Q-09 PARTICULATE CONTAMINATION: SUB-VISIBLE PARTICLES

Particulate matter in parenteral preparations consists of, mobile undissolved substances, other than gas bubbles, that may originate from various sources and are unintentionally present in the preparations. The level of particulate matter should be minimised and controlled, independent of its type.

For the determination of particulate contamination 2 procedures, Method 1 (Light Obscuration Particle Count Test) and Method 2 (Microscopic Particle Count Test), are specified hereinafter. When examining injections and infusions for sub-visible particles, Method 1 is preferably applied.
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, the test is carried out according to Method 2. Emulsions, colloids, and liposomal preparations are examples. Similarly, preparations that produce air or gas bubbles when drawn into the sensor may also require specific precautions during sample preparation and/or 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 particle-free solvent 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 contamination 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 characterise the level of particulate contamination 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 apparatus is calibrated using suitable certified reference materials consisting of dispersions of spherical particles of known sizes between 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

The test is carried out under conditions limiting particulate contamination, 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 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, the following test is carried out: determine the particulate contamination of 5 samples of *particle-free water R*, 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 directly represents the product fill. 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.

Powders for parenteral administration are reconstituted with *particle-free water R* or with an appropriate particle-free solvent 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. The number of test specimens 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.

Remove 4 portions, each of approximately 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 average number of particles from the remaining portions of the preparation to be examined. Smaller volumes 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), testing with the volume of 1 to 5 mL may be acceptable.

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 average number of particles 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 the average number of particles present in each unit tested does not exceed 25 per millilitre equal to or greater than 10 µm and does not exceed 3 per millilitre equal to or greater than 25 µm.

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 the average number of particles present in each unit 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. 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.

METHOD 2. MICROSCOPIC PARTICLE COUNT TEST

Use a suitable binocular microscope, filter assembly for retaining particulate contamination 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, 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 2.9.19.-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 for retaining particulate contamination 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.

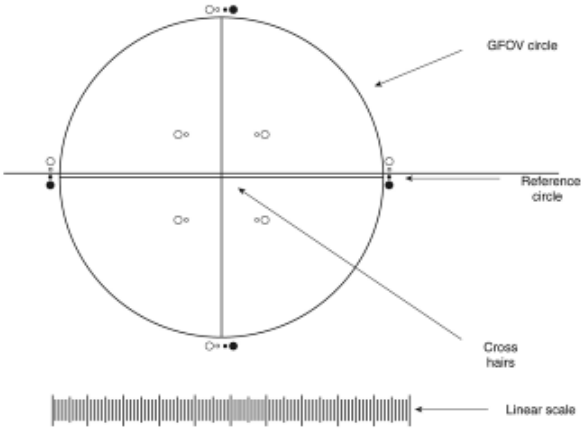

Figure 1. – *Circular diameter graticule*

General precautions

The test is carried out under conditions limiting particulate contamination, 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 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, the following test is carried out: determine the particulate contamination of a 50 mL volume of *particle-free water R* 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

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 directly represents the product fill. 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.

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

The number of test specimens 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 the last addition of sample, begin rinsing 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 greater than 10 µm and the number of particles that are equal to or greater than 25 µm. Alternatively, partial filter count and determination of the total filter count by calculation is allowed. Calculate the average 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 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 the average number of particles present in each unit tested does not exceed 12 per millilitre equal to or greater than 10 µm and does not exceed 2 per millilitre equal to or greater than 25 µm.

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 the average number of particles present in each unit 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. 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.