

Use of mass photometry for accurate and fast quantification of empty, partially filled, and full adeno-associated virus capsids for AAV-based gene therapies

By Kassiani Kytidou, Senior R&D Scientist, Eurofins BPT Netherlands and

Sabine van der Sanden, Head of Department, Viral Safety & Cell Banking, Eurofins BPT Netherlands.

AAV-based gene therapies: A rapidly expanding field

Adeno-associated viruses (AAVs) are small, non-enveloped viruses with a single-stranded DNA genome. They are increasingly used as vehicles for gene transfer in gene therapies. AAV-based gene therapies have emerged as a novel therapeutic modality in the past few decades, with over 200 clinical trials and six EMA/FDA approved therapies. AAV-based clinical trials target five main therapeutic areas: blood disorders, eye disorders, central nervous system disorders, neuromuscular disorders, and additional disease areas that are still under clinical trials, showing promising results and the significant impact on the patient community.

Product quality challenges in AAV manufacturing

AAVs are the most promising gene transfer viral vectors that show established long-term gene expression in different tissues. Constant improvements to recombinant AAV cassettes and capsids contribute to optimal gene delivery and successful therapeutic outcomes. However, inconsistency in AAV preparations during manufacturing processes leads to product heterogeneity and negatively affect the gene delivery. Additionally, impurities can influence bioavailability and biodistribution of the particles, potentially causing undesired immunogenic reactions.

The need for reliable capsid characterisation

To ensure the quality and regulatory compliance of AAV preparations, fast, robust, reliable, and GMP-compliant analytical methods are needed. A revolutionary method using Mass Photometry (MP) is now being implemented for GMP-compliant measurement of empty, full, and partially filled AAV viral particles, to ensure that the product falls within the justified release specifications for the amount of empty or partially filled particles that can impact the therapeutic outcome, as directed by FDA/EMA guidelines.

How MP works?

MP is a light scattering-based technique that detects individual, unlabelled molecules in dilute solutions. A single AAV particle, in contact with a glass coverslip, is exposed to a beam of light and produces a small, but measurable light scattering signal. This signal is directly proportional to the particle's mass. SamuxMP® from Refeyn Ltd. is a mass photometer tailored to the characterisation of AAV particles, using a method/software that is compliant with FDA 21 CFR 11 (US) and EU GMP Annex 11.

Advantages of MP for AAV characterisation

Using MP for AAV characterisation (empty/full/partially filled) has many advantages over the orthogonal techniques, such as analytical ultracentrifugation, transmission electron microscopy, and size exclusion chromatography coupled to multi-angle light scattering.

Monitoring AAV packaging throughout development

- Following AAV packaging (measurements of all subpopulations) in three stages:
 - Vector development stage (packaging efficiency, manufacturability)
 - Manufacturing process: optimisation of purification process
 - Final QC testing

Rapid and sample-efficient analysis

- Rapid analysis, requiring minimal sample amount (10-20 μ L, $1E11$ particles/mL) and sample preparation

Fast Turnaround Time

- Easy measurement with a very low turnaround time (2 min)

Broad applicability across AAV serotypes

- Applicable to all AAV serotypes without method adaptation

Eurofins BPT GMP implementation of MP

Eurofins BioPharma Product Testing Netherlands is now leading the implementation and development of this innovative method under GMP conditions, in accordance with ICH guidelines, to offer it as a new service to customers.

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