Calculate the quantity, in mg, of the analyte in the *Test Preparation* by the formula:

$$W_S(A_U/A_S)(E_U/E_S)$$

in which W_S is the weight, in mg, of *Internal Standard* taken; and E_U and E_S are the proton equivalent weights (i.e., the molecular weights divided by the number of protons giving rise to the reference peak) of the analyte and the *Internal Standard*, respectively.

Relative Method of Quantitation—Where the individual monograph directs that the *Relative Method of Quantitation* be employed, proceed as follows.

Solvent, NMR Reference, and *Test Preparation*—Use as directed under *Absolute Method of Quantitation*.

Procedure—Transfer an appropriate amount (0.4 to 0.8 mL) of *Test Preparation* to a standard 5-mm NMR spinning tube, and record the spectrum, adjusting the spin rate so that no spinning side bands interfere with the peaks of interest. Measure the area or intensity under each of the peaks specified in the individual monograph by integrating not fewer than five times. Record the average area or intensity resulting from the resonances of the groups designated in the individual monograph as A_1 and A_2.

Calculate the quantity, in mole percent, of the analyte in the *Test Preparation* by the formula:

$$100 \times (A_1/n_1)/[(A_1/n_1) + (A_2/n_2)]$$

in which n_1 and n_2 are, respectively, the numbers of protons in the designated groups.

⟨771⟩ OPHTHALMIC OINTMENTS

Added Substances—Suitable substances may be added to ophthalmic ointments to increase stability or usefulness, unless proscribed in the individual monograph, provided they are harmless in the amounts administered and do not interfere with the therapeutic efficacy or with the responses to the specified assays and tests. No coloring agent may be added, solely for the purpose of coloring the finished preparation, to an article intended for ophthalmic use (see also *Added Substances* under *General Notices* and under *Antimicrobial Effectiveness Testing* ⟨51⟩).

A suitable substance or mixture of substances to prevent the growth of microorganisms must be added to ophthalmic ointments that are packaged in multiple-use containers, regardless of the method of sterilization employed, unless otherwise directed in the individual monograph, or unless the formula itself is bacteriostatic. Such substances are used in concentrations that will prevent the growth of or kill microorganisms in the ophthalmic ointments (see also *Antimicrobial Effectiveness Testing* ⟨51⟩ and *Antimicrobial Agents—Content* ⟨341⟩). Sterilization processes are employed for the finished ointment or for all ingredients, if the ointment is manufactured under rigidly aseptic conditions, even though such substances are used (see also *Parenteral and Topical Preparations* in the section *Added Substances*, under *General Notices*, and *Sterilization and Sterility Assurance of Compendial Articles* ⟨1211⟩). Ophthalmic ointments that are packaged in single-use containers are not required to contain antibacterial agents; however, they meet the requirements for *Sterility Tests* ⟨71⟩.

Containers—Containers, including the closures, for ophthalmic ointments do not interact physically or chemically with the preparation in any manner to alter the strength, quality, or purity beyond the official requirements under the ordinary or customary conditions of handling, shipment, storage, sale, and use.

Metal Particles—Follow the *Procedure* set forth under *Metal Particles in Ophthalmic Ointments* ⟨751⟩.

Leakage—Select 10 tubes of the Ointment, with seals applied when specified. Thoroughly clean and dry the exterior surfaces of each tube with an absorbent cloth. Place the tubes in a horizontal position on a sheet of absorbent blotting paper in an oven maintained at a temperature of 60 ± 3° for 8 hours. No significant leakage occurs during or at the completion of the test (disregard traces of ointment presumed to originate externally from within the crimp of the tube or from the thread of the cap). If leakage is observed from one, but not more than one, of the tubes, repeat the test with 20 additional tubes of the Ointment. The requirement is met if no leakage is observed from the first 10 tubes tested, or if leakage is observed from not more than one of 30 tubes tested.

⟨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*):

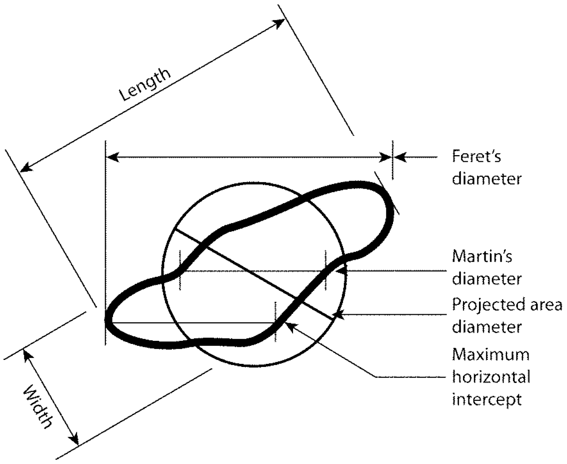

Figure 1. Commonly used measurements of particle size.

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*):

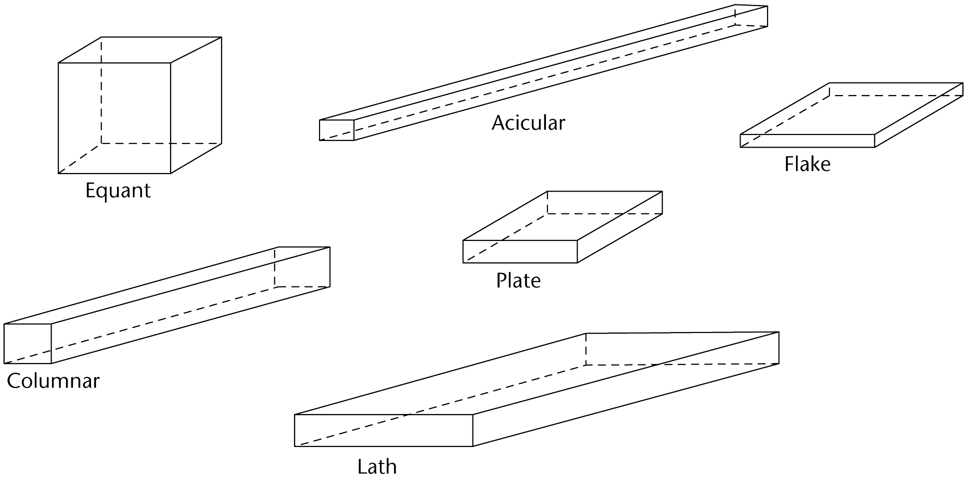

Figure 2. Commonly used descriptions of particle shape.

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.

⟨781⟩ OPTICAL ROTATION

Many pharmaceutical substances are optically active in the sense that they rotate an incident plane of polarized light so that the transmitted light emerges at a measurable angle to the plane of the incident light. This property is characteristic of some crystals and of many pharmaceutical liquids or solutions of solids. Where the property is possessed by a liquid or by a solute in solution, it is generally the result of the presence of one or more asymmetric centers, usually a carbon atom with four different substituents. The number of optical isomers is 2^n, where n is the number of asymmetric centers. Polarimetry, the measurement of optical rotation, of a pharmaceutical article may be the only convenient means for distinguishing optically active isomers from each other and thus is an important criterion of identity and purity.

Substances that may show optical rotatory power are *chiral.* Those that rotate light in a clockwise direction as viewed towards the light source are *dextrorotatory*, or (+) *optical isomers*. Those that rotate light in the opposite direction are called *levorotatory* or (–) optical isomers. (The symbols *d*- and *l*-, formerly used to indicate dextro- and levorotatory isomers, are no longer sanctioned owing to confusion with D- and L-, which refer to configuration relative to D-glyceraldehyde. The symbols R and S and α and *β* are also used to indicate configuration, the arrangement of atoms or groups of atoms in space.)

The physicochemical properties of nonsuperimposable chiral substances rotating plane polarized light in opposite directions to the same extent, *enantiomers*, are identical, except for this property and in their reactions with other chiral substances. Enantiomers often exhibit profound differences in pharmacology and toxicology, owing to the fact that biological receptors and enzymes themselves are chiral. Many articles from natural sources, such as amino acids, proteins, alkaloids, antibiotics, glycosides, and sugars, exist as chiral compounds. Synthesis of such compounds from nonchiral materials results in equal numbers of the enantiomers, *racemates*. Racemates have a net null optical rotation, and their physical properties may differ from those of the component enantiomers. Use of stereoselective or stereospecific synthetic methods or separation of racemic mixtures can be used to obtain individual optical isomers.

Measurement of optical rotation is performed using a polarimeter.* The general equation used in polarimetry is:

$$[\alpha]^{t}_{\lambda} = \frac{100a}{lc}$$

where [α] is the specific rotation at wavelength λ, *t* is the temperature, *a* is the observed rotation in degrees (°), *l* is the pathlength in decimeters, and *c* is the concentration of the analyte in g per 100 mL. Thus, [α] is 100 times the measured value, in degrees (°), for a solution containing 1 g in 100 mL, measured in a cell having a pathlength of 1.0 dm under defined conditions of incident wavelength of light and temperature. For some Pharmacopeial articles, especially liquids such as essential oils, the optical rotation requirement is expressed in terms of the observed rotation, a, measured under conditions defined in the monograph.

Historically, polarimetry was performed using an instrument where the extent of optical rotation is estimated by visual matching of the intensity of split fields. For this reason, the D-line of the sodium lamp at the visible wavelength of 589 nm was most often employed. Specific rotation determined at the D-line is expressed by the symbol:

$$[\alpha]^{25}_{D} \text{ or } [\alpha]^{20}_{D}$$

and much of the data available are expressed in this form. Use of lower wavelengths, such as those available with the mercury lamp lines isolated by means of filters of maximum transmittance at approximately 578, 546, 436, 405, and 365 nm in a photoelectric polarimeter, has been found to provide advantages in sensitivity with a consequent reduction in the concentration of the test compound. In general, the observed optical rotation at 436 nm is about double and at 365 nm about three times that at 589 nm. Reduction in the concentration of the solute required for measurement may sometimes be accomplished by conversion of the substance under test to one that has a significantly higher optical rotation. Optical rotation is also affected by the solvent used for the measurement, and this is always specified.

It is now common practice to use other light sources, such as xenon or tungsten halogen, with appropriate filters, because these may offer advantages of cost, long life, and broad wavelength emission range, over traditional light sources.

Change to read:

Specific Rotation—The reference *Specific Rotation* ⟨781S⟩ in a monograph signifies that specific rotation is to be calculated from observed optical rotations in the *Test solution* ▲or *Sample solution*▲*USP35* obtained as directed therein. Unless otherwise directed, measurements of optical rotation are made at 589 nm at 25°. Where a photoelectric polarimeter is used, a single measurement, corrected for the solvent blank, is made. Where a visual polarimeter is employed, the average of no fewer than five determinations, corrected for the reading of the same tube with a solvent blank, is used. Temperature, which applies to the solution or the liquid under test, should be maintained within 0.5° of the stated value. Use the same cell for sample and blank. Maintain the same angular orientation of the cell in each reading. Place the cell so that the light passes through it in the same direction each time. Unless otherwise specified, specific rotation is calculated on the dried basis where *Loss on Drying* is specified in the monograph, or on the anhydrous basis where *Water* is specified.

Optical rotation of solutions should be determined within 30 minutes of preparation. In the case of substances known to undergo racemization or mutarotation, care should be taken to standardize the time between adding the solute to the solvent and introduction of the solution into the polarimeter tube.

Angular Rotation—The reference *Angular Rotation* ⟨781A⟩ in a monograph signifies, unless otherwise directed, that the optical rotation of the neat liquid is measured in a 1.0-dm tube at 589 nm at 25°, corrected for the reading of the dry empty tube.

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*Suitable calibrators are available from the Office of Standard Reference Materials, National Institute of Standards and Technology (NIST), Gaithersburg, MD 20899, as current lots of Standard Reference Materials, Dextrose and Sucrose. Alternatively, calibration may be checked using a Polarization Reference Standard, which consists of a plate of quartz mounted in a holder perpendicular to the light path. These standards are available, traceable to NIST, from Rudolph Research Analytical, 354 Route 206, Flanders, NJ 07836, or from Rudolph Instruments, Inc., 40 Pier Lane, Fairfield, NJ 07004-2113.