AAPS Interlaboratory Study

Tier 2 Method: Resonant Mass Measurement (RMM)

Introduction

This protocol describes Resonant Mass Measurement (RMM) analysis of subvisible and sub-micron particles present in mAb protein solutions used in this study. The primary reported results are the particle size distribution of particles in the size range between 300 nm to 4 µm. This document briefly describes sample handling, instrument set up, and data reporting of the results associated with RMM analysis.

Important Notes: This protocol is not a Standard Operating Protocol and assumes the required instrument is in good working order and the analysis is performed by experienced user(s). Required calibration or check standards should be run according to the manufacturer instructions. This protocol does not contain all the details of the analysis. The analyst should rely on their best judgment, routine practices, and knowledge of the technique to conduct the study; this protocol should be used as a guideline for the analysis. Samples should be analyzed immediately after thawing and preparation.

These samples are time sensitive with regards to particles but depending on the time requirement, analyst, and instrument availability, it might be possible to do some measurements simultaneously (i.e. run the samples prepared at the same time on light obscuration and MFI/ FlowCam simultaneously). Please coordinate if it is a general practice to do these measurements concurrently.

Equipment and Materials List

- RMM instrument and Hi Q Micro Sensor and associated consumables (please specify software etc.)
- Protein samples and formulation buffers supplied by sample originators
    - Buffers, aliquots of unstressed, stir stressed, light stressed from Shipping #1
    - Stir-stressed samples from Shipping #2
- Standard control sample- 1 µm polystyrene latex beads
- Particle free/de-ionized ultrafiltered water
- Disposable 1 mL silicone-free syringes
- Microcentrifuge tubes
- 0.02 µm filters, syringes, needles for filtering buffers
- Detergents or cleaning solutions for cleaning between runs

Clarification added Sept 20th regarding filters for RMM:
Preferable to use 0.02 µm filters to filter buffer if using it to prepare the sample.

Reagents and Solutions

Table 1: The following samples and their buffers will be provided by sample originators. Buffers, aliquots of unstressed, stir stressed and light stressed from Shipping #1 and stir-stressed samples from Shipping #2 should be used for this analysis. All stressed material were generated at nominally 1 mg/mL.

Proteins	Formulations	Samples
Amgen IgG2	10 mM sodium acetate, pH 5.0	Unstressed, 1 mg/mL Stir stressed, 1 mg/mL Light stressed, 1 mg/mL
NISTmAb IgG1	12.5 mM L-histidine, 12.5 mMol L-histidine HCl, pH 6.0	Unstressed, 1 mg/mL Stir stressed, 1 mg/mL Light stressed, 1 mg/mL

Procedures

Instrument setup

1. Follow routine setup procedures provided by the manufacturer. Prepare reference solutions and perform sensor calibration according to manufacturer instructions. Use the most recent software version available for collecting and analyzing data. Cleaning solutions and buffer blanks should be run to ensure the instrument is free of particles. Confirm the accuracy of the instrument by analyzing 1 µm polystyrene latex beads.

Sample preparation

1. Thaw the samples from Shipping #1 and aliquot the Tier 2 vials (for the unstressed, stir, and light stressed mAb samples) as described in the instructions into smaller aliquots for the various methods and freeze until ready to use. Be sure to follow the procedure for vigorously mixing the sample prior to aliquoting.

2. For this analysis, thaw the aliquots designated for RMM measurements (prepared and frozen in #1) by bringing them to room temperature for no more than 15 minutes. Thaw 1 vial each of the Amgen mAb and NISTmAb pre-diluted stir stressed samples from Shipping #2 by bringing them to room temperature for 30 minutes.

3. **These samples are time sensitive with respect to particle concentration.** After thawing, particle concentrations may change with time, even if the sample is maintained at (2-6)°C. This change may be mitigated by analyzing the samples immediately (within 2 hours) of thawing. It is recommended to perform all control measurements before the protein samples are fully thawed.

4. A proper mixing of the samples prior to analyzing is important to eliminate particle clumping. Prior to dilution or analysis, mix the aliquot in the following manner: pipet each sample with a 200 uL tip near the bottom of the sample tube 10 times in different directions but not touching the bottom or creating bubbles. Remove sample from near

the bottom of the vial, from about ¾ of the depth of the tube from the top of the liquid before dispensing the sample into another container containing the buffer.

5. Filter the formulation buffers with a 0.02 µm filters before making any dilutions.
6. Under laminar flow hoods, prepare 3 mL of each sample into standard PP-tubes for RMM analysis by diluting the samples 100-fold into the diluent provided for each protein. Take care to avoid formation of air bubbles and prevent evaporation of the sample by closing the tubes. The pre-diluted stir stressed samples do not need to be diluted.

7. It may be necessary to centrifuge and/or perform subsequent dilutions (if any) for some of the samples. Centrifugation is important as it will prevent clogging of the orifice with large particles. The "supernatant" of the centrifuged sample should be carefully separated from the vial and should be used for analysis. Here, we define the "supernatant" as 70% of the filling volume.

Sample measurement

1. Review the **RMM Data** reporting template for the requested information before beginning the measurements.

2. Determine the LOD for the measurements. Ensure the same LOD is used for each sample replicates. LODs may be slightly different for each sample. Measure each sample in triplicates (n = 3), by loading a fresh sample aliquot for every replicate. Between each sample type, perform any specific cleaning operations and run cleaning/detergent solutions to ensure there is no clogging between runs. Once the sensor appears clean, insert the next sample for analysis. For each analysis, note the percent coincidence, which represents the frequency with which two particles pass the sensor at the same time and are counted as one.

3. Automatically stop the run when either 1000 particles have been counted or the run has been going on for 30 min, whichever comes first. Record all instrument parameters and data into the **RMM Data** reporting template. Adjust the final counts or concentrations based on the dilution factor.

4. User may allow the software to automatically recalculate the buffer density and viscosity over the course of the run.

Clarification added Sept 20th regarding RMM:

- Load sample and can do 3 replicates of the same sample with 3 new aliquots. Wash between sample types.
- Enable the automatic recalculation of the density and viscosity after acquisition

Table 2. Recommended Instrument Parameters

Coincidence	< 10 %
Aliquot	500 µL
Particle limit/Run duration	1000 particles or 30 min
Particle Density	1.34 g/cm^3
Solution Density	1.0 g/cm^3
Replicates	3 (fresh aliquots) measurements

Understanding Results

Results from RMM should inform us about the particle size distribution of proteinaceous particles spanning the size range of 300 nm to 4 µm in each of the stressed and unstressed mAb samples.

Instrument biases can depend strongly on the instrument configuration. Document instrument type, model, and configuration and settings. Export the data as recommended by the instrument manufacturer.

Record instrument parameters and the final particle size distribution data into **RMM Data** Excel sheet. Only the negatively buoyant particles will be included in the analysis. Any dilutions made to the samples during analysis should be accounted for in the final Excel sheet.

Troubleshooting

Reference: M. Hubert at el/ Journal of Pharmaceutical Sciences 109 (2020): 830-844

Particles adhering to the walls of sample chamber can cause increases in apparent counts. Follow manufacturer's recommendations for removal of stuck particles. It is important to ensure that the instrument is cleaned sufficiently between runs to prevent carryover from an earlier sample. Always confirm sample cell cleanliness with blank runs, and then recommence sample measurements.

Further Information

For any specific questions regarding this method, please contact Shawn Cao at scao@amgen.com. Please also copy aapsinterlab@nist.gov on your email.