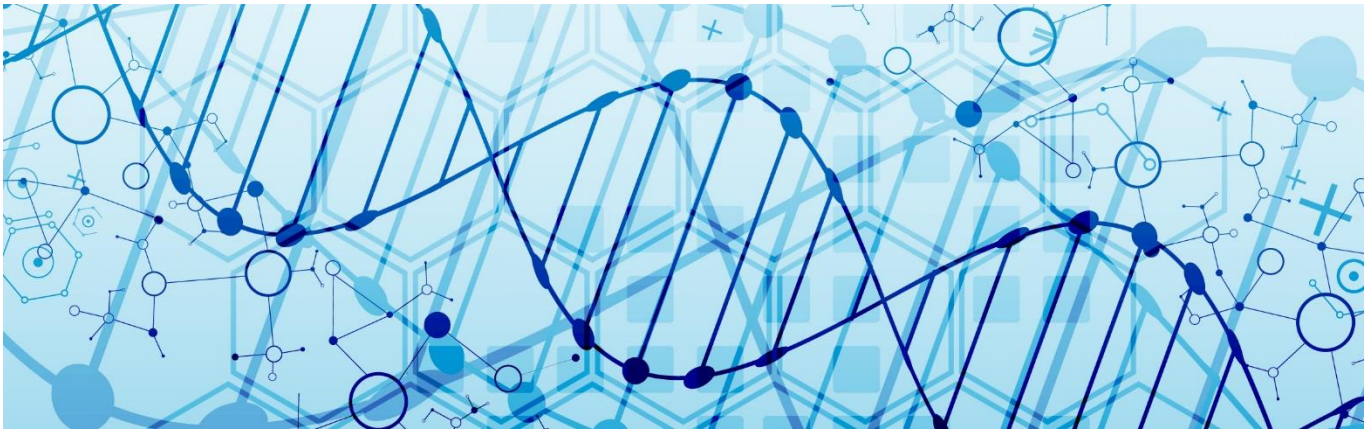

MEASUREMENT OF PARTICULATES IN SINGLE-USE SYSTEMS FOR CELL AND GENE THERAPIES MANUFACTURING

PART 1: MISAPPLICATION OF USP <788>

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ABSTRACT

As the manufacturing of antibody and vaccine therapies using single-use systems (SUS) now has near universal acceptance, a new set of challenges arise in determining the suitability and qualification requirements for application of SUS in manufacturing cell and gene therapies (CGT). One of the key challenges of cell therapy manufacturing lies in the inability to apply sterile filtration operations, since filtration will remove the cells, the active ingredient in the drug product. Sterile filtration not only removes microbiological contamination, but also any particulate contamination, in both the visible (> 100 µm) and subvisible (10-100 µm) size ranges. Most SUS manufacturers claim their products "meet USP <788>" specifications for particulates. Here we argue that this is a clear misapplication of USP <788> Particulate Matter in Injections, which only describes a test method for measurement of subvisible particles in injectable drug products, not SUS. This historical misapplication of USP <788> to SUS must be stopped, and test methods which not only describe the extraction of particulates from SUS, but also measure both subvisible and visible particulates, need to be applied to SUS. Only with dedicated methods for measurement of particulate in SUS will the critical challenges required for clean manufacturing of cell and therapies using SUS be achieved.

INTRODUCTION

Particulates refer to mobile, undissolved particles other than gas bubbles that are unintentionally present in an injectable drug product. Patient safety is impacted by the amounts and types of particulate present in a drug product [*Ref 1*]. Particulates vary in nature (e.g., metal, glass, dust, fiber, rubber, polymer, mold, degradant precipitate) and can be divided into three categories:

1. *Intrinsic (native) particulates:* Particles that are derived from, or formed because of, the manufacturing equipment or process, product formulation, or container system.
2. *Inherent particulates (formulation): Particles that are an innate product characteristic (e.g., adjuvants in vaccines, LNPs as drug delivery vehicles)*.

3. *Extrinsic (foreign) particulates:* Particles that originate from the manufacturing environment and are foreign to the manufacturing process.

Regulatory requirements for final drug products and biopharmaceutical manufacturing processes are clear, and spell out the expectations for equipment, surfaces, and any particulate that may be contained on the surfaces of processing equipment. US FDA Drug cGMP, 21 CFR 211.65 states:

> *"Equipment shall be constructed so that surfaces that contact components, in-process materials, or drug products shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements."*

US FDA cGMP for Biologicals, 21 CFR 600.11 similarly states:

> *"All surfaces that come in contact with products shall be clean and free of surface solids, leachable contaminants, and other materials that will hasten the deterioration of the product or otherwise render it less suitable for the intended use…"*

ICH Q7 Good manufacturing practice for active pharmaceutical ingredients - guideline states:

> *"Equipment should be constructed so that surfaces that contact raw materials, intermediates, or APIs do not alter the quality of the intermediates and APIs beyond the official or other established specifications."*

Typically, multi-use process tanks are rinsed with water for injection (WFI) as the last cleaning step or prior to charging with process fluids. Rinsing with WFI usually reduces the surface particulate load significantly, especially after long storage periods.

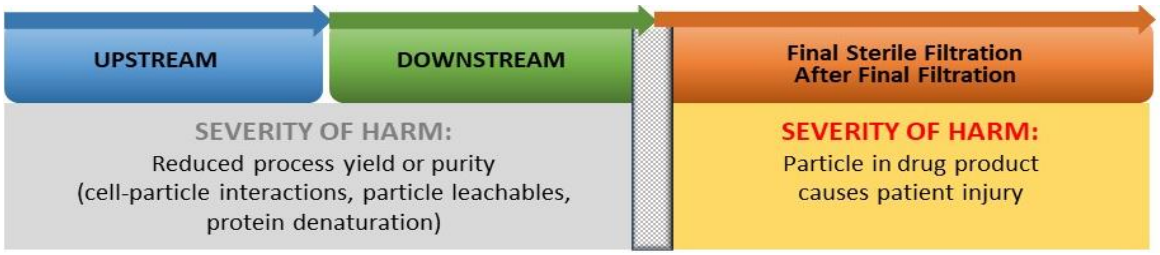
A clear benefit of SUS has surely been the enablement of closed system biopharmaceutical production processing with no Clean-In-Place (CIP) or Steam/Sterilization-In-Place (SIP) steps required, which has greatly reduced the risks from environmental, water, and batch-to-batch contaminants in drug products.

However, risks from particulates may increase when applying SUS, since in the manufacturing of SUS rinsing is typically not used to remove particulates from SUS. As the BPSA paper on particulates indicates [*Ref 2*], SUS

Production of SUS equipment in a cleanroom

manufacturers have addressed these particulate risks by performing a large majority of the manufacturing and assembly processes in ISO-classified clean rooms. SUS manufacturing requirements have steadily improved and currently it is common to manufacture SUS in ISO 7 clean rooms with continuous monitoring and product testing.

Both multi-use and single-use biopharmaceutical production processes control particulate risks with purification processes that implement 0.2 µm filters at



Biopharmaceutical Manufacturing: Probability of Occurrence (Binary Risk Scenario)

critical points within the process and for final drug product filtration. However, appropriate validated procedures need to be executed when filters are used. As defined in the US FDA 21 CFR 210 on cGMPs [*Ref 3*]:

> *"(6) Nonfiber releasing filter means any filter, which after appropriate pretreatment such as washing or flushing, will not release fibers into the component or drug product that is being filtered."*

Drug product manufacturing using sterile 0.2 µm filters works well to remove particulates, including those that result from the manufacturing process (e.g., storage vessels, peristaltic pump tubing, etc.), and thus the areas of concern are reduced to final drug product fill/finish activities and those therapies that cannot be filtered (e.g., vaccines). Final drug products are monitored for visible particulates (> 100 µm) by 100% visual inspection of vials and syringes per USP <1> Injections and USP <790> Visible Particulates in Injections while subvisible particulates (< 100 µm) are monitored by light obscuration and membrane microscopy, per the USP <788> Particulate Matter in Injections.

However, the monitoring and control of particulates in Cell Therapies manufacturing presents new challenges. Cell therapies are manufactured with complex multistep processes, with many potential sources for introduction of particulate matter [*Ref 4*]. Cell therapies are suspensions of living cells (typically between 10 and 30 µm) and application of 0.2 µm sterile filters will remove the active ingredient (the cells), thus there is no opportunity to take advantage of filtration processes to remove unwanted particulates. Another challenge is that cell suspensions are often not fully transparent which limits the effectiveness of visual inspection processes. Also, light obscuration for subvisible particle monitoring is unable to differentiate cells from extrinsic particulates. Particulate contamination present in CGT products can present significant risks to patient safety, as many of these products are administered through intravenous injection. It is thus especially crucial to limit particulates from all possible sources, including particulates from the SUS process equipment used to manufacture cell therapies.

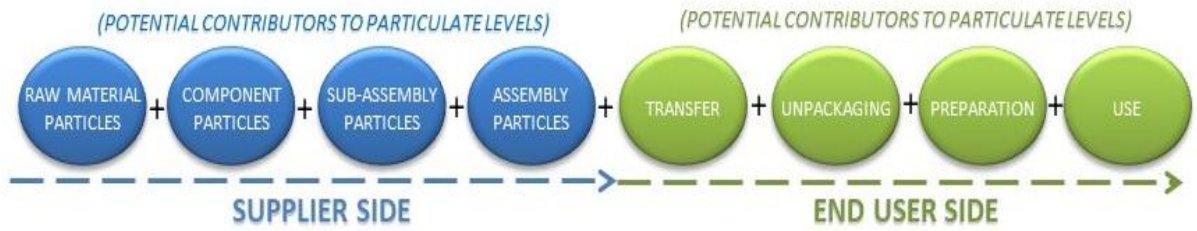
Visual inspection of SUS process equipment for visible particles >100µm

WHAT IS USP <788> AND WHY IS IT APPLIED TO PARTICULATES IN SUS

No standardized test methods exist for the measurement of particulate matter in SUS. The absence is compensated for by "applying" the USP <788> test method for injectable drug products to SUS. However, the forced application of USP <788> to SUS, while convenient, will not detect both the subvisible and visible particulates in SUS, and thus will not give a realistic measure of particulate.

USP <788> describes two test methods for measurement of particulate matter in parenteral drug products:

In Method 1 Light Obscuration Particle Count Test, the drug product flows through a sensor composed of a flow cell, a laser light beam, and a photodiode detector. A particle is counted and sized when it absorbs and/or scatters the light beam, which is registered by the photodetector as an attenuated signal. Via a calibration of the signal attenuation obtained from spherical reference particles of known diameter, the signal from the unknown particle is converted into an "effective" spherical diameter. The LO technique (LO) is quick, automated, and robust, with a nearly 40-year history of



use in the measurement of particles in parenteral drug products.

In an alternate test method described in USP <788> called Method 2 Microscopic Particle Count Test, the drug product is filtered through a membrane filter and, under specific illumination conditions, the particles on the membrane filter are compared by an operator with circular reticules 10 µm and 25 µm in diameter. This technique is essentially a visual inspection of particles on the surface of a membrane filter, aided by the magnification provided by a microscope. The operator visually compares a particle with the circular reticules and decides if the particle is larger or smaller than the reticule diameter. An advantage of membrane microscopy is that the particulates are isolated from the solution and given sufficient size can be characterized further after filtration and counting (e.g., by FTIR/Raman microscopy or SEM-EDX). The nominal pore size of the membrane filter sets the lower limit on particle size trapped upon filtration. The upper particle size limit is only limited by the diameter of the membrane filter (usually 25 or 47 mm). Thus, particles in the visual size range (> 100 µm) are detected.

DISCUSSION: WHY USP <788> IS NOT SUITABLE FOR THE APPLICATION TO SINGLE-USE SYSTEMS

The intended use of USP <788> is parenteral drug products - not SUS

The titles of USP <788>, USP <789> and USP <787>: *Particulate Matter in Injections, Particulate Matter in Ophthalmic Solutions,* and *Subvisible Particulate Matter in Therapeutic Protein Injections*, respectively) make it clear that these guidance chapters are applicable to particulate matter in parenteral drug products. These chapters were not intended for, nor do they address measurement of, particulates in single-use systems.

There is no description of particulate extraction procedures for SUS in USP <788>

The liquid extraction of particulates from the surfaces of SUS is arguably the most critical step for a reliable characterization and/or monitoring of the particulate population present in the systems. The effectiveness of the extraction method is influenced by the wetting and suspension properties of the test liquid, temperature, energy input (rinsing, agitation) and the amount of time the surfaces of the test article are exposed to the test liquid as well as the properties of the single-use system. However, USP <788> does not give any guidance or procedures applicable to the extraction of particulate matter from single-use systems. This lack of a standardized extraction procedure potentially results in test samples not representative of the actual particulate risk in CGT products.

The light obscuration technique in USP <788> has limitations when applied to SUS

Light Obscuration (LO)

According to USP <788>, LO is one of the most common analytical techniques in pharmaceutical quality control labs. LO has been a mandatory standard method for quality control and release of parenteral drug products for decades and is accepted as the de facto standard for subvisible particle analysis among regulators. Due to widespread use and acceptance, most pharmaceutical QC professionals are familiar with LO and the required instrumentation is readily available in most labs run under a cGMP environment.

As indicated in the guidance given in USP <1788> and <1788.1>, the LO technique is limited to the measurement of particles < 300 µm depending upon instrument configuration.

Since LO is an indirect measurement of particles size, for fibers longer than the illumination zone, LO will report a diameter closer to the diameter of the fiber than the length of the fiber. Also, for particles with low contrast to the liquid medium, the particle size reported will be smaller than the actual diameter or might not be detected at all. LO will not only detect solid particles, but also detect liquid droplets and air bubbles, but is not able to differentiate between them. While some LO instrument manufacturers claim detection of particles up to 600 µm in size, according to T. A. Barber, the upper limit on particle size measured with LO depends not only on sensor dimensions, but also upon particle size, density, and morphology [*Ref 5*]. As particle size, aspect ratio (ratio of length to width) and density increase, sampling errors increase since larger particles may not be reliably siphoned up and flow through the sensor. While LO is a well-accepted technique for the measurement of subvisible particles (< 100 µm), LO will therefore not reliably detect all potential particles in the visible size range (> 100 µm).

Membrane Microscopy

As indicated in the guidance provided in USP <1788> and <1788.2>, manual membrane microscopy is labor intensive and fatiguing, especially when many particles are present on the membrane filter. While manual membrane microscopy is a well-accepted technique for the detection of and size classification of particles in both the subvisible and visible size ranges, the reliance on manual counting and sizing large numbers of particulates makes it subject to human error. A more efficient technique with a higher degree of automation would be needed.

Reporting and acceptance criteria in USP <788> are not appropriate for SUS

To effectively assess risk to the final drug product, there is a need for particulate counts per component/ assembly or per surface area in contact with the fluid together with total fill volume or total fluid path contact area.

USP <788> requires that any detected particle counts are reported per milliliter of drug product for large volume parenteral products. It is the authors' opinion that this type of reporting is not appropriate for SUS and is not fit for the intended use of the data. The non-standardized force-fit of USP <788> to single-use systems allows for "creative" adaptations. For example, increasing the volume of liquid used to extract particles from the surfaces of single-use systems will lead to a lower reported particle count if the particles are reported per mL of liquid applied in the extraction. Calculation in this manner simply "dilutes" the particles and is not representative of the particle load of that SUS component. A suggested approach is adding a particulate requirement similar to USP <161> Medical Devices—Bacterial Endotoxin and Pyrogen Tests where it utilizes the procedures from USP <85> Bacterial Endotoxins for the assays but reports the limits per medical device.

Furthermore, the acceptance criteria in USP <788> are intended for, and apply to, final drug product and are thus inadequate for particulate monitoring in SUS. This absence of applicable method and/or material specifications is scientifically and regulatorily problematic. While these assessment(s) are generally performed by the component suppliers, the incorporation of these compendial standards are likely incorrectly applied to SUS commonly used during cell therapy qualification and manufacturing activities. The BPSA paper on particulates clearly discusses the risks from both the manufacture and use of SUS.

CONCLUSION

In summary, light obscuration testing citing USP <788> is often applied to SUS as a checkbox activity to satisfy customers and regulators, instead of following scientific best practice and risk- and evidence-based testing strategies. USP <788> and, by extension, associated and harmonized guidance chapters such as USP <787>/<788>/<789>, USP <1788> and Ph. Eur. 2.09.19, clearly have significant shortcomings when applied to control of particulates in SUS. While analytical technology like light obscuration or membrane microscopy can be used for the measurement of particulates extracted from SUS, USP <788> and the chapters mentioned above were never intended for– nor are they applicable to–single-use systems.

Moreover, the lack of a suitable extraction procedure, as well as inadequate reporting and acceptance criteria, may lead to misrepresentations of the particle load and thus severely misrepresent the associated risks to patient safety.

Therefore, it is the authors' opinion that a standardized method which generates SUS particulate data that is fit for purpose (such as automated microscopy) is desperately needed. In subsequent papers we will be addressing best practices for the measurement of particulates in single-use systems, focusing on development and validation of an extraction procedure, best methods for detection of both subvisible and visible particles, and formats for exchanging essential particulates data between suppliers and end-user.

In addition to a recently published standard practice on extraction of particulates from single-use systems (ASTM E3230-20), multiple task groups in the ASTM E55 committee on Biopharmaceutical Processing are drafting standards to address the gaps in measurement and reporting of particulates in single-use systems.

REFERENCES

Ref 1. Bukofzer S, Ayres J, Chavez A, Devera M, Miller J, Ross D, et al. Industry Perspective on the Medical Risk of Visible Particles in Injectable Drug Products. PDA Journal of Pharmaceutical Science and Technology 2015;69:123–39.

Ref 2. Bio-Process Systems Alliance. BPSA 2020 Recommendations For Testing, Evaluation, and Control of Particulates From Single-Use Process Equipment https://www.scribd.com/document/486229412/BPSA-2020-Recommendations-for-Testing-Evaluation-and-Control-of-Particulates-from-Single-Use-Process-Equipment

Ref 3. 21 CFR Part 210 -- Current Good Manufacturing Practice for Finished Pharmaceuticals. 21CFR 210.3.b.6

Ref 4. Clarke D, Stanton J, Powers D, Karnieli O, Nahum S, Abraham E, et al. Managing particulates in cell therapy: Guidance for best practice. Cytotherapy 2016;18:1063–76.

Ref 5. T. A. Barber, Control of Particulate Contamination in Healthcare Manufacturing, CRC Press, 1999, ISBN: 1574910728

