystalline; amorphous or an agglomerate. Particles may be associated. This degree of association may be described by the following terms:
Lamellar—Stacked plates.
Aggregate—Mass of adhered particles.
Agglomerate—Fused or cemented particles.
Conglomerate—Mixture of two or more types of particles.
Spherulite—Radial cluster.
Drusy—Particle covered with tiny particles.
Particle condition may be described by the following terms:
Edges—Angular, rounded, smooth, sharp, fractured.
Optical—Color (using proper color-balancing filters), transparent, translucent, opaque.
Defects—Occlusions, inclusions.
Surface characteristics may be described as:
Cracked—Partial split, break, or fissure.
Smooth—Free of irregularities, roughness, or projections.
Porous—Having openings or passageways.
Rough—Bumpy, uneven, not smooth.
Pitted—Small indentations.