USP <787> Testing Therapeutic Protein Injections

AccuSizer® Syringe Injection System (SIS)

Figure 1. AccuSizer SIS system.

OVERVIEW

The USP <787> Subvisible Particulate Matter in Therapeutic Protein Injections[1] test is very similar to USP <788>[2] for parenteral drugs. The standard AccuSizer SIS meets and/or exceeds all requirements in USP <787>, but new improvements have been added to accommodate specific requirements of this new sample type. Lower sample volume and the ability to dilute samples are the major differences. The Entegris AccuSizer SIS system is uniquely qualified to generate the best possible particle size and count data for USP <787> testing.

INTRODUCTION

USP <787> is meant for therapeutic protein injections, making changes for smaller test product volumes and smaller test aliquots. Here is a summary of how to test a sample following the new USP <787> procedure:

Follow the same standardization steps as described in USP <1788>:[3]

- Volume accuracy
- Flow rate accuracy
- Calibration
- Sensor resolution
- Count accuracy (optional)

SAMPLE PREPARATION

- Test individual units If there is enough volume
- If the 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

ANALYSIS

- Analyze four aliquots
- Count the particles in the size range of interest, including >10 and 25 µm
- Disregard the first result and average the next three results

PASS/FAIL CRITERIA

- If the container volume <100 mL; less than 6000 particles/container >10 µm and and less than 600 particles/container >25 µm.
- If the container volume >100 mL; less than 25 particles/mL >10 µm and 3 particles/mL >25 µm. Also not to exceed the per container limits for the <100 mL criteria above.

Many of these system preparation and measurement steps are identical to USP <788>. The standard AccuSizer SIS system (Figure 1) is perfectly suited for performing these tests. One important difference is the acknowledgement that sample volumes may be as small as 0.2 mL (200 µL). The SIS sampler can accurately measure samples as small 150 µL, making this uniquely suited for analysis of small volume protein samples. The LE400 sensor has the widest dynamic range available (0.5 – 400 µm), extending the range into the submicron region.

LIGHT BLOCKAGE AND SINGLE PARTICLE OPTICAL SIZING (SPOS)

Both USP <788> and <787> describe testing subvisible particles using a light blockage/obscuration instrument – "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."[2] The Entegris AccuSizer employing the model LE400 sensor (see Figure 2) is based on the principle of single particle optical sizing (SPOS).

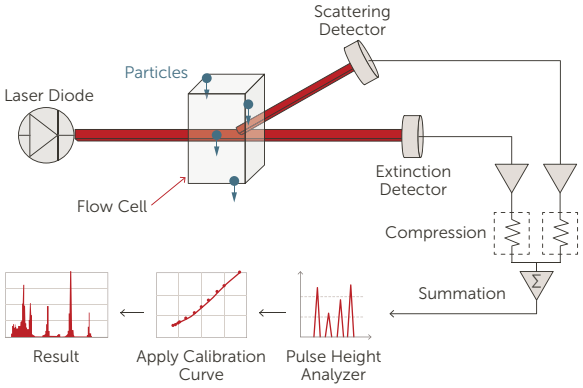

Figure 2. LE400 sensor and AccuSizer schematic.

This sensor contains both an extinction (also called blockage or obscuration) and a scattering detector. Light blockage sensors and systems can only detect down to around 1.5 µm. The scattering detector was added to extend the dynamic range down to 0.5 µm. The AccuSizer can operate in two modes of operation – extinction and summation. In extinction mode only the extinction detector is used. In summation mode both the extinction and scattering detectors are used. All AccuSizer systems are delivered with two calibration curves – extinction and summation. Customers can choose between operating in extinction or summation mode in the protocol window shown in Figure 4. Even when operating in summation mode, at 10 and 25 µm the AccuSizer is measuring in the light blockage region. Therefore, either mode of operation is appropriate for USP 787 testing.

SUBMICRON PARTICLE COUNTING IN PROTEIN INJECTIONS

In August 2014 the FDA issued a Guidance for Industry document *Immunogenicity Assessment for Therapeutic Protein Products*.[4] Within this document the FDA comments that "it has been recognized that subvisible particulates in the size range of 0.1 – 10 microns have a strong potential to be immunogenic, but are not precisely monitored by currently employed technologies... As more methods become available, sponsors should strive to characterize particles in smaller (0.1 – 2 microns) size ranges." The desire to measure at these smaller sizes is driven not only by FDA suggestion, but also by common understanding that investigating protein aggregation phenomenon requires measuring well below the 10 and 25 µm sizes required for USP <787> testing.

Standard light obscuration sensors operate between approximately 1.2 – 150 µm. This covers the most important sizes of 10 and 25 µm but has no submicron detection capability. The Entegris LE400 sensor has a dynamic range of 0.5 – 400 µm, exceeding all USP 787 requirements and extending the range into the submicron region. This extended range facilitates quantifying protein aggregation in the submicron region.

SUBMICRON TESTING: EXPERIMENTAL

NIST reference material 8671[5] is an IgG monoclonal antibody with a mean diameter of 9.96 nm as measured by dynamic light scattering. This RM is intended primarily for use in evaluating the performance of methods for determining physicochemical and biophysical attributes of monoclonal antibodies. The RM is packaged and supplied to the user in internal threaded polypropylene cryovials. Each vial contains 800 µL of 10 mg/mL IgG1κ monoclonal antibody. The RM is shipped on dry ice to remain frozen during shipment. The material was stored frozen immediately upon receipt and then thawed at room temperature for two hours before making any measurements.

A vial of NIST 8671 was thawed and analyzed on the AccuSizer SIS liquid particle counter. The sample was then heat stressed at 60°C for four hours and tested again. The sample was re-frozen and stored for several months, thawed, and tested again. The sample volume for all measurements was 250 µL at a flow rate of 15 mL/min. The results are shown in Figure 3.

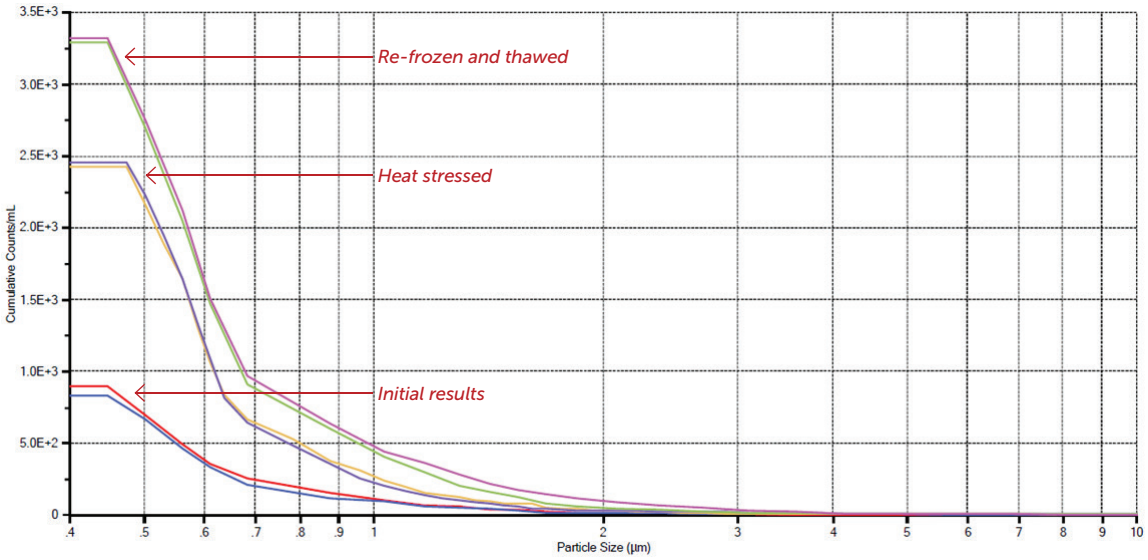

Figure 3. NIST 8671 tested at different conditions.

The submicron particle count/mL results track the increase as a function of sample stress as expected for protein aggregation behavior. This additional valuable information can be generated while performing standard USP <787> testing. Notice there is no obvious increase in concentration in the 10 and 25 µm size ranges and all samples passed the USP <787> pass/fail criteria detailed above.

LOW VOLUME SAMPLE ACCURACY

USP <787> allows for sample volumes as low as 150 – 200 µL, helping to preserve valuable sample. This becomes critical in the R&D and stability testing phases. Different sample syringe volumes can be used in the AccuSizer SIS system. A 10 mL syringe is standard when measuring the typical 5 mL volume for routine USP <788> measurements. A 1 mL syringe volume is used for USP <787> testing when customers wish to minimize sample volume analyzed. Additional protocol settings (Figure 3) available in only the Entegris AccuSizer software optimize the measurement of low sample volumes.

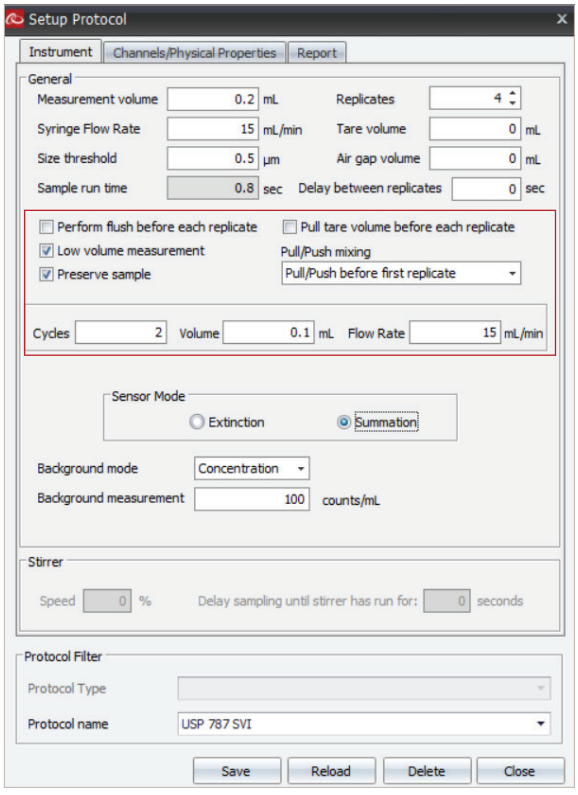

Figure 4. Protocol for USP <787> testing.

Unique protocol settings include:

Air gap volume: Introduces an air gap between the flush liquid and sample to avoid cross contamination. In Figure 4 the air gap is set to zero.

Low volume measurement: Changes the minimum run time requirement and the screen view during data collection to avoid confusion.

Preserve sample: Pushes the sample back into the original sample container instead of to drain. In Figure 4 the system will pull the sample through the sensor for measurement, then push the sample back into the original vessel.

Pull/Push mixing: Uses the syringe to pull and push a small sample volume to mix the sample rather than use stir bars. In Figure 4 the system will push and pull 100 µL twice at a flow rate of 15 mL/min.

LOW VOLUME TESTING: EXPERIMENTAL

The sample used to perform these tests was a 15 µm particle count standard from Micro Measurement Labs, Inc., lot #NK20C. The reference count value for this standard is 3,118 – 4,218 particles/mL >10 µm. All measurements were made on the AccuSizer SIS system equipped with the LE-400 sensor, calibrated and used at a flow rate of 15 mL/min. A 1 mL syringe was installed onto the SIS sampler. The measurement procedure used is described below:

- Flushes of 0.5 mL were performed before and after sampling (an air gap took place after the "before" flush, but before the sampling).
- Fresh 900 uL aliquots were used for each sample, regardless of sampling required.
- An air gap of 0.05 mL was used in each run prior to sampling.
- A tare volume of 0.15 mL was used for each measurement.

Measurements were performed at the following sample volumes: 650, 550, 450, 350, 250, 150 and 50 µL. All measurements were performed in triplicate. The results from the experiments are shown in Figure 5.

Volume	Counts	Counts/mL	Counts average	Average/mL	Std dev	Std. Dev./mL	%RSD
650 µL	2418	3720					
650 µL	2326	3578					
650 µL	2328	3582	2357	3627	53	81	2.2%
550 µL	2016	3665					
550 µL	1947	3540					
550 µL	2050	3727	2004	3644	52	95	2.6%
450 µL	1720	3822					
450 µL	1657	3682					
450 µL	1678	3729	1685	3744	32	71	1.9%
350 µL	1283	3666					
350 µL	1256	3589					

Volume	Counts	Counts/mL	Counts average	Average/mL	Std dev	Std. Dev./mL	%RSD
350 µL	1256	3589	1265	3614	16	45	1.2%
250 µL	907	3628					
250 µL	928	3712					
250 µL	961	3844	932	3728	27	109	2.9%
150 µL	577	3847					
150 µL	580	3867					
150 µL	592	3947	583	3887	8	53	1.4%
50 µL	179	3580					
50 µL	180	3600					
50 µL	187	3740	182	3640	4	87	2.4%

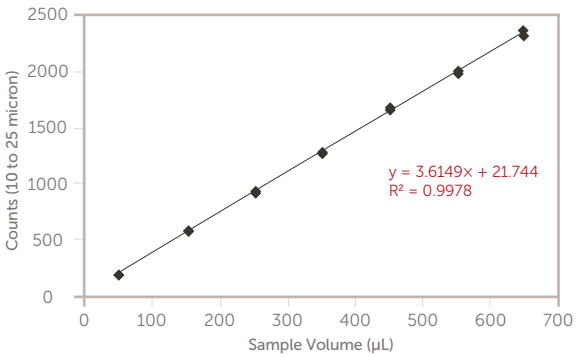

Figure 5. Particle count data at varying sample volumes.

These studies prove that the AccuSizer SIS system can generate accurate size and count data at 10 µm at very low sample volumes. Although the data was accurate down to 50 µL, we suggest using slightly larger sample volumes whenever possible to make the measurement easier on the operator.

SAMPLE DILUTION AUTO-CALCULATION

USP <787> does allow for dilution of samples under certain circumstances, particularly when dealing with high-concentration or high-viscosity protein formulations that may cause issues with testing.

Dilution must be carefully considered and validated to ensure it doesn't introduce artifacts or alter the true particle count.

The AccuSizer software allows for the entry of a dilution factor so that the final calculations are based on actual concentrations in the sample. The operator simply enters the predilution factor (PreDF) in the Run Sample window shown below in Figure 6.

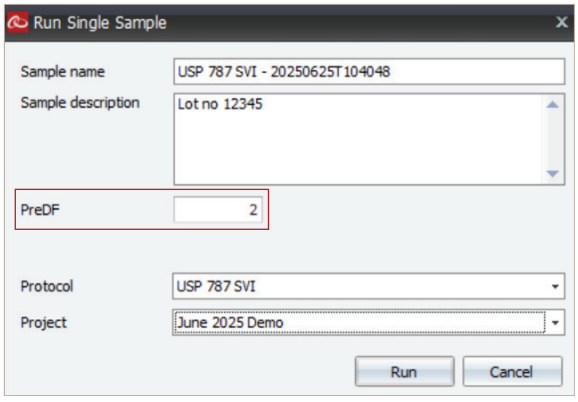

Figure 6. Run sample window.

USP <787> COMPLIANCE

The AccuSizer SIS meets and/or exceeds all requirements as defined in USP <787>, <788>, and <1788> as shown below:

Requirements	Specifications
Based on the principle of light blockage	Both light blockage and single particle optical sizing (SPOS)
Standardization steps such as sample volume accuracy, sample flow rate, sensor resolution, calibration, and particle count accuracy.	All tests performed during IQ/OQ or annual validation visit. Automated reports for sensor resolution and particle count accuracy.
Sample volume accuracy at least 5%	Within 5% of appropriate sample volume
Sensor resolution NMT 10%	Sensor resolution <10%
Calibration by minimum of three NIST-traceable particle size standards or equivalent	Calibrated using 10 – 13 NIST traceable PSL standards
Report particles/mL >10 and 25 µm, and pass/fail results	Automated reports ignore 1st test, average tests 2 – 4, shows average particles/mL >10 and 25 µm plus pass/fail for either SVP or LVP limits.
The dilution factor must be accounted for in the calculation of the final test result.	Automated dilution calculations

ADDITIONAL FEATURES

- Autosampler for high throughput requirements
- 21 CFR part 11 compliant software
- Software validation
- 1024 size channels
- Custom reports can be created by HTML
- Run time data plots to review count rate and sensor voltages
- Protocol import/export utility
- Project import/export utility
- Database backup and export utilities
- Sample explorer search utility
- Calibration maintenance utility to track changes

References

[1] *USP <787> Subvisible Particulate Matter in Therapeutic Protein Injections*

[2] *USP <788> Particulate Matter in Injections*

[3] *USP <1788> Methods for the Determination of Particulate Matter in Injections and Ophthalmic Solutions*

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

[5] *NIST Monoclonal Antibody Reference Material 8671*, available at: https://www.nist.gov/programs-projects/nist-monoclonal-antibody-reference-material-8671

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