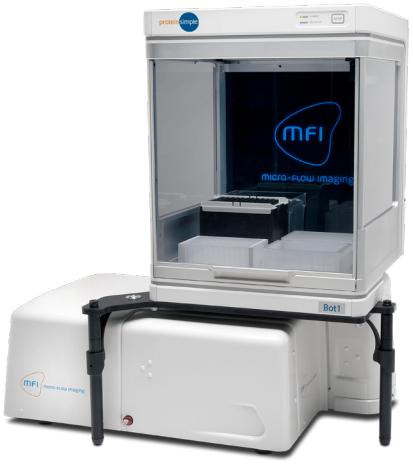
Leveraging Micro-Flow Imaging to the Max



Introduction

Micro-Flow Imaging® (MFI) provides valuable insight into many of the processes involved in developing, manufacturing and delivering a quality biopharmaceutical product. Combining the direct imaging capabilities of digital microscopy with the precise control of microfluidics, MFI gives you more precise counts and sizing with full morphological detail for all subvisible particles in your sample and the complete confidence that you can accurately classify all possible types—from protein aggregates to air bubbles.

As the sample passes through the flowcell's optical window, high-resolution images of the sample capture particles with an 85% sampling efficiency. Each image is then analyzed to create a database of particle count, size, transparency and morphology, providing unique insights into particle characterization and quantification with just a single test. Going beyond the capabilities of pharmacopeial techniques such as light obscuration, MFI's image-based detection allows you to differentiate particle populations within your sample, measure translucent protein aggregates and accurately characterize particle size, concentration and morphology.

In this white paper, we highlight how researchers are leveraging MFI throughout the biomanufacturing process. From monitoring cell culture scale-up to formulation development and stability testing, MFI gives you robust and reproducible results, providing a deeper analysis of your biotherapeutic products.

Cell Culture: Cell Confluency

As cell cultures are scaled-up for production, there is a need to monitor cell growth, viability and confluency accurately. Adherent cell cultures, such as microcarrier-based cultures, can be technically challenging to monitor as the cells have to be first removed from the microcarrier. Traditional methods such as cell counting with a hemocytometer or metabolic and cell toxicity assays can give inaccurate results due to incomplete cell detachment from microcarriers or variations in microcarrier coverage. Analysis with MFI offers direct insight into cell growth in microcarrier cultures. Christopher Farrell and colleagues from Merck leveraged MFI to analyze microcarrier cultures to provide insight into cell confluency on microcarriers, cell morphology and microcarrier defects[1]. **Figure 1** shows an analysis of microcarrier confluency and the percent of microcarriers that fall in each category over time. Cell growth curves can be used to determine when a culture has reached the capacity of a bioreactor (**Figure 1c-f**).

Compared with other methods such as trypsin release and counting using a hemocytometer or metabolic and cell toxicity assays, which provide indirect metrics of cell growth, MFI provides information about cell growth, distribution and culture reproducibility, as well as enabling the inspection of microcarriers for defects. The authors concluded that "…cell confluency analysis by MFI is a simple method that is straightforward to implement" and that the "[s]ame day microcarrier cell confluency results provided upstream process teams with timely, actionable data that can be used to inform bioprocess decisions."

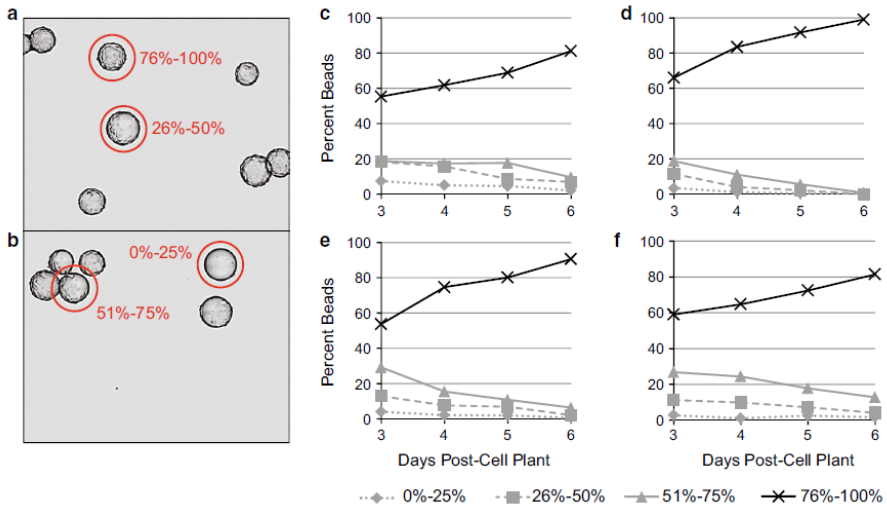


FIGURE 1. MFI image analysis of microcarriers illustrating cell coverage on microcarriers (a,b). Growth curve analysis of microcarrier confluency in four 3-L bioreactors over six days (c–f). Reprinted from ***Cytotechnology***, 68, CJ Farrell, SM Cicalese, HB Davis, B Dogdas, T Shah, T Culp, VM Hoang, Cell confluency analysis on microcarriers by micro-flow imaging, 2469–78, Copyright (2016), with permission from Springer Science+Business Media Dordrecht.

Formulation Development and Manufacturing

As biotherapeutics move through the formulation and manufacturing process, external environmental factors, including temperature fluctuations, exposures to air-liquid interfaces and mechanical stress can influence the relative stability of the therapeutic. One of the most common challenges is protein aggregation, which can lead to immunogenicity and adverse reactions in patients. Traditional techniques such as size exclusion chromatography or light obscuration are insensitive to subvisible particles, which can be an early indicator of changes in product quality[2,3]. MFI provides the ideal tool for characterizing these critical particles.

ULTRAFILTRATION/DIAFILTRATION

Ultrafiltration followed by diafiltration (UF/DF) is commonly used in biopharmaceutical manufacturing to concentrate and purify the final drug substance. This provides a process for establishing the final drug concentration, removing any impurities and establishing the formulation buffer. The shear forces present in UF/DF not only introduce the possibility of exposing the product to air-liquid interfaces but can also cause protein aggregation and influence the stability of the therapeutic[4].

Abhiram Arunkumar and colleagues from Bristol-Myers Squibb used MFI to quantify the impact of UF/DF on product quality as assessed by subvisible particle measurements[5]. In a study to examine the effect of feed screens of a UF cassette module on product quality, they were able to determine that the Pellicon D filter was a better choice for their process development, as measured by lower levels of subvisible particles (**Figure 2**). By leveraging MFI as a process analysis tool, they were able to understand how screen-channel induced shear can impact particulate formulation and optimize their biologic process development to produce a better-quality product with lower particle levels.

AGGREGATION DURING LYOPHILIZATION

Lyophilization offers a number of advantages including increased long-term product stability and ease of reconstitution. However, the lyophilization process can have destabilizing effects on the biotherapeutic. To determine the stability of lyophilized sucrose formulations, Janice Davis and her team from Althea Technologies, Legacy BioDesign LLC, Colorado State University and Roche prepared eight lyophilized formulations of an IgG1 monoclonal antibody (mAb), each containing increasing levels of sucrose[6]. Sorbitol was added at a level of 5% w/w relative to sucrose in three of the samples to determine if sorbitol increased the storage stability of the IgG antibody. The samples were stored for up to 4 weeks at 40 °C and,

upon reconstitution, the levels of subvisible particles were measured using MFI. Davis and her colleagues found that the addition of sucrose decreased the number of subvisible particles (**Figure 3**, blue bars). The addition of sorbitol further decreased the number of subvisible particles (**Figure 3**, yellow bars). MFI enabled Davis and her colleagues to optimize the lyophilized formulation of their mAb.

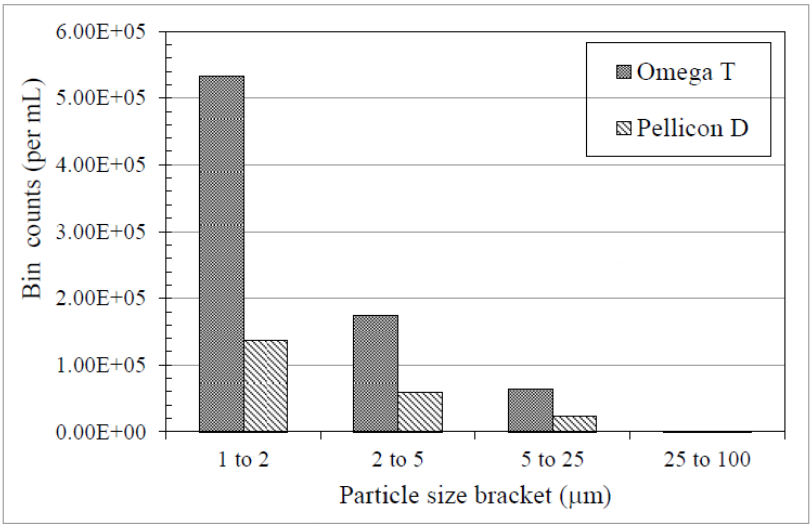


FIGURE 2. MFI analysis showed a distinct difference in the levels of subvisible particles formed when the product was filtered using the two different cassettes. Reprinted from ***Journal of Membrane Science***, 514, A Arunkumar, N Singh, EG Schutsky, M Peck, RK Swanson, MC Borys, ZJ Li, Effect of channel-induced shear on biologics during ultrafiltration/diafiltration (UF/DF), 671–83, Copyright (2016), with permission from Elsevier.

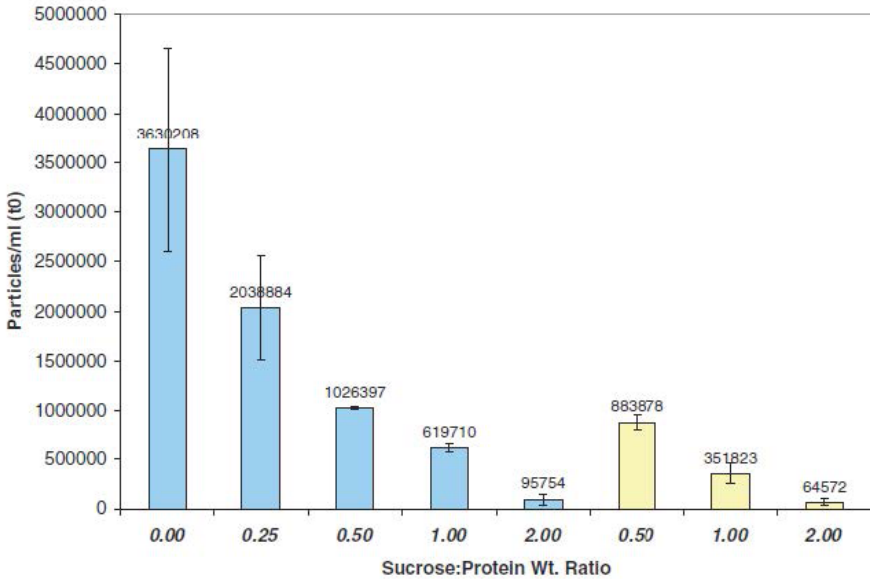


FIGURE 3. Optimizing the ratio of sucrose to protein was important for minimizing subvisible particle formation in the product. The yellow bars represent formulations where sorbitol (5% w/w relative to sucrose) was added. Reprinted from ***Pharmaceutical Development and Technology***, 18, JM Davis, N Zhang, RW Payne, BM Murphy, AM Abdul-Fattah, JE Matsuura, AC Herman, MC Manning, Stability of lyophilized sucrose formulations of an IgG1: subvisible particle formation, 883–96, Copyright (2013), with permission from Taylor & Francis.

STABILITY TESTING AND SHIPPING STRESS

To examine the impact of shipping stress on biotherapeutics, scientists at MedImmune developed a shipping stimulator model[7]. This model of agitation stress was used to study transportation-induced degradation of six mAb formulations. While the study compared the ability of multiple assays to monitor the impact of agitation on mAb formulations, MFI's detection of subvisible particles was the only effective method that enabled a comparison of agitation methods. By studying a range of formulation and configuration parameters, they were able to determine that their shipping simulator mimicked real-time shipment counts of particles (**Figure 4A**), suggesting this transportation model, coupled with monitoring of subvisible particles with MFI, could be used to predict real-time shipment with a high probability of success. They also used their model to develop an efficient, low-resource method to optimize polysorbate concentration to control particle formation (**Figure 4B**).

Conclusions

MFI provides deeper insight into the nature of your particles, supplying you with the sensitive detection of protein aggregates you need to support your New Drug Application or regulatory filing. With its image-based approach, you get quantifiable morphological parameters, allowing you to differentiate subvisible particle populations from each other and classify them accordingly. Leverage MFI throughout your biomanufacturing process to provide a deeper analysis of your biotherapeutic product, to ultimately make a better product.

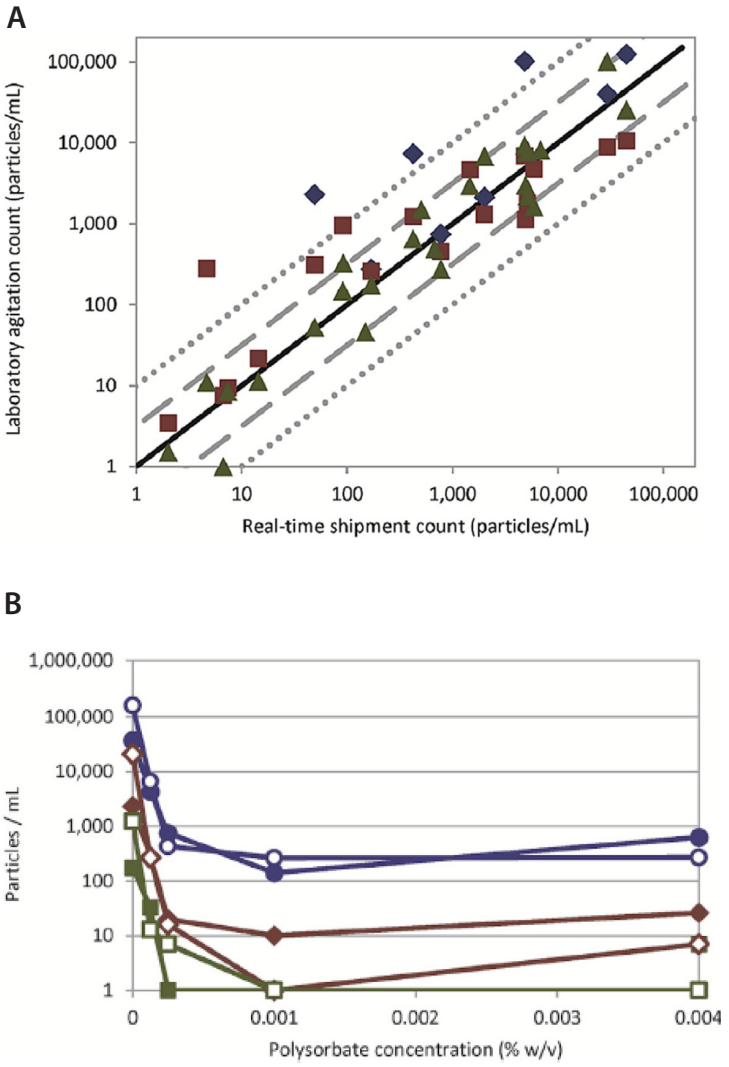


FIGURE 4. Subvisible particle measurements with MFI examining the correlation between different methods of agitation and the particle measurements taken from shipped samples (A). The results are shown for vortexed samples (blue diamonds), rotated samples (red squares) and samples which experienced their shipping simulator (green triangles). The shipping simulator (green triangles) demonstrated a strong correlation with results from actual shipped samples. Predictive results of the shipping simulator show the formation of particles in size bins corresponding to ≥2um (blue circles), ≥10um (red diamonds) and ≥25um (green squares) (B). The results for the simulator are represented in open symbols and the results for shipped samples are represented by filled symbols. Reprinted from *Journal of Pharmaceutical Sciences*, 106, ML Fleischman, J Chung, EP Paul, RA Lewus, Shipping-induced aggregation in therapeutic antibodies: utilization of a scale-down model to assess degradation in monoclonal antibodies, 994–1000, Copyright (2017), with permission from Elsevier.

References

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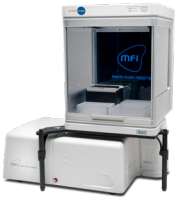
7. Shipping-induced aggregation in therapeutic antibodies: utilization of a scale-down model to assess degradation in monoclonal antibodies, ML Fleischman, J Chung, EP Paul, RA Lewus, *Journal of Pharmaceutical Sciences*, 2017; 106:994–1000.

Product Highlights

MFI 5100
Particle analysis for sizes ranging from 2-300 µm

MFI 5200
Particle analysis for sizes ranging from 1-70 µm



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