Novel Software Analysis of Protein Aggregation and Particle Analysis using Micro-Flow Imaging (MFI)

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Introduction

Many features of protein-based pharmaceuticals limit the ability of standard particle analysis methods to characterize them. For example, high particle concentrations, heterogeneous particle types, viscosity, and a low refractive index are known to reduce the detection and sizing accuracy of light obscuration and membrane microscopy.

As reviewed in this poster, Micro-Flow Imaging (MFI) technology can analyze many types of challenging protein samples using direct, imaging-based particle measurement. Particle types detected include semi-transparent protein fragments, air bubbles, and contaminants such as silicone oil micro-droplets. Moreover, MFI detection is largely independent of a particle's optical properties; thus it can handle concentrated antibody solutions, as well as viscous samples.

Micro-Flow Imaging

MFI systems, from ProteinSimple, combine the flexibility and visual verification of manual microscopy with speed, statistical accuracy and quantitation.

To monitor protein formulations, MFI uses direct image-based detection of particles above 1 micron in size. For particles greater than 2 microns, morphologic parameters can be uniquely customized to delineate sub-groups of individual particles commonly found in biopharmaceuticals.

Results and Discussion

Representative images of protein aggregates

Representative images of protein particles, ranging in size from 25 to 354 µm, were evaluated by MFI in protein-based pharmaceuticals. Protein particles observed were heterogeneous in shape, from small dense fibers to large ribbon-like aggregates (D.K. Sharma et al., 2010), as shown in Figures 1, 2, and 3.

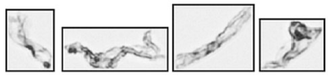

Figure 1: Small dense fibers

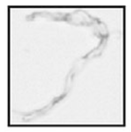

Figure 2: Ribbon-like aggregates

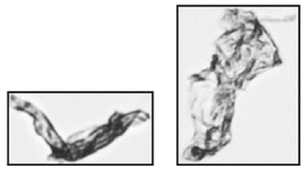

Figure 3: Large, complex aggregates

Increased sensitivity for transparent protein aggregates

MFI shows increased sensitivity for transparent particles such as protein aggregates in Figures 4 and 5. In Figure 4, MFI image filtering distinguishes protein aggregates from silicone micro-droplets and air bubbles (D.K. Sharma et al., 2010). Figure 5 shows representative screenshots of MFI analysis of (a) silicone oil in filtered IgG-B solution, (b) particles of IgG-B subjected to heat/shaking stress, (c) particles of IgG-B subjected to pH stress, and (d) particles of IgG-B subjected to freeze/thawing stress (R. Strehl et al., 2012).

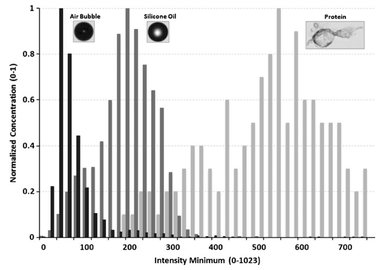

Figure 4: Sensitive detection of protein aggregates

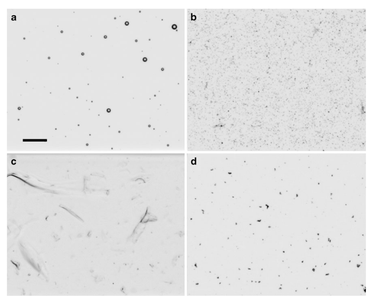

Figure 5: Image analysis of protein fragments

Accurate particle sizing in highly concentrated and/or viscous solutions

MFI provides better particle sizing under conditions of high particle concentration (>100,000 P/mL) as well as high viscosity.

Figure 6 compares MFI to a light obscuration (LO) method and shows particle analysis of an IgG1 monoclonal antibody solution at 90 mg/mL. For particles in the 5 to >40 µm range, the MFI method of detection resulted in ~5 to 10 fold more sub-visible particles (K. Wuchner et al., 2010).

In the example shown in Figure 7, high viscosity has little impact on the sizing accuracy of MFI. Beads of specific sizes were added both to water and to 40% ethylene glycol. With increased viscosity due to ethylene glycol, the particle contrast is reduced. MFI accurately sized the beads for both solutions (D.K. Sharma et al., 2010).

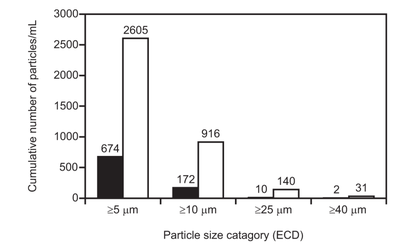

Figure 6: Sub-visible particle numbers in IgG1 mAb compared as a function of size. MFI measurements (white) versus LO (dark grey).

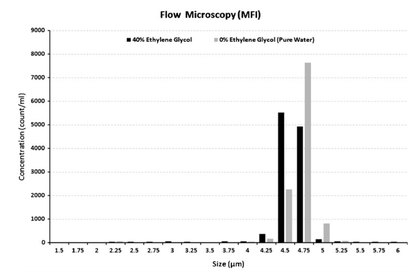

Figure 7: Accurate particle sizing with high viscosity

Conclusions

MFI's direct, imaging-based particle measurement provides critical benefits for particle analysis of biopharmaceuticals over indirect methods such as obscuration or scatter.

- Images provide quantitative information (sizing, count, and concentration) as well as qualitative shape.
- Increased sensitivity to semi-transparent protein fragments
- Categorize particle sub-populations with customized filters
- Accurate particle sizing for concentrated or high viscosity samples
- Pre-set objectives require no user calibration and control variation between samples

References : Micro-flow imaging: flow microscopy applied to sub-visible particulate analysis in protein formulation. D.K. Sharma, D. King, P. Oma, C. Merchant. AAPS Journal, (12)3:455-464, 2010. Development of a microflow digital imaging assay to characterize particulates during storage of a high concentration monoclonal IgG1 solution. K. Wuchner et al, J of Pharmaceutical Science, (99)8: 3343-3361, 2010. Discrimination between silicone oil droplets and protein aggregates in biopharmaceuticals: A novel multi-parametric image filter for sub-visible particles in microflow imaging analysis. R. Strehl, V. Rombach-Riegraf et al., Pharmaceutical Research, 29:594-602, 2012. Sub-visible Particle Counting Provides a Sensitive Method of Detecting and Quantifying Aggregation of Monoclonal Antibody Caused by Freeze-Thawing: Insights Into the Roles of Particles in the Protein Aggregation Pathway. Barnard JG, Singh S, Randolph TW, Carpenter JF. Journal of Pharmaceutical Sciences, 2010.