AAPS Interlaboratory Study

Tier 1 Method: Nanoparticle Tracking Analysis

Introduction

This protocol describes Nanoparticle Tracking Analysis (NTA) of sub-micron particles present in various protein solutions. This document briefly describes sample handling, instrument set up, and data reporting of the results associated with NTA analysis. The primary reported results are the particle size distributions of particles in the size range between 100 nm and 1000 nm.

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

- NTA equipment and associated consumables (specify laser wavelengths, magnification, syringe pump information, autosampler, if available, 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
- Control sample typically used by lab to verify the instrument's performance
- Particle free/de-ionized ultrafiltered water
- Disposable 1 mL silicone-free syringes
- 0.02 µm filters, syringes, needles for filtering buffers
- Detergent or cleaning solutions for cleaning between runs

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 set-up procedures provided by the manufacturer. Use the most recent software version available for collecting and analyzing data. Cleaning solutions and buffer blanks can be run to ensure the instrument is free of particles. Follow general lab practices to confirm the sizing and counting accuracy of the instrument (e.g. 200 nm polystyrene beads).

Sample Preparation

1.) Thaw the samples from Shipping #1 and aliquot the Tier 1 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 NTA 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 each aliquot in the following manner: pipet 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 NTA 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.

Sample Measurement

1.) Review the **NTA Data Reporting Template** for the requested information before beginning the measurements.
2.) Inject samples into the sample chamber using silicone oil-free syringes. It may be necessary to make further dilutions using filtered buffer to optimize the measurement counts for your instrument. Aim for an optimal number of particles per frame depending on your instrument. Too few or too many particles may prevent accurate particle tracking.
3.) Measurements should be taken at room temperature (25˚C) with an assumed viscosity of 8.90×10^{-4} Pa·s. Whenever possible, use automatic settings to adjust shutter, minimum track length, gain, and camera levels. Appropriately set the detection threshold to include as many particles as possible for analysis. During injection, avoid formation of air bubbles.
4.) Measure each sample in triplicates (n=3) by loading new sample aliquots for every replicate. Between each mAb sample, run cleaning/detergent solutions to ensure there are no particles stuck in the sample chamber and being carried over into the next run. Once the sample chamber is verified to be clean, insert the next sample for analysis. Record all instrument parameters in the accompanying **NTA Data Collection Template** Excel sheet. Adjust the final counts or concentrations based on the dilution factor.

Understanding Results
Results from NTA should inform us about the particle size distribution of proteinaceous particles spanning the size range of 100 nm to 1 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 (e.g., cell thicknesses, illumination, laser, etc), and settings (blur, MEPS, minimum track length, etc.). Export the data as recommended by the instrument manufacturer.

Report instrument parameters and the average size and concentration with standard deviations into the **NTA Data Collection Template**. The final data should be adjusted for the dilution factors.

Troubleshooting
Reference: M. Hubert et al./ Journal of Pharmaceutical Sciences 109 (2020): 830-844

Particles adhering to the walls of the 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 Kurt Benksteain at kurt.benksteain@nist.gov. Please also copy aapsinterlab@nist.gov on this email.