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Spotlight

AAV Thermostability Assessment with Micro-Flow Imaging

The presence of aggregates and particulates in AAV-based gene therapy products can negatively impact their safety and efficacy. These impurities can result from the manufacturing process and a host of environmental conditions including temperature[1]. Classified as sub-visible particles (SVPs), aggregates in AAV-based products need to be visualized and accounted for as per the guidelines in USP General Chapters <787>, <788>, and <1787>.

Light obscuration (LO) and membrane microscopy are analytical methods detailed by USP <787> and <788> for the analysis of SVPs. However, these methods are limited in their ability to provide critical details such as particle morphology, size distribution, analysis of transparent particles and more. To address these drawbacks, automated and sophisticated techniques such as micro-flow imaging (MFI) are increasingly becoming the choice for SVP analysis.

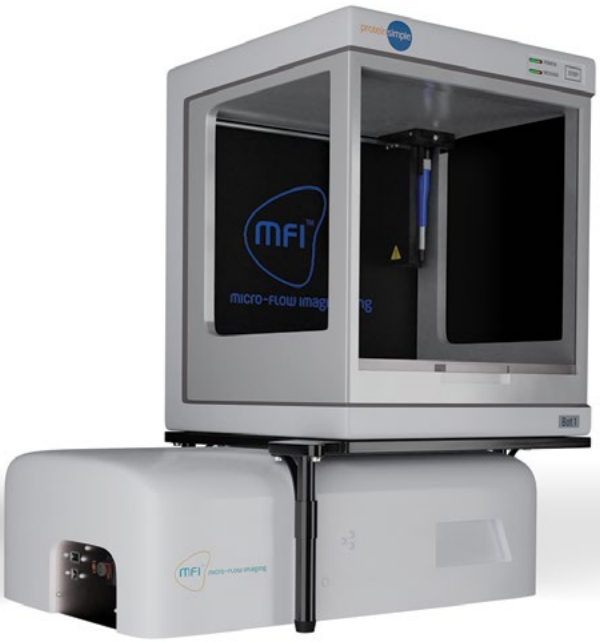
This spotlight demonstrates the use of the micro-flow imaging (MFI) system in evaluating the purity and stability of AAV9 particles subjected to heat stress at two different temperatures: 37°C and 75°C.

Key takeaways:

- The MFI 5200 system was used to evaluate the thermostability of AAV9.
- Subvisible particles (1-100 µm) were evaluated for heat-stressed samples, providing critical insights into sample stability and purity.
- The MFI system provides real-time visualization of different subvisible particles and offers several benefits compared to other USP-accepted methods, allowing scientists to make informed decisions while developing AAV-based gene therapies.

Benefits of MFI

- Make key decisions on purity and formulation by visualizing different particle types in real time.
- Get information on particle size distribution and shape simultaneously.
- Characterize different particle populations based on morphology.
- Save time and labor by automating sample handling and flow control.



Materials and Methods

AAV9 samples (1 x 10^9 VP/mL) encapsulating the eGFP gene were used in this study*. One set of AAV9 samples was stored at 37°C for two hours, and the other set was stored at 75°C for two hours. All samples were analyzed on the MFI 5200 using a 100 µm Flow Cell, 1.6 mm SP3, Silane Coating (Bio-Techne, PN 4002-002-001). Prior to analysis, the instrument was calibrated with 10 µm NIST Certified Particle Size Standard (Bio-Techne, PN 4004-002-001) and with 5 µm Particle Concentration Standard (Bio-Techne, PN 4004-003-002) to ensure correct instrument operation. The volume dispensed was 0.9 mL; the sample purge volume was set to 0.2 mL. The sample volume analyzed was 0.61 mL. Optimization illumination was performed with water, and a water baseline was established before running samples. A water flush was run between samples, and after running samples containing AAVs, the system was flushed with 10% bleach. Data acquisition was enabled with the MFI View System Software (MVSS) version 5.1. Data processing and custom filter generation were done with the MFI Image Analysis software version 1.1.

*The samples were procured from our customers, therefore details on sample preparation are considered confidential.

TABLE 1. Analysis of heat-stressed AAV9 samples using MFI.

SVP Count in Heat-Stressed AAV9 Samples Grouped by Particle Size (ECD)										
ECD (µm)	1-2	2-4	5-9	10-14	15-24	25-39	40-49	50-69	70-100	Total Count/ mL
-80°C	40391	14263	2282	242	69	16	3	0	0	57267
37°C	66649	35447	10745	2330	1249	328	92	39	16	116894
75°C	31559	8815	1678	324	213	75	10	3	0	42718

Table 1. Analysis of heat-stressed AAV9 samples using MFI. Samples were heat-stressed at 37°C and 75°C and compared with a control sample stored at the recommended temperature (-80°C). A decrease in the number of SVPs is observed at the highest temperature.

Results

Table 1 provides a breakdown of particle counts detected after heat-induced degradation of AAV9 samples. The particles are grouped according to size, i.e., equivalent circular diameter (ECD). These results were compared with SVPs detected in AAV9 control samples stored at -80°C, which is the recommended storage temperature.

The observed increase in overall particle count at 37°C is expected and is suggestive of aggregate formation. Interestingly, at the highest temperature (75°C), an overall decrease in particle count was observed. Denaturation of AAV9 has been reported at 76.2°C through differential scanning fluorimetry (DSF) experiments in another study[2], which is a plausible explanation of the low particle count observed with MFI. The bar graph (**Figure 1**) illustrates a comparison of the difference in particle counts between the control and heat-stressed samples.

The MFI system enables in-depth characterization of various SVPs through the MFI Image Analysis Software, which can filter particles based on ten different morphological characteristics. As a result, you can easily distinguish between silicon oil droplets, air bubbles, protein aggregates, and glass shards among many other common SVPs. **Figure 2** shows different particles captured by the MFI system across different size groups in this study for both sets of heat-stressed samples.

FIGURE 1. Bar graph of the number of SVPs detected with MFI.

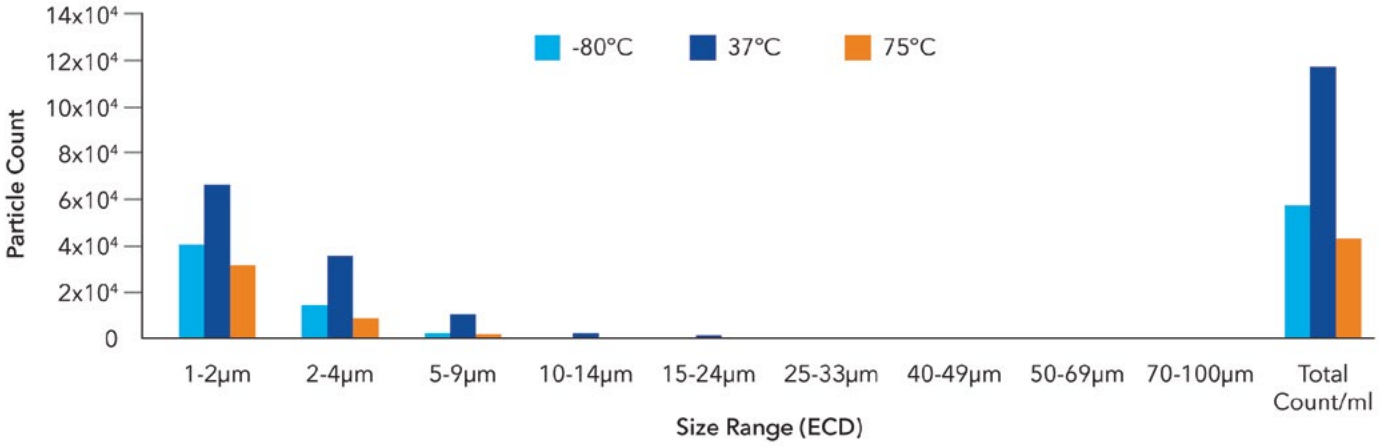


Figure 1. Graphical representation of the number of SVPs detected with MFI. A sharp increase in particle number is visible as the temperature increases to 37°C, followed by a significant decrease when heated to 75°C, indicative of sample denaturation.

FIGURE 2. Images of subvisible particles captured by the MFI system.

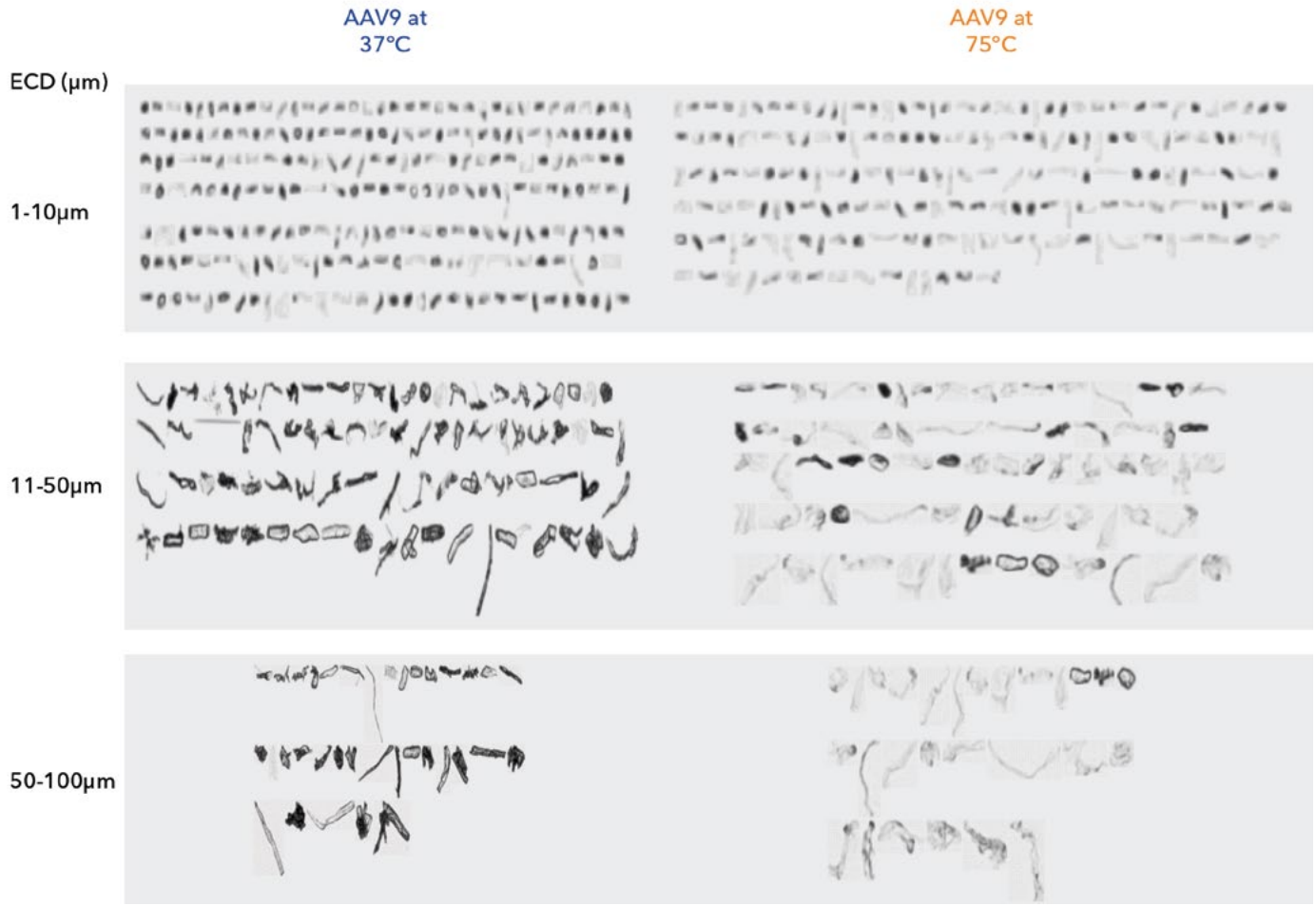


Figure 2. Images of different subvisible particles captured by the MFI system. The example above shows how the MFI Image Analysis software enables the classification of particles based on size (ECD). More importantly, the different types of particles are clearly visible within each size group, providing insights into aggregates, other impurities, and sample degradation resulting from temperature stress.

Conclusion

The thermostability of AAV samples is known to be serotype dependent[3]. During development, subjecting AAVs and other biotherapeutic samples to different temperatures and analyzing them for impurities and stability is routine, especially for establishing formulation conditions and shelf-life. Monitoring them for particulates is also critical to ensure efficacy and reduce the risk of immunogenicity. The study described in this spotlight evaluated the thermostability of AAV9 samples by treating them at two different temperatures, 37°C and 75°C, and analyzing them for the formation of aggregates. While aggregates and other SVPs were detected at 37°C, a temperature as high as 75°C didn't result in a high number of aggregates or SVPs, possibly due to sample degradation.

The MFI system is an orthogonal technique to light obscuration and membrane microscopy for the analysis of SVPs, which are crucial in formulation development and stability studies. By letting you get information on particle size, distribution, shape, and classification on several morphological parameters, MFI lets you make informed decisions on the stability and purity of your AAV-based gene therapeutics. To learn more about MFI, visit our website.

References

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