that such charging is not influencing the analysis. An antistatic agent, such as colloidal silicon dioxide and/or aluminum oxide, may be added at a 0.5 percent (m/m) level to minimize this effect. If both of the above effects cannot be eliminated, an alternative particle-sizing technique must be selected.

Agitation Methods—Several different sieve and powder agitation devices are commercially available, all of which may be used to perform sieve analyses. However, the different methods of agitation may give different results for sieve analyses and endpoint determinations because of the different types and magnitude of the forces acting on the individual particles under test. Methods using mechanical agitation or electromagnetic agitation, and that can induce either a vertical oscillation or a horizontal circular motion, or tapping or a combination of both tapping and horizontal circular motion are available. Entrainment of the particles in an air stream may also be used. The results must indicate which agitation method was used and the agitation parameters used (if they can be varied), because changes in the agitation conditions will give different results for the sieve analysis and endpoint determinations, and may be sufficiently different to give a failing result under some circumstances.

Endpoint Determination—The test sieving analysis is complete when the weight on any of the test sieves does not change by more than 5% or 0.1 g (10% in the case of 76-mm sieves) of the previous weight on that sieve. If less than 5% of the total specimen weight is present on a given sieve, the endpoint for that sieve is increased to a weight change of not more than 20% of the previous weight on that sieve.

If more than 50% of the total specimen weight is found on any one sieve, unless this is indicated in the monograph, the test should be repeated, but with the addition to the sieve nest of a more coarse sieve, intermediate between that carrying the excessive weight and the next coarsest sieve in the original nest, i.e., addition of the ISO series sieve omitted from the nest of sieves.

SIEVING METHODS

Mechanical Agitation

Dry Sieving Method—Tare each test sieve to the nearest 0.1 g. Place an accurately weighed quantity of test specimen on the top (coarsest) sieve, and replace the lid. Agitate the nest of sieves for 5 minutes. Then carefully remove each from the nest without loss of material. Reweigh each sieve, and determine the weight of material on each sieve. Determine the weight of material in the collecting pan in a similar manner. Reassemble the nest of sieves, and agitate for 5 minutes. Remove and weigh each sieve as previously described. Repeat these steps until the endpoint criteria are met (see *Endpoint Determination* under *Test Sieves*). Upon completion of the analysis, reconcile the weights of material. Total losses must not exceed 5% of the weight of the original test specimen.

Repeat the analysis with a fresh specimen, but using a single sieving time equal to that of the combined times used above. Confirm that this sieving time conforms to the requirements for endpoint determination. When this endpoint has been validated for a specific material, then a single fixed time of sieving may be used for future analyses, providing the particle size distribution falls within normal variation.

If there is evidence that the particles retained on any sieve are aggregates rather than single particles, the use of mechanical dry sieving is unlikely to give good reproducibility, and a different particle size analysis method should be used.

Air Entrainment Methods

Air Jet and *Sonic Sifter Sieving*—Different types of commercial equipment that use a moving air current are available for sieving. A system that uses a single sieve at a time is referred to as air jet sieving. It uses the same general sieving methodology as that described under the *Dry Sieving Method,* but with a standardized air jet replacing the normal agitation mechanism. It requires sequential analyses on individual sieves starting with the finest sieve to obtain a particle size distribution. Air jet sieving often includes the use of finer test sieves than those used in ordinary dry sieving. This technique is more suitable where only oversize or undersize fractions are needed.

In the sonic sifting method, a nest of sieves is used, and the test specimen is carried in a vertically oscillating column of air that lifts the specimen and then carries it back against the mesh openings at a given number of pulses per minute. It may be necessary to lower the sample amount to 5 g, when sonic sifting is employed.

The air jet sieving and sonic sieving methods may be useful for powders or granules when mechanical sieving techniques are incapable of giving a meaningful analysis.

These methods are highly dependent upon proper dispersion of the powder in the air current. This requirement may be hard to achieve if the method is used at the lower end of the sieving range (i.e., below 75 µm), when the particles tend to be more cohesive, and especially if there is any tendency for the material to develop an electrostatic charge. For the above reasons endpoint determination is particularly critical, and it is very important to confirm that the oversize material comprises single particles and is not composed of aggregates.

INTERPRETATION

The raw data must include the weight of test specimen, the total sieving time, and the precise sieving methodology and the set values for any variable parameters, in addition to the weights retained on the individual sieves and in the pan. It may be convenient to convert the raw data into a cumulative weight distribution, and if it is desired to express the distribution in terms of a cumulative weight undersize, the range of sieves used should include a sieve through which all the material passes. If there is evidence on any of the test sieves that the material remaining on it is composed of aggregates formed during the sieving process, the analysis is invalid.

⟨788⟩ PARTICULATE MATTER IN INJECTIONS

This general chapter is harmonized with the corresponding texts of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia.* These pharmacopeias have undertaken not to make any unilateral change to this harmonized chapter. Portions of the present general chapter text that are national USP text, and therefore not part of the harmonized text, are marked with symbols (✦✦) to specify this fact.

Particulate matter in injections and parenteral infusions consists of extraneous mobile undissolved particles, other than gas bubbles, unintentionally present in the solutions.

✦As stated in *Injections* ⟨1⟩, solutions for injection administered by the intramuscular or subcutaneous route must meet the requirements of *Particulate Matter in Injections* ⟨788⟩. Parenterals packaged and labeled exclusively for use

as irrigating solutions are exempt from the requirements of *Particulate Matter in Injections* ⟨788⟩. Radiopharmaceutical preparations are exempt from the requirements of *Particulate Matter in Injections* ⟨788⟩. Parenteral products for which the labeling specifies use of a final filter prior to administration are exempt from the requirements of *Particulate Matter in Injections* ⟨788⟩, provided that scientific data are available to justify this exemption.✦

For the determination of particulate matter, two procedures, *Method 1 (Light Obscuration Particle Count Test)* and *Method 2 (Microscopic Particle Count Test),* are specified hereinafter. When examining injections and parenteral infusions for subvisible particles, *Method 1* is preferably applied. However, it may be necessary to test some preparations by the *Light Obscuration Particle Count Test* followed by the *Microscopic Particle Count Test* to reach a conclusion on conformance to the requirements.

Not all parenteral preparations can be examined for subvisible particles by one or both of these methods. When *Method 1* is not applicable, e.g., in the case of preparations having reduced clarity or increased viscosity, the test should be carried out according to *Method 2*. Emulsions, colloids, and liposomal preparations are examples. Similarly, products that produce air or gas bubbles when drawn into the sensor may also require microscopic particle count testing. If the viscosity of the preparation to be tested is sufficiently high so as to preclude its examination by either test method, a quantitative dilution with an appropriate diluent may be made to decrease viscosity, as necessary, to allow the analysis to be performed.

The results obtained in examining a discrete unit or group of units for particulate matter cannot be extrapolated with certainty to other units that remain untested. Thus, statistically sound sampling plans must be developed if valid inferences are to be drawn from observed data to characterize the level of particulate matter in a large group of units.

METHOD 1
LIGHT OBSCURATION PARTICLE COUNT TEST

Use a suitable apparatus based on the principle of light blockage that allows for an automatic determination of the size of particles and the number of particles according to size. The definition for *particle-free water* is provided in *Reagent Specifications* under *Reagents, Indicators, and Solutions.*

The apparatus is calibrated using dispersions of spherical particles of known sizes between 10 μm and 25 μm. These standard particles are dispersed in *particle-free water*. Care must be taken to avoid aggregation of particles during dispersion. ✦System suitability can be verified by using the USP Particle Count RS.✦

General Precautions

The test is carried out under conditions limiting particulate matter, preferably in a laminar flow cabinet.

Very carefully wash the glassware and filtration equipment used, except for the membrane filters, with a warm detergent solution, and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use, rinse the equipment from top to bottom, outside and then inside, with *particle-free water.*

Take care not to introduce air bubbles into the preparation to be examined, especially when fractions of the preparation are being transferred to the container in which the determination is to be carried out.

In order to check that the environment is suitable for the test, that the glassware is properly cleaned, and that the water to be used is particle-free, the following test is carried out: determine the particulate matter in 5 samples of *particle-free water,* each of 5 mL, according to the method described below. If the number of particles of 10 μm or greater size exceeds 25 for the combined 25 mL, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware, and water are suitable for the test.

Method

Mix the contents of the sample by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using a jet of *particle-free water* and remove the closure, avoiding any contamination of the contents. Eliminate gas bubbles by appropriate measures such as allowing to stand for 2 minutes or sonicating.

For large-volume parenterals, single units are tested. For small-volume parenterals less than 25 mL in volume, the contents of 10 or more units are combined in a cleaned container to obtain a volume of not less than 25 mL; the test solution may be prepared by mixing the contents of a suitable number of vials and diluting to 25 mL with *particle-free water* or with an appropriate particle-free solvent when *particle-free water* is not suitable. Small-volume parenterals having a volume of 25 mL or more may be tested individually.

Powders for parenteral use are reconstituted with *particle-free water* or with an appropriate particle-free solvent when *particle-free water* is not suitable.

The number of test specimens must be adequate to provide a statistically sound assessment. For large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, fewer than 10 units may be tested, using an appropriate sampling plan.

Remove four portions, not less than 5 mL each, and count the number of particles equal to or greater than 10 μm and 25 μm. Disregard the result obtained for the first portion, and calculate the mean number of particles for the preparation to be examined.

Evaluation

For preparations supplied in containers with a nominal volume of more than 100 mL, apply the criteria of *Test 1.A*.

For preparations supplied in containers with a nominal volume of less than 100 mL, apply the criteria of *Test 1.B*.

For preparations supplied in containers with a nominal volume of 100 mL, apply the criteria of *Test 1.B*. [NOTE—*Test 1.A* is used in the *Japanese Pharmacopeia*.]

If the average number of particles exceeds the limits, test the preparation by the *Microscopic Particle Count Test*.

Test 1.A *(Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of more than 100 mL)*—The preparation complies with the test if the average number of particles present in the units tested does not exceed 25 per mL equal to or greater than 10 μm and does not exceed 3 per mL equal to or greater than 25 μm.

Test 1.B *(Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of less than 100 mL)*—The preparation complies with the test if the average number of particles present in the units tested does not exceed 6000 per container equal to or greater than 10 μm and does not exceed 600 per container equal to or greater than 25 μm.

METHOD 2
MICROSCOPIC PARTICLE COUNT TEST

Use a suitable binocular microscope, a filter assembly for retaining particulate matter, and a membrane filter for examination.

The microscope is adjusted to 100 ± 10 magnifications and is equipped with an ocular micrometer calibrated with an objective micrometer, a mechanical stage capable of holding and traversing the entire filtration area of the mem-

brane filter, and two suitable illuminators to provide episcopic illumination in addition to oblique illumination.

The ocular micrometer is a circular diameter graticule (see *Figure 1*)

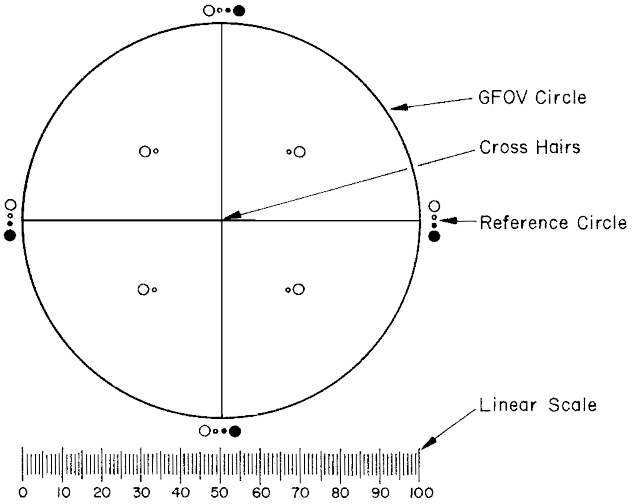

Fig. 1. Circular diameter graticule. The large circle divided by crosshairs into quadrants is designated the graticule field of view (GFOV). Transparent and black circles having 10-µm and 25-µm diameters at 100× are provided as comparison scales for particle sizing.

and consists of a large circle divided by crosshairs into quadrants, transparent and black reference circles 10 µm and 25 µm in diameter at 100 magnifications, and a linear scale graduated in 10-µm increments. It is calibrated using a stage micrometer that is certified by either a domestic or international standard institution. A relative error of the linear scale of the graticule within ±2% is acceptable. The large circle is designated the graticule field of view (GFOV).

Two illuminators are required. One is an episcopic brightfield illuminator internal to the microscope, the other is an external, focusable auxiliary illuminator that can be adjusted to give reflected oblique illumination at an angle of 10° to 20°.

The filter assembly for retaining particulate matter consists of a filter holder made of glass or other suitable material, and is equipped with a vacuum source and a suitable membrane filter.

The membrane filter is of suitable size, black or dark gray in color, nongridded or gridded, and 1.0 µm or finer in nominal pore size.

General Precautions

The test is carried out under conditions limiting particulate matter, preferably in a laminar flow cabinet.

Very carefully wash the glassware and filter assembly used, except for the membrane filter, with a warm detergent solution, and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use, rinse both sides of the membrane filter and the equipment from top to bottom, outside and then inside, with *particle-free water*.

In order to check that the environment is suitable for the test, that the glassware and the membrane filter are properly cleaned, and that the water to be used is particle-free, the following test is carried out: determine the particulate matter of a 50-mL volume of *particle-free water* according to the method described below. If more than 20 particles 10 µm or larger in size or if more than 5 particles 25 µm or larger in size are present within the filtration area, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware, membrane filter, and water are suitable for the test.

Method

Mix the contents of the samples by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using a jet of *particle-free water* and remove the closure, avoiding any contamination of the contents.

For large-volume parenterals, single units are tested. For small-volume parenterals less than 25 mL in volume, the contents of 10 or more units are combined in a cleaned container; the test solution may be prepared by mixing the contents of a suitable number of vials and diluting to 25 mL with *particle-free water* or with an appropriate particle-free solvent when *particle-free water* is not suitable. Small-volume parenterals having a volume of 25 mL or more may be tested individually.

Powders for parenteral use are constituted with *particle-free water* or with an appropriate particle-free solvent when *particle-free water* is not suitable.

The number of test specimens must be adequate to provide a statistically sound assessment. For large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, fewer than 10 units may be tested, using an appropriate sampling plan.

Wet the inside of the filter holder fitted with the membrane filter with several mL of *particle-free water*. Transfer to the filtration funnel the total volume of a solution pool or of a single unit, and apply a vacuum. If needed, add stepwise a portion of the solution until the entire volume is filtered. After the last addition of solution, begin rinsing the inner walls of the filter holder by using a jet of *particle-free water*. Maintain the vacuum until the surface of the membrane filter is free from liquid. Place the membrane filter in a Petri dish, and allow the membrane filter to air-dry with the cover slightly ajar. After the membrane filter has been dried, place the Petri dish on the stage of the microscope, scan the entire membrane filter under the reflected light from the illuminating device, and count the number of particles that are equal to or greater than 10 µm and the number of particles that are equal to or greater than 25 µm. Alternatively, partial membrane filter count and determination of the total filter count by calculation is allowed. Calculate the mean number of particles for the preparation to be examined.

The particle sizing process with the use of the circular diameter graticule is carried out by estimating the equivalent diameter of the particle in comparison with the 10 µm and 25 µm reference circles on the graticule. Thereby the particles are not moved from their initial locations within the graticule field of view and are not superimposed on the reference circles for comparison. The inner diameter of the transparent graticule reference circles is used to size white and transparent particles, while dark particles are sized by using the outer diameter of the black opaque graticule reference circles.

In performing the *Microscopic Particle Count Test,* do not attempt to size or enumerate amorphous, semiliquid, or otherwise morphologically indistinct materials that have the appearance of a stain or discoloration on the membrane filter. These materials show little or no surface relief and present a gelatinous or film-like appearance. In such cases, the interpretation of enumeration may be aided by testing a sample of the solution by the *Light Obscuration Particle Count Test*.

Evaluation

For preparations supplied in containers with a nominal volume of more than 100 mL, apply the criteria of *Test 2.A.*

For preparations supplied in containers with a nominal volume of less than 100 mL, apply the criteria of *Test 2.B.*

For preparations supplied in containers with a nominal volume of 100 mL, apply the criteria of *Test 2.B.* [NOTE—*Test 2.A* is used in the *Japanese Pharmacopeia.*]

Test 2.A *(Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of more than 100 mL)*—The preparation complies with the test if the average number of particles present in the units tested does not exceed 12 per mL equal to or greater than 10 μm and does not exceed 2 per mL equal to or greater than 25 μm.

Test 2.B *(Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of less than 100 mL)*—The preparation complies with the test if the average number of particles present in the units tested does not exceed 3000 per container equal to or greater than 10 μm and does not exceed 300 per container equal to or greater than 25 μm.

⟨789⟩ PARTICULATE MATTER IN OPHTHALMIC SOLUTIONS

Particulate matter consists of mobile, randomly sourced, extraneous substances, other than gas bubbles, that cannot be quantitated by chemical analysis because of the small amount of material they represent and because of their heterogeneous composition. Ophthalmic solutions should be essentially free from particles that can be observed on visual inspection. The tests described herein are physical tests performed for the purpose of enumerating extraneous particles within specific size ranges.

Every ophthalmic solution for which the monograph includes a test for *Particulate matter* is subject to the particulate matter limits set forth for the test being applied, unless otherwise specified in the individual monograph. When higher limits are appropriate, they will be specified in the individual monograph. Ophthalmic preparations that are suspensions, emulsions, or gels are exempt from these requirements, as are medical devices. Refer to the specific monograph when a question of test applicability occurs.

Light obscuration and microscopic procedures for the determination of particulate matter in ophthalmic solutions are identical to those for injections; therefore, where appropriate, *Particulate Matter in Injections* ⟨788⟩ is cross-referenced. This chapter provides a test approach in two stages. The ophthalmic solution is first tested by the light obscuration procedure (stage 1). If it fails to meet the prescribed limits, it must pass the microscopic procedure (stage 2) with its own set of test limits. Where for technical reasons the ophthalmic solution cannot be tested by light obscuration, microscopic testing may be used exclusively. Documentation is required, demonstrating that the light obscuration procedure is incapable of testing the ophthalmic solution or that it produces invalid results.

It is expected that most articles will meet the requirements on the basis of the light obscuration test alone; however, it may be necessary to test some articles by the light obscuration test followed by the microscopic test to reach a conclusion on conformance to requirements. Any product that is not a pure solution having a clarity and a viscosity approximating those of water may provide erroneous data when analyzed by the light obscuration counting method. Such materials may be analyzed by the microscopic counting method. In some instances, the viscosity of a material to be tested may be sufficiently high so as to preclude its analysis by either test method. In this event, a quantitative dilution with an appropriate diluent may be made to decrease viscosity, as necessary, to allow the analysis to be performed.

In the tests described below, the results obtained by examining a discrete unit or group of units for particulate matter cannot be extrapolated with certainty to other units that remain untested. Thus, sampling plans based on known operational factors must be developed if valid inferences are to be drawn from observed data to characterize the level of particulate matter in a large group of units. Sampling plans need to be based on consideration of product volume, particle numbers historically found to be present in comparison to limits, particle size distribution of particles present, and variability of particle counts between units.

LIGHT OBSCURATION PARTICLE COUNT TEST

This test applies to ophthalmic solutions, including solutions constituted from sterile solids, for which a test for *Particulate matter* is specified in the individual monograph. The test counts suspended particles that are solid or liquid.

Test Apparatus, Instrument Standardization, Test Environment, Test Procedure, and Calculations—Proceed as directed for *Light Obscuration Particle Count Test* under *Particulate Matter in Injections* ⟨788⟩.

Interpretation—The ophthalmic solution meets the requirements of the test if the average number of particles present in the units tested does not exceed the appropriate value listed in *Table 1*. If the average number of particles exceeds the limit, test the article by the *Microscopic Particle Count Test.*

Table 1. Light Obscuration Test Particle Count

	Diameter	
	≥ **10** μm	≥ **25** μm
Number of particles	50 per mL	5 per mL

MICROSCOPIC PARTICLE COUNT TEST

Some articles cannot be tested meaningfully by light obscuration. In such cases, individual monographs clearly specify that only a microscopic particle count is to be performed. The microscopic particle count test enumerates subvisible, essentially solid, particulate matter in ophthalmic solutions, after collection on a microporous membrane filter. Some ophthalmic solutions, such as solutions that do not filter readily because of their high viscosity, may be exempted from analysis using the microscopic test.

When performing the microscopic test, do not attempt to size or enumerate amorphous, semiliquid, or otherwise morphologically indistinct materials that have the appearance of a stain or discoloration on the membrane surface. These materials show little or no surface relief and present a gelatinous or film-like appearance. Because in solution this material consists of units on the order of 1 μm or less, which may be counted only after aggregation or deformation on an analytical membrane, interpretation of enumeration may be aided by testing a sample of the solution by the light obscuration particle count method.

Test Apparatus, Test Environment, Test Procedure, and Enumeration of Particles—Proceed as directed for *Microscopic Particle Count Test* under *Particulate Matter in Injections* ⟨788⟩.

Interpretation—The ophthalmic solution meets the requirements of the test if the average number of particles present in the units tested does not exceed the appropriate value listed in *Table 2.*