Particle Counting in Injectable Solutions

This paper discusses the requirements laid out by US (USP), European (EP), and Japanese (JP) Pharmacopoeia standards and includes the most recent USP <788> (USP 42 2018), EP 5.1 and JP 17 release information. These standards demand that injectable solutions are effectively monitored for microcontamination, specifically non-soluble particulates.

Past notable changes have occurred to all of the three standards, namely:

- USP <788>, updated in April 2007, undertakes a new course of testing.
- EP changed to include SVI products in 2005.
- JP released its 17th edition in 2016.

Injectable Solutions

Pharmaceutical companies are manufacturers of both solid and liquid formulations. Solid formulations are tablets, dry powers, confectionery, and some solid injectables. Liquid formulations, historically known as parenteral solutions, are now described as injectable solutions or injectables. They include opthalmics, ointments, I.V., vaccines, and others. Injectable solutions are packed as Large Volume Injectable (LVI) solutions, Small Volume Injectable (SVI) solutions, and dry powders requiring reconstitution as either LVI or SVI but most commonly as SVI.

LVIs are typically packaged as bags or bottles containing large volumes of intravenous (IV) solutions. Common uses of LVI solutions without additives include: 1) correction of electrolyte and fluid balance disturbances; 2) nutrition; 3) a vehicle for administering other drugs. Large volume parenteral solutions are packaged in containers holding 100 ml or more (≥ 100 ml) and can be packaged in one of the three types of containers: glass bottle with an air vent tube, glass bottle without an air vent tube, or plastic bags.

Small volume parenteral (SVI) solutions are usually less than 100 ml (< 100 ml) and are packaged depending on the intended use. SVIs are typically packed as ampoules, vials, small bags, and pre-filled syringes.

If the solution is a sterile formulation it must be free of all visible particulate material as well as of smaller particles. Particulate material refers to mobile solids unintentionally present in parenteral products. These solids may consist of individual components or mixtures of cellulose, glass, or rubber cores from vials, metal, or plastic fragments. Sterile suspensions may have particulate material but these are usually the active drug or an ingredient, not contaminants.

Potential sources of particulate contamination:

- Manufacturing environment and equipment
- Manufacturing personnel
- Packaging components

History of Control over Particles in Injectables

When we look for the rational as to why are we controlling the limits and number of particles a patient may be exposed to we must ask: "What effect might the particles have?" and "How many can a patient safely be subjected to?"

Primarily, it is important to have control over the limits of these non-soluble particles as they can prove to be deleterious to human health for several reasons, including:

1. Chemical Reaction – The burden of particles are chemically incompatible with the arterial system of the body, essentially poisoning the patient.
2. Pulmonary Aneurism – The particle is of sufficient size that it becomes entrapped in the arterial system, causing a physiological effect.
3. Hypertension – The body rejects the foreign matter and encourages the immune system to work excessively causing secondary effects.

We must gauge how many particles can be deemed to be a safe limit. Two factors are important, the physiological form of the patient and the anticipated tolerances to extraneous particles and the testing limits of the available technology to make such measurements. The physiological form is a huge variable and difficult to realize and may be in excess of the tolerance posed by the instrumentation.

Therefore, let us consider the tolerances on measurement of the injectable solution.

There have been several studies reviewing the testing limits of liquid particle counters [Sizing accuracy of Particle Counters, Fujishita, Sendo, Hisazumi, Otsubo, Aoyama & Oishi. Coincidence Model for Particle Counters, Knapp, Lieberman & Abramson]. These papers looked at defining the maximum concentration allowed by particle counters and anticipated accuracies of measuring devices. The conclusions of these studies were:

- Sizing accuracy should be defined by either a half count value of the voltage threshold (sensor resolution) or by determining the Gaussian distribution of particles by computer program.
- Maximum permitted concentration is a function of Doublet/Triplet particles resident in the laser simultaneously.

The effects of the above are twofold:

- Over-counting and possible false failure of limit testing due to doublet/triplet particles encroaching on upper channel.
- Under-counting and false passing of smaller size channel as multiple particles are counted as single, larger particles.

Limits were defined based upon the original particle counting efficiencies of available technology. USP 22 originally defined the limits for test <788> as:

- 10,000 counts per container @ 10 µm
- 1,000 counts per container @ 25 µm

Following the studies, the limits were reviewed for maximum counting efficiency for optical particle counters and determined that due to false counts and allowing for acceptable errors, the limits for USP 23 (and the current 42) <788> should be:

- 6,000 counts per container @ 10 µm
- 600 counts per container @ 25 µm

Counting Particles to Specifications

The devices used to administer IV products also create potential particle contamination and fall under a different requirement for medical device testing and proof of control. Particle measurements of 50 µm or larger can be detected by visual inspection. To detect particles less than 50 µm an APSS 2000 particle counting system is recommended. International limits apply to the number of particles which can be present in parenteral formulations (USP Test Section <788>).

FIGURE 1 APSS 2000 SAMPLER SYSTEM WITH VALIDATION DOCUMENTATION

The United States Pharmacopoeia (USP) Test Section <788> defines the allowable limits of noninfectious contaminants, i.e., those particulate materials which may be present and, therefore, considered safe for IV administration. Specific limits are set forth for particles above 10 and 25 µm. The USP directive applies to all large volume solutions intended for single-dose infusion that is ready for use from the manufacturer.

The original European Pharmacopoeia (EP) standard 5.0 states in Section 2.9.19 that preparations greater than 100 ml must also be tested to meet defined limits of particulate concentration. These limits are defined in Table 1. This definition created questions about small volume parenterals. SVIs had defined limits in the EP 5.0 for preparation but had no requirement for testing. This was, therefore, done as a function of quality control validation and not because it was specified in EP 5.0. This changed in April 2005 with the release of EP 5.1. The section now requires that "For preparations for human use, solutions for infusion or solutions for injection (must) comply with this test." Therefore, all preparations (LVI and SVI) must meet the EP 5.0 requirement. It also identified that intramuscular and subcutaneous products are allowed higher limits, and that radiopharmaceuticals and those with final filtration are exempt from testing.

Japanese Pharmacopoeia (JP) requires that insoluble particles are tested for either in the finished products or the transportation media is tested independently, but all large and small volume injectables must conform to the test.

	Small Volume Parenterals	Large Volume Parenterals	Dry Powders
US Pharmacopoeia	< 6000 @ 10 µm < 600 @ 25 µm	< 25/ml @ 10 µm < 3/ml @ 25 µm	None
European Pharmacopoeia	< 6000 @ 10 µm < 600 @ 25 µm	< 25/ml @ 10 µm < 3/ml @ 25 µm	< 10,000 @ 10 µm < 1,000 @ 25 µm
Japanese Pharmacopoeia	< 6000 @ 10 µm < 600 @ 25 µm	< 25/ml @ 10 µm < 3/ml @ 25 µm	None

TABLE 1 PHARMACOPOEIA PARTICLE STANDARDS

Apparatus and Methodologies

The Pharmacopoeia tests allow for the determination of the particulate content of LVI samples to be performed by two different methodologies: the "Light Obscuration Particle Count Test" and the "Microscopic Particle Count Test". Not all injection formulations can be analyzed by both these methods; light obscuration is not always applicable to solutions having a different color, viscosity or clarity than water and may give erroneous results. The Microscopic method is unsuitable for solutions which may contain gelatinous constituents that agglomerate on a filter paper. Additional evaluation may be required in these instances to support the release of a product. When a product is unsuitable for testing by Method 1 (light blockage) it is allowable to dilute the sample to achieve either a clarity or viscosity to achieve testing.

The automation of particle counting predominantly is performed by an optical laser particle counter system. Two criteria are defined for the performance of an automated system:

1. Sensor Concentration Limits: the concentration at which the sensor coincidence count rate is 10% at the 10 µm size limit. The APSS 2000 System has a maximum concentration of 10,000 /ml.
2. Sensor Dynamic Range: the dynamic range of the instrument which must include the smallest size to be enumerated. For the USP this is 10 and 25 µm. The APSS 2000 System is typically configured to sample dynamically from 2 to 125 µm, and can be configured for particles as small as 0.1 µm.

FIGURE 2 LIQUILAZ® II LIQUID PARTICLE COUNTER

The LiQuilaz II liquid particle counter in the APSS 2000 system operates on the principle that the light extinguished by a particle in a liquid within a classical laser beam is a direct function of its area. Particles obscure the laser beam during transit through the beam. The pulses produced by electronically detecting the total laser light minus the light obscured by the particle are used to size the particle. These pulses are measured by an analog to digital converter in the sensor. The liquid is presented to the optical system through a rectangular capillary.

The capillary has a window attached to both the front and back sides, which are coated to reduce reflections. The whole system is controlled by a central software application, which controls the hardware, analyzes the data, and stores data for future interpretation. The variation in light caused by the passing of a particle is electronically detected by the photodetector. This signal is then amplified and converted to its digital equivalent. The value of this digital signal is converted into an equivalent particle size in a microprocessor. The different size particles are counted and stored in the microprocessor and made available for transmission to the data display system upon request.

To ensure that the LiQuilaz II particle sensor, the LS- 2000 Sampler, and the SamplerSight-Pharma Control System Software are acceptable and validated to perform the Pharmacopoeia tests with defined acceptance criteria.

These IQ and OQ (Installation and Operational Qualification) tests challenge the following areas:

Sample Volume Accuracy. As the total number of particles is based upon a known volume of sample measured, it is critical that the sample volume is accurate. The LS-2000 Syringe Sampler is available with syringes of 1, 5, 10, and 25 ml and has sample accuracy greater than the required 5%.

Sample Flow Rate. The sample flow rate importance is based upon the speed at which a particle moves through the optical chamber. This determines the duration of the shadow the particle presents to the photodiode. The LiQuilaz II is calibrated at two sample flow rates, 10 and 20 ml/min. The sample flow rate is timed, for a fixed volume, from when the syringe starts moving to the point at which it resets to its origination point.

Running the internal self-calibration program performs the calibration of the APSS-2000 system. The program systematically requests particles sizes of 2, 5, 10, 15, 20, 25, and 30 µm. These particles include the USP specified 10, 15, and 25 µm sizes (EP specifies 10 and 25 µm). The algorithm built into the system micro-controller comes with a Particle Measuring Systems' certificate of conformity. JP17 requires the system meet a specified tolerance for the instrumentation, where as EP and now USP require only that the instrument be "suitably" calibrated. The specification for JP17 is:

Measurement principle	Light Obscuration
Calibration sizes required	5 µm and 10 µm, 25 µm PSL particles
Sample accuracy	5%
Resolution	10%
Sizing accuracy	5%
Sample flow	Within the manufacturer's published range

Once the sizing capabilities have been challenged in the calibration, the counting accuracy is performed during the site PQ (Performance Qualification) procedures. A known concentration of particles is sampled by the system and the results verified as being within < 10% the total count in solution at both 10 and 15 µm.

Taking Measurements

The system is now ready to use and measure the particle count levels in the parenteral sample. The sample measurement is performed by first preparing a sample of known volume of the solution for analysis. This may be a single large volume parenteral, or an agglomeration of smaller vials into one single sample. In either case it is representative of the released product.

The LiQuilaz II particle sensor and LS-2000 liquid sampler are easily configured in the SamplerSight- Pharma software to match the criteria of testing required and a recipe can be defined and saved for future runs of a similar product or batch. The sample is presented to the APSS-2000 system and a magnetic stirrer is used to constantly agitate the sample to ensure that any potential particulate contamination is evenly distributed and does not settle to the bottom of the sample vessel. The syringe is used to draw a known volume of sample for analysis through the LiQuilaz II particle sensor. The particle count data is recorded and a number of measurements taken for the sample. Data can be interpreted as either raw counts, counts per ml, counts per container, a ratio of counts in each channel, or as an average of pooled measurements for a single sample. The average of the results is then assessed by the software for compliance to known standards.

The data is stored in a database managed by SamplerSight-Pharma software and results can be printed immediately or saved for future data interpretation. To comply with 21 CFR Part 11 regulatory guidelines the data files are stored in an encrypted format to ensure that data cannot be modified. Software security features built into the sampling system restrict system operation and data interpretation to authorized system personnel only.

The data can then used to prove the particle matter contamination values for the product. When this data is used in conjunction with additional data from other sources proving sterility, pyrogen-free or otherwise, stability, pH, and osmotic pressure the solution can be released for approval and use.

Conclusion

USP, EP and JP have strict requirements for parenteral sterility. The APSS-2000 system from Particle Measuring Systems clearly meets these standards and provides a simple methodology for users to demonstrate compliance.

Author

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