MFI for Particle Characterization of Biopharmaceuticals Today

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Regulatory agencies currently require sub-visible particles to be well characterized in biopharmaceutical formulations due to concerns about undesired side effects and reduced efficacy. Because of this, sub-visible particle characterization is a key step in evaluating the quality attributes of complex biopharmaceuticals. Development of protein therapeutics is challenging as the individual proteins in these formulations can change conformation and clump together eventually growing from tiny, nanometer-sized aggregates into larger insoluble protein particles. Biopharmaceuticals contain other types of particle contaminants as well, including silicone microdroplets, polyethylene, and cellulose fibers.

Initial publications pose the question of immunogenicity and reduced therapeutic efficacy for insoluble protein aggregates in the sub-visible range (1–10 microns), while noting that the required particle analysis techniques are poor at detection of protein aggregates.[1,2]

Subsequently, a proposed revision to USP guidelines <788> suggests the use of newer analysis technologies such as Micro-Flow Imaging (MFI) to address sub-visible particles.

As a result, the biopharmaceutical industry has focused on how to limit these potential safety and efficacy risks by monitoring particle size and distribution with a view towards using newer particle analysis techniques such as MFI. Micro-Flow Imaging technology uses dynamic imaging for direct particle detection, which enables rapid quantification of particle size and shape for tens of thousands of particles per sample, and the sensitivity to detect translucent protein particles. Emerging regulatory guidelines consider MFI a validated method for particle size distribution, and an approved method for particle characterization of a wide range of contaminants.[2, 3]

Particle Characterization in Biopharmaceutical Development

The main focus of testing protein formulations is often on protein identification and activity, or on purity analysis. However, because the presence of specific types of contaminating particles can impact safety and efficacy, particle characterization is now critical in biopharmaceutical development. Preliminary studies suggested that protein particulates could enhance immunogenicity to the protein alone, or perhaps by coating of non-protein particles.[1,4] However, the exact mechanism remains unclear because the level of protein particles required to induce immunogenicity is still unknown. More recent studies have found a link between the presence of sub-visible particles and clinical immunogenicity with repeated dosing. Apparently the sub-visible particles promote the formation of neutralizing antibodies to the biopharmaceutical, which then eliminates the drug's effectiveness.[5] These risks were sufficient enough that industry now controls and monitors the types and amounts of all particles present in a formulation, including sub-visible particles. The fragile nature of proteins and the challenges of the compounding process can destabilize them. Therefore, although they can occur at any point in the process, it is important to monitor whether contaminants are introduced during the process of mixing the formulation, in manufacturing, or in storage. Given this, optimizing a biopharmaceutical to eliminate the various types of contaminants is a significant activity. In fact, industry reports state that it is necessary to develop a particle characterization method that can be used throughout development to help solve issues as they arise, and inform process and formulation development on ways to minimize the formation of aggregates.[2,4] Tapan Das noted this benefit in a recent review of particle characterization

technologies; "This [MFI] may allow superior imaging of highly irregular shaped particulates and monitoring the dynamic behavior of particulates if the size distribution is changing over time. Such information is valuable during formulation of biologics to characterize particulates and find potential preventative measures."[5]

Ideally, as this type of testing becomes standardized, the process of particle characterization becomes traceable through all stages, from process development to commercial quality control.

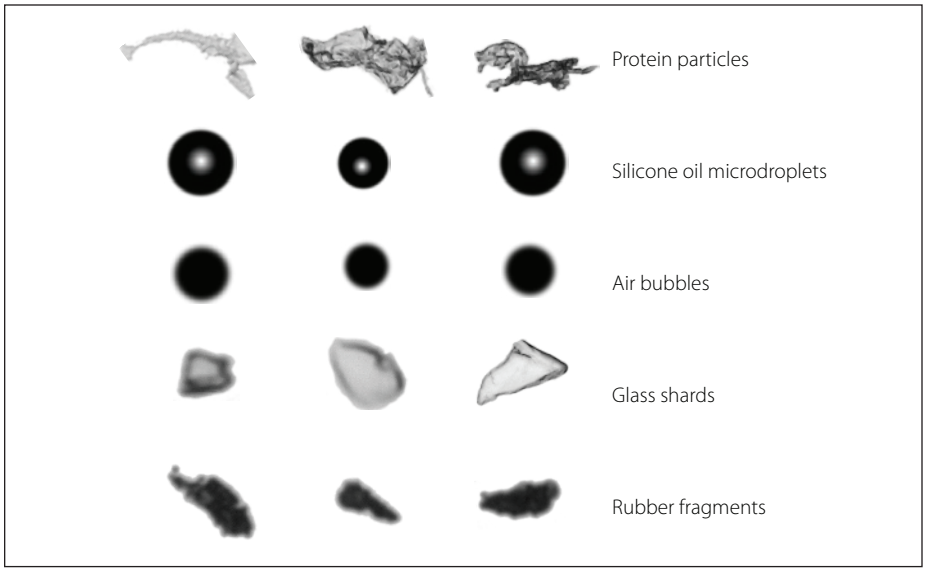

FIGURE 1. MFI detects protein particles, silicone oil microdroplets, air bubbles and other contaminants, from 1–300 µm in size.

Role of MFI Technology in USP-Defined Particle Analysis

A diverse array of technologies is used to characterize formulation samples, and each is suited to a particle size range. Individual formulations must be characterized for both the types and numbers of particle present, in addition to particle sizing. Current FDA requirements in USP <787> regulate particles in the visible range, specifically at greater than 10 micron and greater than 25 micron. However, due to the emerging concern over immunogenicity with sub-visible particles,[4] regulatory agencies now also request data on sub-visible particles (SVP). No limits for SVPs have been set as of the date of this review.

At an industry presentation at WCBP 2013, Maria Toler of Pfizer notes, "Dynamic flow imaging can provide particle counts and images showing morphology, stability (to shear and other mechanical stresses), intensity (transparency) over the size limits of the instrument."[7]

Imaging technologies such as MFI are well suited for sub-visible particle characterization (**Figure 1**). Technologies required by USP <787>, such as light obscuration (LO) which enumerates particles one at a time or manual light microscopy, were early techniques initially designed for small, chemical-based drugs. They are limited when evaluating protein based formulations that are more impacted by refractive index as protein concentration increases.[8] LO and light microscopy are also not as sensitive to sub-visible particles in the 2–10 µm range, because they are designed to detect particles at sizes greater than 10 microns, and the translucent nature of these cause them to become invisible due to loss of contrast.

In addition, both techniques are incompatible with concentrated and viscous protein samples that require sample dilution.

Because MFI uses a quantitative imaging format, it can detect particles more accurately in both the sub-visible and visible range.[14]

For these reasons, MFI has been described as an orthogonal technique for sub-visible particle characterization in the recent draft revision to USP guidelines for parenteral formulations.[9] Recent publications in analytical testing have combined MFI with other particle analysis systems, showing that it is possible to cover the required particle size range from sub-micron, to sub-visible and visible, with the use of only two instruments, versus having to use three or more systems. MFI has been paired with LO, membrane microscopy techniques, and more recently with a sub-micron particle detection system, to ensure detection of the common particle types and the broader size range commonly associated with biopharmaceuticals.[10] However, MFI is the only technology in

these published examples which provides morphology for particle characterization.

Case Study Overviews: Practical Use of MFI in Protein Formulation Evaluation

Today's biopharmaceutical formulations are highly diverse products, from humanized antibodies to antibody-drug conjugates or small peptide-based compounds. They also vary broadly in terms of concentration and viscosity. MFI is able to address a diverse range of formulation types, even concentrated monoclonal antibody products ranging from 50–150 mg/mL. MFI is now commonly used to monitor sub-visible particle formation in biopharmaceuticals.[14,15] This technique can be used to evaluate many different questions relating to product quality and stability — from comparing different types of protein products to the impact that changes in formulation or storage conditions may have on them (**Table 1**).

COMPARING STABILITY BETWEEN DIFFERENT PROTEINS

The ability to form sub-visible particles appears to be an unpredictable attribute, and it varies for each protein. **Figure 2** shows a simple example of this. Comparison of three proteins (IgG1, IgG4 and a glycoprotein) tested using MFI indicates different responses to agitation in terms of SVP formation.[11]

APPLICATION	DESCRIPTION
USP <787>	Addresses USP revision requirements for sub-visible particle analysis
Monitoring and process optimization	Optimize mAb formulations to limit formation of SVPs Evaluate and control process variables on particle formation
Stability testing	Detect impact of storage conditions and excipients on SVPs
Particle characterization of contaminants	Distinguish silicone micro-droplets from protein aggregates
Lyophilized product	Characterize formulations after reconstitution of lyophilized product
Microparticles (drug delivery)	Sizing of microparticles from 1–10 µm

TABLE 1. Biopharmaceutical applications for MFI.

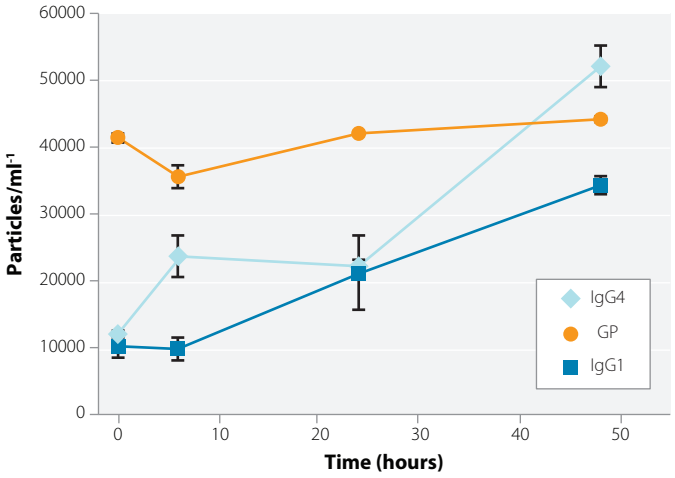

FIGURE 2. Sub-visible particle counts as a function of agitation time for three proteins. The two antibodies of subtype IgG1 and IgG4 show an increase in SVP formation during 48 hours of agitation, while the glycoprotein (GP) shows no change.[10]

COMPARING THE OCCURRENCE OF SVPS TO CLINICAL SIDE EFFECTS IN DIFFERENT THERAPEUTICS

Other recent examples confirm the significance of evaluating the number of SVPs and their type (protein aggregate, silicone micro-droplets, etc.) in biopharmaceuticals.

For example, aggregated therapeutic antibodies containing high numbers of sub-visible particles with partially folded proteins have the potential to induce innate immune responses based on a recent in vitro model.[17] Their presence can have clinical implications, as shown in a comparison of three versions of commercial β-interferon (Betaseron, Rebif and Avonex). MFI reported greater levels of protein aggregates

and SVP in Betaseron and Rebif, while Avonex had the least, and most of its particles were silicone oil microdroplets. Notably, Betaseron and Rebif were associated with higher production of neutralizing antibodies (NAbs) clinically, at 22–47% and 5–35% respectively, versus Avonex, which only showed 2–13% incidence rates for NAbs.[6] The authors considered that protein aggregates and particle content are key quality attributes to evaluate for clinical outcomes. MFI's ability to identify novel shape parameters (morphology) can discriminate groups of particles from each other and readily established differences in SVPs.

IMPACT OF STORAGE CONDITIONS ON FORMULATION STABILITY

Storage conditions can dramatically alter the generation of particles in protein formulations. MFI was used to determine the effect of storage temperature. Higher temperature ranges were found to induce larger numbers of visible particles greater than 25 microns in size, while the numbers of sub-visible particles largely remained the same. MFI can also monitor the effectiveness of excipients (formulation additives) on product stability or production of protein aggregates at levels where the excipient does not cause opalescence.[8]

CHARACTERIZATION OF MICROPARTICLES FOR DRUG DELIVERY AND VACCINATION

An emerging and novel area involves the use of microparticles in drug delivery and vaccination. Microparticles are non-spherical, micron-sized particles, and are currently are under evaluation for their ability to deliver minute quantities of drugs or vaccines. A recent publication on this application showed that MFI reliably identifies particle size and aspect ratios of 10 µm microparticles, indicating successful particle characterization for this unique material.[13]

DETECTION OF PROTEIN AGGREGATES DURING PROTEIN CRYSTALLIZATION

Protein aggregation can occur throughout the protein development process, even during protein crystallization. However the evaluation of crystallization conditions has been hampered by a lack of cost-effective methods to rapidly screen samples for protein aggregates in a high-throughput fashion. New research suggests that MFI is suited for this type of screening, and its analysis parameter for mean light intensity quantitatively distinguishes protein crystals from amorphous product aggregates. The authors noted that this feature may provide a key measure of product quality for protein crystallization.[16]

Conclusion

MFI is a sensitive, simple and automated method for the analysis of sub-visible particles and protein aggregates. Its ability to provide particle size, count and morphology enables researchers to discriminate groups of particles from each other, and monitor how these groups change over time. These benefits support its use as an orthogonal technique in the draft revision to USP <787> for parenteral formulations. As a result, MFI is now an established technology for many biopharmaceutical applications, and Biopharma development and quality groups increasingly rely on MFI to evaluate product quality and stability.

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