APPLICATION NOTE

Optimizing and monitoring AAV capsid purification using mass photometry

The Samux^{MP} mass photometer can quickly measure the empty/full capsid ratio during AAV downstream purification, making it ideal to use when optimizing and monitoring AAV purification processes. As the Samux^{MP} requires very little sample at low concentrations, it can be applied even from the earliest stages of process development – when sample amounts and concentrations are particularly limited.

With growing interest in AAV-based gene therapies comes an urgent need for efficient ways to optimize AAV production, particularly downstream purification processes. Optimal AAV purification processes would maximize the yield of loaded capsids while minimizing the amount of empty capsids and impurities. Achieving this requires the ability to determine, at each stage of the purification process, what proportion of the sample contains full vs. empty capsids as well as impurities. Such measurements are difficult at present because existing technologies tend to be costly and slow, require prohibitively large amounts of sample, or have limited accuracy. As a result, it is challenging to gather the data required to optimize the downstream processing of AAVs – particularly early in process development, when there is often very little sample available.

In this application note, our collaborators at the CDMO Pharmaron Gene Therapy show that the Samux^{MP}, a mass photometer optimized for AAV analysis, can provide information that is crucial for process development and monitoring downstream purification – quantification of the proportions of empty and full capsids, and detection of impurities. The Samux^{MP} features mass photometry technology, which is ideal for quantifying AAV loading because it measures the mass of individual particles without being affected by particle size or shape. The Samux^{MP} provides results quickly using little sample.

Pharmaron Gene Therapy used the Samux^{MP} to analyze AAV samples collected at various stages during their development of upstream and downstream purification processes, from harvest to chromatographic column separation (Fig. 1).

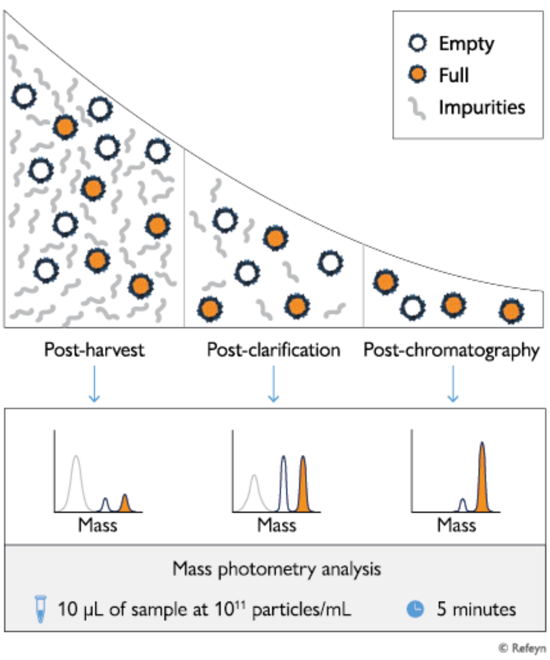

Fig. 1 Mass photometry provides valuable information throughout downstream purification processes. At each of the AAV purification steps covered in this application note (top row), mass photometry could be used to quantify empty and full capsids as well as to detect cellular debris.

Samux^{MP} showed AAV enrichment after clarification

The Samux^{MP} mass photometer was used to characterize the product after harvest and clarification. The resulting mass histograms indicate that the clarification step removed cellular debris, increasing the proportion of AAV capsids (Fig. 2). The peaks at the lower mass range (<2.0 MDa) correspond

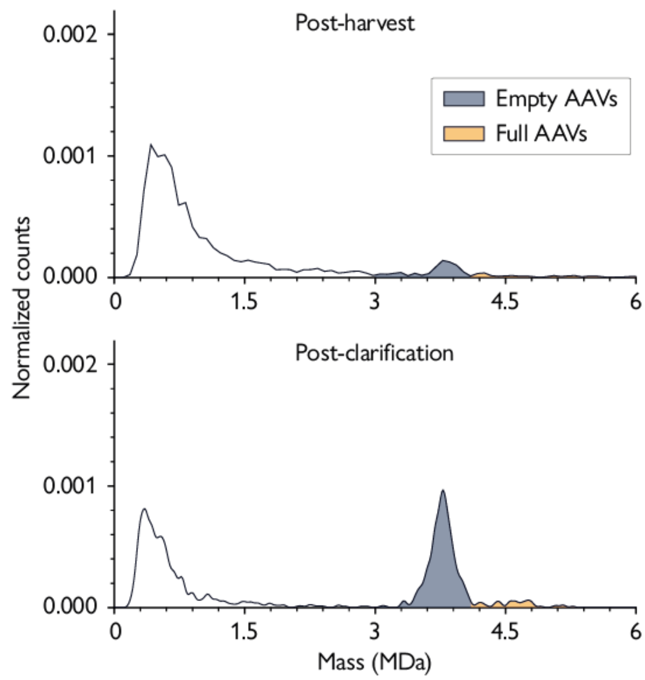

Fig. 2 Mass photometry was used to characterize the product after harvest and clarification. The mass histograms obtained showed that the proportion of particles in the AAV capsid mass range (>3.5 MDa) increased dramatically after clarification relative to the lower-mass particles (likely impurities). Data were obtained by the CDMO Pharmaron Gene Therapy using the Samux^{MP}.

to impurities, while the peaks in the higher mass range (>3.5 MDa) correspond to AAV capsids with varying payloads.

The Samux^{MP} confirmed column binding

The Samux^{MP} was successfully applied to confirm the binding of the AAV capsids to the chromatography column. Analysis of the flowthrough using the Samux^{MP} showed an absence of particles in the AAV capsid mass range (>3.5 MDa) and a large peak around 355 kDa, indicating flow-through of the cellular impurities (Fig. 3).

The Samux^{MP} resolved empty vs. full capsids

The Samux^{MP} was next used to monitor elution from the chromatography column. Seven elution fractions were collected from the column and samples from each were analyzed (Fig. 4). The resulting mass histograms revealed variability in the percentage of full capsids over the course of the elution, with the fourth elution fraction being clearly the most enriched for full capsids in this case. The earliest and later fractions also contained some lower-mass particles. This information, which was quick to obtain, could be readily used to select which elution fractions to pool for further processing.

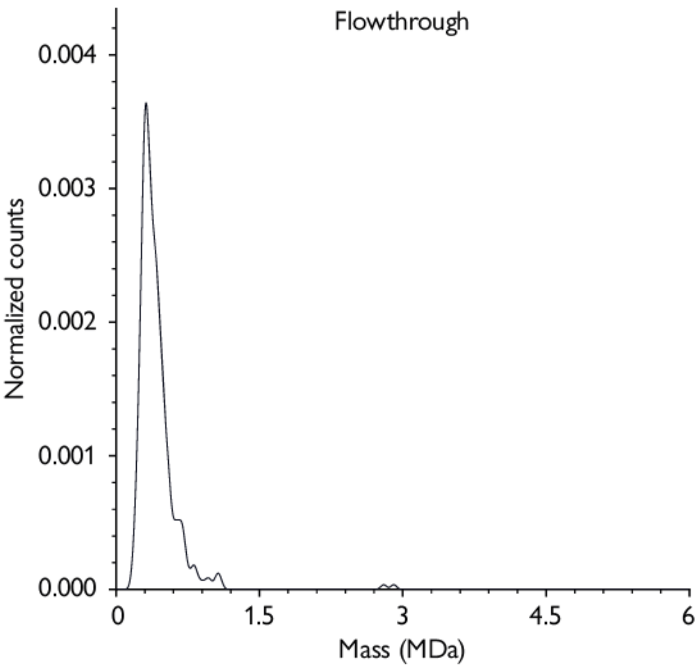

Fig. 3 Mass photometry confirmed column binding. Mass photometry analysis of the flowthrough from a chromatography column used for AAV capsid purification showed an absence of particles in the AAV capsid mass range, confirming that the capsids had bound to the column. Data were obtained by the CDMO Pharmaron Gene Therapy using the Samux^{MP}.

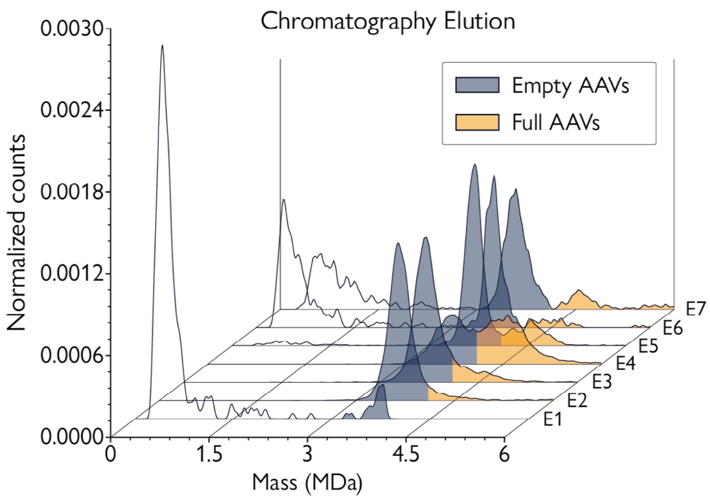

Fig. 4 Mass photometry quantified the empty-to-full ratio during the elution. Mass histograms were obtained for samples from each of seven elution fractions (E1–E7) from an unoptimized downstream purification process. They show that the amounts of empty capsids, full capsids and lower-mass particles varied across the fractions. Data were obtained by the CDMO Pharmaron Gene Therapy using the Samux^{MP}.

Conclusion

The Samux^{MP} mass photometer can clearly quantify the proportions of empty and full capsids present in AAV samples obtained at various stages during downstream purification, and can detect impurities. This information is valuable for characterizing AAVs products – enabling the monitoring of capsid purification and supporting process development. The Samux^{MP} makes it possible, for example, to quickly and easily identify which elution fractions are most enriched for full capsids, and compare purification conditions.

The Samux^{MP}'s accuracy in measuring empty-to-full ratios is comparable to the gold-standard techniques analytical ultracentrifugation (AUC) and cryogenic transmission electron microscopy (cryoTEM),[1] and the Samux^{MP} offers numerous advantages. First, it requires very little sample (10-20 µL) at a low concentration (10^{11} particles/mL), so it can be used even in initial optimization of process development, when sample volumes and concentrations are often limited. Also, the Samux^{MP} takes just a few minutes to complete a measurement, it fits on a benchtop, it requires minimal training and its operational costs are very low. Overall, the Samux^{MP} is an ideal tool for monitoring AAV downstream purification and guiding process development.

[1] *Refeyn, AAV analytics with mass photometry [Brochure] 2022.*

康龙化成 PHARMARON

Unit 9, Trade City, Sandy Lane West, Oxford OX4 6FF, United Kingdom

For information on products, demos and ordering, write to info@refeyn.com

refeyn.com
@refeynit
Refeyn
Refeyn