Sensitive Discrimination of Protein Aggregates and Sub-Visible Particle Types with Micro-Flow Imaging and Morphologic Analysis

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Abstract

Concentration and morphologic distributions of particle sub-populations is required to ensure product quality of biopharmaceuticals. However, current pharmacopeial methods are not optimized for transparent protein aggregates which can lead to undercounting. MFI addresses this technology gap, combining sensitive image capture and morphologic analysis that can discriminate translucent protein particles from silicone micro-droplets and other particles. Particle characterization is particularly challenging, because the optical properties of the particle sub-groups versus the surrounding buffer are different for each formulation, and no one classification format will work for all products. MFI resolves this issue by tailoring the analysis to the specific formulation itself. As a result MFI is a valuable tool for counting, monitoring and characterizing sub-visible particles throughout biopharmaceutical development. This poster describes how to create custom morphologic filters in MFI View Analysis Software (MVAS) that accurately discriminate specific particle groups from each other. Results from morphologic analysis show the diversity in morphology and particle size across a range of formulations, and how custom filters can be used to tailor a specific analytical approach.

Introduction

Pharmacopeial methods are not optimized for biopharmaceuticals

- Pharmacopeial methods fail to detect variation in particle sub-types between samples
  - Cannot discriminate between same-sized particles that differ in intensity and morphology
  - Lack of sensitivity for translucent protein aggregates leads to undercounting
  - Cannot separate air bubbles from silicone oil micro-droplets
- Do not address demand for higher throughput and standardization

MFI is essential for particle classification of formulation

- Proven ability to track changes in particle sub-types between lots
- Separates particle sub-types on basis of size, intensity and morphologic parameters
- Provides quantitative measurement of particle morphology (size, shape, intensity)
- Sensitive detection of translucent protein aggregation
- Automated format increases throughput and provides standardization

MVAS solves the requirement for particle characterization

- Rapid visual identification of particle populations in formulation
- Morphologic parameters robustly discriminates particle sub-types.
- Classifies MVSS data using automatic or user-defined filters:
  - Select visual images to create filter (find similar feature)
  - Custom filter lets user create own parameters
  - Test separation (filter manager)

Methods

Sample Preparation of Monoclonal Antibody Solution

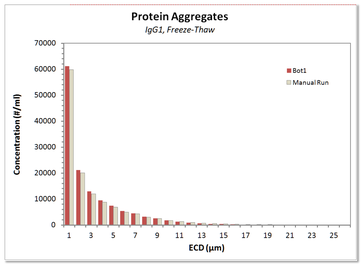
- A 1 mg/ml mAb IgG1 solution in 10mM Citrate buffer provided by Eli Lilly and Company (Indianapolis, IN, USA) was used during this study.
- Aggregates generated using a freeze-thaw method, and suspended in a filtered phosphate buffered saline (PBS) solution[2]
- Silicone oil emulsion (Refractive Index of 1.4) diluted with PBS buffer at 0.2%
- Air bubbles introduced prior to sampling by vigorous agitation

MFI Operation

- MVSS method configured with Sample Dispensed of 0.9 mL, and a 0.2 mL purge
- Optimize Illumination performed using 0.22 µm of sample on the Bot1, and 0.22 µm of filtered PBS sample buffer on the MFI 5200 in manual mode

Aggregates Formed Following Freeze-thaw Stress

Protein Aggregates Analyzed by the MFI 5200 in Manual and Automated Mode



IgG1 Aggregates	Manual Concentration	Bot1 Concentration
R1	131567	135790
R2	129417	142581
R3	139788	134681
Mean	133591	137684
RSD	4.1%	3.1%

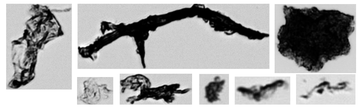
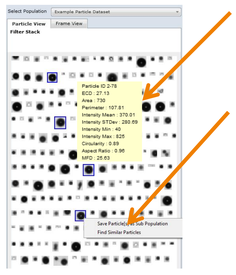


Figure 1. Example of IgG1 aggregates from 1 mg/ml formulation measured on an MFI 5200 in manual mode and automated mode. Either format provides the ability to measure particle size, concentration and morphology while the automated format also offers higher throughput and unattended operation.

Creation of Morphologic Filters Using MVAS

Fast classification based on visual images: "Find Similar Particles"



Hover over particle to obtain summary of morphological parameters

Select multiple particles by holding down shift or control keys

Right-click to save selected particles as a sub-population, or to perform a quick filter using "Find Similar Particles"

View updated table for sub-population filter results

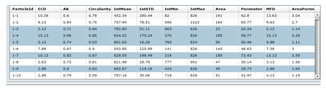


Figure 2. The MVAS feature "Find Similar Particles" provides rapid classification based on the unique visual features of particles such as protein aggregates, silicone micro droplets. Quickly select the particles of interest and automatically create a filter. Then apply the filter to the entire mixed population for sub-group isolation. The quantitative filter images and the tabular format will instantly update.

Create and test user-defined filters: Filter Manager

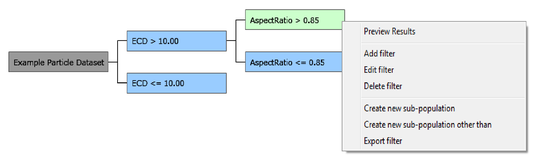
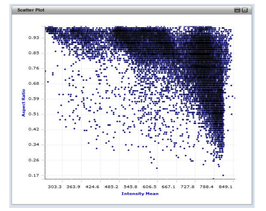


Figure 3. The MVAS Filter Manager module can be used to create and test filters.
In this example, the particles of interest tend to be round, so a circularity filter was created (e.g. Aspect Ratio > 0.8). F or each filter expression, 2 nodes are created: filter and its inverse.
The filter can be adjusted based on the particles included or the particles missed, after reviewing data in both nodes. You can import the filter into other MVAS projects as an overall analysis template.

Testing Particle Separation of MVAS Filters

Single Parameter Analysis of Silicone Oil Droplet vs Protein



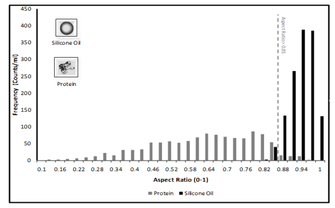


Figure 4. Use of filter manager functions to test a single parameter analysis**.** Based on the scatterplot shown on the left, aspect ratio varied in this sample, suggesting its use as a filter parameter. A single filter analysis was then created on the right, using the aspect ratio for particles >5 micron in size. 96% of the particles were correctly classified as protein, out of a population of 2417. These results showed that the aspect ratio filter was an effective option to classify protein from silicone oil, for this specific formulation.

Multiparameter Analysis of Silicone Oil Droplet vs Protein vs Air Bubbles

A

Size Range	Protein Aggregates: Intensity Min Filter Value	Protein Aggregates: Filter Success (#)	Protein Aggregates: Total Particles (#)	Silicone Oil: Filter Error (#)	Silicone Oil: Total Particles (#)	Air Bubbles: Filter Error (#)	Air Bubbles: Total Particles (#)
10+ µm	≥ 260	112	119	0	704	0	1748
		Filter Success: 94.1 %		Filter Success: 100 %		Filter Success: 100 %	

B

Size Range	Silicone Oil: Intensity Min Filter Value	Silicone Oil: Filter Success (#)	Silicone Oil: Total Particles (#)	Air Bubbles: Intensity Min Filter Value	Air Bubbles: Filter Success (#)	Air Bubbles: Total Particles (#)
10+ µm	≥ 75	552	704	< 75	1560	1748
	Filter Success: 78.4 %			Filter Success: 89.24 %		

C

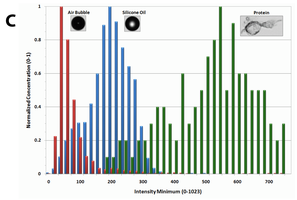


Figure 5. Use of filter manager to test a multi-parameter analysis in a complex sample. Complex particle mixtures are a challenge for particle characterization. For samples with many particle sub-groups, sequentially applied filters provide optimal separation, as shown here. An overall filter of aspect ratio for particles >5 micron in size was first applied to all particles. A second filter shown in panel (A) was then created using an intensity min >260 and particle size >10 micron, yielding a filter success level of 94% for protein aggregates. To isolate silicone oil, intensity parameters are often used. In bottom panel (B), air bubbles are separated from silicone oil using an Intensity Min Filter of >75. This led to ~90% correct classification of air bubbles, an optimal result for a complex mixture. In (C), multiparameter analysis data is displayed as Intensity versus Concentration for each sub-group.

Conclusion

- Micro-Flow Imaging (MFI) measures particle size and count but also provides images that allow you to discriminate particle sub-populations from one another.
- MVAS quickly creates and applies accurate filters that allow the user to classify particle sub-groups based on their image intensity and morphologic characteristics
  - Addresses the key challenge of particle characterization: effective classification
- MFI is ideally suited for routine analysis and characterization of biopharmaceuticals.
  - Sensitive to translucent protein aggregates
  - Easily distinguishes particle sub-groups with customized filters
  - Designed for a wide range of protein concentrations
  - Provides automation for higher throughput and standardization
  - Allows determination of protein aggregates throughout biopharmaceutical development