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

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Abstract

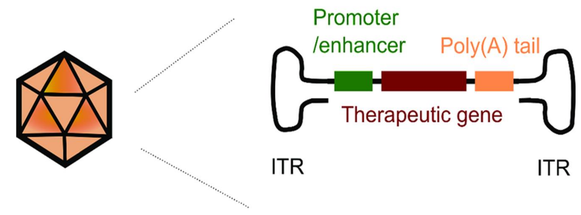
Adeno-associated viruses (AAVs) are small, non-enveloped viruses with a single-stranded DNA genome, commonly used as gene transfer vehicles. AAV-based gene therapies have emerged as a novel therapeutic modality in the past few decades, with over 200 clinical trials and 6 EMA/FDA approved therapies. Undoubtfully, 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 affects gene delivery. Additionally, impurities can influence bioavailability and biodistribution of the particles, potentially causing undesired immunogenic reactions.

To ensure the quality and regulatory compliance of AAV preparations, fast, robust, reliable, and GMP-compliant analytical methods are needed. The accurate measurement of empty, full, and partially filled AAV viral populations has become increasingly important in regulatory assessments by the FDA and EMA. A novel method employing Mass Photometry (MP) is now available for this specific measurement, showing important advantages over orthogonal techniques. MP is a light scattering-based technique that detects individual, unlabeled 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. The signal is directly proportional to the particle's mass. We present the comprehensive validation of this novel Mass Photometry (MP)-based method for GMP-compliant quantification of empty, full, and partially filled AAV viral particles, in accordance with ICH guidelines and FDA/EMA directives.

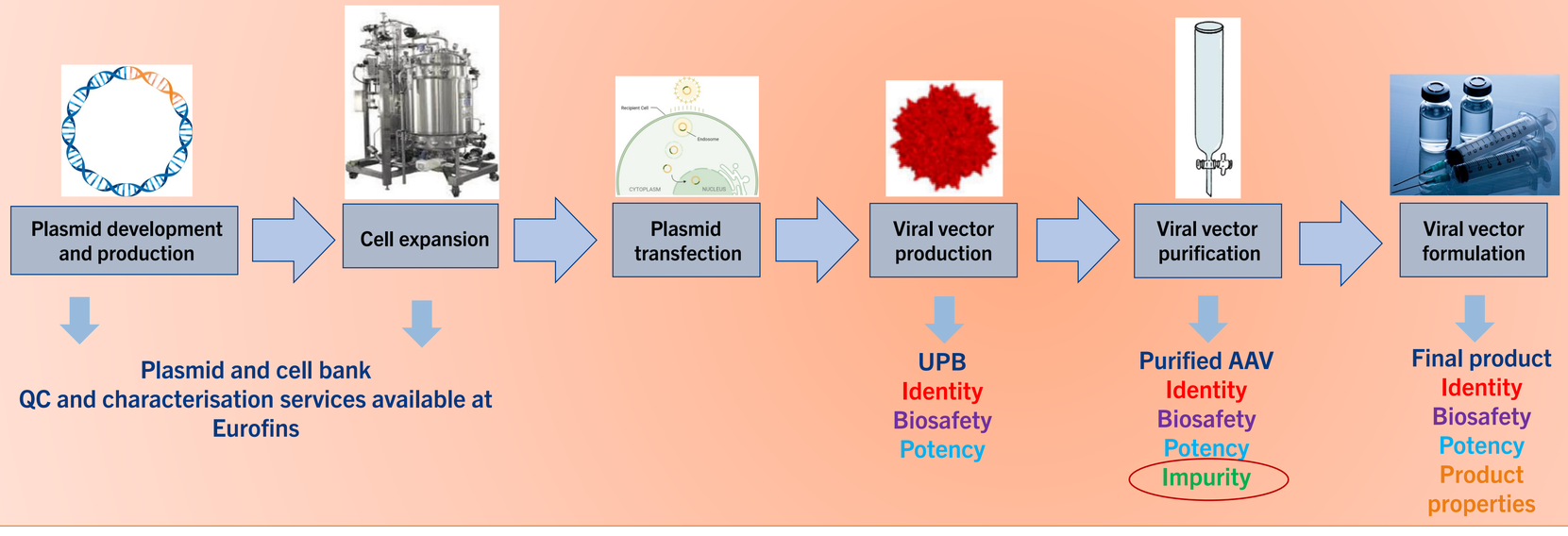
Using SamuxMP, a mass photometer from Refeyn, we show data on linearity, robustness and reproducibility among different AAV serotypes. Additionally, we demonstrate its application in final quality control (QC) testing, employing a fully validated and GMP-compliant method.

AAV Gene Therapies & Their Challenges

Adeno-associated viruses (AAVs) have become leading vectors in gene therapy due to their ability to deliver genetic material with high specificity and low immunogenicity. However, a major challenge in AAV production is achieving a high proportion of full capsids, those that contain the therapeutic DNA, versus empty capsids which lack genomic payload and contribute to unwanted immune responses and reduced efficacy. During manufacturing, achieving optimal genome packaging is complicated by the inherent inefficiency of the viral replication and encapsidation process. Several analytical techniques are currently employed to distinguish and quantify empty versus full AAV capsids, including analytical ultracentrifugation (AUC), transmission electron microscopy (TEM), and ion-exchange chromatography (IEC). Although AUC is considered as the gold standard for its resolution, it is time-consuming and low-throughput; TEM offers visual validation but it is qualitative and subject to operator bias; and IEC provides scalability but lacks the resolution to differentiate partially filled capsids accurately. These limitations highlight the need for more precise, scalable, and high-throughput analytical methods in AAV quality control.



AAV Quality Testing & Safety Requirements

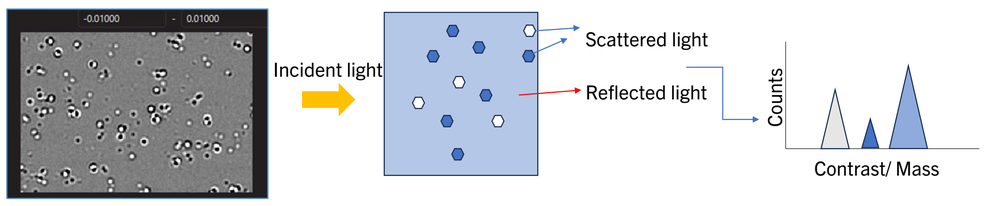


Regulatory references

- European Pharmacopoeia (Ph. Eur.) 5.14
- EMA guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products
- ICH Topic Q6B
- ICH Topic Q 5A
- ICH Topic Q5D
- USP ⟨1047⟩
- FDA Guideline for gene therapy manufacturing and testing (1225)

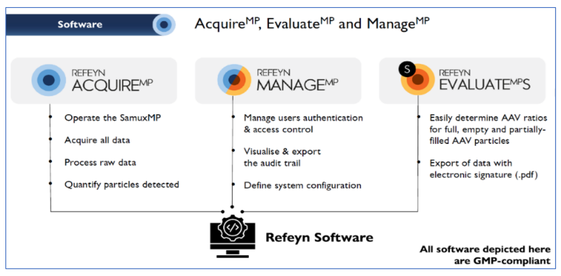
Mass Photometry for AAV Capsid Characterisation

Mass photometry (MP) is a cutting-edge, label-free technique that allows rapid quantification of AAV capsid populations based on mass differences. By measuring the light scatter of individual particles landing on a glass surface, MP can distinguish empty, full, and partially filled AAV capsids in solution with high accuracy and minimal sample volume. Unlike traditional methods like AUC or TEM, MP provides results within minutes, requires no staining or extensive preparation, and works at near-native conditions. Its main limitations are lower throughput and the need for calibration for different serotypes, but its speed, sensitivity, and simplicity make MP an attractive tool for AAV quality control in research and production settings.

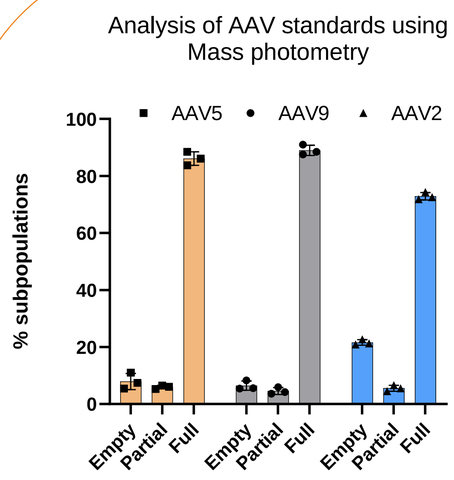


Key Advantages of Using Mass Photometry for AAV

- Rapid analysis, requiring minimal sample amount (10-20µL, of 2E+11vp/mL) and sample preparation
- Easy measurement with a very low turnaround time (2 min)
- Applicable to all AAV serotypes without method adaptation
- **GMP-compliant software (FDA 21 CFR 11 and EU GMP Annex 11)**
- Following AAV packaging (empty/full) in three stages:
  - 1) Vector development stage (packaging efficiency, manufacturability)
  - 2) Manufacturing process: optimisation of purification process
  - 3) Final DS QC testing



Measurements of 3 Different AAV Capsids

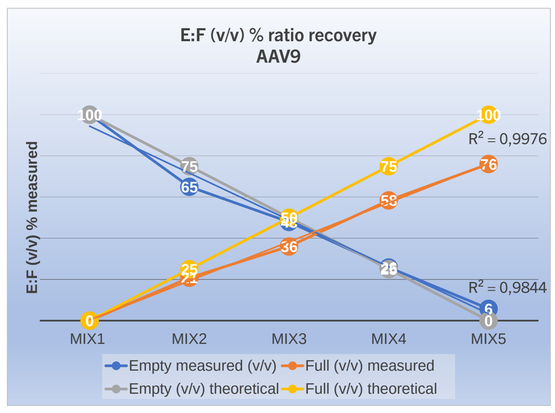


- Accurate/ Precise/ Robust
- Data in alignment with Orthogonal techniques

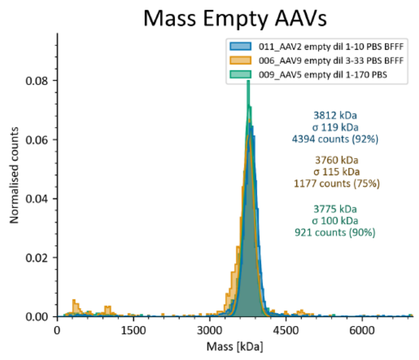
Orthogonal methods	AAV5 Standard	AAV9 Standard	AAV2 Standard
Full ratio Mass photometry	86%	89%	73%
Full ratio TEM	83 %	NA	NA
Full ratio-DLS-SLS-UV/Vis	84%	78%	58%
Full ratio-CDMS	79%	84%	75%

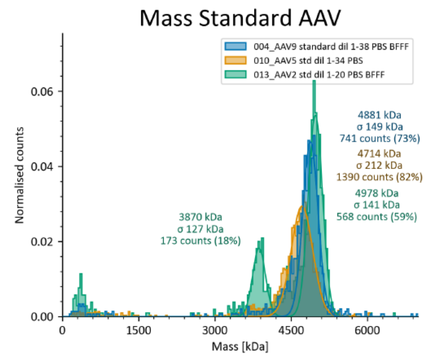
- Linearity of recovery; Empty / Full (E/F) ratios

Mixes (v/v) E/F ratio	Empty (v/v) measured	Full (v/v) measured	Empty (v/v) theoretical	Full (v/v) theoretical
Mix1	100	0	100	0
Mix2	65	21	75	25
Mix3	48	36	50	50
Mix4	26	58	25	75
Mix5	6	76	0	100



- Precise mass measurements with CV < 2%





Method Validation According to ICH (Q2) R2 Guidelines

Type of measured product attribute / Analytical Procedure Performance Characteristics to be demonstrated (2)	Identity	Impurity (Purity) Other quantitative measurements (1)		Assay content/potency Other quantitative measurements (1)
		Quantitative	Limit	
Specificity (3) Specificity Test	+	+	+	+
Working Range Suitability of Calibration model	-	+	-	+
Lower Range Limit verification	-	QL (DL)	DL	-
Accuracy (4) Accuracy Test	-	+	-	+
Precision (4) Repeatability Test	-	+	-	+
Intermediate Precision Test	-	+ (5)	-	+ (5)

Impurity Quantitative test (parameters for method validation)
Specificity
Linearity
Robustness
Accuracy
Precision (repeatability and intermediate precision)

Conclusions

Mass Photometry and the Refeyn SamuxMP allow:

- Easy determination of AAVs' Empty/Full ratio, including partially or overfilled populations (GMP)
- Enables a rapid estimate of the titer of different AAV sub-populations (non-GMP)
- Estimates the sample purity and degradation and observes the homogeneity of the AAV populations (non-GMP)
- Characterises and performs quality control (QC) of samples at multiple stages of the purification process (GMP)
- Mass photometry E/F AAV data align with orthogonal techniques

Eurofins Biopharma Product Testing Netherlands, Eurofins Microsafe Laboratories are leading the implementation and development of the method under GMP conditions, following ICH guidelines.