Minimizing the impact of stability studies on gene therapy batch yield.

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USP Stakeholder Forum

22nd Feb 2024

Inspired by **patients.**
Driven by **science.**

What makes GT analytical package unique?

1) Product is NOT the API

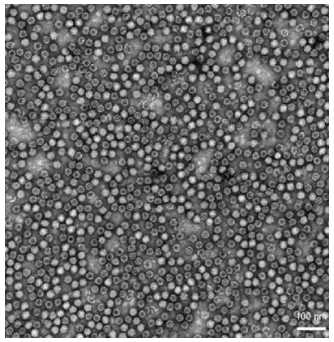
- Unprecedented complexity…

Mabs = ~20k atoms

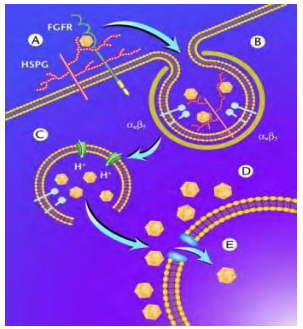
rAAV= > 190k atoms

- Raises new questions…

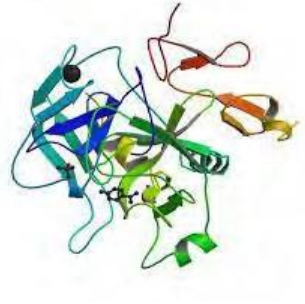
Is my input (plasmid DNA) of good quality?



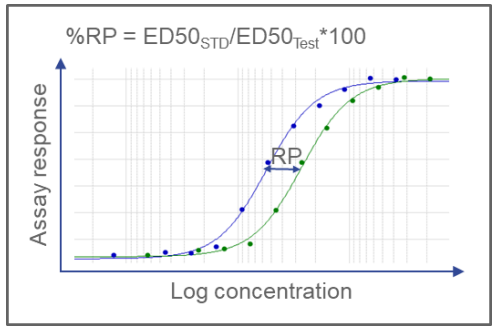
- How much viral particles in my product?
- Are they full, partially filled or empty?



Is my rAAV product infectious?



Is my rAAV product producing the API?



Is the produced therapeutic compound biologically active?

… that require additional tools in the analytical package

What makes GT analytical package unique?

2) Batch size is different

Typical batch size: GT **<!** mAbs

GT batch size (~500mL)

mAbs batch size (~20L)

Sample volume for in-process testing, DS & DP release/stability: QC GT ≥ QC mAbs (e.g.; DS release ~15mL)

… proportionally impact on batch yield is multiplied

QC impact on **GT** batch yield

QC impact on **mAbs** batch yield

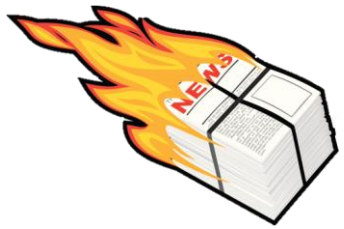
Mitigation strategies to limit QC/stability impact on batch yield

➔ More product to treat more patients!

Biophorum ATMP Workstream

Biophorum Position Paper

If you want to find out more about BioPhorum, feel free to contact Simon Walker's (simon.walker@biophorum.com), Steven Wall's (Steven.Wall@biophorum.com) or Rich Harrison's (Rich.Harrison@biophorum.com) and to visit Biophorum LinkedIn page



Position paper released the 5th of July 2023!

Mitigation strategies to limit QC/stability impact on batch yield

➔ More product to treat more patients!

Supportive Studies e.g., freeze/thaw, intermediate storage condition

- Use representative non-GMP material such as pilot or engineering batches

Sample Volumes

- Pooling strategy
  - Multiple assays share the same container (e.g., appearance, pH, osmolality)
  - Concurrent testing scheduling
  - Limit the number of testing sites
- Streamline the overages
  - Use a % of the total number of containers for duration of study as opposed to for each assay/timepoint
- Use of scaled down model for Drug Substance containers
  - Limit the excess volume that would not be needed to complete the testing at each stability timepoint

Adapted from BioPhorum. *Minimizing the impact of stability testing on gene therapy batch yield.* (2023)

Mitigation strategies to limit QC/stability impact on batch yield

➔ More product to treat more patients!

Study Protocol

- Length of DS stability studies should be limited to the time required prior to DP fill rather than set at an arbitrary number of years
- Attributes unlikely to change in the short term tested annually, not at every timepoint
- Where applicable, reduce the number of time points and temperatures evaluated

Analytical Methods

- Streamline stability package
  - Identify & limit assessment to stability indicating assays
- Focus on high sample consuming items
  - Support Pharmacopeias in adapting current compendial methods to GT specificities & constraints (e.g., sub-vis particles, bioburden, sterility, …)
  - Use surrogate approaches not consuming product (e.g., CCIT performed annually on "surrogate vials" in lieu of sterility)
  - Consider/switch to low sample volume technologies

Adapted from BioPhorum. *Minimizing the impact of stability testing on gene therapy batch yield.* (2023)

Mitigation strategies to limit QC/stability impact on batch yield

Impact on a model rAAV stability study

Baseline protocol

	GMP batch	
	DS	DP
Storage conditions (°C)	**Stability time points**	
<-60	1, 3, 6, 9,12,18, 24, 36 months	1, 3, 6, 9, 12, 18, 24, 36 months
-15 to -25	1, 2, 3 months	1, 2, 3 months
2 to 8	N/A	1, 2, 4 weeks
Total volume requirements	**81.9mL**	**412 vials**
% batch yield	**33%**	**82%**

~2-fold reduction

Optimized protocol

	GMP batch	
	DS	DP
Storage conditions (°C)	**Stability time points**	
<-60	3, 6, 9,12 months	3, 6, 9, 12, 18, 24, 36 months
-15 to -25	1, 2, 3 months	N/A
2 to 8	N/A	1, 2, 4 weeks
Total volume requirements	38.5mL	190 Vials
Savings (optimized vs. baseline)	**43.4 mL**	**222 Vials**
Re-calculated % batch yield [vs baseline %]	15% [vs baseline 33%]	38% [vs baseline 82%]

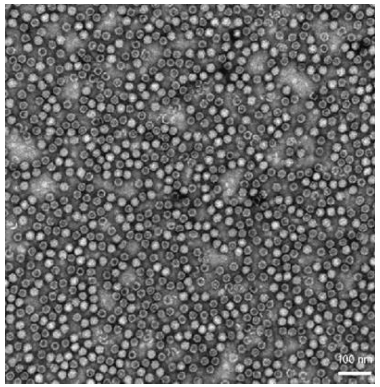
BioPhorum working group recommends that organizations discuss these approaches internally and with regulatory agencies to collaboratively identify efficient pathways for the development of CGTs.

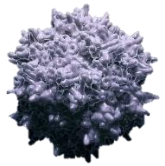
Inspired by patients. Driven by science.

Adapted from BioPhorum. *Minimizing the impact of stability testing on gene therapy batch yield.* (2023)

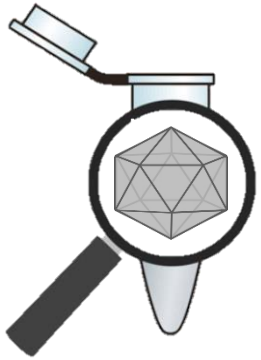
BioPhorum

Low sample volume technology: capsid distribution

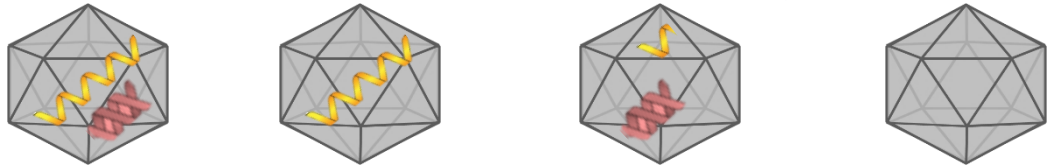


ssDNA + capsid = rAAV 

What does capsid distribution mean?



DNA Containing Capsids



Oversized Capsids	Full Capsids	Partially-filled Capsids	Empty Capsids
Encapsidated DNA is larger than 1 copy of the correct transgene	Correct transgene packaging	Packaging of partial transgene or unwanted packaged DNA (host cells, plasmids, …)	No DNA packaging

The impact/clinical outcome of partially filled & empty capsids is unclear
➔ Having good « sensors » to analyze capsid distribution is a key driver!

Capsid distribution assessment is part of the stability indicating package
➔ Having low sample volume technology would have a significant impact on the batch yield

AAV Empty Capsids: For Better or for Worse?, Molecular Therapy, 2014
Gimpel et al. Molecular Therapy, Methods and Clinical Development, Vol 20, March 2021
DHC White Paper i(Jan 2023) n public consultation untill April 2023

What are the most popular capsid distribution analytical tools?

Industry benchmark from the Biophorum "Full/Empty sub-team" (n = 20 respondents)

Most popular does not necessarily mean most appropriate technique for all applications

Most popular approaches: pro/cons

Characteristics	Vg/capsid titer ratio (PCR/ELISA)	AUC	SEC-MALS	TEM
Throughput	(+) High			
Ease of implementation	(+) Easy (part of the "standard" analytical package)			
Ease of Analysis	(+) simple ratio from Vg and Capsid titer data			
GMP QC readiness	(+) Software 21CFR part 11 compliant			
Sample volume requirements	(++) no additional volume to what used for Vg and capsid titer assessment			
Sample conc./purity requirements	(+) E10 capsids/mL, FFP from clarified harvest			
Partially filled capsid characterization	(--) Can't resolve partial capsids			
Assay performance	(--) Combined variability of two methods			

Most popular approaches: pro/cons

Characteristics	Vg/capsid titer ratio (PCR/ELISA)	AUC	SEC-MALS	TEM**
Throughput	(+) High	(--) limited number of Sample (4) / day	(+) High (12 samples per day)	(-) requires sample staining and is low throughput
Ease of implementation	(+) Easy (part of the "standard" analytical package)	(--): specialized equipment, specific skillset	(-) specialized equipment, specific skillset	(--) specialized equipment, specific skillset
Ease of Analysis	(+) simple ratio from Vg and Capsid titer data	(--) complex data treatment	(-) complex data treatment	(-) image analysis is challenging**
GMP QC readiness	(+) Software 21CFR part 11 compliant	(--) Software 21CFR part 11 compliant module NOT available	(+) Software 21CFR part 11 compliant	(--) Software 21CFR part 11 compliant module NOT available
Sample volume requirements	(++) no additional volume to what used for Vg and capsid titer assessment	(--) 100 to 500µl/sample	(+) 100µl/sample	(+) 3-20µl/sample
Sample conc./purity requirements	(+) E10 capsids/mL, FFP from clarified harvest	(-) E12/13 capsids/mL, FFP from affinity step	(-) E12 capsids/mL, FFP from affinity step	Not described**, cell debris can interfere with results
Partially filled capsid characterization	(--) Can't resolve partial capsids	(++) Quantitative measurement of partial capsids based on density	(--) Can't resolve partial capsids	(--) Can't resolve partial capsids
Assay performance	(--) Combined variability of two methods	(++) Limited variability	(+) Limited variability but result depends on an appropriate extinction coefficient (ε)	(-) small sample size can impact statistical significance and accuracy

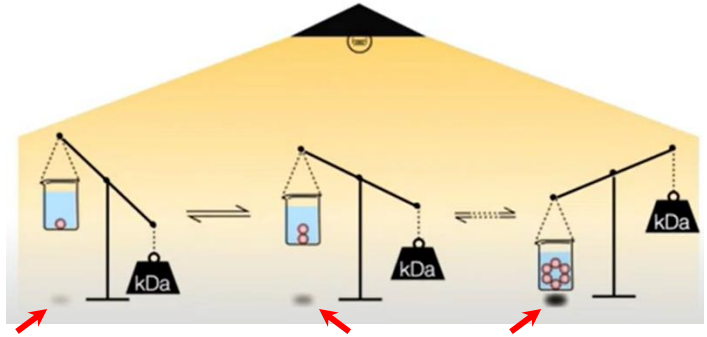
No 1-size-fits-all type of assay…

e.g., AUC = excellent characterization tools but less suitable for QC GMP release / SEC-MALS = excellent QC GMP tool but not able to resolve partial capsids

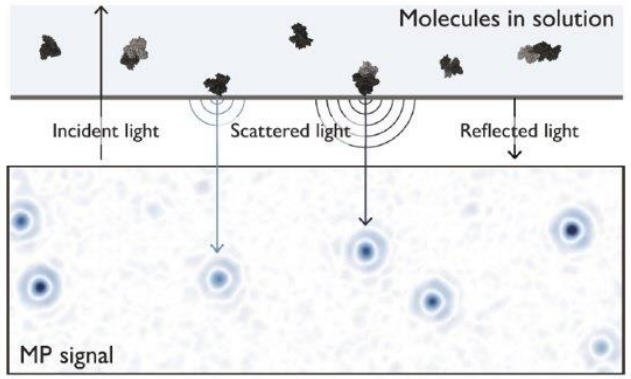
** Assessment adapted from Werle, et. al., "Comparison of Analytical Techniques to Quantitate the Capsid Content of Adeno-Associated Viral Vectors" (2021) and Gimpel, et. al., "Analytical methods for process and product characterization of recombinant adeno-associated virus-based gene therapies" (2021).

Emerging technology: Mass Photometry

Principle: weighting molecule with light

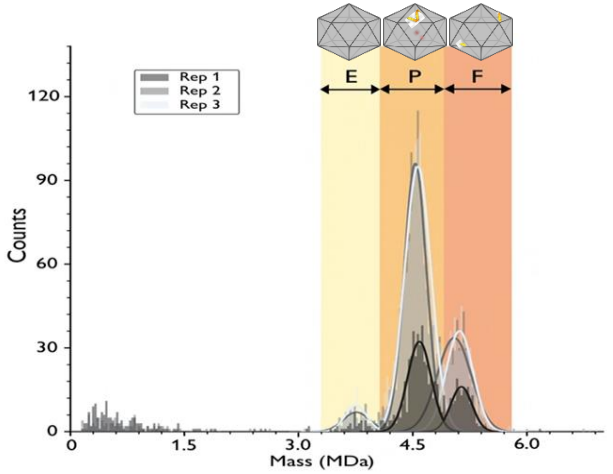


- *Molecules in solution*
- *You shine light on molecules*
- *The strength of the shadow is correlated with the mass of the molecule*



The light scattered by a molecule that has landed on a measurement surface interferes with light reflected by that surface. The interference signal is quantitated and scales linearly with mass.

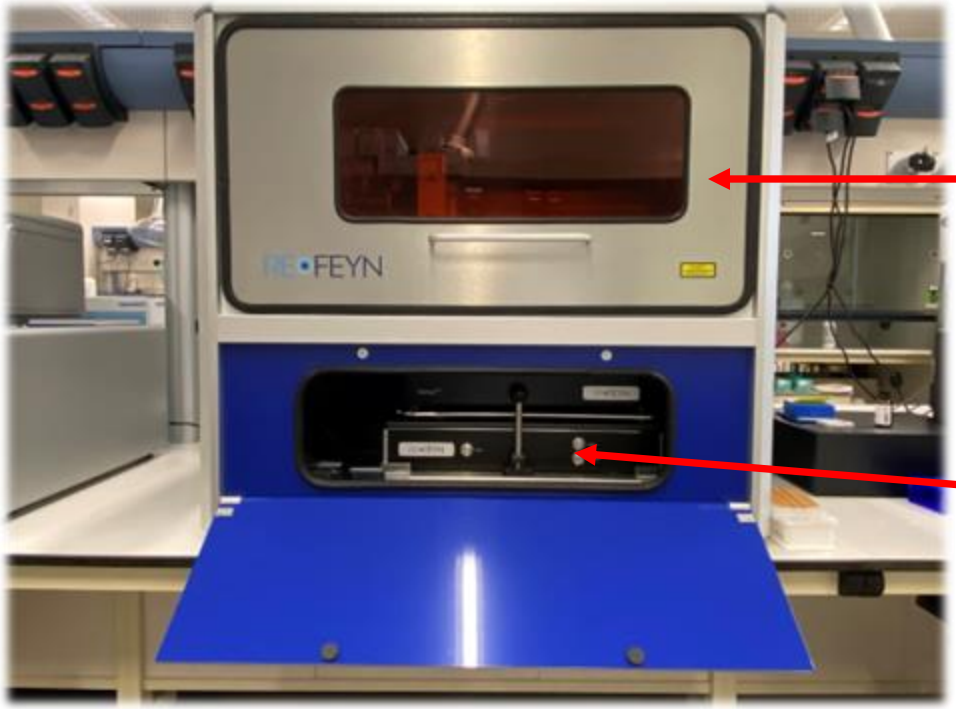
Mass photometry can be applied for the characterization of full, partially filled and empty capsids as it measures the mass of individual AAV particles in solution



Refeyn Mass photometer

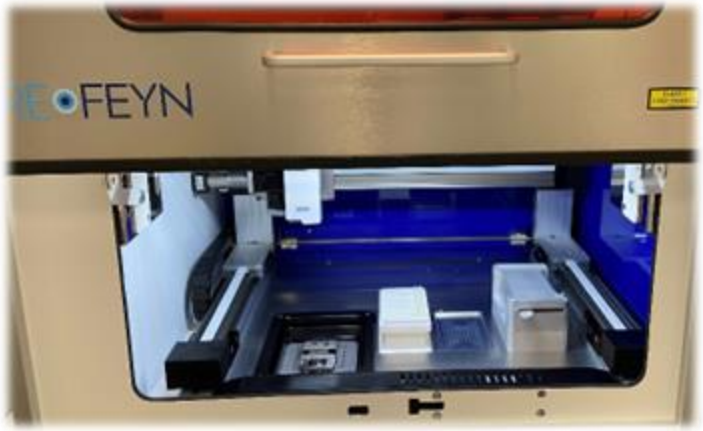
[Webinar] Measuring Molecules with Light - Prof. Philipp Kukura, University of Oxford - YouTube

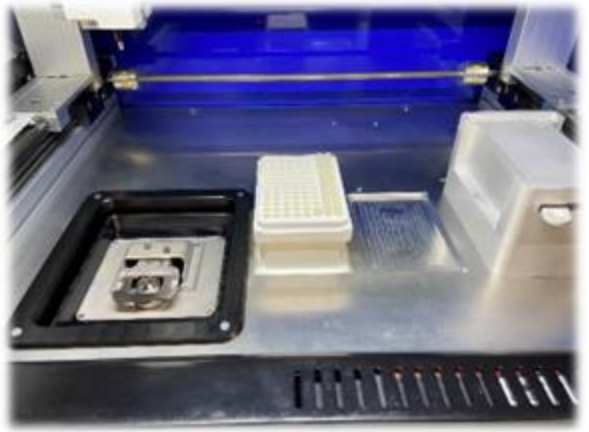
Emerging technology: Mass Photometry



Liquid handling system

Samux^{mp} reader





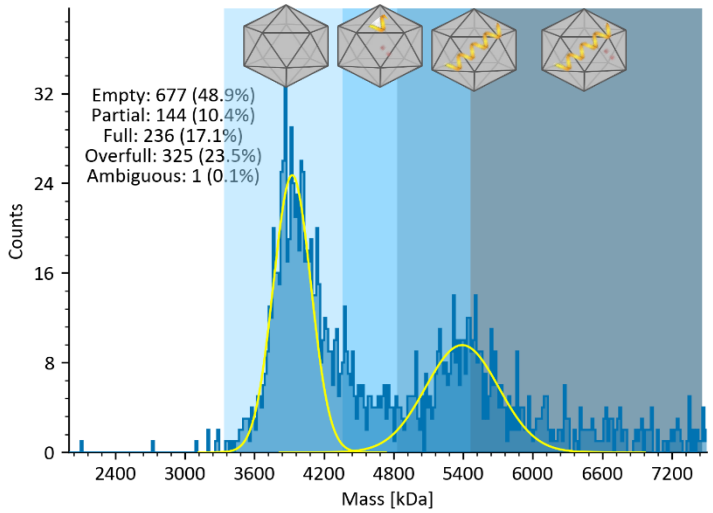
24 sample well cassettes (24 measurements in ~90 min)

Mass Photometry preliminary results

PRELIMINARY

Objective: Assess method performance on « control samples » (on 1 serotype at this stage)

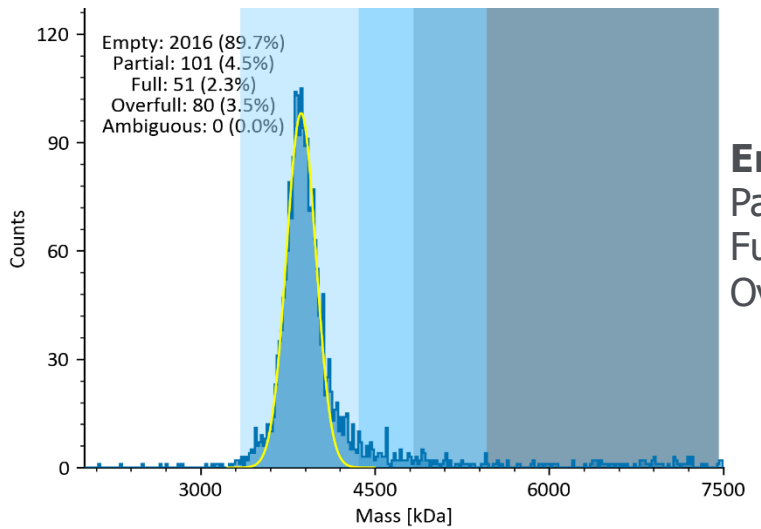
Affinity load (AEX load)



Average Mass:

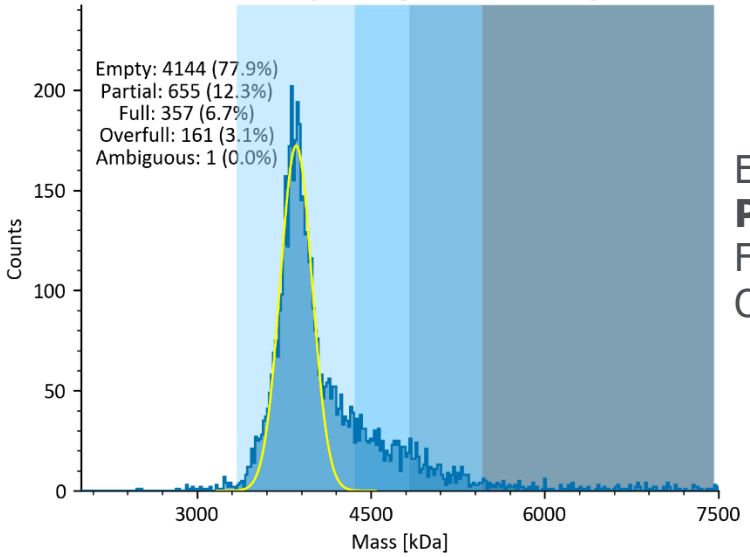
- Empty AAV= ~3.7 MDa
- Full AAV = ~5.2 MDa

Empty capsids (AEX fraction)



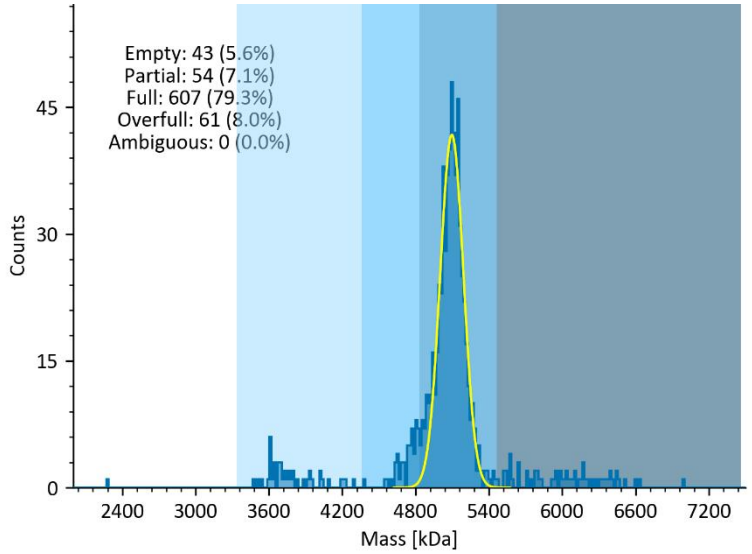
Empty 90%
Partial 5%
Full 2%
Overfull: 4%

Partial capsids (AEX fraction)



Empty 78%
Partial 12%
Full 7%
Overfull: 3%

Full capsids (AEX eluate)



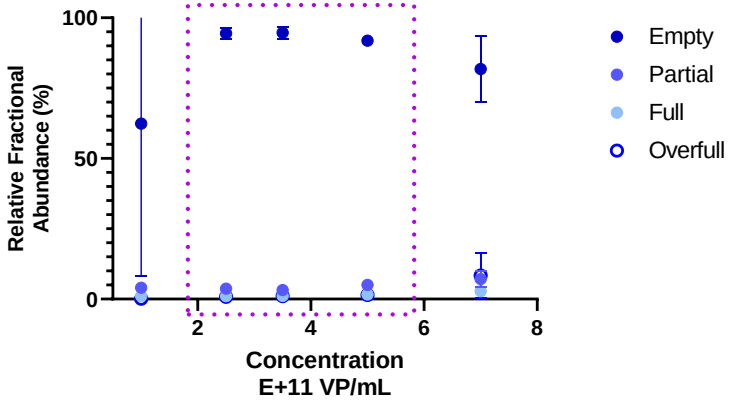
Empty 6%
Partial7%
Full 79%
Overfull: 8%

Method optimization

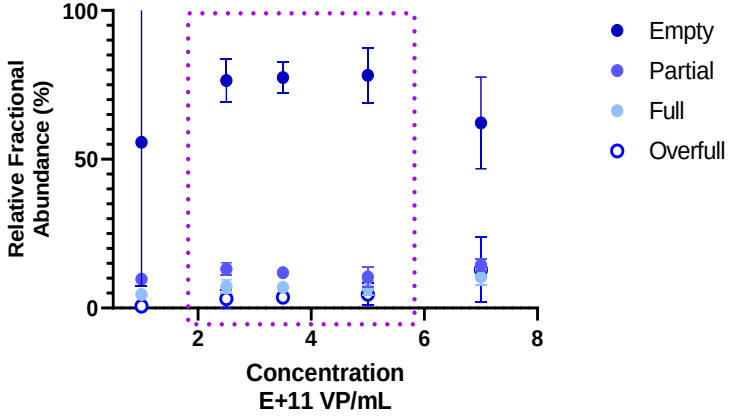
PRELIMINARY

Impact of the capsid load (from 1 to 7 E+11 Vp/mL) on the result (n=4)

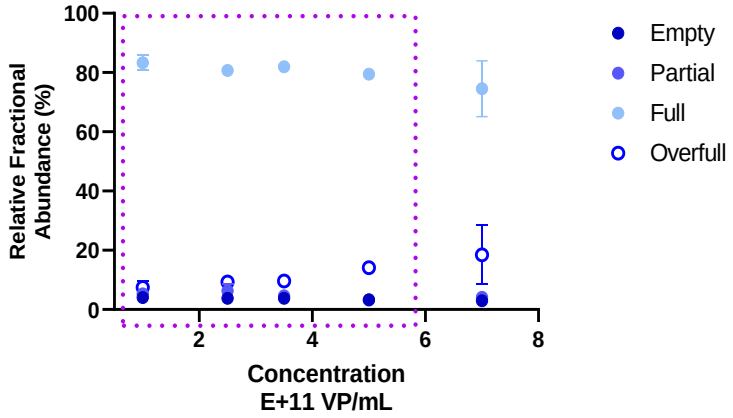
Empty capsids (AEX fraction)



Partial capsids (AEX fraction)



Full capsids (AEX eluate)



→ Consistent results observed across 2.5 – 5 E+11 Vp/mL

Preliminary precision (n=4) at 2 capsid load

Sample type	Capsid load			
	3.5E+11 (Vp/mL)		5E+11 (Vp/mL)	
	%Average	%CV	%Average	%CV
Empty capsids (AEX fraction)	94.7%	2.3%	91.9%	2.1%
Partial capsids (AEX fraction)	11.9%	15.6%	10.4%	31.5%
Full capsids (AEX eluate)	82.0%	1.7%	79.5%	0.4%

Good level of precision for the quantification of full and empty capsids. More variability observed for the quantification of partial capsids

Mass Photometry vs AUC & SEC-MALS

Characteristics	AUC	SEC-MALS	Mass Photometry (MP)
Sample volume	100 to 500µl/sample	100µl/sample	10µl/sample
Throughput	4 samples per day	~ 12 samples per day	Up to 42 samples per day (based on 3 runs per day using automated robot)
Time to result	Slow (equilibration step required, complex data treatment)	Slow (equilibration and chromatographic step required, data integration)	Fast (virtually no sample prep, quick data analysis)
Ease of implementation	• Specific technology • High expertise for data analysis • Low transferability	• Specific technology • High expertise for data analysis • Moderate transferability	• Specific technology • Data analysis with limited expertise • High transferability
GMP QC readiness	Not 21CFR part 11 compliant	21CFR part 11 compliant	21CFR part 11 compliant software released in 2023 and being tested now
Partially filled capsid characterization	Quantitative measurement of partial capsids	Quantifies DNA containing capsids (can't distinguish partial/full capsids)	Qualitative view on partial capsids

Assessment performed from internal data and supported by consultation-document---Characterisation-of-the-particle-population-in-AAV-products

Mass Photometry vs AUC & SEC-MALS

Characteristics	AUC	SEC-MALS	Mass Photometry (MP)	MP improvement
Sample volume	100 to 500µl/sample	100µl/sample	10µl/sample	10-50X less volume
Throughput	4 samples per day	~ 12 samples per day	Up to 42 samples per day (based on 3 runs per day using automated robot)	3-10X higher throughput
Time to result	Slow (equilibration step required, complex data treatment)	Slow (equilibration and chromatographic step required, data integration)	Fast (virtually no sample prep, quick data analysis)	Faster time to result
Ease of implementation	• Specific technology • High expertise for data analysis • Low transferability	• Specific technology • High expertise for data analysis • Moderate transferability	• Specific technology • Data analysis with limited expertise • High transferability	Easy data analysis and transferability
GMP QC readiness	Not 21CFR part 11 compliant	21CFR part 11 compliant	21CFR part 11 compliant software released in 2023 and being tested now	Currently, advantage to SEC-MALS in terms of data integrity but might be comparable based on the outcome of the on-going data integrity assessment
Partially filled capsid characterization	Quantitative measurement of partial capsids	Quantifies DNA containing capsids (can't distinguish partial/full capsids)	Qualitative view on partial capsids	Qualitative view on partial capsids due to mass range acquisition of current MP systems

Assessment performed from internal data and supported by consultation-document---Characterisation-of-the-particle-population-in-AAV-products

Mass Photometry : Next steps

- Comparison MP/SEC-MALS and AUC
  - Considering technology limitations (e.g. SEC-MALS measuring total DNA containing capsids)

- In-depth assessment of method Precision/Accuracy including:
  - ≠ capsid serotypes
  - ≠ process steps

Take Home Message

- QC impact on GT batch yield is HIGH
- Our goal: Deploy mitigation strategies to provide more product to treat more patients
- Implement strategies to streamline stability design & adapt sample consuming compendial test to GT CMC constraints
- Mass photometry is a promising low sample volume technology to study rAAV capsid distribution that contributes reducing sample impact on batch yield

Special thanks!

Wallonie

BioPhorum