See What Light Obscuration Misses with Micro-Flow Imaging

Introduction

Subvisible particles are a critical quality attribute for pharmaceutical products as protein aggregates can elicit an immunogenic response that affects the therapeutic's efficacy.[1] Regulatory agencies require a full analysis of any particles present in a therapeutic product, including quantitative measurements of size and count and information on the type of the particles. Compendial methods like Light Obscuration (LO) don't give you complete profiles due to gaps in their analysis, creating more risk during QC or when you need to file New Drug Applications (NDAs). Micro-Flow Imaging (MFI®) on the MFI 5000 Series system fills in those gaps because it sees particles LO can't — making it the perfect complement for your regulatory submissions. The system classifies proteins and non-protein particles and gives you direct, image-based detection of size, count and shape for sub-visible particles between 1-300 microns in solution.

Measuring protein aggregation is a common way to get an indication of stability, and MFI is widely used in formulation studies and stability studies where aggregation measurements are needed.[2] Measuring small and potentially translucent protein aggregates with MFI gives you early insight into the efficacy of a formulation and the long-term stability of your product. Observing protein aggregation is also critical when understanding the safety of your biotherapeutic. Protein aggregation alters the physical and chemical properties of biologic drugs, potentially making them immunogenic.[3,4]

The Bot1 Autosampler for MFI systems also gives you an automated way to handle the throughput needed in these studies, and lets you evaluate up to 90 samples with no manual intervention.

In this white paper, we'll highlight the differences between MFI and LO using published literature to help you learn more about how MFI can improve your particle analysis.

MFI sees translucent particles LO misses

Size matters when it comes to particle analysis, and both MFI and LO provide information in the range regulatory agencies need. This includes the ranges regulated by USP 788; particles greater than 10 µm and 25 µm, and particles in the 2-10 µm range stated in USP 787 that have become increasingly important for biopharmaceutical products (**Figure 1**).

Translucent particles are one of the bigger challenges for particle analysis because of the sensitivity you need to see them. LO isn't sensitive enough to detect them, but MFI can.

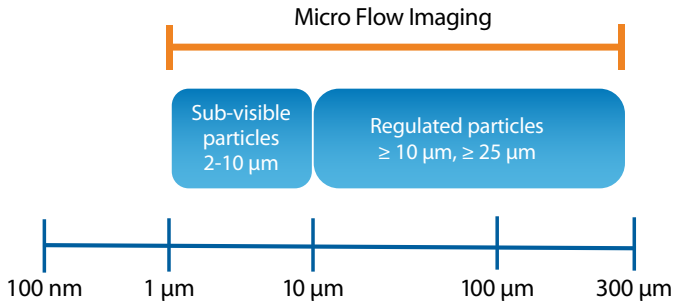

FIGURE 1. Particle ranges regulatory agencies require information on for biopharmaceutical products.

A study comparing the detection methods of LO and MFI done by the National Institute of Standard and Technology (NIST) clearly demonstrates how MFI is more sensitive when detecting these hard-to-see particles. They titrated their sample matrix and matched that refractive index to the refractive index of the silica beads (Delta n= 0.00) they were studying (**Figure 2**).[5] When the two refractive indexes matched, the particles were invisible to both technologies. But as the refractive index of the matrix changed, the difference in the technologies became clear. MFI reported a diameter of 16 µm when measuring 20 µm silica beads when there was a 2% change in refractive index compared to LO which reported a diameter of only 2 µm. When refractive index difference increased to ~4%, MFI measurements were within 10% of the nominal diameter of the beads while LO measurements were only 20-40% of the nominal diameter.

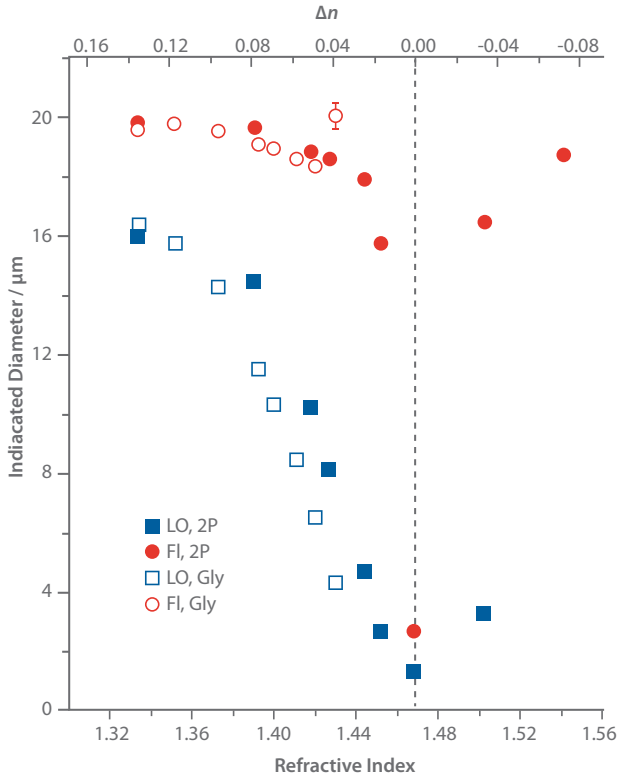

FIGURE 2. MFI provides quantitative information on particle size even when there's a small refractive index difference between the particle and the surrounding matrix.[5] This ultra-sensitive detection threshold makes MFI ideal for detecting translucent particles.

Sensitivity Matters

Accurately characterizing all the aggregated protein in a sample is really important as it conveys crucial information about the product. Protein aggregates can be highly translucent in nature, and are low in optical contrast from the surrounding matrix. These aggregates occur in both the regulated size ranges of >10 µm and >25 µm, but also happen frequently in the 2-10 µm range (**Figure 3**). MFI is more sensitive compared to LO, so you'll be able to see protein aggregates that would be otherwise invisible.

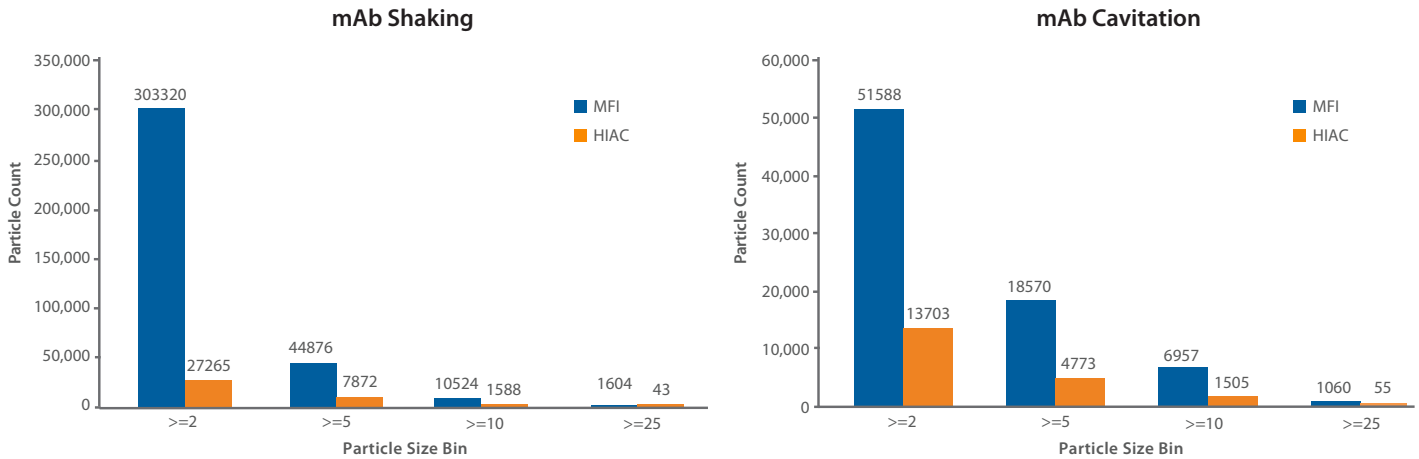

FIGURE 3. MFI detects up to 10-fold more aggregates than LO (HIAC) when comparing solutions of stressed monoclonal antibodies.

Morphology

In addition to size and count, MFI also gives you morphological parameters LO can't, like particle shape and intensity (**Figure 4**). Classifying particles in this way gives you a better understanding of the nature of the particles detected so you can determine the risk to patients and how to mitigate them by making changes in your production process.

Because of this, regulatory agencies have developed terminology to categorize these different particle types (**Figure 5**):

Inherent - Particles that come from the active pharmaceutical ingredient or another part of the formulation. These are particles that form from materials that make up the pharmaceutical product, either the Active Pharmaceutical Ingredient (API), or part of the formulation. Protein aggregates are common examples of inherent particles.

Intrinsic - Intrinsic particles are contaminants that come from the manufacturing process and are constituted by materials that shouldn't be in the final pharmaceutical product. These materials can include rubber, silicon oil, glass, plastic or metal.

Extrinsic - Extrinsic particles are materials that aren't part of the drug production process but have entered the product. Common examples of extrinsic particles include human hair, insect parts and clothing fibers.

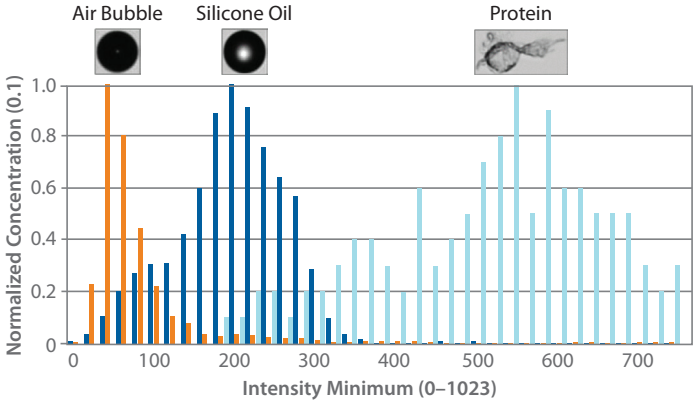

FIGURE 4. MFI differentiates between multiple particles based on image intensity. The translucent protein aggregate scores very highly on the intensity-minimum parameter, while the darker silicon oil and air bubbles score much lower.

Not only does MFI help you classify particles, it also makes it simple to do. The MFI software suite streamlines analysis, letting you create filters that'll automatically classify particles in a sample.[6] You can create filters for just one morphological parameter at a time, or expand your filters to use multiple parameters in combination to get a highly-sophisticated classification.[7]

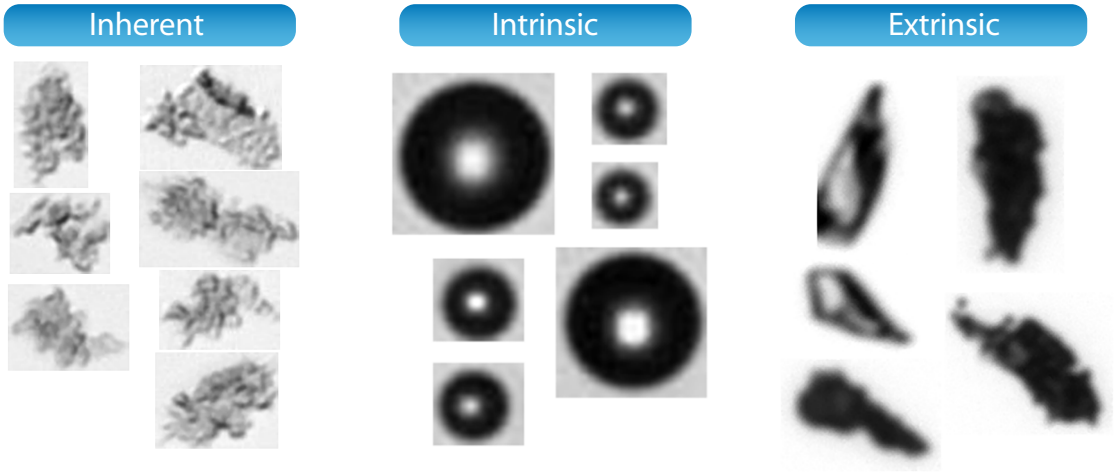

FIGURE 5. MFI particle classifications based on conventions formalized by Pharmacopeial agencies. These morphological measurements help pharmaceutical companies and regulators assess the quality and safety of biopharmaceutical products.

MORPHOLOGY IMPACTS FORMULATION DECISIONS

Scientists at Amgen performed a full battery of analytical tests during development of a biosimilar to Humira[8] in a pre-filled syringe that included:

- Subvisible particle concentrations by light obscuration
- Subvisible particle concentrations and morphology by MFI
- Submicron particle profile by DLS
- Submicron particle profile by FFF-LS
- Aggregate profile by AUC-SV
- Aggregate profile by SE-HPLC with light scattering

This included a full characterization of particles and aggregates in both the subvisible and submicron ranges. Subvisible range analysis was performed using the LO measurements mandated by USP 788's prescribed limits for particles >10 µm and >25 µm. They also supported the LO observations with MFI measurements.

ANALYTICAL TESTING/ ATTRIBUTES	ABP 501 [RANGE (*n*)]	ADALIMUMAB (US) [RANGE (*n*)]	ADALIMUMAB (EU) [RANGE (*n*)]
LO/particles-size (particles/mL)			
≥2 µm	5140-23,748 (10)	4560-31,000 (7)	9447-15,820 (7)
≥5 µm	1000-7630 (10)	1057-13,600 (7)	3577-7587 (7)
≥10 µm	93-1525 (10)	107-3727 (7)	570-2284 (7)
≥25 µm	0-14 (10)	4-97 (7)	3-60 (7)
MFI/non-spherical particles-size ≥5 µm (particles/mL)	24-172 (10)	18-139 (7)	7-183 (7)
CHO cell protein by ELISA (ppm)	0-46 (10)	129-168 (3)	87-171 (3)

TABLE 1. LO and MFI results for the three Amgen products tested. MFI's morphological information indicated that most particles in the products were silicon oil.[8]

Injectable drugs often have traces of silicon oil in their formulation that has leached off the syringe. Amgen was able to discern the proportion of circular particles to amorphous particles using MFI's image-based analysis, which gave them the ability to judge the contribution silicon oil had to the overall particle load (**Table 1**). The morphological information derived from the images indicated most of the particles present in the final product were in fact silicon, and this was consistent between the originator molecule and the biosimilar. Had they only performed analysis with LO, the lack of information would have caused them to mischaracterize the silicon oil as protein aggregates. Knowing that there were two distinct particle populations within their formulation gave them more insight into the stability and safety of their product.

Conclusion

MFI's combination of sensitivity and morphological information makes it a crucial tool in the effort to more fully characterize particles and protein aggregates in biopharmaceuticals. It also gives you a deeper level of insight when it comes to sub-visible particle analysis compared to LO because of its ability to detect highly translucent protein particles and distinguish them from intrinsic and extrinsic contaminants.

In fact, MFI gives you the sub-visible particle data requested in FDA letters and immunogenicity guidelines. Regulatory agencies often request particle data beyond a single QC check: *"It is also recommended that in addition to USP <788> particulate testing, sub-visible particles <10 µm in size be characterized at release and at regular intervals in the drug product stability program including under accelerated and/or stressed condition."* Because MFI's analysis is image-based, you can identify the specific classes of particulates you have so you'll also know how to minimize them — something LO just can't do. That's why a 2011 joint regulatory/industry panel listed MFI as an orthogonal technique to LO for particle sizing, and stated its morphologic assessment can be used for characterization.9 So with an MFI 5000 Series system in your arsenal, you'll be able make smarter formulation decisions and cover all your QC bases.

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