PDA - Aseptic Processing Training Program

Liquid Particulate Counting Evaluations

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Outline
USP <788> - Particulate Matter in Injections

Introduction

USP 30 Verbatim harmonized text as proposed in the European and Japanese pharmacopoeias

Became official on April 1, 2007

7 pages -> 2 pages

Particulate matter – "mobile undissolved particles, other than gas bubbles, unintentionally present in the solution."

Light Obscuration Particle Count Test

NOTE: Tests in square brackets ("[. .]") were deleted from the harmonized <788>

Required for

All large-volume injections (< 100 ml)

Small volume injections for which the monograph specifies

Test Apparatus

Liquid-borne particle counting light-obscuration sensor

[Sensor Concentration Limit]

Instrument Standardization

[Sample Volume Accuracy]

[Sample Flow Rate]

[Calibration]

Manual Method, Automated Method, Electronic Method

[Sensor Resolution]

Manual Method, Automated Method, Electronic Method

Particle Counting Accuracy – ONLY NATIONAL USP TEXT

Method 1 – USP Particle Count Reference Standard (PCRS)

[Method 2 – Homemade standards]

Test Environment

Particle-free water –

"water filtered through a membrane with a pore size of 0.22 um."

Glassware check (in 5 x 5-mL samples of *particle-free water*)

25 particles > 10 um - for the combined 25 mL

[2 particles > 25 um]

Statistically sound sampling plan – TBD by the user

Test Procedure

[Test Preparation]

Measure 4 samples, each at least 5ml samples. Discard the first.

Product Determination

Liquid Preparation

Volume in Containers < 25 mL

Pool at least 10 containers, volume at least 25 mL

Dilution is now explicitly allowed

Volume in Containers 25 mL or more

Dry or Lyophilized Preparations

Products Packaged with Dual Compartments Constructed to Hold the Drug Product and a Solvent in Separate Compartments

Products Labeled "Pharmacy Bulk Package Not for Direct Infusion"

Light Obscuration Particle Count Test (cont.)

Calculations

Pooled Samples (Small-Volume Injections)

Individual Samples (Small-Volume Injections)

Individual Unit Samples (Large-Volume Injections)

Interpretation

	>10 um	>25 um	
Small Volume Injections	6000	600	**per container**
Large Volume Injections	25	3	per mL

** Small volume limit is "per container" – Assumes the patient is getting the total contents of the container.

Two-stage test

1) Light obscuration
2) If it fails (1), Microscopy
   a. "Where for technical reasons the injection cannot be tested by light obscuration, microscopic testing may be used exclusively."

Microscopic Particle Count Test

Test Apparatus
Test Environment
Test Procedures
Enumeration of Particles
Interpretation

	>10 um	>25 um	
Small Volume Injections	3000	300	per container
Large Volume Injections	12	2	per mL

** About ½ of that for Light Obscuration Sensor

Optical Particle Counting

Light Obscuration Sensor

Obscuration = Blockage = Extinction
NOT Scatter sensor

Specifications

- Calibration
  - Critical to accurate operation
  - Function of flow rate
- Sample flow rate
- Sensor Concentration limit
- Sample Volume accuracy
- Count accuracy
- Particle counting accuracy

Interferences

- Bubbles
  - Viscous solutions
  - Bicarbonate
  - Protein solutions
- Contamination
  - Water
  - Glassware
- Settling of large particles

Sources of Contamination

- bubbles
- tubing
- precipitates
- glassware
- incompatible samples

Particle Counting Issues

Flakes
Fibers

Changing calibration curve – "Effect of Change of Calibration on Counts"

Problem samples

- Lyophilized samples
- Prefilled syringes
- Iodine solutions
- Emulsions

Particle Counting in Injectables

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- presented at PDA TRI
- Bethesda, MD
- Nov. 14, 2008

Optical Particle Counter

- **Single particle counter**
  - **Counts and sizes particles**
- **Three components of all OPCs**
  - **Sensor, Sampler, Counter**
- **Based on light interaction**
  - **Light extinction (blockage, obscuration)**
  - **Light scatter**

Particle Counting

- *Light Extinction*
  - *Also called Obscuration, Blockage*
  - *1 um – very large*
- *Light Scatter*
  - *0.1 um – 10-25 um*

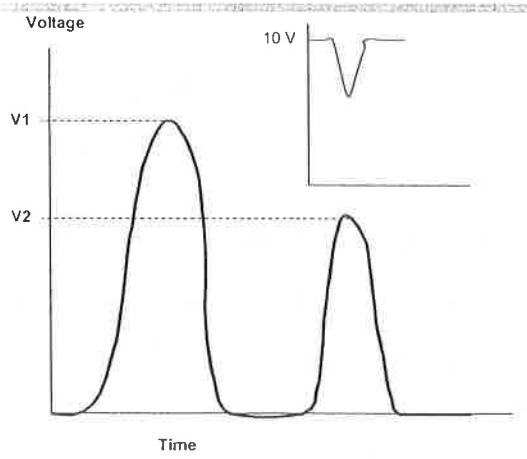
Operation of an OPC

- *Different than other analytical instruments*
  - *Optically "equivalent projected area diameter"*
- *Calibration*
  - *Critical for operation*
  - *Basis of getting accurate results*

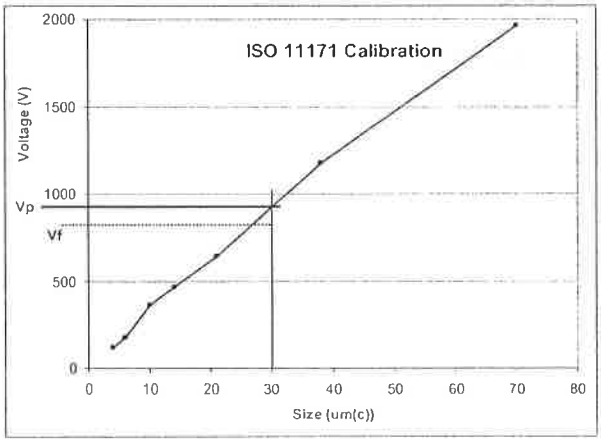
Operation of an OPC

- *Let's make an OPC based on light extinction*

Operation of an OPC



Calibration Curve



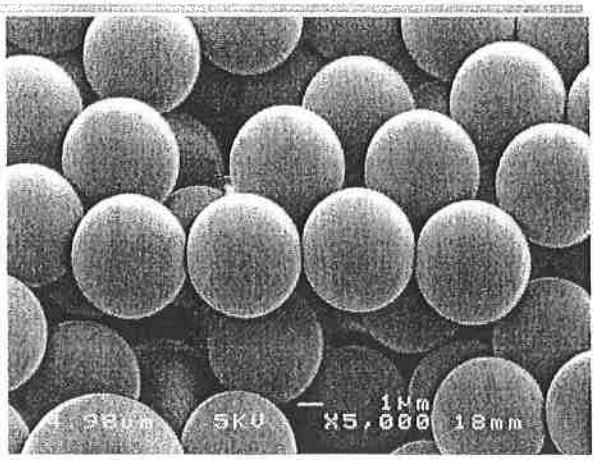
Optical Particle Counter

- *Precision and accuracy*
  - Precision – How closely grouped is the data
  - Accuracy – How close to the true value
- *Particle Counters are very precise*
  - *Requires operator's knowledge to be accurate*
- *Require good, careful methods*

Calibration - Size Calibration

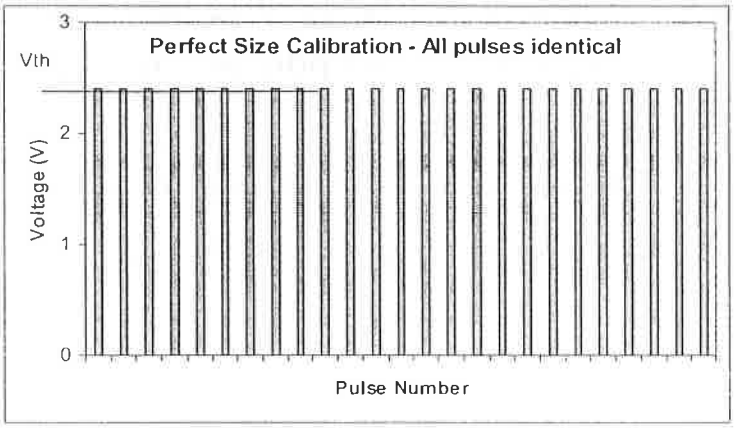
- *Typically "polystyrene latex spheres"*
  - monodispersed polymer microspheres
- *NIST traceable*
- *Known size and narrow distribution*
- *Determine the voltage response*

Size Calibration Particles

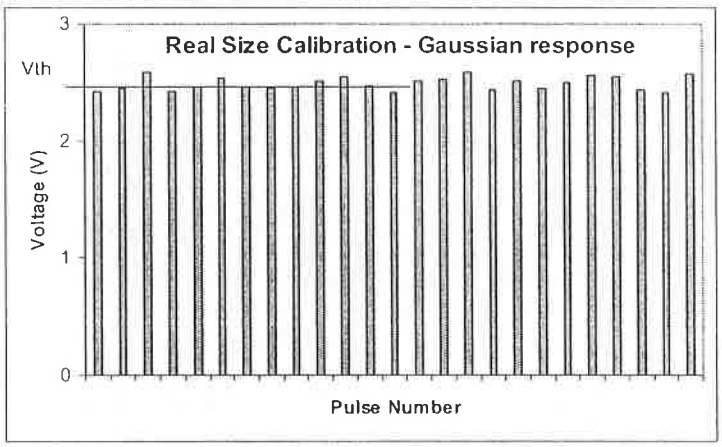


from Duke Scientific Corp., www.dukescientific.com

Size Calibration - Ideal



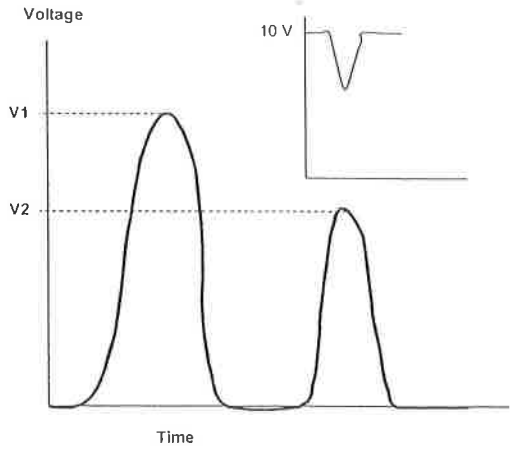
Size Calibration - Real



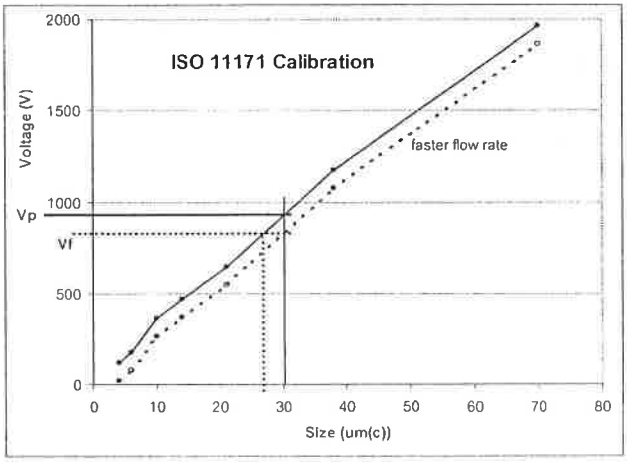
Calibration accuracy

- *Volume Accuracy*
- *Flow rate*
- *Sensor contamination*

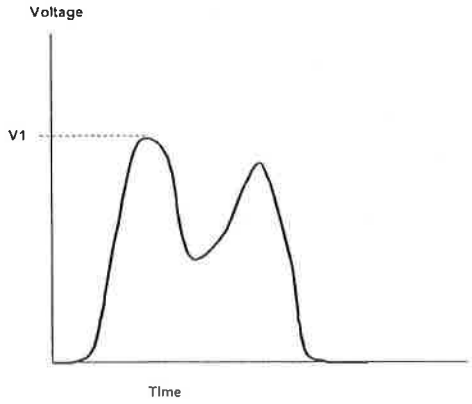
Flow Rate Effects



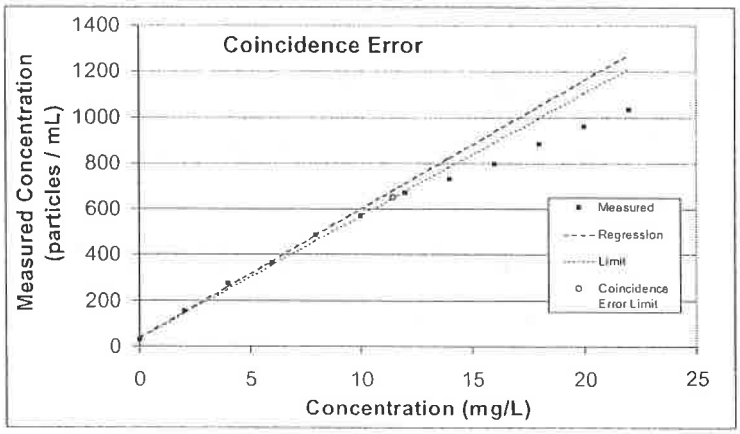
Calibration "Accuracy"



Particle Coincidence



Concentration Limit Test



Interferences

- *Bubbles*
- *Settling*
- *Aggregation*
- *Sample contamination*
- *Dirty sample lines*

Data analysis

- Plot of data – log-log
- "Straight line" -> power X^n
  - Counts ~ A / Size ^ n
  - log(Counts) = log(A) - n * log (Size)
  - Typically, slope (n) 2-3

Conclusions

- Particle counting is a rapid, reproducible method for determining the contamination level of fluids
- Care must be taken to get accurate particle count data
- Knowledge of particle counting methodology is critical to getting good results

Interim Revision Announcement

Notice of Harmonized Text: USP-NF General Chapter <788> Particulate Matter in Injections

March 30, 2007

The harmonized text for General Chapter <788> has been approved by the Parenteral Products—Industrial Expert Committee, Dr. Steven L. Nail, Chair and will become official on April 1, 2007. The changes include verbatim harmonized text as proposed in the European and Japanese pharmacopoeias, and reflect alignment with the 2004 signed-off text of the Pharmacopoeial Discussion Group (PDG).

The harmonized chapter, which becomes official via an Interim Revision Announcement in PF 33(2), references a definition for particle-free water in Reagents, Indicators and Solutions. That definition will read "Particle-Free Water: Filter water through a membrane with a pore size of 0.22 µm."

Questions should be directed to Dr. Desmond Hunt, Scientific Liaison, PPI Expert Committee (dgh@usp.org or 301-816-8341).

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⟨788⟩ PARTICULATE MATTER IN INJECTIONS

Particulate matter consists of mobile, randomly-sourced, extraneous substances, other than gas bubbles, that cannot be quantitated by chemical analysis due to the small amount of material that it represents and to its heterogeneous composition. Injectable solutions, including solutions constituted from sterile solids intended for parenteral use, is essentially free from particulate matter that can be observed on visual inspection. The tests described herein are physical tests performed for the purpose of enumerating subvisible extraneous particles within specific size ranges.

Microscopic and light obscuration procedures for the determination of particulate matter are given herein. This chapter provides a test approach in two stages. The injection 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 injection cannot be tested by light obscuration, microscopic testing may be used exclusively. Documentation demonstrating that the light obscuration procedure is incapable of testing the injection or produces invalid results is required in each case. 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.

All large-volume injections for single-dose infusion and those small-volume injections for which the monographs specify such requirements are subject to the particulate matter limits set forth for the test being applied, unless otherwise specified in the individual monograph. Excluded from the requirements of this chapter are injections intended solely for intramuscular and subcutaneous administration.

Not all injection formulations can be examined for particles by one or both of these tests. 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 method. Emulsions, colloids, and liposomal preparations are examples. Similarly, products that produce air or gas bubbles when drawn into the sensor, such as bicarbonate-buffered formulations, may also require microscopic testing. Refer to the specific monographs when a question of test applicability occurs. Higher limits are appropriate for certain articles and will be specified in the individual monographs.

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 for large-volume and small-volume injections, 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 based upon 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 should be based on consideration of product volume, numbers of particles 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

USP Reference Standards ⟨11⟩—*USP Particle Count RS.*

The test applies to large-volume injections labeled as containing more than 100 mL, unless otherwise specified in the individual monograph. It counts suspended particles that are solid or liquid. This test applies also to single-dose or multiple-dose small-volume injections labeled as containing 100 mL or less that are either in solution or in solution constituted from sterile solids, where a test for particulate matter is specified in the individual monograph. Products for which the individual monograph specifies that the label states that the product is to be used with a final filter are exempt from these requirements.

Test Apparatus

The apparatus is an electronic, liquid-borne particle counting system that uses a light-obscuration sensor with a suitable sample-feeding device. A variety of suitable devices of this type are commercially available. It is the responsibility of those performing the test to ensure that the operating parameters of the instrumentation are appropriate to the required accuracy and precision of the test result, and that adequate training is provided for those responsible for the technical performance of the test.

It is important to note that for Pharmacopeial applications the ultimate goal is that the particle counter reproducibly size and count particles present in the injectable material under investigation. The instruments available range from systems where calibration and other components of standardization must be carried out by manual procedures to sophisticated systems incorporating hardware- or software-based functions for the standardization procedures. Thus, it is not possible to specify exact methods to be followed for standardization of the instrument, and it is necessary to emphasize the required end result of a standardization procedure rather than a specific method for obtaining this result. This section is intended to emphasize the criteria that must be met by a system rather than specific methods to be used in their determination. It is the responsibility of the user to apply the various methods of standardization applicable to a specific instrument. Critical operational criteria consist of the following.

Sensor Concentration Limits—Use an instrument that has a concentration limit (the maximum number of particles per mL) identified by the manufacturer that is greater than the concentration of particles in the test specimen to be counted. The vendor-certified concentration limit for a sensor is specified as that count level at which coincidence counts due to simultaneous presence of two or more particles in the sensor view volume comprise less than 10% of the counts collected for 10-µm particles.

Sensor Dynamic Range—The dynamic range of the instrument used (range of sizes of particles that can be accurately sized and counted) must include the smallest particle size to be enumerated in the test articles.

Instrument Standardization

The following discussion of instrument standardization emphasizes performance criteria rather than specific methods for calibrating or standardizing a given instrument system. This approach is particularly evident in the description of calibration, where allowance must be made for manual methods as well as those based on firmware, software, or the use of electronic testing instruments. Appropriate instrument qualification is essential to performance of the test according to requirements. Since different brands of instruments may be used in the test, the user is responsible for ensuring that the counter used is operated according to the manufacturer's specific instructions; the principles to be followed to ensure that instruments operate within acceptable ranges are defined below.

The following information for instrument standardization helps ensure that the sample volume accuracy, sample flow rate, particle size response curve, sensor resolution, and count accuracy are appropriate to performance of the test. Conduct these procedures at intervals of not more than six months.

SAMPLE VOLUME ACCURACY

Since the particle count from a sample aliquot varies directly with the volume of fluid sampled, it is important that the sampling accuracy is known to be within a certain range. For a sample volume

determination, determine the dead (tare) volume in the sample feeder with filtered distilled or deionized water that has been passed through a filter having a 1.2-µm or finer porosity. Transfer a volume of filtered distilled or deionized water that is greater than the sample volume to a container, and weigh. Withdraw through the sample feeding device a volume that is appropriate for the specific sampler, and again weigh the container. Determine the sample volume by subtracting the tare volume from the combined sample plus tare volumes. Verify that the value obtained is within 5% of the appropriate sample volume for the test. Alternatively, the sample volume may be determined using a suitable Class A graduated cylinder (see *Volumetric Apparatus* ⟨31⟩). [NOTE—Instruments of this type require a variable tare volume. This is the amount of sample withdrawn prior to counting. This volume may be determined for syringe-operated samplers by setting the sample volume to zero and initiating sampling, so that the only volume of solution drawn is the tare. Subtract the tare volume from the total volume of solution drawn in the sampling cycle to determine the sample volume.]

SAMPLE FLOW RATE

Verify that the flow rate is within the manufacturer's specifications for the sensor used. This may be accomplished by using a calibrated stopwatch to measure the time required for the instrument to withdraw and count a specific sample volume (i.e., the time between beginning and ending of the count cycle as denoted by instrument indicator lights or other means). Sensors may be operated accurately over a range of flow rates. Perform the *Test Procedure* at the same flow rate as that selected for calibration of the instrument.

CALIBRATION

Use one of the following methods.

Manual Method—Calibrate the instrument with a minimum of three calibrators, each consisting of near-monosize polystyrene spheres having diameters of about 10, 15, and 25 µm, in an aqueous vehicle.* The calibrator spheres must have a mean diameter of within 5% of the 10-, 15-, and 25-µm nominal diameters and be standardized against materials traceable to NIST standard reference materials. The number of spheres counted must be within the sensor's concentration limit. Prepare suspensions of the calibrator spheres in water at a concentration of 1000 to 5000 particles per mL, and determine the channel setting that corresponds to the highest count setting for the sphere distribution. This is determined by using the highest count threshold setting to split the distribution into two bins containing equal numbers of counts, with the instrument set in the differential count mode (moving window half-count method). Use only the central portion of the distribution in this calculation to avoid including asymmetrical portions of the peak. The portion of the distribution, which must be divided equally, is the count window. The window is bounded by threshold settings that will define a threshold voltage window of ±20% around the mean diameter of the test spheres. The window is intended to include all single spheres, taking into account the standard deviation of the spheres and the sensor resolution, while excluding noise and aggregates of spheres. The value of 20% was chosen based on the worst-case sensor resolution of 10% and the worst-case standard deviation of the spheres of 10%. Since the thresholds are proportional to the area of the spheres rather than the diameter, the lower and upper voltage settings are determined by the equations:

$$V_L = 0.64V_S$$

in which V_L is the lower voltage setting and V_S is the voltage at the peak center, and

$$V_U = 1.44V_S$$

in which V_U is the upper voltage setting.

Once the center peak thresholds are determined, use these thresholds for the standards to create a regression of log voltage versus log particle size, from which the instrument settings for the 10- and 25-µm sizes can be determined.

Automated Method—The calibration (size response) curve may be determined for the instrument-sensor system by the use of validated software routines offered by instrument vendors; these may be included as part of the instrument software or used in conjunction with a microcomputer interfaced to the counter. The use of these automated methods is appropriate if the vendor supplies written certification that the software provides a response curve equivalent to that attained by the manual method and if the automated calibration is validated as necessary by the user.

Electronic Method—Using a multichannel peak height analyzer, determine the center channel of the particle counter pulse response for each standard suspension. This peak voltage setting becomes the threshold used for calculation of the voltage response curve for the instrument. The standard suspensions to be used for the calibration are run in order, and median pulse voltages for each are determined. These thresholds are then used to generate the size response curve manually or via software routines. The thresholds determined from the multichannel analyzer data are then transferred to the counter to complete the calibration. If this procedure is used with a comparator-based instrument, the comparators of the counter must be adjusted accurately beforehand.

SENSOR RESOLUTION

The particle size resolution of the instrumental particle counter is dependent upon the sensor used and may vary with individual sensors of the same model. Determine the resolution of the particle counter for 10-µm particles using the 10-µm calibrator spheres. The relative standard deviation of the size distribution of the standard particles used is not more than 5%. Acceptable methods of determining particle size resolution are (1) manual determination of the amount of peak broadening due to instrument response; (2) using an electronic method of measuring and sorting particle sensor voltage output with a multichannel analyzer; and (3) automated methods.

Manual Method—Adjust the particle counter to operate in the cumulative mode or total count mode. Refer to the calibration curve obtained earlier, and determine the threshold voltage for the 10-µm spheres. Adjust 3 channels of the counter to be used in the calibration procedure as follows:

Channel 1 is set for 90% of the threshold voltage.
Channel 2 is set for the threshold voltage.
Channel 3 is set for 110% of the threshold voltage.

Draw a sample through the sensor, observing the count in Channel 2. When the particle count in that channel has reached approximately 1000, stop counting, and observe the counts in Channels 1 and 3. Check to see if the Channel 1 count and the Channel 3 count are 1.68 ± 10% and 0.32 ± 10%, respectively, of the count in Channel 2. If not, adjust Channel 1 and Channel 3 thresholds to meet these criteria. When these criteria have been satisfied, draw a sample of suspension through the counter until the counts in Channel 2 have reached approximately 10,000, or until an appropriate volume (e.g., 10 mL) of the sphere suspension has been counted. Verify that Channel 1 and Channel 3 counts are 1.68 ± 3% and 0.32 ± 3%, respectively, of the count in Channel 2.

Record the particle size for the thresholds just determined for Channels 1, 2, and 3. Subtract the particle size for Channel 2 from the size for Channel 3. Subtract the particle size for Channel 1 from the size for Channel 2. The values so determined are the observed standard deviations on the positive and negative side of the mean count for the 10-µm standard. Calculate the percentage of resolution of the sensor by the formula:

$$100\left(\sqrt{S_O^2 - S_S^2/D}\right)$$

in which S_O is the highest observed standard deviation determined for the sphere; S_S is the supplier's reported standard deviation for the

* ASTM standard F658-87 provides useful discussions pertaining to calibration procedures applying near-monosize latex spheres.

spheres; and D is the diameter, in µm, of the spheres as specified by the supplier. The resolution is not more than 10%.

Automated Method—Software that allows for the automated determination of sensor resolution is available for some counters. This software may be included in the instrument or used in conjunction with a microcomputer interfaced to the counter. The use of these automated methods is appropriate if the vendor supplies written certification that the software provides a resolution determination equivalent to the manual method and if the automated resolution determination is validated as necessary by the user.

Electronic Method—Record the voltage output distribution of the particle sensor, using a multichannel analyzer while sampling a suspension of the 10-µm particle size standard. To determine resolution, move the cursor of the multichannel analyzer up and down the electric potential scale from the median pulse voltage to identify a channel on each side of the 10-µm peak that has approximately 61% of the counts observed in the center channel. Use of the counter size response curve to convert the mV values of these two channels to particle sizes provides the particle size at within 1 standard deviation of the 10-µm standard. Use these values to calculate the resolution as described under *Manual Method*.

PARTICLE COUNTING ACCURACY

Determine the particle counting accuracy of the instrument, using *Method 1* (for small-volume injections) or *Method 2* (for large-volume injections).

Method 1—

Procedure—Prepare the suspension and blank using the USP Particle Count RS. With the instrument set to count in the cumulative (total) mode, collect counts at settings of greater than or equal to 10 µm and greater than or equal to 15 µm. Mix the blank by inverting 25 times within 10 seconds, and degas the mixture by sonicating (at 80 to 120 watts) for about 30 seconds or by allowing to stand. Remove the closure from the container, and gently stir the contents by hand-swirling or by mechanical means, taking care not to introduce air bubbles or contamination. Stir continuously throughout the analysis. Withdraw directly from the container three consecutive volumes of not less than 5 mL each, obtain the particle counts, and discard the data from the first portion. [NOTE—Complete the procedure within five minutes.] Repeat the procedure, using the suspension in place of the blank. From the averages of the counts resulting from the analysis of the two portions of the suspension at greater than or equal to 10 µm and from the analysis of the two portions of the blank at greater than or equal to 10 µm, calculate the number of particles in each mL by the formula:

$$(P_S - P_B) / V$$

in which P_S is the average particle count obtained from the suspension; P_B is the average particle count obtained from the blank; and V is the average volume, in mL, of the 4 portions tested. Repeat the calculations, using the results obtained at the setting of not less than 15 µm.

Interpretation—The instrument meets the requirements for *Particle Counting Accuracy* if the count obtained at greater than or equal to 10 µm and the ratio of the counts obtained at greater than or equal to 10 µm to those obtained at greater than or equal to 15 µm conform to the values that accompany the USP Particle Count RS. If the instrument does not meet the requirements for *Particle Counting Accuracy*, repeat the procedure using the remaining suspension and blank. If the results of the second test are within the limits given above, the instrument meets the requirements of the test for *Particle Counting Accuracy*. If on the second attempt the system does not meet the requirements of the test, determine and correct the source of the failures, and retest the instrument.

Method 2—

Procedure—Using standard calibrator spheres having a nominal diameter of 15 to 30 µm, prepare a suspension containing between 50 and 200 particles per mL. Degas the suspension by sonicating (at 80 to 120 watts) for about 30 seconds or by allowing to stand. Properly suspend the particles by stirring gently, and perform five counts on 5-mL volumes of the suspension, using the particle counter 10-µm size threshold. Obtain the mean cumulative particle count per mL. Pipet a volume of this suspension containing 250 to 500 particles into a filter funnel prepared as described for *Filtration Apparatus* under *Microscopic Particle Count Test*. After drying the membrane, count the total number of standard spheres collected on the membrane filter. This count should be within 20% of the mean instrumental count per mL for the suspension.

Test Environment

Perform the test in an environment that does not contribute any significant amount of particulate matter. Specimens must be cleaned to the extent that any level of extraneous particles added has a negligible effect on the outcome of the test. Preferably, the test specimen, glassware, closures, and other required equipment are prepared in an environment protected by high-efficiency particulate air (HEPA) filters, and nonshedding garments and powder-free gloves are worn throughout the preparation of samples.

Cleanse glassware, closures, and other required equipment, preferably by immersing and scrubbing in warm, nonionic detergent solution. Rinse in flowing tap water, and then rinse again in flowing filtered distilled or deionized water. Organic solvents may also be used to facilitate cleaning. [NOTE—These steps describe one way to clean equipment; alternatively, particulate-free equipment may be obtained from a suitable vendor.] Finally, rinse the equipment in filtered distilled or deionized water, using a hand-held pressure nozzle with final filter or other appropriate filtered water source, such as distilled or deionized water passed through a capsule filter having a 1.2-µm or finer porosity.

To collect blank counts, use a cleaned vessel of the type and volume representative of that to be used in the test. Place a 50-mL volume of filtered distilled or deionized water in the vessel, and agitate the water sample in the cleaned glassware by inversion or swirling. [NOTE—A smaller volume, consistent with the article to be counted, can be used.] Degas by sonicating (at 80 to 120 watts) for about 30 seconds or by allowing to stand. Swirl the vessel containing the water sample by hand or agitate by mechanical means to suspend particles. Withdraw and obtain the particle counts for three consecutive samples of not less than 5 mL each, disregarding the first count. If more than 10 particles of 10-µm or greater size, or more than 2 particles of 25-µm or greater size are observed in the combined 10-mL sample, the environment is not suitable for particulate analysis: the filtered distilled or deionized water and glassware have not been properly prepared or the counter is generating spurious counts. In this case, repeat the preparatory steps until conditions of analysis are suitable for the test.

Test Procedure

TEST PREPARATION

Prepare the test specimens in the following sequence. Outside of the laminar enclosure, remove outer closures, sealing bands, and any loose or shedding paper labels. Rinse the exteriors of the containers with filtered distilled or deionized water as directed under *Test Environment*. Protect the containers from environmental contamination until analyzed. Withdraw the contents of the containers under test in a manner least likely to generate particles that could enter the sample. Contents of containers with removable stoppers may be withdrawn directly by removing the closures. Sampling devices having a needle to penetrate the unit closure may also be employed. Products packaged in flexible plastic containers may be sampled by cutting the medication or administration port tube or a corner from the unit with a suitably cleaned razor blade or scissors.

Dry or lyophilized products may be constituted either by removing the closure to add diluent or by injecting diluent with a hypodermic syringe having a 1.2-µm or finer syringe filter. If test specimens are to be pooled, remove the closure and empty the contents into a clean container.

The number of test specimens must be adequate to provide a statistically sound assessment of whether a batch or other large group of units represented by the test specimens meets or exceeds the limits. If the volume in the container is less than 25 mL, test a solution pool of 10 or more units. Single small-volume injection units may be tested if the individual unit volume is 25 mL or more.

For large-volume injections, individual units are tested. For large-volume injections or for small-volume injections where the individual unit volume is 25 mL or more, fewer than 10 units may be tested, based on the definition of an appropriate sampling plan.

PRODUCT DETERMINATION

Depending upon the dosage form being tested, proceed as directed under the appropriate category below.

Liquid Preparations—

Volume in Container Less than 25 mL—Prepare the containers as directed under *Test Preparation*. Mix and suspend the particulate matter in each unit by inverting the unit 20 times. [NOTE—Because of the small volume of some products, it may be necessary to agitate the solution more vigorously to suspend the particles properly.] In a cleaned container, open and combine the contents of 10 or more units to obtain a volume of not less than 20 mL. Degas the pooled solution by sonicating for about 30 seconds or by allowing the solution to stand undisturbed until it is free from air bubbles. Gently stir the contents of the container by hand-swirling or by mechanical means, taking care not to introduce air bubbles or contamination. Withdraw a minimum of three aliquots, each not less than 5-mL in volume, into the light obscuration counter sensor. Discard the data from the first portion. [NOTE—For some products, a pool of 15 or more units may be necessary to achieve a pool volume sufficient for three 5-mL sample aliquots. Smaller sample aliquots (i.e., less than 5 mL) can be used if the assay result obtained with the smaller aliquots is validated to give an assessment of batch suitability equivalent to that obtained with the 5-mL aliquots specified above.]

Volume in Container 25 mL or More—Prepare the containers as directed under *Test Preparation*. Mix and suspend the particulate matter in each unit by inverting the unit 20 times. Degas the solution by sonicating for about 30 seconds or by allowing the solution to stand undisturbed until it is free from air bubbles. Remove the closure of the unit or effect entry by other means so that the counter probe can be inserted into the middle of the solution. Gently stir the contents of the unit by hand-swirling or by mechanical means. Withdraw not fewer than three aliquots, each not less than 5 mL in volume, into the light obscuration counter sensor. Discard the data from the first portion.

Dry or Lyophilized Preparations—Prepare the containers as directed under *Test Preparation*. Open each container, taking care not to contaminate the opening or cover. Constitute as directed under *Test Preparation*, using the specified volume of filtered water or an appropriate filtered diluent if water is not suitable. Replace the closure, and manually agitate the container sufficiently to ensure dissolution of the drug. [NOTE—For some dry or lyophilized products, it may be necessary to let the units stand for a suitable interval, and then agitate again to effect complete dissolution.] After the drug in the constituted sample is completely dissolved, mix and suspend the particulate matter present in each unit by inverting it 20 times prior to analysis. Proceed as directed for the appropriate unit volume under *Liquid Preparations*, and analyze by withdrawing a minimum of three aliquots, each not less than 5 mL in volume, into the light obscuration counter sensor. Discard the data from the first portion.

Products Packaged with Dual Compartments Constructed to Hold the Drug Product and a Solvent in Separate Compartments—Prepare the units to be tested as directed under *Test Preparation*. Mix each unit as directed in the labeling, activating and agitating so as to ensure thorough mixing of the separate components and drug dissolution. Degas the units to be tested by sonicating or by allowing the solution to stand undisturbed until it is free from any air bubbles. Proceed as directed for the appropriate unit volume under *Liquid Preparations*, and analyze by withdrawing a minimum of three aliquots, each not less than 5 mL in volume, into the light obscuration counter sensor. Discard the data from the first portion.

Products Labeled "Pharmacy Bulk Package Not for Direct Infusion"—Proceed as directed for *Liquid Preparations* where the volume is 25 mL or more. Calculate the test result on a portion that is equivalent to the maximum dose given in the labeling. For example, if the total bulk package volume is 100 mL and the maximum dose volume is 10 mL, then the average light obscuration particle count per mL would be multiplied by 10 to obtain the test result based on the 10-mL maximum dose. [NOTE—For the calculations of test results, consider this maximum dose portion to be the equivalent of the contents of one full container.]

Calculations

Pooled Samples (Small-Volume Injections)—Average the counts from the two or more aliquot portions analyzed. Calculate the number of particles in each container by the formula:

$$PV_T/V_A n$$

in which P is the average particle count obtained from the portions analyzed; V_T is the volume of pooled sample, in mL; V_A is the volume, in mL, of each portion analyzed; and n is the number of containers pooled.

Individual Samples (Small-Volume Injections)—Average the counts obtained for the 5-mL or greater aliquot portions from each separate unit analyzed, and calculate the number of particles in each container by the formula:

$$PV/V_A$$

in which P is the average particle count obtained from the portions analyzed; V is the volume, in mL, of the tested unit; and V_A is the volume, in mL, of each portion analyzed.

Individual Unit Samples (Large-Volume Injections)—Average the counts obtained for the two or more 5-mL aliquot portions taken from the solution unit. Calculate the number of particles in each mL of Injection taken by the formula:

$$P/V$$

in which P is the average particle count for an individual 5 mL or greater sample volume; and V is the volume, in mL, of the portion taken.

For all types of product, if the tested material has been diluted to effect a decrease in viscosity, the dilution factor must be accounted for in the calculation of the final test result.

Interpretation

The injection meets the requirements of the test if the calculated number of particles present in each discrete unit tested or in each pooled sample 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

	≥10 µm	≥25 µm
Small-Volume Injections	6000	600 per container
Large-Volume Injections	25	3 per mL

MICROSCOPIC PARTICLE COUNT TEST

The microscopic particulate matter test may be applied to both large-volume and small-volume injections. This test enumerates subvisible, essentially solid, particulate matter in these products on a per volume or per container basis, after collection on a microporous membrane filter. Some articles cannot be tested meaningfully by light obscuration. In such cases, individual monographs specify only this microscopic assay. Solutions exempted from analysis using the microscopic assay are identified on a monograph basis. Examples are solutions that do not filter readily because of high viscosity (e.g., concentrated dextrose, starch solutions, or dextrans). In performance of the microscopic assay 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. Since in

on this material consists of units on the order of 1 µm or less, ch 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

Microscope—Use a compound binocular microscope that corrects for changes in interpupillary distance by maintaining a constant tube length. The objective and eyepiece combination of lenses must give a magnification of 100 ± 10×. The objective must be of 10× nominal magnification, a planar achromat or better in quality, with a minimum numerical aperture of 0.25. In addition, the objective must be compatible with an episcopic illuminator attachment. The eyepieces must be of 10× magnification. In addition, one eyepiece must be designed to accept and focus an eyepiece graticule. The microscope must have a mechanical stage capable of holding and traversing the entire filtration area of a 25-mm or 47-mm membrane filter.

Illuminators—Two illuminators are required. One is an external, focusable auxiliary illuminator that can be adjusted to give incident oblique illumination at an angle of 10° to 20°. The other is an episcopic brightfield illuminator internal to the microscope. Both illuminators must be of a wattage sufficient to provide a bright, even source of illumination and may be equipped with blue daylight filters to decrease operator fatigue during use.

Circular Diameter Graticule—Use a circular diameter graticule (see *Figure 1*) matched to the microscope model objective and eyepiece such that the sizing circles are within 2% of the stated size in the plane of the stage.

Micrometer—Use a stage micrometer, graduated in 10-µm increments, that is certified by NIST.

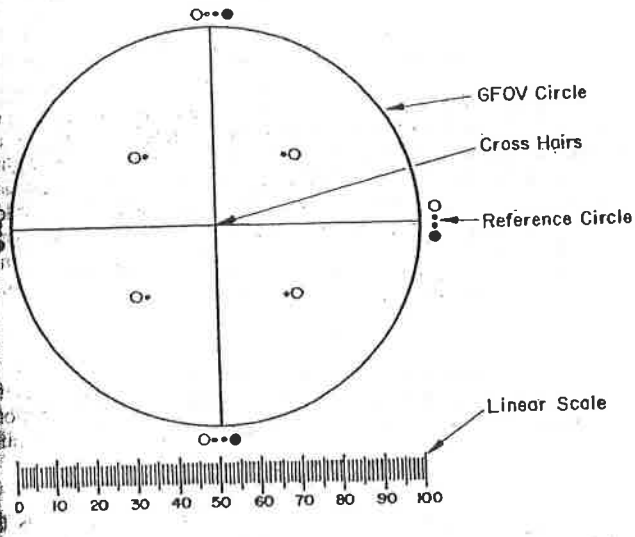

Fig. 1. Circular diameter graticule. The large circle divided by crosshairs into quadrants is designated the graticule field of view (GFOV). Transparent and black circles having 10-µm and 25-µm diameters at 100× are provided as comparison scales for particle sizing.

Filtration Apparatus—Use a filter funnel suitable for the volume to be tested, having a minimum diameter of about 21 mm. The funnel is made of plastic, glass, or stainless steel. Use a filter support made of stainless steel screen or sintered glass as the filtration diffuser. The filtration apparatus is equipped with a vacuum source, a solvent dispenser capable of delivering solvents filtered at 1.2-µm or finer retention rating at a range of pressures from 10 psi to 80 psi, and membrane filters (25 mm or 47 mm nongridded or gridded, black or dark gray, or of suitable material compatible with the product, with a 1.0-µm or finer porosity). Use blunt forceps to handle membrane filters.

Test Environment

Use a laminar flow hood or other laminar airflow enclosure, having a capacity sufficient to envelop the area in which the analysis is prepared, and containing HEPA-filtered air having not more than 100 particles (0.5 µm or larger) per cubic foot. For the blank determination, deliver a 50-mL volume of filtered distilled or deionized water to the filtration funnel. Apply vacuum, and draw the entire volume of water through the membrane filter. Remove the membrane from the filter funnel base, and place atop a strip of double-sided tape in a Petri slide or Petri dish. After allowing the membrane to dry, examine it microscopically at a magnification of 100×. If not more than 20 particles 10 µm or larger in size and 5 particles 25 µm or larger are present within the filtration area, the background particle level is sufficiently low for performance of the microscopic assay.

Throughout this procedure, it is preferable to use powder-free gloves and thoroughly clean glassware and equipment. Prior to conducting the test, clean the work surfaces of the laminar flow enclosure with an appropriate solvent. Glassware and equipment should be rinsed successively with a warm, residue-free solution of detergent, hot water, filtered distilled or deionized water, and isopropyl alcohol. [NOTE—Prior to use, pass the distilled or deionized water and the isopropyl alcohol through filters having a 1.2-µm or finer porosity.] Perform the rinsing under the laminar flow enclosure equipped with HEPA filters. Allow the glassware and filtration apparatus to dry under the hood, upstream of all other operations. Preferably, the hood is located in a separate room that is supplied with filtered air-conditioned air and maintained under positive pressure with respect to the surrounding areas.

MICROSCOPE PREPARATION

Place the auxiliary illuminator close to the microscope stage, focusing the illuminator to give a concentrated area of illumination on a filter membrane positioned on the microscope stage. Adjust the illuminator height so that the angle of incidence of the light is 10° to 20° with the horizontal. Using the internal episcopic brightfield illuminator, fully open the field and aperture diaphragms. Center the lamp filament, and focus the microscope on a filter containing particles. Adjust the intensity of reflected illumination until particles are clearly visible and show pronounced shadows. Adjust the intensity of episcopic illumination to the lowest setting, then increase the intensity of episcopic illumination until shadows cast by particles show the least perceptible decrease in contrast.

OPERATION OF CIRCULAR DIAMETER GRATICULE

The relative error of the graticule used must initially be measured with an NIST-certified stage micrometer. To accomplish this, align the graticule micrometer scale with the stage micrometer so that they are parallel. (Compare the scales, using as large a number of graduations on each as possible.) Read the number of graticule scale divisions, *GSD*, compared to stage micrometer divisions, *SMD*. Calculate the relative error by the formula:

$$100[(GSD - SMD)/SMD].$$

A relative error of ±2% is acceptable. The basic technique of measurement applied with the use of the circular diameter graticule is to transform mentally the image of each particle into a circle and then compare it to the 10- and 25-µm graticule reference circles. The sizing process is carried out without superimposing the particle on the reference circles; particles are not moved from their locations within the graticule field of view (the large circle) for comparison to the reference circles. Compare the area of the particle being sized to that of the black or transparent circles. Use the area of the clear graticule reference circles to size white or transparent particles. Use the area of the black reference circles to size dark particles.

Rotate the graticule in the right microscope eyepiece so that the linear scale is located at the bottom of the field of view, bringing the graticule into sharp focus by adjusting the right eyepiece diopter ring while viewing an out-of-focus specimen. Focus the microscope on a

specimen, looking through the right eyepiece only. Then, looking through the left eyepiece, adjust the left eyepiece diopter to bring the specimen into sharp focus.

PREPARATION OF FILTRATION APPARATUS

Preferably, wash the filtration funnel, base, and diffuser in a solution of liquid detergent and hot water. Rinse with hot water. Following the hot water rinse, apply a second rinse with filtered distilled or deionized water, using a pressurized jet of water over the entire exterior and interior surfaces of the filtration apparatus. Repeat the pressurized rinse procedure using filtered isopropyl alcohol. Finally, using the pressurized rinser, rinse the apparatus with filtered distilled or deionized water.

Remove a membrane filter from its container, using ultracleaned blunt forceps. Use a low pressurized stream of filtered purified water to wash both sides of the filter thoroughly by starting at the top and sweeping back and forth to the bottom. Assemble the cleaned filtration apparatus with the diffuser on top of the filtration base, placing the clean membrane filter on top of the diffuser. Place the funnel assembly on top of the filtration base, and lock it into place.

Test Procedure

TEST PREPARATIONS

Proceed as directed for *Test Preparation* under the *Light Obscuration Particle Count Test*, beginning with "Prepare the test specimens in the following sequence," and ending with "For large-volume injections, individual units are tested." For small-volume injections containing a volume of 25 mL or more and tested individually and for large-volume injections, the entire unit volume is tested. For large-volume injections or for small-volume injections where the individual unit volume is 25 mL or more, fewer than 10 units may be tested, based on the definition of an appropriate sampling plan.

PRODUCT DETERMINATION

Depending upon the dosage form being tested, proceed as directed under the appropriate category below.

Liquid Preparations—Thoroughly mix the units to be tested by inverting 20 times. Open the units in a manner consistent with the generation of the lowest possible numbers of background particles. For products less than 25 mL in volume, open and combine the contents of 10 or more units in a cleaned container. Filter large-volume injection units individually. Small-volume injection units having a volume of 25 mL or more may be filtered individually.

Transfer to the filtration funnel the total volume of a solution pool or of a single unit, and apply vacuum. If the volume of solution to be filtered exceeds the volume of the filtration funnel, add, stepwise, a portion of the solution until the entire volume is filtered. If the partial count procedure is to be used (see *Partial Count Procedure* under *Enumeration of Particles*), do not allow the liquid volume in the filtration funnel to drop below approximately one-half of the funnel volume between refills. [NOTE—Use a filter funnel appropriate to the volume of solution if a partial count procedure is to be employed. This is necessary to ensure even distribution of particles on the analytical membrane.]

After the last addition of solution, begin rinsing the walls of the funnel by directing a low-pressure stream of filtered distilled or deionized water in a circular pattern along the walls of the funnel, and stop rinsing the funnel before the volume falls below about one-fourth of the fill level. Maintain the vacuum until all the liquid in the funnel is gone.

Remove the filtration funnel from the filtration base while maintaining vacuum, then turn the vacuum off, and remove the filter membrane with blunt forceps. Place the filter in a Petri dish or similar container, secure in place with double-sided tape, and label with sample identification. Allow the filter to air-dry in the laminar-flow enclosure with the cover ajar.

Dry or Lyophilized Preparations—To test a dry powder vial or similar container of drug powder, constitute the material with an appropriate diluent, using the method least likely to introduce extraneous contamination, as directed for *Test Preparation* under *Light Obscuration Particle Count Test*. Using a solution pool of 10 or more units, or the desired number of individual units, proceed as directed for *Liquid Preparations*.

Products Packaged with Dual Compartments Constructed to Hold the Drug Product and a Solvent in Separate Compartments—Activate each unit as directed in the labeling, agitating the contents sufficiently to ensure thorough mixing of the separate components, and then proceed as directed for *Liquid Preparations*.

Pharmacy Bulk Packages or Multiple-Dose Containers—For *Products Labeled "Pharmacy Bulk Package—Not for Direct Infusion,"* or for multiple-dose containers, proceed as directed for *Liquid Preparations*, filtering the total unit volume.

Calculate the test result on a portion that is equal to the maximum dose given in the labeling. Consider this portion to be the equivalent of the contents of one full container. For example, if the total bulk package volume is 100 mL and the maximum dose listed is 10 mL, the microscopic total unit volume count test result would be multiplied by 0.1 to obtain the test result for the 10-mL dose volume. [NOTE—For calculation of the test result, consider this portion to be the equivalent of the contents of one full container.]

Enumeration of Particles

The microscopic test described in this section is flexible in that it can count, in particles per mL, specimens containing 1 particle per mL as well as those containing significantly higher numbers of particles per mL. This method may be used where all particles on an analysis membrane surface are counted or where only those particles on some fractional area of a membrane surface are counted.

TOTAL COUNT PROCEDURE

In performance of a total count, the graticule field of view (GFOV) defined by the large circle of the graticule is ignored, and the vertical crosshair is used. Scan the entire membrane from right to left in a path that adjoins but does not overlap the first scan path. Repeat this procedure, moving from left to right to left until all particles on the membrane are counted. Record the total number of particles that are 10 µm or larger and the number that are 25 µm or larger. For large-volume injections, calculate the particle count, in particles per mL, for the unit tested by the formula:

$$P/V$$

where P is the total number of particles counted; and V is the volume, in mL, of the solution. For small-volume injections, calculate the particle count, in particles per container, by the formula:

$$P/n$$

in which P is the total number of particles counted; and n is the number of units pooled (1 in the case of an individual unit).

PARTIAL COUNT PROCEDURE

If a partial count of particles on a membrane is to be performed, the analyst must first ensure that an even distribution of particles is present on the membrane. This is assessed by rapid scanning in order to look for clumps of particles. None should be present. Count the 10-µm or larger particles in one GFOV at the edge of the filtration area as well as those in the center of the membrane. The number of ≥10-µm or larger particles in the GFOV with the highest total particle count is not more than twice that of the GFOV with the lowest particle count. Reject a filter failing these criteria, and prepare another if a partial count procedure is used, or, alternatively, analyze this membrane by the total count method.

The normal number of GFOV counted for a partial count is 20. If a smaller confidence interval about the result is desired, a larger number of fields and particles may be counted. Count all particles

that have a circular area diameter of 10 µm or larger and 25 µm or larger within the GFOV and those that are in contact with the right side of the GFOV circle. Do not count particles outside of the GFOV. Ignore those that touch the left side of the GFOV circle. The dividing line between right and left sides of the GFOV circle is the vertical cross hair. [NOTE—Make the best possible judgment on particle size without changing the microscope magnification or illumination.]

To perform a partial count of the particles on a membrane, start at the right center edge of the filtration area and begin counting adjacent GFOVs. When the left edge of the filtration area is reached, move one GFOV toward the top of the filter and continue counting GFOVs by moving in the opposite direction. Moving from one GFOV to the next can be accomplished by one of two methods. One method is to define a landmark (particle or surface irregularity in the filter) and move over one GFOV in relation to the landmark. A second method is to use the vernier on the microscope stage to move 1 mm between GFOVs. To facilitate the latter, adjust the microscope x- and y-stage positioning controls to a whole number at the starting position at the center right edge of the filtration area, then each GFOV will be one whole division of movement of the x-stage positioning control. If the top of the filtration area is reached before the desired number of GFOVs is reached, begin again at the right center edge of the filtration area one GFOV lower than the first time. This time move downward on the membrane when the end of a row of GFOVs is reached. Continue as before until the number of GFOVs is complete.

For large-volume injections, if a partial count procedure for the ≥10-µm and ≥25-µm size ranges is used, calculate the particles per mL by the formula:

$$PA_T/A_PV$$

in which P is the number of particles counted; A_T is the filtration area, in mm², of the membrane; A_P is the partial area counted, in mm², based on the number of graticule fields counted; and V is the volume, in mL, of solution filtered. For a solution pool (for small-volume injection units containing less than 25 mL) or for a single unit of a small-volume injection, calculate the number of particles per unit by the formula:

$$PA_T/A_Pn$$

in which n is the number of units counted (1 in the case of an individual unit); and the other terms are as defined above.

For all types of product, if the tested material has been diluted to effect a decrease in viscosity, the dilution factor must be accounted for in the calculation of the final test result.

Interpretation

The injection meets the requirements of the test if the number of particles present (actual or calculated) in each discrete unit tested or in each pooled sample tested does not exceed the appropriate value listed in *Table 2*.

Table 2. Microscopic Method Particle Count

	≥10 µm	≥25 µm
Small-Volume Injections	3000	300 per container
Large-Volume Injections	12	2 per mL

⟨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⟩.

Explanatory Note

Erratum to <788> Particulate Matter in Injections and the USP Particle Count Reference Standard

August 1, 2007
This Erratum inserts national text into harmonized General Chapter <788> to inform users that the USP Particle Count Reference Standard can be used to determine system suitability of light obscuration equipment.

The current version of General Chapter <788> Particulate Matter in Injections (amended via direct IRA in Pharmacopeial Forum 33 (2), and later updated via a Revision Bulletin posted on the USP Web site April 4, 2007) indicates the use of the USP Particle Count Reference Standard as a calibration standard, which is an incorrect usage for that standard. The USP Particle Count Reference Standard is a system suitability standard that can be used to verify the calibration of the light obscuration equipment, and is not used for calibration.

The complete text (79KB), which also will be published as an Interim Revision Announcement in PF 33(6), is official as of this date and is included in this web posting. The text now includes the original harmonized text followed by the national text that states that the USP Particle Count Reference Standard can be used to determine system suitability.

For further information, contact Desmond Hunt, Ph.D., liaison to the Parenteral Products Industrial Expert Committee (dgh@usp.org).

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U.S. Pharmacopeia
The Standard of Quality℠

USP Certificate

PARTICLE COUNT SET
LOT J0D067

LABEL TEXT

For use with specified USP-NF Tests. Not for use as a drug. Read USP29 before using. For Calibration Use Only.

USP REFERENCE STANDARD

PARTICLE COUNT-SUSPENSION 25 mL

Contains 15 µm polystyrene spheres in aqueous suspension. Particle must be resuspended before use. Do not open until ready to begin test. Store in a cool place. PROTECT FROM FREEZING. Acceptance values: Counts at 10 µm: between 3381 and 4298 counts/mL. Ratio of counts at 10 µm to the counts at 15 µm: between 1.294 and 3.242.

CAT. NO. 1500502 USP ROCKVILLE, MD LOT J0D067

For use with specified USP-NF Tests. Not for use as a drug. Read USP29 before using. For Calibration Use Only.

USP REFERENCE STANDARD

PARTICLE COUNT - BLANK 25 mL

Contains the aqueous phase without added particles. Do not open until ready to begin test. Store in a cool place. PROTECT FROM FREEZING.

CAT. NO. 1500502 USP ROCKVILLE, MD LOT J0D067

PARTICLE COUNTING ACCURACY

Determine the particle counting accuracy of the instrument for small-volume injections as described by the following method:

Procedure— Prepare the suspension and blank using the USP Particle Count RS. With the instrument set to count in the cumulative (total) mode, collect counts at settings of greater than or equal to 10 µm and greater than or equal to 15 µm. Mix the blank by inverting 25 times within 10 seconds, and degas the mixture by sonicating (at 80 to 120 watts) for about 30 seconds or by allowing to stand. Remove the closure from the container, and gently stir the contents by hand-swirling or by mechanical means, taking care not to introduce air bubbles or contamination. Stir continuously throughout the analysis. Withdraw directly from the container three consecutive volumes of not less than 5 mL each, obtain the particle counts, and discard the data from the first portion. [NOTE—Complete the procedure within five minutes.] Repeat the procedure, using the suspension in place of the blank. From the averages of the counts resulting from the analysis of the two portions of the suspension at greater than or equal to 10 µm and from the analysis of the

two portions of the blank at greater than or equal to 10 µm, calculate the number of particles in each mL by the formula:

$$(P_S - P_B) / V$$

in which P_S is the average particle count obtained from the suspension; P_B is the average particle count obtained from the blank; and V is the average volume, in mL, of the 4 portions tested. Repeat the calculations, using the results obtained at the setting of not less than 15 µm.

Interpretation— The instrument meets the requirements for Particle Counting Accuracy if the count obtained at greater than or equal to 10 µm and the ratio of the counts obtained at greater than or equal to 10 µm to those obtained at greater than or equal to 15 µm conform to the values that accompany the USP Particle Count RS. If the instrument does not meet the requirements for Particle Counting Accuracy, repeat the procedure using the remaining suspension and blank. If the results of the second test are within the limits given above, the instrument meets the requirements of the test for Particle Counting Accuracy. If on the second attempt the system does not meet the requirements of the test, determine and correct the source of the failures, and retest the instrument.

USP certifies that the USP Reference Standards Committee, in accordance with their rules and procedures, determined that this USP Reference Standard lot is suitable to assess compliance with the monograph standards for which it is specified. The critical characteristics of this lot are usually determined independently in three or more laboratories, including USP, government, academic, and industrial collaborators.

QA Director

Calculation Value

Unless otherwise stated on the Reference Standard label, a value of 100.0% should be used in USP or NF compendial applications for which the use of this Reference Standard is intended. Please refer to the specific Reference Standard label for further information.

Expiration

Current lots are identified in the Official USP Reference Standards catalog. In some cases, the previous lot may still be considered official. If so, it is identified in the column marked "Previous Lot/Valid Use Date." Ordinarily, the previous lot is carried in official status for about one year after the current lot enters distribution.

It is the responsibility of each user to determine that this lot is current when used. To ensure up-to-date information, USP publishes the Official USP Reference Standards Catalog, which contains official lot designations. This information is also available on the USP web site, at www.usp.org, as well as in the bimonthly subscription publication, *Pharmacopeial Forum*.

Instructions for Use

Follow the instructions in the appropriate USP or NF Monographs and General Requirements for Tests and Assays of the current *USP–NF*. In the event that instructions on the label of this lot differ from those found in the current *USP–NF*, those on the label supersede any instructions listed in Chapter <11>.

Non-Monograph Use

The suitability of this Reference Standard for use in non-compendial applications is solely the responsibility of the user.

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<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 mobile undissolved particles, other than gas bubbles, unintentionally present in the solutions.

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 sub-visible 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 sub-visible particles by one or both of these methods. When Method 1 is not applicable, e.g. in 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 which allows 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 Solution section.

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 (<11>).✦

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 min 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 is 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, based on an appropriate sampling plan.

Remove four portions, each of not less than 5 ml, 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 Pharmacopoeia]

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, filter assembly for retaining particulate matter and membrane filter for examination.

The microscope 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 membrane filter, two suitable illuminators to provide episcopic illumination in addition to oblique illumination, and is adjusted to 100 ± 10 magnifications.

The ocular micrometer is a circular diameter graticule (see Figure 1) 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 per cent 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 adjustable 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, non-gridded 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 is combined in a cleaned container; where justified and authorized, 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, based on an appropriate sampling plan.

Wet the inside of the filter holder fitted with the membrane filter with several milliliter of particle-free water. Transfer to the filtration funnel the total volume of a solution pool or of a single unit, and apply 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 membrane 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 transforming mentally the image of each particle into a circle and then comparing it to the 10 µm and 25 µm graticule reference circles. 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, semi-liquid, 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 Pharmacopoeia]

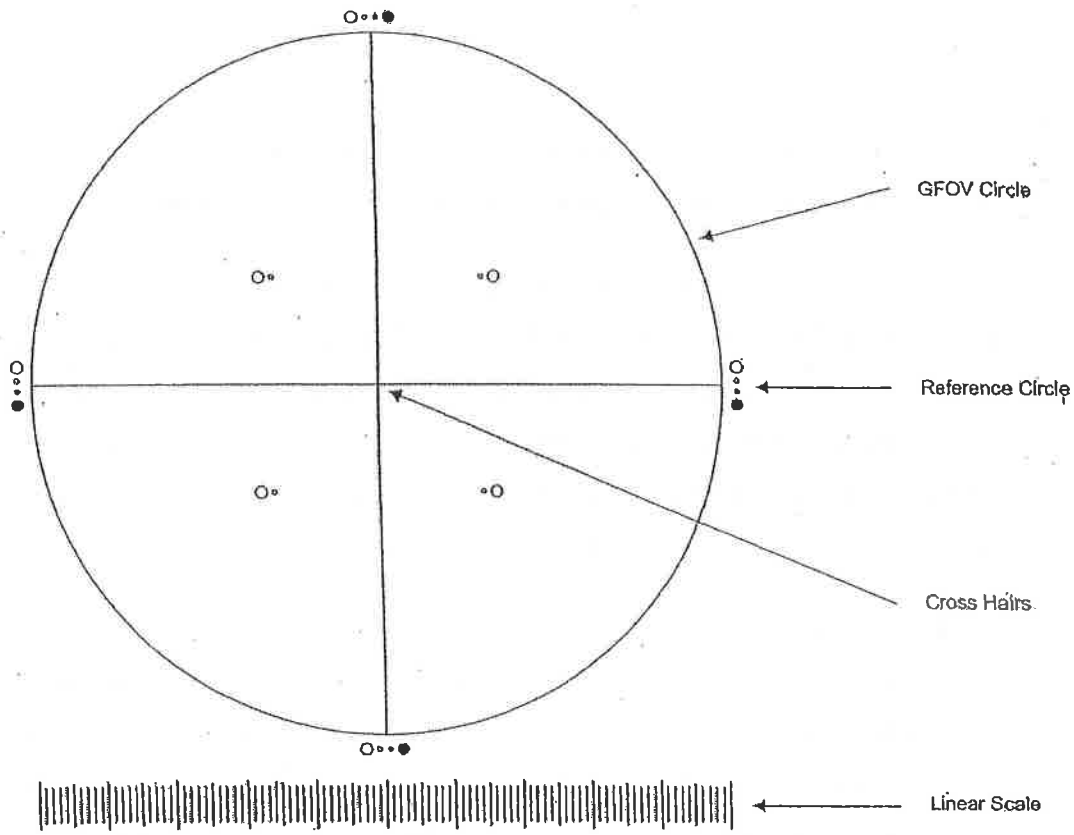
Test 2.A — Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of more than 100 mL.

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.

1. Circular diameter graticule



<788> PARTICULATE MATTER IN INJECTIONS

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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 sub-visible 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 sub-visible particles by one or both of these methods. When Method 1 is not applicable, e.g. in 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 which allows 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 Solution section.

The apparatus is calibrated using dispersions of spherical particles of known sizes between 10 µm and 25 µm, USP Particle Count Reference Standard. These standard particles are dispersed in particle-free water. Care must be taken to avoid aggregation of particles during dispersion.

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 min 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 is 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, based on an appropriate sampling plan.

Remove four portions, each of not less than 5 mL, 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.

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, filter assembly for retaining particulate matter and membrane filter for examination.

The microscope 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 membrane filter, two suitable illuminators to provide episcopic illumination in addition to oblique illumination, and is adjusted to 100 ± 10 magnifications.

The ocular micrometer is a circular diameter graticule (see Figure 1) 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 per cent 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 adjustable 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, non-gridded 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 is combined in a cleaned container; where justified and authorized, 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, based on an appropriate sampling plan.

Wet the inside of the filter holder fitted with the membrane filter with several milliliters of particle-free water. Transfer to the filtration funnel the total volume of a solution pool or of a single unit, and apply 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 membrane 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 and 25 um 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, semi-liquid, 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.

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.

Explanatory Note

Erratum to <788> Particulate Matter in Injections and the USP Particle Count Reference Standard

August 1, 2007
This Erratum inserts national text into harmonized General Chapter <788> to inform users that the USP Particle Count Reference Standard can be used to determine system suitability of light obscuration equipment.

The current version of General Chapter <788> Particulate Matter in Injections (amended via direct IRA in Pharmacopeial Forum 33 (2), and later updated via a Revision Bulletin posted on the USP Web site April 4, 2007) indicates the use of the USP Particle Count Reference Standard as a calibration standard, which is an incorrect usage for that standard. The USP Particle Count Reference Standard is a system suitability standard that can be used to verify the calibration of the light obscuration equipment, and is not used for calibration.

The complete text (79KB), which also will be published as an Interim Revision Announcement in PF 33(6), is official as of this date and is included in this web posting. The text now includes the original harmonized text followed by the national text that states that the USP Particle Count Reference Standard can be used to determine system suitability.

For further information, contact Desmond Hunt, Ph.D., liaison to the Parenteral Products Industrial Expert Committee (dgh@usp.org).

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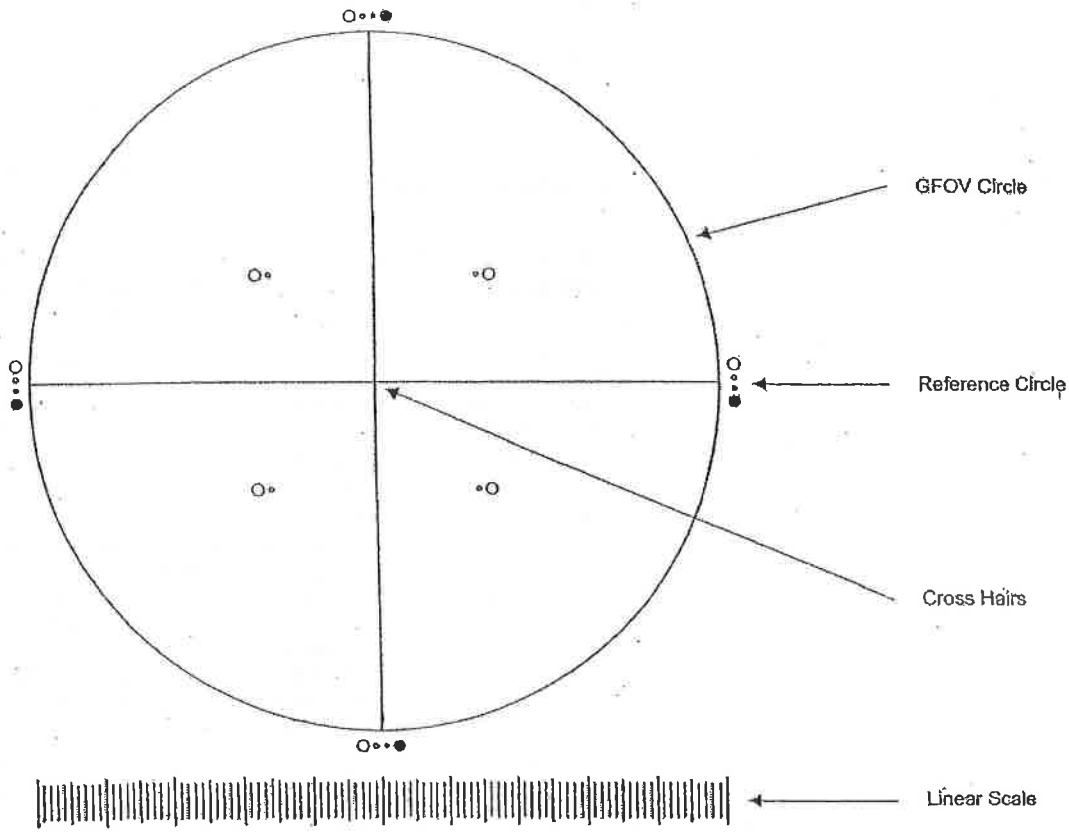
1. Circular diameter graticule



Effect of Calibration Change on the Measured Counts

Technical Report # 304
December 1993

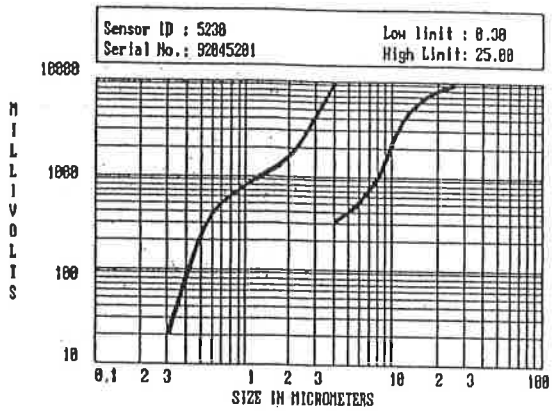
Dr. Charles Montague
Pacific Scientific Company
HIAC/ROYCO DIVISION
11801 Tech Road
Silver Spring, Maryland 20904

Introduction

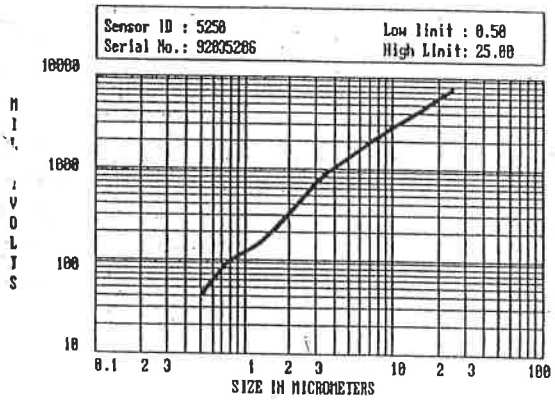
What effect will a change in the calibration curve have on the measured counts? There is no simple answer to this question. The magnitude of the effect depends on many factors. The primary factors are the sensor used, the sizes measured, the size distribution of the contamination, and the composition of the contaminant.

This paper estimates the effects for three sensors. The calculations ar approximate and provide an estimate of the effects for some of the abov factors. Typical calibration curves for the sensors and typical size distributions for contaminants are assumed. The results are shown for two aerosol sensors (5230 and 5250) and one liquid sensor (HRLD-150). The contaminants are assumed to be described by a power law distribution. Even in this simplified case, it will be seen that the effects are a complex function of the sensor and size.

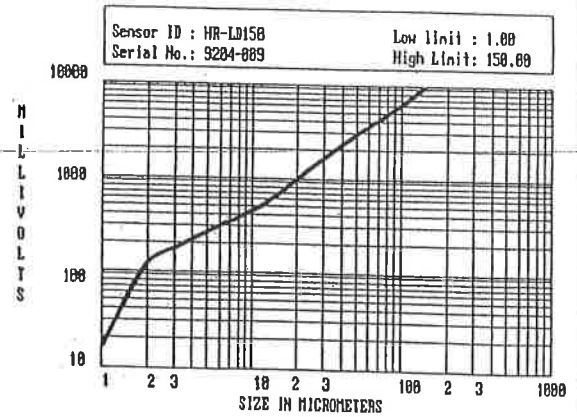
Figure 1.



a. Calibration curve for a 5230 aerosol particle counter. NOTE: There are two gain stages in the 5230 to get a range of 0.3 to 25 µm. This causes the two segments of the calibration curve.



b. Calibration curve for a 5250 aerosol particle counter. Both the 5230 and the 5250 use scattering to detect particles.



c. Calibration curve for an HRLD-150 sensor. This sensor uses extinction to detect particles.

Calculations

The distributions of contaminants are assumed to follow a power law distribution described by the power P. In this distribution, when the measured size, D, changes by a factor R, the number of counts at the new size is the counts at size D multiplied by R raised to some power, p. The value of p is a constant characteristic of the contaminant. Distributions of this type are linear when the cumulative counts are plotted against size on log-log paper. If the particle count at size D1 is Cts(D1), the counts at another size D2 is:

$$Cts\ (D2) = Cts\ (D1) * (D1/D2)^P$$

To find the size at which the particle count will change by a factor F:

$$Cts\ (D2) = F * Cts\ (D1) = Cts\ (Dl) * (D1/D2)^P$$

$$D2 = Dl\ /\ (F^{1/P}).$$

To determine how much the calibration curve of the sensor must change for the count to change by a factor F, the threshold voltages of both D1 and D2, Vth(Dl) and Vth(D2), respectively, must be calculated. This requires a calibration curve for the sensor.

The percentage change, L, in the calibration curve that will cause a change in particle count of F will be:

$$L = 100 * (Vth(D1) - Vth(D2))\ /\ Vth(D1).$$

A change in the calibration curve by L will result in the measured count changing by a factor F.

Examples For Three Sensors

Calibration curves from typical sensors are used to calculate the threshold voltages. The calibration curves are shown in Figure 1. For aerosol counters, the effects are evaluated at 0.3 µm, 0.5 µm, 5 µm, 10 µm, and 25 µm (0.3 µm is not evaluated for the 5250, which has a sensitivity limit of 0.5 µm). For the liquid sensor, the effect is shown for 2 µm, 5 µm, 10 µm, and 25 µm. These are the sizes measured for the USP and the BP tests for particles in pharmaceuticals. The changes in the calibration curves that cause a change of 10% or 20% are calculated.

For the calculations, assume that the distribution of contaminants in the aerosol is described by a power of 2.2. (See Figure 2a.) This distribution is used in Federal Standard 209E, which defines cleanliness class levels for clean rooms. For liquids, the distribution of contaminants is assumed to be described by a power of 3. (See Figure 2b.) This value is typical for contaminants in many liquids.

For Aerosols (power = 2.2):

Sizes at which the particle count changes by X %

	X = 10 %		X = 20 %	
Size	**decrease**	**increase**	**decrease**	**increase**
0.3 µm	0.315 µm	(0.287 µm)	0.332 µm	(0.276 µm)
0.5	0.525	(0.479)	0.553	0.460
5.0	5.25	4.79	5.53	4.60
10.0	10.49	9.58	11.07	9.21
25.0	(26.23)	23.94	(27.67)	23.01

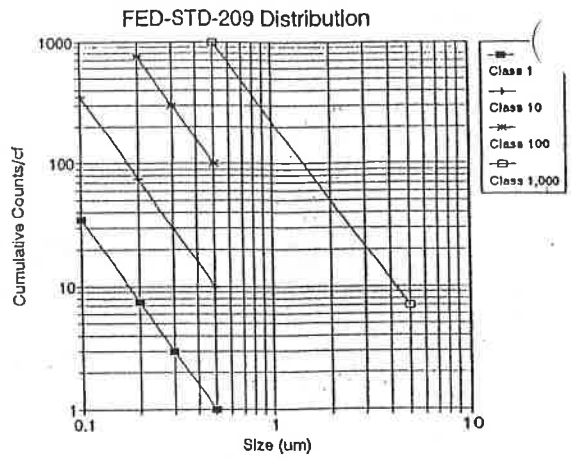
NOTE 1: The sizes in parenthesis are outside of the range of the sensors.

For liquids (power = 3):

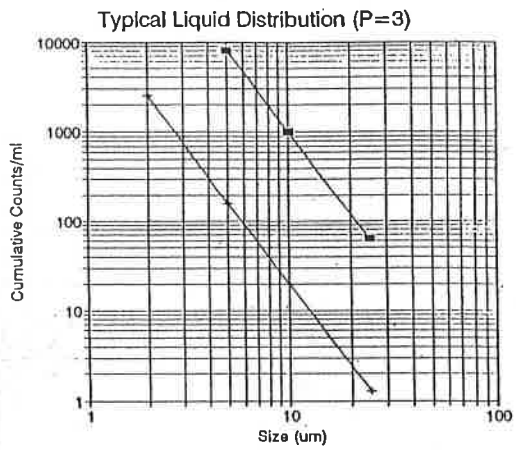
Sizes at which the counts change by X %

	X = 10 %		X = 20 %	
Size	**decrease**	**increase**	**decrease**	**increase**
2.0 µm	2.07 µm	1.94 µm	2.15 µm	1.88 µm
5.0	5.18	4.84	5.39	4.71
10.0	10.36	9.69	10.77	9.41
25.0	25.89	24.22	26.93	23.53

Figure 2.



a. Class limits specified by FED-STD-209E. These numbers are based on a power law with P = 2.2.



b. An ideal power law distribution with P = 3. Two distributions are shown, one with 20 particles > 10 µm/ml, and another with 1000 particles > 10 µm/ml.

Results

The threshold voltages of the above sizes are calculated for each sensor. The ratio L defines the change in the calibration curve that causes the listed effect.

5250 Aerosol Sensor

Threshold change, L, which changes the counts by X %

	X = 10 %		X = 20 %	
Size	**decrease**	**increase**	**decrease**	**increase**
0.5 µm	7.9 %	-10.8 %	14.7 %	-23.4 %
5.0	3.7	-4.5	7.0	-10.5
10.0	15.1	-18.2	27.4	-39.0
25.0	10.6	-12.5	19.2	-27.6

5230 Aerosol Sensor

Threshold change, L, which changes the counts by X %

	X = 10 %		X = 20 %	
Size	**decrease**	**increase**	**decrease**	**increase**
0.3 µm	20.3 %	-29.0 %	34.7 %	-69.5 %
0.5	14.8	-17.4	27.7	-36.3
5.0	3.8	-4.9	6.9	-11.8
10.0	14.7	-17.8	26.6	-38.6
25.0	2.2	-3.0	4.2	-6.9

HRLD-150 Liquid Sensor

Threshold change, L, which changes the counts by X %

	X = 10 %		X = 20 %	
Size	**decrease**	**increase**	**decrease**	**increase**
2.0 µm	5.4 %	-5.3 %	10.5 %	-10.6 %
5.0	2.8	-3.1	5.3	-6.5
10.0	2.5	-2.9	4.7	-6.5
25.0	4.1	-4.7	7.7	-10.2

Discussion

As can be seen, the changes in threshold voltage that cause similar effects vary greatly. They are a function of size and sensor model. Even though the 5230 and 5250 are similar in design, the effects at 25 µm are very different while the effects at 0.5, 5, and 10 µm are similar. These calculations do not include any effects that might be introduced by the distribution or composition of contaminants. Thus, it is very difficult to assign, *a priori*, an error to a change in threshold voltage.

To understand part of the variation in the values above, note that all calibration curves are very different. They all have wiggles in them, and different slopes. The largest values in the above tables for L occur when the slope of the sensor calibration curve is the largest. At these sizes, large changes in threshold voltage have only small changes in size. The values L are the smallest for the HRLD-150, whose calibration curve has the lowest slope.

One final note about the effect of voltage changes. The 8000A, 8000, 5250, and 5230 counters all have the "Quick-Adjust" feature which verifies the accuracy of the electronics in the counter. This feature checks the voltages set in the counter against a precision voltage reference. If a set voltage cannot be adjusted within 0.5% of the true voltage, a "Quick-Adjust Error" will be indicated. This assures the user that the counter electronics, used for sorting the sensor signals, do not drift.

FINISHED PRODUCT TESTING
PDA-TRI ASEPTIC PROCESSING COURSE- ??? 200?

Steven Messick
Pharm.Specialist
Genentech, Inc.

References & Regulations

Particulate Testing

- USP <1>, 100% Inspection, reject visible foreign matter
- USP <788>, 'essentially free, extraneous, foreign' w/ limits
- USP <789> opthalmics
- Pharm.Euro.
- JP Forum Vol. 5

Container/Closure Integrity

- USP <1207>
- PDA Tech. Report #27 (1998)
- FDA Guidance Doc. Jan. 1998 "Container Closure in lieu of Sterility Testing…"
- FDA Guidance Doc. May 1999 "Container Closure…Drugs/Biologics"
- PDA Journal Sept/Oct. 1997 "CCI Testing…"

Final Product/Release Testing

- Sterility: USP <71>, 21CFR 610.12
- Potency: cGMPs
- General Safety: 610.11
- pH: USP <791>
- Endotoxin: USP <85>, <151>, CFRs 211.167a, 610.13b
- Identity: cGMPs
- Purity: cGMPs
- Stability: CFR 211.166, FDA Draft Guidance (June '98)
- ICH Guidelines for Stability, Drug Specifications, Impurities

Web Sites

pda.org
fda.gov (cGMPs on line)
usp.org
ich.org

Particulate Matter in Injections<788> USP 24

Steve Messick
&
John Ramirez

Module Agenda

- Compendia Requirements
- Potential Sources of Particulate Matter in Injections
- Maintenance and Validation of Particulate Control
- Demonstration of Liquid Particle Counter

Compendia Requirements

- Injections <1>
- Particulate Matter in Injections <788>
- EP Supplement 2000

Injections <1>

- "Container is made of material that permits inspection of the contents."
- "Good pharmaceutical practice requires also that each final container of Injection be subjected individually to a physical inspection, whenever the nature of the container permits and that every container whose contents show evidence of contamination with visible ***foreign*** material be rejected."

Particulate Matter in Injections <788>

- Light Obscuration Particle Count Test
- Microscopic Particle Count Test

Light Obscuration

- Limited by Solution Product characteristics
  - Products that are not true solutions having a clarity and viscosity approximately that of water
    - Emulsions
    - Colloids
    - Liposomal preparations

Light Obscuration

- Limited by Container design
  - Pre-filled syringes
  - Cartridges
- Products requiring the use of a final filter during administration

Small Volume Parenterals

- Light Obscuration
  - (> or = 10 microns) NMT 6,000 per container
  - (> or = 25 microns) NMT 600 per container

Small Volume Parenterals

- Microscopic Particle Count
  - (> or = 10 microns) NMT 3,000 per container
  - (> or = 25 microns) NMT 300 per container

Potential Sources of Particulate Matter

- Product incompatibility with the Container Closure system
- Preparation of the Container Closure components
- Filling Equipment
- Personnel
- Filling Environment

Product Incompatibility with the Container Closure system

- Glassware
- Elastomeric Closures
- Preparation of the Container Closure components
- Cleaning of Equipment
- Water Filtration Systems
- Transport Containers
- Environmental Conditions
- Filling Equipment
- Equipment Cleaning
- Proper Alignment
- Pumps
- Filling Needles

Personnel

- Design of Protective Clothing
- Good Gowning Practices
- Proper Aseptic Technique

Filling Environment

- Class 100 Conditions
  - LAF

Maintenance of Particulate Control

- Compliance with SOP's
- Control of Cleaning Supplies
- HVAC System Maintenance
  - Air Supply
  - Air Return
  - HEPA Filter Integrity

Validation of Particulate Control

- Similarity to Sterility for an Aseptically Produced Product
  - Processes of Elimination
  - Human Intervention
  - Sample size is not statistically valid

Validation of Inspection Processes

- Human Visual Inspection
  - Estimated acuity is 50 to 100 microns
  - Probabilistic not Deterministic

Electronic Inspection

- Validated versus Human Visual Inspection