⟨787⟩ SUBVISIBLE PARTICULATE MATTER IN THERAPEUTIC PROTEIN INJECTIONS

This chapter can be used as an alternative to *USP* general chapter *Particulate Matter in Injections* ⟨788⟩. It specifically addresses therapeutic protein injections and related preparations, allowing use of smaller test product volumes and smaller test aliquots to determine particulate matter content, with sample-handling instructions that take into account the issues associated with the analysis of these materials. Although the methodology and limits presented in this chapter are preferred for therapeutic protein injections, the use of regulatory-acceptable alternative analytical methods with adequately developed subvisible particulate limits is acceptable.

Therapeutic protein injections are biotechnology-derived products of protein or peptides and other therapeutic protein injections such as naturally sourced therapeutic proteins, including their final infusion preparations. Certain preparations that are not amenable to these tests, such as prophylactic vaccines, are not included in this guidance.

Particulate matter in therapeutic protein injections consists of mobile undissolved substances that may originate from various sources. The particles may be (a) truly foreign, or "extrinsic", e.g., unexpected foreign material, such as cellulose; (b) "intrinsic" resulting from addition or by insufficient cleaning during manufacturing, such as tank metals or gaskets, lubricants, filling hardware, or resulting from instability, e.g., changes over time, such as insoluble drug salt forms or package degradation; and (c) "inherent", such as particles of the protein or formulation components. All of these particle types may be detected and counted in the test method described in this chapter. In addition, for light obscuration (LO) methods, gas bubbles are commonly counted during particulate matter testing and are counting artifacts. For the determination of subvisible particles in therapeutic protein injections, the *Light Obscuration Particle Count Test* is the preferred method. The *Microscopic Particle Count Test* similar to that described in ⟨788⟩ may be useful in cases where the particles being analyzed are primarily nonproteinaceous, or in cases where examination of particle characteristics may be important. The results from this test are not equivalent to those from the LO test.

Not all therapeutic protein injections can be examined directly for subvisible particles by the LO method. When the test method is not directly applicable to specific test samples, as indicated by issues with method verification (reproducibility, linearity), a quantitative dilution with an appropriate diluent can be made to allow analysis by light obscuration. Method verification may be required to ensure the appropriateness of the sample handling procedures and the performance of the method for each drug product.

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 to support valid inferences from observed data in order to characterize the level of particulate matter in a large group of units. Sampling plans should be based on considerations of product volume, particle numbers historically found in comparison to limits, particle size distribution, and variability of particle counts between units. Products that are used with a final filter during administration (in-line) are exempt from these requirements, providing that scientific data are available to justify the exemption.

Change to read:

LIGHT OBSCURATION PARTICLE COUNT TEST

Analysts should use a suitable instrument that is based on the principle of light blockage and that allows an automatic determination of particle size and number.

The sensor selected must be appropriate for the intended particle size range and anticipated particle count. Several standardization steps such as sample volume accuracy, sample flow rate, sensor resolution, calibration, and particle count accuracy are important for the proper operation of the apparatus and are further reviewed in general information chapter ▲*Methods for the Determination of Subvisible Particulate Matter* ⟨1788⟩▲ (CN 1-May-2021).[1]

For equipment calibration purposes, use of NIST-traceable particle size standards or equivalent traceable standards, see ⟨1788⟩. These standards generally are between 2 and 100 µm. Analysts should verify the performance of the apparatus using the USP Particle Count RS dispersed in particle-free water at an appropriate volume. Care must be taken to avoid agglomeration of particles during dispersion in the calibration process. The definition for *Particle-Free Water* is provided in the introductory section of *Reagents, Indicators, and Solutions*.

General Precautions

Carry out the test under conditions that limit ingress of particulate matter, preferably in a laminar-flow cabinet. The glassware and equipment used should be properly cleaned and handled to remain particle free. Care should be taken to minimize shaking and other stresses to the preparations (for liquids or reconstituted powders) and to prevent introduction of air bubbles into the preparation under examination. This is particularly important when preparations are combined or transferred to the container in which the determination will be carried out.

[1] For informational purposes only, analysts can refer to ⟨1788⟩, which may be a helpful but not mandatory resource.

Preparatory Steps

Glassware selected for the method should accommodate the volume of blank and sample aliquots, allow easy mixing, and be amenable to repeated cleaning used to prevent any significant contribution to particle counts. Typically, separate vessels are utilized and retained specifically for this test only. Cleaning and rinsing of the method glassware should be in close proximity to the counting area.

The work area and equipment should be separated from general lab traffic. A good supply of filtered water (to comply with *Particle-Free Water*) and support equipment should be maintained and available for the routine work. Refer to general information chapter ⟨1788⟩.[1]

Two types of system controls are recommended: a *Blank Test* and *System Suitability Verification*.

1. The *Blank Test* involves the following:
    A. Run at the start of the day or analysis to ensure that proper care has been used in preparing the site and apparatus for use (*Environmental Blank*).
    B. Any additional blanks run throughout the day (such as between product families) should also comply with the *Blank Test* requirements (*Procedural Blank*).
2. The *System Suitability Verification* is run to verify proper count/mL and ratio for the current USP Particle Count RS or equivalent commerical standard. See general information chapter ⟨1788⟩.[1]

Blank Test

In order to check that (a) the environment is suitable for the test, (b) the glassware is properly cleaned, and (c) the water or suitable solvent that will be used is particle free (b and c constitute the "blank"), the following test is carried out. Determine the count in five aliquots of degassed particle-free water or suitable solvent, representative of the product test volume (but NLT 1 mL). If the number of particles 10 µm or greater in size exceeds 1 particle per mL for the combined aliquot volume, the precautions taken for the test are not sufficient. Repeat the preparatory steps until the environment, glassware, and water are suitable for the test determination. The *Blank Test* must be run initially each day (environment) and is recommended throughout the daily routine, especially between product families (procedural).

System Suitability Verification

To verify system suitability, *Particle-Free Water* and the USP Particle Count RS or equivalent commercial standard aqueous suspension are tested from the same or similar type of container as product samples, are degassed in the same manner, and generally are handled like the test article. Ensure that the system can meet the *Blank Test* requirement. Determine the particulate matter in a sample of the USP Particle Count RS that corresponds to the product test volume, according to the method described below. Results from the Standard preparation should fall within the stated specifications of the Reference Standard.

Method

Product samples are tested in the manner that most suitably represents delivery (e.g., expelled syringe contents). For parenterals that have a sufficient volume (i.e., volume large enough to facilitate testing), testing of individual units is often diagnostic. For parenteral products 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 (generally 0.2–5.0 mL). If a dilution is performed, ensure that the blank container has sufficient volume for product and diluents and that few, if any, particles are introduced in the process. Open each unit cautiously, remove the sealing closure, and avoid contamination of the contents before sampling, diluting, or, if necessary, pooling.

When a product solvent is specified, e.g., for lyophilized solids or powders for parenteral use, the reconstitution or dilution must be performed with the appropriate amount of specified solvent. In this case, the solvent itself may be tested to ensure that it is not a significant source of particles. Subtraction of the solvent particle count from the total count is not allowed.

Eliminating gas bubbles is a key step, especially for proteinaceous products that readily entrain gas. Two methods are recommended: either allowing the product fluid to stand under ambient pressure or applying a gentle (e.g., 75 Torr) vacuum. Other methods may be used when demonstrated to be suitable. Sonication should be avoided.

Once the samples have been degassed, they must be remixed gently to suspend all particles by mixing the contents of the sample gently but thoroughly by an appropriate means, e.g., slow swirling of the container by hand. Inversion to mix the product fluid is not recommended at any time.

Immediately after mixing, withdraw NLT four aliquots, each of a volume appropriate for the instrument's capacity (generally 0.2–5.0 mL). Count the number of particles over the selected size range, including particles equal to or greater than 10 and 25 µm. Disregard the result obtained for the first aliquot, and calculate the mean number of particles at each size range for the remaining aliquots of the preparation being tested. A tare volume also can be applied to control sampling that should be representative of the sensor dynamic range and needle volume.

General Considerations

Dilution is allowed as long as the diluent and methods are demonstrated to be appropriate and the smallest level of dilution that allows for reproducible testing is used. Under certain circumstances dilution of samples may be required to obtain reliable results. This may be the case with high-concentration products and/or high-viscosity products that cannot be drawn into the instrument properly; products that cause saturation of the instrument sensor due to high counts, especially in the smaller than 10-µm size ranges; and cases where the high concentration results in very small differences in refractive index between the

solution and the protein aggregates, etc. In situations where sample dilution is conducted, the underlying rationale for the dilution as well as the suitability of the selected dilution scheme including choice of diluent must be demonstrated. Suitability of the dilution scheme and of the diluent may be assessed by demonstrating consistency of results over a dilution range. Bridging studies with and without dilution at size ranges intended for particle counting should be considered. Studies exploring the ability of dilution to decrease the number of aggregates, or to increase particles due to changes in the ratio of protein to excipients, should also be performed. An orthogonal technique may be used to justify the suitability of the method by verifying the impact of dilution.

Evaluation

The values below are derived historically from general chapters ⟨788⟩ and ⟨789⟩. If specifications are different from those stated below, they will be indicated in an individual monograph where available.

For parenteral products that are therapeutic protein injections for infusion or injection supplied in containers with a nominal content of less than or equal to 100 mL:

The average number of particles present in the units tested should not exceed 6000 per container equal to or greater than 10 µm and should not exceed 600 per container equal to or greater than 25 µm.

For therapeutic protein injections supplied in containers with a nominal content of more than 100 mL, and parenteral infusion preparations or injections with a nominal content of more than 100 mL:

The average number of particles present in the units tested should not exceed 25/mL equal to or greater than 10 µm and should not exceed 3/mL equal to or greater than 25 µm. Also, total particle load should not exceed 6000 per container equal to or greater than 10 µm and should not exceed 600 per container equal to or greater than 25 µm.

Products that are used with a final filter during administration (in-line) are exempt from these requirements, providing that scientific data are available to justify the exemption. However, filtrates are expected to comply with the guideline. For products supplied or first reconstituted in <100 mL, and then diluted for infusion in a volume >100 mL, particle content should be assessed both before and after dilution and evaluated based on their final volume.

MICROSCOPIC PARTICLE COUNT TEST

As noted, the LO method is the preferred method for therapeutic protein injections and parenteral infusions. However, the microscopic method may be used when appropriate, such as determination of extrinsic and intrinsic particle types only. It should be demonstrated, however, that particular classes of particles (e.g., inherent) are also being counted when using this method. For the determination of product acceptability, apply the limits for the membrane microscopic test in general chapter ⟨788⟩. Because of the interference of some protein particles and their physical characteristics (fragile or translucent), the results of the *Microscopic Particle Count Test* are not equivalent to those of the *Light Obscuration Particle Count Test*, and the two methods cannot be considered interchangeable.