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Standard Guide for Subvisible Particle Measurement in Biopharmaceutical Manufacturing Using Dynamic (Flow) Imaging Microscopy[1]

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1. Scope

1.1 Biotherapeutic drugs and vaccines are susceptible to inherent protein aggregate formation which may change over the product shelf life. Intrinsic particles, including excipients, silicone oil, and other particles from the process, container/closures, equipment or delivery devices, and extrinsic particles which originate from sources outside of the contained process, may also be present. Monitoring and identifying the source of the subvisible particles throughout the product life cycle (from initial characterization and formulation through finished product expiry) can optimize product development, process design, improve process control, improve the manufacturing process, and ensure lot-to-lot consistency.

1.2 Understanding the nature of particles and their source is a key to the ability to take actions to adjust the manufacturing process to ensure final product quality. Dynamic imaging microscopy is a useful technique for particle analysis and characterization (proteinaceous and other types) during product development, in-process and commercial release with a sensitive detection and characterization of subvisible particles at ≥2 and ≤100 micrometers (although smaller and larger particles may also be reported if data are available). In this technique brightfield illumination is used to capture images either directly in a process stream, or as a continuous sample stream passes through a flow cell positioned in the field of view of an imaging system. An algorithm performs a particle detection routine. This process is a key step during dynamic imaging. The digital particle images in the sample are processed by image morphology analysis software that quantifies the particles in size, count, and other morphological parameters. Dynamic imaging particle analyzers can produce direct determinations of the particle count per unit volume (that is, particle concentration), as a function of particle size by dividing the particle count by the volume of imaged fluid (see Appendix X1).

1.3 This guide will describe best practices and considerations in applying dynamic imaging to identification of potential sources and causes of particles during biomanufacturing. These results can be used to monitor these particles and where possible, to adjust the manufacturing process to avoid their formation. This guide will also address the fundamental principles of dynamic imaging analysis including image analysis methods, sample preparation, instrument calibration and verification and data reporting.

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E2589 Terminology Relating to Nonsieving Methods of Powder Characterization

2.2 *ISO Standards:*[3]

ISO 2859 Sampling Procedures for Inspection by Attributes

ISO 8871 Elastomeric Parts for Parenterals and for Devices for Pharmaceutical Use

ISO 9276-6 Representation of Results of Particle Size Analysis Part 6: Descriptive and Quantitative Representation of Particle Shape and Morphology

2.3 *Other Standards:*

ANSI/ASQ Z1.4-2003 Sampling Procedures and Tables for Inspection by Attributes[3]

ASME BPE-2014 Bioprocessing Equipment[4]

[1] This guide is under the jurisdiction of ASTM Committee E55 on Manufacture of Pharmaceutical and Biopharmaceutical Products and is the direct responsibility of Subcommittee E55.03 on General Pharmaceutical Standards.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

[4] Available from American Society of Mechanical Engineers (ASME), ASME International Headquarters, Two Park Ave., New York, NY 10016-5990, http://www.asme.org.

BS 6001-1:1999+A1:2011 Sampling procedures for inspection by attributes. Sampling schemes indexed by acceptance quality limit (AQL) for lot-by-lot inspection[5]
USP <787> Subvisible Particulate Matter in Therapeutic Protein Injections[6]
USP <788> Particulate Matter in Injections[6]
USP <1663> Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems[6]
USP <1664> Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging Delivery Systems[6]
USP <1787> Subvisible Particulate Matter in Therapeutic Protein Injections[6]

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this standard, refer to Terminology E2589.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *brightfield illumination, n*—a method of providing light into a measurement space whereby the illuminated objects are located between the light source and the viewing receiver.

3.2.2 *circularity, n*—degree to which a particle (or its projection area) is similar to a circle.

3.2.3 *cumulative particle size distribution, n*—a representation, as a table, graph, or mathematical function, that gives the total fraction or concentration of particles greater than or less than a set of specified size values.

3.2.3.1 *Discussion*—Cumulative particle size distributions may be expressed as either mass, volume, area, number, or concentration values.

3.2.4 *depth of field, n*—depth of field is the distance between the nearest and farthest objects that are in acceptably sharp focus in an image.

3.2.5 *dynamic imaging, n*—particle size and shape analysis using computer image analysis techniques on instantaneously captured still frame projected images of particles in motion (also referred to as *flow imaging*, *flow microscopy*, *direct imaging*).

3.2.6 *equivalent diameter, n*—the diameter of a sphere or circle that is equal to the measured diameter obtained by a particle sizing instrument.

3.2.6.1 *Discussion*—For dynamic imaging, the reported diameter is based on the projected area of a measured particle.

3.2.7 *extrinsic particle, n*—a particle introduced from sources that are foreign or external to the manufacturing process.

3.2.8 *Feret diameter, F, n*—apparent diameter of an object determined from the distance between two parallel tangents on opposite sides of a binary object.

3.2.8.1 *Discussion*—There are an infinite number of Feret's diameters; the maximum and the minimum Feret's find most use within imaging.

3.2.9 *field of view, n*—the two dimensional, lateral extent of the imaged area.

3.2.10 *frequency distribution, n*—a representation, as a table, graph, or mathematical function, that gives the frequency or count of values within a set of specified intervals.

3.2.11 *inherent particle, n*—a particle made entirely of components of the formulated drug product or its manufacturing intermediate, arising from the product itself.

3.2.12 *intrinsic particle, n*—a particle composed of materials that the product or intermediate contacts or is mixed with during the manufacturing process or during storage in primary packaging components.

3.2.13 *particle size distribution (PSD), n*—a frequency or volume distribution of the concentration of particles versus particle size.

3.2.13.1 *Discussion*—Dynamic imaging particle analyzers of use to the biopharmaceutical industry report the PSD as the concentration of particles per unit volume within specified size ranges, where the size is most commonly the equivalent diameter but may be another morphological size attribute. See Appendix X1.

3.2.14 *subvisible particle, n*—a particle with a measured equivalent diameter within the approximate range 1 µm to 100 µm.

NOTE 1—When it is necessary to specify an exact size range, the range should be defined explicitly rather than by such adjectives as subvisible.

3.2.14.1 *Discussion*—The term particle may be used to designate any self-contained object that is optically distinguishable from the background image, including liquid droplets and gas-phase bubbles.

3.2.14.2 *Discussion*—The 100 µm upper limit is based on the historical definition of subvisible particle as used in the field of drug inspection. Particles of 20 µm or smaller of sufficient optical contrast are readily visible under bright illumination, especially when present in numerous quantity.

3.2.15 *threshold, n*—the minimum quantitative change in intensity (of either positive or negative sign) from the background pixel value for a pixel to be identified as a possible particle.

3.2.16 *volume distribution, n*—a frequency distribution that gives the distribution of particle volume as a function of particle size.

4. Significance and Use

4.1 This guide will encompass considerations for manufacturers regarding sources and potential causes of subvisible particles in biomanufacturing operations and the use of dynamic imaging particle analyzers as a suggested common method to monitor them. The guide will address the following components of particle analysis using dynamic imaging microscopy: fundamental principles, operation, image analysis methods, sample handling, instrument calibration, and data reporting.

[5] Available from British Standards Institution (BSI), 389 Chiswick High Rd., London W4 4AL, U.K., http://www.bsigroup.com.

[6] Available from U.S. Pharmacopeial Convention (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.

5. Types of Particles

5.1 USP <1787> defines three subcategories of particles related to their source or nature. When combined with appropriate strategies for characterizing particle types, this categorization scheme provides a framework for assessing the root cause and acceptable concentrations of different types of particles.

5.1.1 Inherent particles are related to the product formulation (for example, chemical and physical properties and concentration of the Active Pharmaceutical Ingredient (API) proteins, excipients, API solid suspensions, emulsions, adjuvant aluminum salts added to vaccines). Packaging of the product and external stresses (including temperature, mechanical shock or movement, light exposure, and interaction with liquid/solid and liquid/air interfaces) can all have substantial impact on the concentration and characteristics of protein aggregates. Protein aggregates may change over time, in both concentration and characteristics, and some levels of protein degradation or related aggregation, or both, may be expected. Inherent particles must be well characterized and monitored over the product shelf-life.

5.1.2 Intrinsic particles include product contact materials from the manufacturing process or primary packaging components (that is, silicone oil, glass, stainless steel, rubber closure, polymer tubing, semi-solid silicone lubricant, process related fibers, etc.). This category also includes stability-indicating particles found predominantly during development or stability studies (formulation degradation, container closure-related, glass delamination, stopper degradation, etc.). The presence of intrinsic particle types must be minimized, and if they are stability-indicating, they should be eliminated whenever possible.

5.1.3 Extrinsic particles comprise any particles not sourced from the manufacturing process or product contact materials including particles of a biological source (that is, external environmental fibers, hair, airborne particles, etc.). Extrinsic particle types should be a rare occurrence and eliminated.

6. Sources of Particles

6.1 Subvisible particles may be generated by a number of sources during the manufacturing process. In analyzing the risk of particle generation introduced by various process steps, it is useful to understand the sensitivity of the drug product or substance to a variety of stresses known to promote particle formation.

6.2 *Sources of Inherent Particles:*

6.2.1 Stresses which may cause inherent particle changes may include:

6.2.1.1 Interaction with interfaces or other particles.

(1) Increased interfacial transport resulting from agitation, stirring, etc.

(2) Interfacial adsorption: both liquid/vapor and liquid/solid

(3) Nucleation on other particles

(4) Trace metals and other molecules promoting oxidation and aggregation

6.2.1.2 Chemical environment.

(1) Formulation, which may promote or hinder particle generation

(2) Excipients

(3) Impurities

6.2.1.3 Physical environment.

(1) Vibration

(2) Mechanical shock

(3) Cavitation

(4) Temperature and humidity

(5) Environment—contamination

(6) Intense light exposure

6.2.2 The count and characteristics of the particles formed as a result of these stresses will vary in general with the duration of the stress and subsequent storage time and conditions.

6.3 *Sources of Intrinsic Particles:*

6.3.1 Intrinsic particles may be formed when materials in contact with drug substance or product are stressed, such as the shedding of particles by pumps used in fill and finish operations. In other cases, the stresses may be minimal, but the materials are not verified to be sufficiently particle free; an example would be the shedding of particles from a filter. As with inherent particles, the creation of particles depends both on the duration of particular stresses and the time of storage.

6.4 Combinations of particular stresses may arise in different process steps during manufacturing operations, including:

6.4.1 Formulation,

6.4.2 Sterilization,

6.4.3 Storage: conditions, time of storage, and choice of container,

6.4.4 Transport,

6.4.5 pH adjustments,

6.4.6 Viral Inactivation Steps,

6.4.7 UF/DF,

6.4.8 Container or closure siliconization, which may promote aggregation of proteins,

6.4.9 Freeze-thaw,

6.4.10 Mixing, and

6.4.11 Fill/Finish.

6.5 Components in the manufacturing process may contribute particles directly (for example, polymer particles shed by a single use system component or other flexible system components), or may contribute to increased particle load indirectly (for example, protein adsorption and subsequent desorption as a particle from a hydrophobic polymer surface). The use of components and filters requires the development of compatibility profiles with the product and solutions to assure leachable substances are not a concern as discussed in USP <1663> and USP <1664>. The therapeutically active drug substance (small or large molecule) would have to be shown not to bind to the filter system as evidence by loss of potency or any indications of API degradation. Process steps may either increase or decrease particle concentrations, or a combination thereof. For example, filtration will remove inherent particles but may introduce intrinsic particles shed from the filtration media or even promote further growth in inherent particles by nucleating interfacial growth of protein aggregates. ISO 8871