PHARMACOPOEIAL DISCUSSION GROUP

SIGN-OFF DOCUMENT
CODE: Q-09
NAME: PARTICULATE CONTAMINATION

REVISION 2

It is understood that sign-off covers the technical content of the draft and each party will adapt it as necessary to conform to the usual presentation of the pharmacopoeia in question; such adaptation includes stipulation of the particular pharmacopoeia's reference materials and general chapters.

Harmonised provisions:

Provision	EP	JP	USP
Introduction	+	+	+
Method 1	+	+	+
Method 2	+	+	+

Legend
+ will adopt and implement; — will not stipulate

Non-harmonised provisions:

1) The requirements for preparations supplied in containers with a nominal value of 100 mL

Local requirements

EP	None
JP	Section of calibration of the apparatus includes more detailed information.
USP	Added as national text in Particulate Matter in Injection <788>: • Exemption of radiopharmaceutical preparations from the requirements of USP General Chapter <788>. • Solution for injections administered by intramuscular or subcutaneous route must meet the requirements of <788>.

	• Parenterals packaged and labeled exclusively for use as irrigating solutions are exempt from the requirements of <788>. • Parenteral products for which the labeling specifies use of a final filter prior to administration are exempt from the requirements of <788> provided the scientific data are available to justify this exemption. • This chapter addresses subvisible particles, including extrinsic (e.g., foreign material like cellulose), intrinsic (e.g., manufacturing residues or instability-related particles), and inherent (e.g., API or formulation components). Test methods detect all these particle types, though light obscuration methods may also count gas bubbles as artifacts, see *⟨1788⟩ Methods for the Determination of Subvisible Particulate Matter* • For the purpose of this chapter, small volume parenteral is synonymous with small volume injection and large volume parenteral is synonymous with large volume injection. • System suitability can be verified by using the USP Particle Count RS • For pharmacy bulk packages for parenteral use labeled “Not for Direct Infusion”, proceed as directed for small-volume parenterals when 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 particle count per mL would be multiplied by 10 to obtain the test result based on the 10-mL maximum dose. [Note—For the calculation of test results, consider this maximum dose portion to be equivalent to the contents of one full container.] • Products packaged with dual compartments meant to hold a drug product and a solvent should be prepared and tested as directed for large-volume parenterals or small-volume parenterals, depending on container volume. Mix each unit as directed in the labeling, activating and agitating to ensure thorough mixing of the separate components and drug dissolution. • More stringent limits may be more appropriate for individual products based on available lot data, clinical safety data, and route of administration.

European Pharmacopoeia

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Q-09 PARTICULATE CONTAMINATION: SUB-VISIBLE PARTICLES

Unintended particulate matter in parenteral preparations consists of mobile undissolved substances, other than gas bubbles, that may originate from various sources such as contamination . The level of particulate matter must be minimised and controlled, independent of its type.

For the determination of sub-visible particulate matter 2 procedures are described hereafter: Method 1 (Light Obscuration Particle Count Test) and Method 2 (Microscopic Particle Count Test). It is preferable to use Method 1 when examining the parenteral preparation for sub-visible particles.

However, not all parenteral preparations can be examined directly 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 (some emulsions, suspensions, colloids, and liposomal preparations are examples), the test is carried out according to Method 2. Similarly, specific precautions during sample preparation and/or the use of the microscopic particle count method may also be required for preparations that produce air or gas bubbles when drawn into the sensor. If the viscosity of the preparation to be tested precludes its examination by either procedure, a quantitative dilution with an appropriate particle-free diluent may be made to decrease viscosity to a value that allows the analysis to be performed.

Because the methods measure different characteristics, the results of the *Light Obscuration Particle Count Test* are not equivalent to those of the *Microscopic Particle Count Test* and the two methods cannot be considered interchangeable.

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

METHOD 1. LIGHT OBSCURATION PARTICLE COUNT TEST

Use a suitable apparatus based on the principle of light blockage that allows an automatic determination of the size of particles and the number of particles according to size.

The apparatus is calibrated using suitable certified reference materials consisting of dispersions of spherical particles of known sizes at about 10 µm and 25 µm. These standard particles are dispersed in *particle-free water R*. Care must be taken to avoid agglomeration of particles during dispersion.

General precautions

Carry out the test under conditions limiting further contamination with particles, preferably in a laminar-flow cabinet.

Very carefully wash glassware and the equipment used in the test procedure by cleaning with a warm detergent solution and rinsing with abundant amounts of water to remove

all traces of detergent. Immediately before use, rinse the fluid path with *particle-free water R*.

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, carry out the following test: determine the particulate matter in 5 aliquots of degassed *particle-free water R*, or an alternative particle-free diluent if justified, 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

Clean the outer surfaces of the container(s) using a jet of *particle-free water R* and avoid contamination of the contents. Samples are tested in a manner that most closely represents the product use. For parenteral preparations that have a sufficient volume for a single test, based on instrument capability and properties of the sample, it is preferrable to test individual units to estimate the level and variation of particulate matter in an entire group of units.

For parenteral preparations that do not have a sufficient volume, carefully and thoroughly mix each unit. Then combine the contents of a suitable number of units in a separate container, to obtain the volume required for a single test, based on instrument capability and properties of the sample.

Powders for injections and infusions are reconstituted with *particle-free water R* or an alternative particle-free diluent when *particle-free water R* is not suitable.

Eliminate gas bubbles by appropriate measures such as allowing to stand, applying a gentle vacuum, or sonicating. Preparations containing proteins should not be sonicated. After sample treatment, the sample should be remixed gently but thoroughly to suspend the particles, taking care to minimize the generation of bubbles.

The number of test samples must be adequate to provide a statistically sound assessment. For large and small volume parenterals, an adequate volume of test sample must be provided for analysis; however, single units may be tested in a statistically sound sampling plan.

Remove 4 portions, each of approximately 5 mL, and count the number of particles equal to or larger than 10 µm and 25 µm. Disregard the result obtained for the first portion and calculate the average number of particles from the remaining portions of the preparation to be examined. The number of particles per unit is calculated from the number of particles per volume. Volumes smaller than 5 mL can also be tested provided that this amount is appropriately justified. In general, for parenteral products that do not have a sufficient volume (e.g. less than 25 mL), it may be acceptable to carry out the test with a volume of 1 to 5 mL.

Evaluation

For preparations supplied in units that contain a nominal volume of more than 100 mL, apply the criteria of test 1.A.

For preparations supplied in units that contain a nominal volume of less than 100 mL,

apply the criteria of test 1.B.

For preparations supplied in units that contain a nominal volume of 100 mL, apply the criteria of test 1.A (JP requirements) or those of test 1.B (Ph. Eur. and USP requirements).

If the number of particles in a tested unit exceeds the limits, test the preparation by the microscopic particle count test.

Test 1.A – Preparations for infusion or injection supplied in units that contain *a nominal content of more than 100 mL*

The preparation complies with the test if , in each unit tested, the number of particles present per unit that are 10 µm or larger in size does not exceed 25 per millilitre and the number of particles that are 25 µm or larger in size does not exceed 3 per millilitre.

Test 1.B – Preparations for infusion or injection supplied in units that contain a nominal content of less than 100 mL

The preparation complies with the test if, in each unit tested, the number of particles present per unit that are10 µm or larger in size does not exceed 6000 per container and the number of particles that are 25 µm or larger in size and does not exceed 600 per container. Where combining units is required, the average number of particles present in the combined sample is used to calculate the number of particles in one container.

When justified and authorized for example in cases of highly viscous preparations, dilution of samples may be required to obtain reliable results. Dilution is allowed provided that the diluent and methods are demonstrated to be appropriate and the smallest level of dilution that allows for reproducible testing is used.

METHOD 2. MICROSCOPIC PARTICLE COUNT TEST

Use a suitable binocular microscope and a filter assembly to retain particulate matter on a membrane filter.

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, 2 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).

2 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 10-20°.

The filter assembly 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 grey in colour, non-gridded or gridded, and 1.0 µm or finer in nominal pore size.

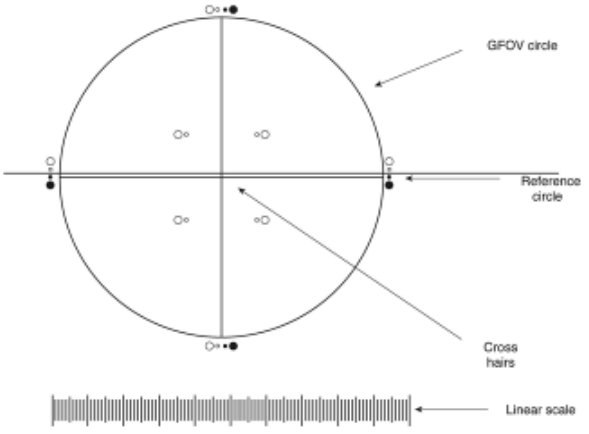

Figure 1. – *Circular diameter graticule*

General precautions

Carry out the test under conditions limiting further contamination with particles, preferably in a laminar-flow cabinet.

Very carefully wash the glassware and filtration 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 R*.

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, carry out the following test: determine the level of particulate matter of a 50 mL volume of *particle-free water R* following the procedure 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

Clean the outer surfaces of the container(s) using a jet of *particle-free water R* and avoid contamination of the contents. Samples are tested in a manner that most closely represents the product use. For parenteral preparations that have a sufficient volume for a single test, based on instrument capability and properties of the sample, testing of individual units is often preferred to estimate the level and variation of particulate matter in an entire group of units.

For parenteral preparations that do not have a sufficient volume, carefully and thoroughly mix each unit. Then combine the contents of a suitable number of units in a

separate container to obtain the volume required for a single test based on instrument capability and properties of the sample.

Lyophilized solids or powders for injections and infusions are reconstituted with *particle-free water R* or an alternative particle-free diluent when *particle-free water R* is not suitable.

The number of test samples must be adequate to provide a statistically sound assessment. For large and small volume parenterals, an adequate volume of sample must be provided for analysis; however, single units may be tested in a statistically sound sampling plan.

Wet the inside of the filter holder fitted with the membrane filter with several millilitres of *particle-free water R*. Transfer to the filtration funnel the total volume of a sample pool or of a single unit and apply vacuum. If needed, add stepwise a portion of the sample until the entire volume is filtered. After adding the last portion, rinse the inner walls of the filter holder by using a jet of *particle-free water R*. Maintain the vacuum until the surface of the membrane filter is free from liquid. Place the filter in a Petri dish and allow the filter to air-dry with the cover slightly ajar. After the 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 larger than 10 µm and 25 µm. Determine the number of particles per unit. Where particle distribution on the filter is uniform, partial filter count and determination of the total filter count by calculation is allowed.

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. For elongated fibers size should be assigned based on the longest dimension.

In performing the microscopic particle count test care should be taken to ensure that individual particles are discernible. 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 are flat, show little or no surface relief, no shadow when illuminated from the side, and present a gelatinous or film-like appearance (for instance protein or fatty acid particles). In such cases, the interpretation of enumeration may be aided by testing a sample of the preparation by the light obscuration particle count test.

Evaluation

For preparations supplied in units that contain a nominal volume of more than 100 mL, apply the criteria of test 2.A.

For preparations supplied in units that contain a nominal volume of less than 100 mL, apply the criteria of test 2.B.

For preparations supplied in units that contain a nominal volume of 100 mL, apply the criteria of test 2.A (JP requirements) or those of test 2.B (Ph. Eur. and USP requirements).

Test 2.A – Preparations for infusion or injection supplied in units that contain *a nominal*

content of more than 100 mL

The preparation complies with the test if, in each unit tested, the number of particles present per unit that are10 µm or larger in size does not exceed 12 per millilitre and the number of particles that are 25 µm or larger in size does not exceed 2 per millilitre.

Test 2.B – Preparations for infusion or injection supplied in units that contain *a nominal content of less than 100 mL*

The preparation complies with the test if, in each unit tested, the number of particles present per unit that are10 µm or larger does not exceed 3000 per container and the number of particles that are 25 µm or larger in size does not exceed 300 per container. Where combining units is required, the average number of particle present in the combined sample is used to calculate the number of particles in one container.