A Comparison Study of Manual and Automated Particle Characterization using Micro-Flow Imaging (MFI)

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Abstract

Micro-Flow Imaging (MFI) has become a standard application for particle analysis of biopharmaceuticals because of its ability to easily detect particle size and morphology for a diverse range of particle contaminants, including translucent protein fragments and silicone micro-droplets (in-process revision <787>, 2013, and L Narhi, 2012). Automating particle analysis by MFI offers key advantages, through reproducible sample handling and higher throughput with less hands-on time.

In this poster we demonstrate the comparability of a MFI manual and automated method, using a model protein system comprised of 1% BSA. The manual and automated modes were evaluated for counting and concentration accuracy. A minimum of 8 replicates for each condition, at two separate laboratories (ProteinSimple in USA and Takeda in Denmark) were performed with no statistically relevant differences observed between the two modes.

Introduction

MFI systems, from ProteinSimple, are well-suited to particle characterization of biopharmaceuticals, providing quantitative data on particle size, count, and shape for a broad range of protein-based pharmaceuticals.

In the Takeda Pharmaceutical development laboratory, MFI was implemented due to its advantages over conventional techniques (HIAC-based light obscuration used according to Ph. Eur. 2.9.20/ USP <788>) for measurement of sub-visible particles in the µm range (e.g. 2–10 µm). These advantages include:

- Lower sample consumption versus light obscuration
- 1–10 µm particle detection
- Ability to distinguish between particle types based on morphology (Figure 1)
- Automated sample delivery

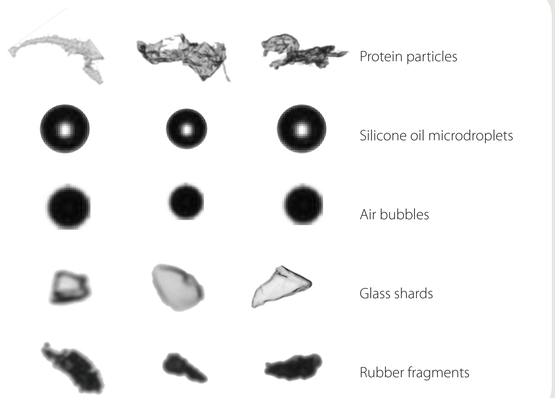

FIGURE 1. MFI detects protein particles, silicone oil microdroplets, air bubbles and other contaminants, from 1–300 µm in size.

Many protein formulations are fragile and highly sensitive to sample preparation and handling techniques. Consistent sample handling is critical to minimize run-to-run and instrument-to instrument variability. An automated sample introduction system can provide these advantages, but without significant changes to the level of particles and protein aggregates.

In this poster we describe optimization of an automated sample delivery method and compare it to results obtained by manual sample delivery. This study was performed at two separate locations (customer site and ProteinSimple), with different operators. The joint study was initiated to allow Takeda to transfer all MFI methods to from manual to automated analysis.

Materials & Methods

Materials

Protein test solution

- 1% BSA, heat-treated overnight

Wash solutions

- ddH_20, 10% Triton X-100

Methods

- Analysis of 1% BSA using manual or automated format: automated method optimization shown in Figure 2, below.
- Each sample was run at least eight times in both the manual and automated modes at two different laboratories.
- Average mean and %CV of replicates

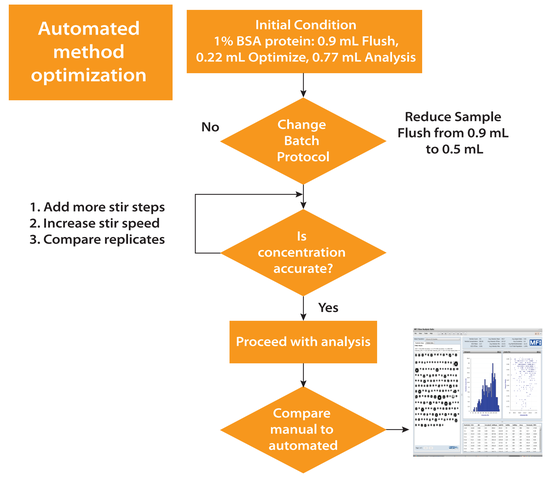

FIGURE 2. Methods are easily optimized by evaluation of stirring and sample volume parameters via a simple flow chart.

Results and Discussion

Method Transfer & Optimization

Manual to automated protocol transfer requires modification of sample handling.

Below are the key differences:

- **Sample introduction differences**
  Automated pipettors mix the sample differently than manual techniques such as manual pipetting or introduction via syringe barrel with stirrer.
- **Sample and purge volumes**
  Volumes may change due to and fluid path (see Figure 2).

Optimized Methods User for Comparison Study

The method parameters for the original manual method and the optimized automated method are shown in Table 1.

	MANUAL OPERATION	AUTOMATED OPERATION
Bot1 Flush with Sample	N/A	0.5 mL
Sample Volume Dispensed (defined in Method)	0.9 mL	0.77 mL
Sample Purge Volume (defined in Method)	0.2 mL	0.0 mL
Optimized Illumination Volume and Liquid Type	0.22 mL of buffer (MFI 5200)	0.22 mL of sample (MFI 5200)
Dead Volume	0.1 mL	0.03 mL
Total Sample Volume	1.2 mL	1.52 mL

TABLE 1. Method used for the comparison study.

Analysis of variability in manual versus automated format on the Bot1

The automated format produced consistent counts and concentration data, compared to the manual format with 1% BSA protein solution. The results for both sites are shown in Figure 3. The %CV achieved for both methods at both sites were within the expected range of variability.

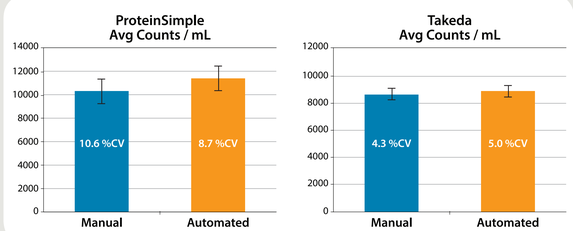

FIGURE 3. Manual to automated method transfer results in similar concentration values for 1% BSA.

ANOVA Multifactor Analysis for Particle Concentration

Employing a standard analysis for statistical variance (ANOVA) indicated that only between 0 and 4% of variance in particle counts can be attributed to method type (automated versus manual, Figure 4), which is not significant. Regression analysis showed no relationship between other input variables such as day-to-day variation, and variance in the data (Figure 5).

Results at Takeda were approximately 2000 counts/mL lower than those at ProteinSimple, likely related to sample quality due to sample stress or degradation in shipment.

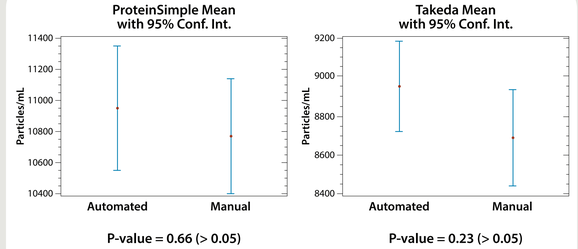

FIGURE 4. ANOVA one-way analysis was used to evaluate impact of method type on concentration. Analysis shows no significant impact on particle concentration based on method type.

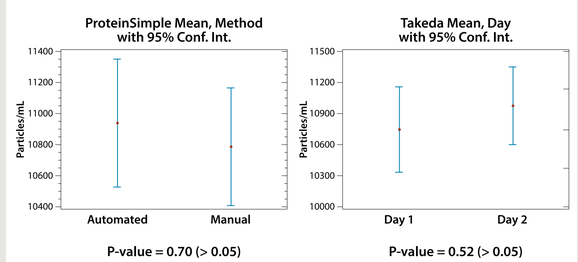

FIGURE 5. Linear regression analysis showed no significant impact of system inputs (automation, day to day variance) on particle concentration.

Conclusions

The MFI 5200 produces the same high quality particle characterization in manual or automated mode. This study showed that method optimization could further reduce the sample volume required without impairing concentration accuracy. Statistical analysis confirmed that these protocols are robust and provide an example of standardization of methods across instrument configurations.

The option to automate provides a key advantage for particle characterization of protein formulations, offering many benefits compared to more common techniques. Automated protocols allow for much greater throughput and less hands-on time, with up to 80 samples per unattended run. Implementation of automation for higher throughput and standardization can help address demand for more rapid and consistent screening methods in particle characterization.

References: AAPS update on USP expert committee for subvisible particle analysis, L Narhi, *Newsletter of the AAPS Aggregation and Biological Relevance Focus Group*, 2012; 3(2).