Subvisible Particulate Matter Testing

Achieving reliable particle count and size analysis of injections, ophthalmic solutions, and lipid emulsions

The United States Pharmacopeia (USP) and other agencies such as EP, JP, and ChP have established test methods to assure the minimization of particle counts in intravenous injections (parenteral drugs) and ophthalmic solutions. Particulate matter is both unwanted contamination and a potential health risk to the patient. Tests are in place for both visible and subvisible particulate matter. Subvisible particulate matter tests include USP <787>,[1] <788>,[2] <789>,[3] and <729>.[4]

USP TEST PROCEDURE SUMMARIES

USP <788>, Particulate Matter in Injections

The USP guidelines are in place for small volume injections (SVI, volume <100 mL) when the monographs specify the requirement and for large volume injections (LVI, volume >100 mL) for single dose infusions unless an exception is noted in the individual monograph. Drugs labeled that a final filter must be used with the product are exempted provided that scientific data supports the exemption. Other exemptions include radiopharmaceutical preparations and parenterals used exclusively for irrigation solutions.

Two Procedures are Specified:

- Method 1 – Light obscuration particle count test
- Method 2 – Microscopic particle count test

Method 1 is the preferred and predominantly used test. Method 2 is used when a sample fails (or comes close to failing) the method 1 test or when the sample material (such as emulsions) may lead to higher counts. This document will only address the method 1 test.

The basic testing procedures and specifications for particle count at 10 and 25 µm are shown in Tables 1 and 2 for both small and large volume injections. The SVI test report results in particles/container while the LVI test report results in particles/mL.

Table 1. SVI test and specifications

Open/combine 10 or more units into a cleaned container for volume NLT 25 mL
Degas (let stand, ultrasonic bath, or vacuum)
Sample 4 times, disregard first, average last three
Count ≥10 and 25 µm
Pass/fail criteria: 6000/container ≥10 µm 600/container ≥25 µm

Table 2. LVI test and specifications

Fewer than 10 units acceptable with appropriate sampling plan
Test individual units
Sample four times, disregard first, average last three
Count ≥10 and 25 µm
Pass/fail criteria: 25/mL ≥10 µm 3/mL ≥25 µm

The AccuSizer® SIS system (Figure 1) is the ideal instrument for performing USP <788> testing.

Figure 1. AccuSizer SIS system.

Measurements and reporting are fully automated in the AccuSizer software. Protocols define sample volume (typically 5 mL), number of analyses (typically four), number of containers pooled and volume/container. The software then calculates the average values for runs 2 – 4 and reports the results including the pass/fail determination as shown in Figure 2.

Sample	Run Date/Time	Containers (#)	Container Vol. (#)	Sample Vol. (mL)	≥ 10 um (#)	≥ 10 um (#/Cont.)	≥ 25 um (#)	≥ 25 um (#/Cont.)
SVIT3 Rep. 2	14:01 02/13/2019	10	25.0	5.0	56	280	2	10
SVIT3 Rep. 3	14:02 02/13/2019	10	25.0	5.0	49	245	2	10
SVIT3 Rep. 4	14:03 02/13/2019	10	25.0	5.0	55	275	3	15
				Mean	53.3	266.6	2.3	11.6

TEST Criteria	RESULT
(Mean #/Container ≥ 10 um) ≤ 6000/Container AND (Mean #/Container ≥ 25 um) ≤ 600/Container (PASS)	PASS

Figure 2. USP <788> report.

USP <787>, Subvisible Particulate Matter in Therapeutic Protein Injections

USP <787> is an alternative for USP <788>, making changes for smaller test product volumes, smaller test aliquots, and additional sample handling instructions. A summary of how to test a sample following the new USP <787> procedure and the pass/fail criteria is shown in Table 3.

Table 3. USP <787> test and specifications

Dilution may be necessary and is allowed, but have supporting data for the rationale and suitability of the selected scheme
Sample preparation
If there is enough volume test individual units
If volume is too small, mix units and combine the contents to obtain the required volume (typically 0.2 – 5.0 mL)
Degas the sample and gently mix again
Pass/fail criteria: **SVI** 6000/container ≥10 µm 600/container ≥25 µm **LVI** 25/mL ≥10 µm 3/mL ≥25 µm

Sample programming, execution, and reporting are all automated by the AccuSizer software as described in the above description of USP <788>.

USP <789>, PARTICULATE MATTER IN OPHTHALMIC SOLUTIONS

This test is different than the previous three because the particles being measured are the drug product (an emulsion), not contamination. The critical size characteristics of lipid injectable emulsions include the mean droplet size and the large diameter tail >5 µm. No single technique or test can adequately measure both parameters, so two methods exist in USP <729>:

Method I — Light Scattering Method

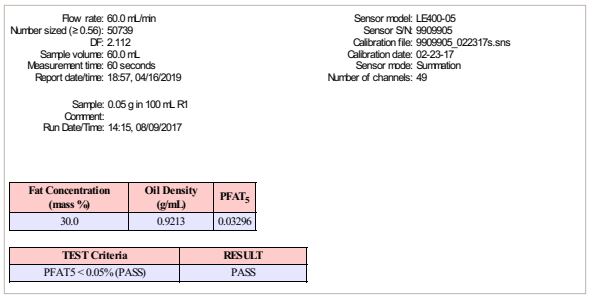
Either dynamic light scattering (DLS) or laser diffraction (referred to as classic light scattering in the method) is used to measure the mean size. The basic testing procedures and specifications for the mean droplet size are shown in Table 5.

Table 5. USP <729> method I test and specifications

Verify system performance with standards at 100, 250, and 400 nm
Dilute the sample to an appropriate concentration
Measure the size with the detector at an angle of 90°
Check that the Chi Square value is acceptably low
Report the intensity mean diameter
Pass/fail criteria: Mean <500 nm (0.5 µm)

The Nicomp® DLS system (Figure 3) is the ideal system to use for method I testing to determine the mean droplet size. The Chi Square calculation is automated in every measurement and the Nicomp multi-modal analysis algorithm is available for samples with multiple peaks. The unique autodilution option automates measurement of high concentration emulsions.

Figure 3. Nicomp DLS system.



Flow rate: 60.0 mL/min
Number sized (≥ 0.56): 50739
DF: 2.112
Sample volume: 60.0 mL
Measurement time: 60 seconds
Report date/time: 18:57, 04/16/2019

Sample: 0.05 g in 100 mL R1
Comment:
Run Date/Time: 14:15, 08/09/2017

Sensor model: LE400-05
Sensor S/N: 9909905
Calibration file: 9909905_022317s.sns
Calibration date: 02-23-17
Sensor mode: Summation
Number of channels: 49

Fat Concentration (mass %)	Oil Density (g/mL)	$PFAT_5$
30.0	0.9213	0.03296

TEST Criteria	RESULT
PFAT5 < 0.05% (PASS)	PASS

Figure 5. USP <729> method II result.

Method II — Light obscuration method

The large diameter droplet tails (PFAT5) is measured using a light obscuration/extinction liquid particle counter that employs the single particle optical sizing (SPOS) technique. The basic testing procedures and specifications for the mean droplet size are shown in Table 6.

Table 6. USP <729> method II test and specifications

Check system performance using two different size standards of ~ 5 and 10 µm
Dilute the sample
Set the lower size limit at 1.8 µm and upper limit at 50 µm
Make two measurements varying the concentration or measurement time so that there is at least a factor of two difference in total number of particles >5 µm between the two runs
Pass/fail criteria: The volume-weighted result >5 µm (PFAT5) must be < 0.05%.

The ideal instrument for making method II measurements is the AccuSizer APS system (Figure 4). The AccuSizer APS system provides automatic dilution, measurements, and USP <729> method II result calculations and reporting (Figure 5).

Figure 4. AccuSizer APS system.

INSTRUMENT GUIDANCE IN USP <1788>

The USP <1788> document,[5] Methods for the Determination of Particulate Matter in Injections and Ophthalmic Solutions provides important standardization and calibration information, recommendations for sample handling, laboratory environment, and operator training. These guidelines can be applied to all light obscuration testing for subvisible particles in injections.

Test Apparatus

The instrument used shall be "The apparatus is a liquid-borne particle counting system that uses a light-obscuration sensor with a suitable sample feeding device to deliver controlled aliquots of sample for analysis."[5] The AccuSizer instrument utilizes a "light obscuration sensor", but it is worth noting that the LE400 sensor is more advanced than required by this USP chapter. Figure 6 shows a diagram of the LE400 sensor and associated electronics used to perform the measurement.

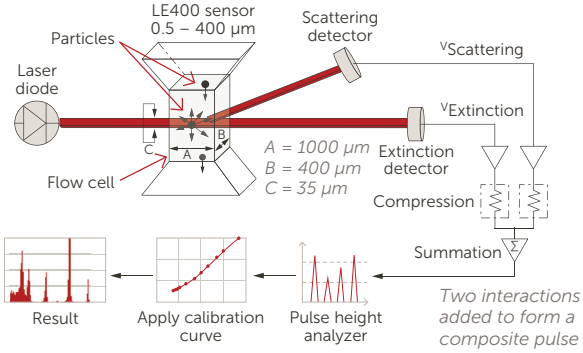


Figure 6. LE400 sensor and counter schematic.

The extinction detector in direct alignment with the incident laser beam performs the light obscuration component of the measurement. The additional scattering detector at the two o'clock position is used to extend the dynamic range down to 0.5 µm by also collecting scattered light from the laser/particle interaction. The AccuSizer instrument can be operated in extinction (obscuration) mode using only the extinction detector or summation mode using both detectors. At the 10 and 25 µm sizes used in these USP tests all measurements are light obscuration measurements since the scattering signal is insignificant at these sizes.

The LE400 sensor has the highest sensitivity specification (0.5 µm) of any system used for USP subvisible particle testing. Although the pass/fail criteria are set at 10 and 25 µm there are significant benefits to being able to detect down into the sub-micron range. If counts are always near zero at 10 µm, having data in smaller size ranges can provide the ability to differentiate between batches that contain more or less total particles. More or less particles at smaller sizes at the time of manufacture could indicate which batches may maintain low counts over the course of the shelf life of the product. Additional data down to 0.5 µm is also valuable both for formulation and USP <787> testing of protein injections by providing insight into protein aggregation. USP guidance documents for protein injections[6] suggest collecting data in smaller size ranges, including down to 0.2 µm – possible when using the FX Nano sensor.

Sensor Concentration Limits and Dynamic Range

All measurements should be performed below the concentration limit of the sensor to avoid coincidence errors (two particles at the same time being present in the sensing zone). The concentration limit of the LE400 sensor is 9,000 – 10,000 particles/mL depending on the beam height of the laser. But this range is unimportant when test results at 10 µm must always be below around 50 particles/mL. When working at higher concentrations for USP <729> testing (or high concentration protein injections) the AccuSizer APS system can accurately and automatically dilute up to 1 million-to-one dilution ratios, and provide results calculated in actual sample particles/mL concentration.

INSTRUMENT STANDARDIZATION TESTS

Sample Volume Accuracy and Flow Rate

These two tests are easily performed with the use of a balance and stopwatch.

Calibration

The procedure requires the sensor be calibrated at a minimum of three sizes, often at 10, 15, and 25 µm. The automated and electronic calibration methods described in USP <1788> are antiquated compared to the AccuSizer software capabilities. The AccuSizer system has advanced calibration routines that assure optimized calibration, resolution, and count standard testing. All LE400 sensors are calibrated using at least twelve sizes/curve points (not a mere three) at the factory before delivery. The same detailed calibration procedure is performed in the field during the biannual verification visit. The AccuSizer software automatically tracks which calibration curve was used for each measurement, when the last calibration was performed, and sensor detector voltages during the measurement. All of this additional data and tracking assures the highest level of data confidence.

Sensor Resolution

The sensor resolution is tested to assure that the sensor does not add significant error to the measurement. The test is performed by calculating the increase in the standard deviation (st dev) of 10 µm polystyrene latex (PSL) particles as defined in Figure 7. The calculated resolution must be less than 10%.

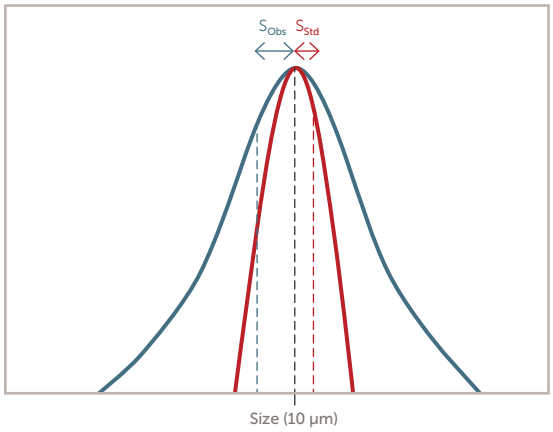
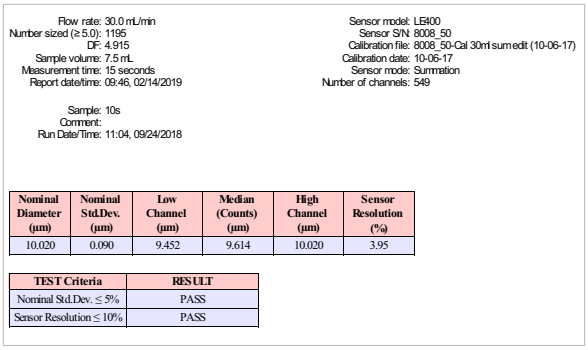


Figure 7. Sensor resolution definition.

The AccuSizer automates the sensor resolution calculations and reports the test results as shown in Figure 8. Having 1024 size channels improves the ability to accurately calculate the sensor resolution. The 10 µm PSL only needs to be analyzed once. The same measurement used to determine the calibration point is also used to calculate sensor resolution.



Flow rate: 30.0 mL/min
Number sized (≥ 5.0): 1195
DF: 4.915
Sample volume: 7.5 mL
Measurement time: 15 seconds
Report date/time: 09:46, 02/14/2019

Sensor model: LE400
Sensor S/N: 8008_50
Calibration file: 8008_50-Cal 30ml sum edit (10-06-17)
Calibration date: 10-06-17
Sensor mode: Summation
Number of channels: 549

Sample: 10s
Comment:
Run Date/Time: 11:04, 09/24/2018

Nominal Diameter (µm)	Nominal Std.Dev. (µm)	Low Channel (µm)	Median (Counts) (µm)	High Channel (µm)	Sensor Resolution (%)
10.020	0.090	9.452	9.614	10.020	3.95

TEST Criteria	RESULT
Nominal Std.Dev. ≤ 5%	PASS
Sensor Resolution ≤ 10%	PASS

Figure 8. Sensor resolution report.

Particle Counting Accuracy

The counting accuracy of the instrument used is tested using a particle count standard available from the USP and other sources.[7,8] The count standards are PSL particles with a mean size of 15 µm. Table 7 lists the basic testing procedure for the count standard test.

Table 7. USP count test procedure

Measure the blank sample at 10 and 15 µm three times
Measure the count standard at 10 and 15 µm three times
Discard the first result, average the second and third
Calculate the average counts at 10 and 15 µm
Subtract the blank counts from the standard counts
Compare the adjusted counts at 10 µm to the expected values
Compare the counts at 10 µm/counts at 15 µm to the expected values

The expected values are provided on the certificate of analysis provided with the count standard. A typical value from a previous lot provided these expected results:

- Counts at 10 µm:
  between 3330 and 4110 counts/mL
- Ratio of counts at 10 µm to the counts at 15 µm:
  between 1.78 and 2.57

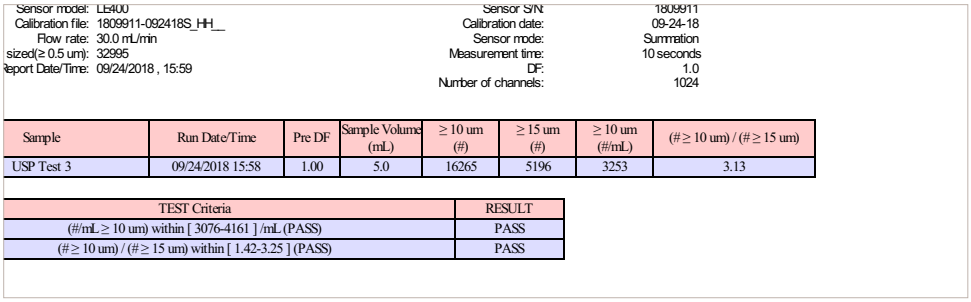
This particular test has caused the most anxiety for users and service engineers due to the expense and limited volume in the USP count standard bottles. The AccuSizer SIS system is ideal for performing the USP count standard test for several reasons:

- The testing, calculation, and reporting is automated in the AccuSizer software (Figure 9)
- The 15 µm calibration curve point can be optimized and adjusted before and after analyzing the count standard
- This way one single set of measurements will always produce acceptable results

A unique feature in the AccuSizer software is the ability to re-analyze data using an updated calibration curve. Most USP count standard failures come from the ratio calculation. A small error in the 15 µm calibration curve point can create a large shift in the ratio calculation. If the test fails the ratio calculation a 15 µm PSL standard is used to ascertain if the calibration point is accurate or requires adjustment. Once this adjustment is made the original count standard data can be recalculated using the new calibration curve.

Test Environment and Blank Testing

Both USP <788> and <1788> discuss how to perform blank measurement to assure the test is carried out in conditions (such as a laminar flow hood) limiting additional particulate matter. The basic testing procedures and specifications for the blank test are shown in Table 8.



Sensor model: LE400
Calibration file: 1809911-092418S_HH__
Flow rate: 30.0 mL/min
sized(≥ 0.5 um): 32995
Report Date/Time: 09/24/2018 , 15:59

Sensor S/N: 1809911
Calibration date: 09-24-18
Sensor mode: Summation
Measurement time: 10 seconds
DF: 1.0
Number of channels: 1024

Sample	Run Date/Time	Pre DF	Sample Volume (mL)	≥ 10 um (#)	≥ 15 um (#)	≥ 10 um (#/mL)	(# ≥ 10 um) / (# ≥ 15 um)
USP Test 3	09/24/2018 15:58	1.00	5.0	16265	5196	3253	3.13

TEST Criteria	RESULT
(#/mL ≥ 10 um) within [3076-4161] /mL (PASS)	PASS
(# ≥ 10 um) / (# ≥ 15 um) within [1.42-3.25] (PASS)	PASS

Figure 9. USP count standard report.

Table 8. Blank test and specifications

Use a cleaned vessel representative of what will be used for the other tests
Fill the vessel with 50 mL filtered water, swirl to mix
Degas the sample by sitting, ultrasonic bath, or vacuum
Measure five samples of 5 mL each
Pass/fail criteria: Total number of particles in 25 mL ≥10 µm must be less than 25 (1 particle/mL average)

Note: For USP <789> testing the number of particles ≥25 µm must not exceed three.

AUTOMATION

AccuSizer systems provide the highest level of calibration, validation, measurement, and reporting automation as already discussed in this document. In addition, the entire measurement sequence can be automated for high throughput sample analysis with the use of two optional autosampler solutions.

The AccuSizer Autosampler (Figure 10) can be used to perform USP <788> or <789> testing on multiple samples – up to 24 for 30 mL sample vials. Greater sample numbers are possible for smaller sample volumes. Sophisticated protocols for the measurement and autosampler functions can mimic manual measurements but provide complete automation after the sample tray is loaded.

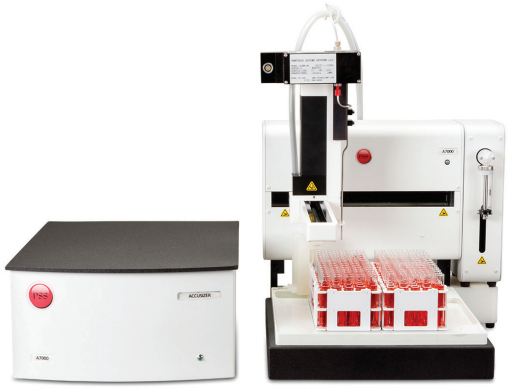


Figure 10. AccuSizer Autosampler.

The AccuSizer A2000 MPA Micro Plate Analyzer (Figure 11) can be used for USP <787> testing on smaller sample volumes as low as 180 µL. One or two 96 well micro plates (well volume = 1 mL) are loaded onto the system, measurement and auto-sampler protocols are defined and then all analyses are completely automated.

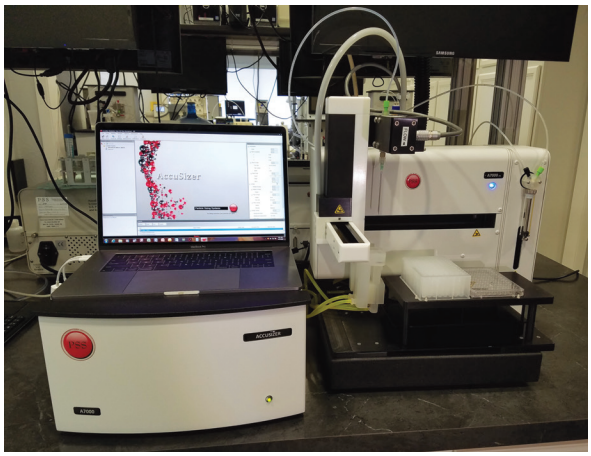


Figure 11. AccuSizer A2000MPA Micro Plate Analyzer.

CONCLUSIONS

Entegris AccuSizer and Nicomp systems are uniquely capable solutions to subvisible particle testing in injections, ophthalmic solutions, and lipid emulsions. Combining the highest specification technology with advanced software and automation options make these the most advanced instruments available for USP particle testing.

ABOUT ENTEGRIS

Entegris is a leader in specialty chemicals and advanced materials solutions for the microelectronics industry and other high-tech industries. Entegris is ISO 9001 certified and has manufacturing, customer service, and/or research facilities in the United States, China, France, Germany, Israel, Japan, Malaysia, Singapore, South Korea, and Taiwan. Additional information can be found at www.entegris.com

References

[1] *USP <787> subvisible particulate matter in therapeutic protein injections*

[2] *USP <788> particulate matter in injections*

[3] *USP <789> particulate matter in ophthalmic solutions*

[4] *USP <729> globule size distribution in lipid injectable emulsions*

[5] *USP <1788> methods for the determination of particulate matter in injections and ophthalmic solutions*

[6] *Guidance for Industry, Immunogenicity Assessment for Therapeutic Protein Products,* August 2014, see https://www.fda.gov/drugs/guidance-compliance-regulatory-information/guidances-drugs

[7] *USP particle count set,* catalog # 1500502, www.usp.org/reference-standards

[8] *Fisher Scientific PHARM-TROL count standards,* www.fishersci.com/shop/products/pharm-trol-count-precision-size-standards/p-4530446

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