LEUKOCARE

formulation expertise meets data science

Analytical methods to support formulation development of Adeno-associated virus

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Introduction

Background:

- Recombinant Adeno-associated viruses (rAAV) are widely used in gene therapy
- The benefit of optimal formulation is well recognized also for such drug products
- Analytical methods for characterization of rAAV are plentiful, typically cover capsid count, percentage of full rAAV particles, particle size, potential aggregate formation, capsid stability and infectivity.

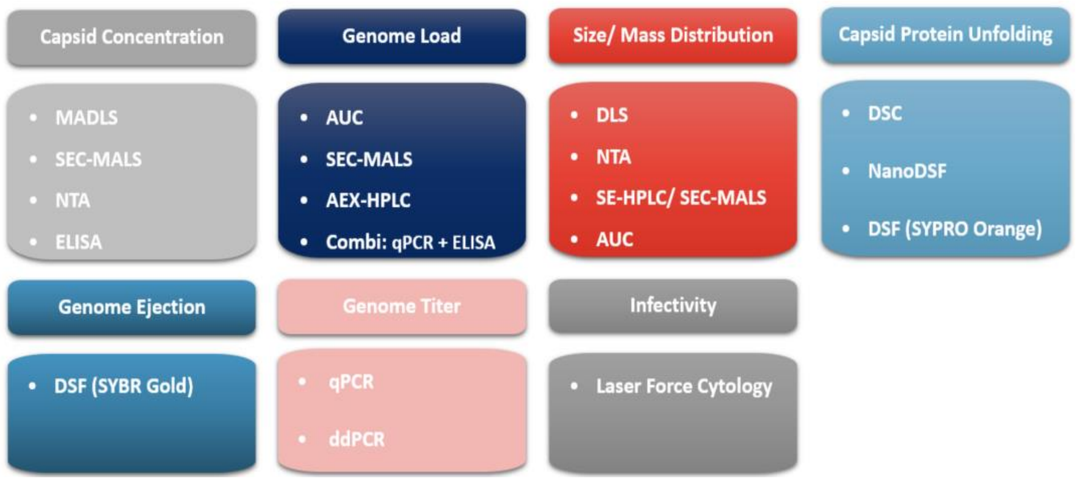
Determination of relevant state-of-the-art analytical techniques for formulation development needs with AAV:

- **Stability indicating & predictive power**
- **Low virus material requirement**
- **High throughput option**

Approach: Accelerated aging at 40 °C in reference buffer PBS/0.001% Poloxamer 188 (Ref) and in up to seven designed formulations (F01-F07).
Model: AAV-2 and AAV-5 with insert Factor IX, a common genome size

Methods

Analytical methods typically used in AAV analysis and tested in this context:



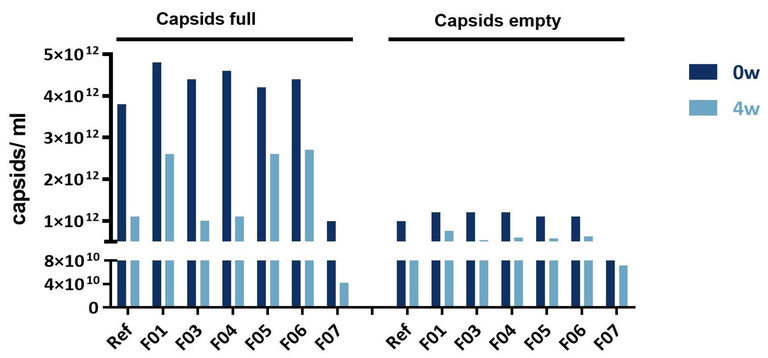
Results

Particle integrity and aggregation: Analysis by AEX-HPLC, SE-HPLC, MALS, MADLS, and AUC as a standard

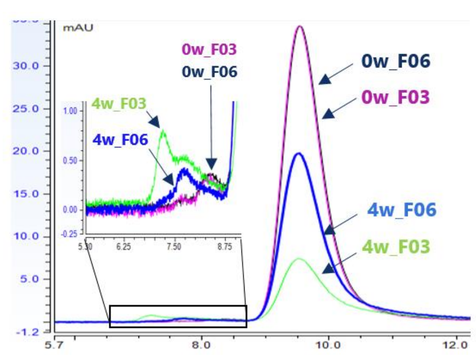
Analysis of AAV in reference buffer and formulations designed by Leukocare before & after thermal stress application (4 weeks at 40 °C):

- SE-HPLC & AUC indicated impact of thermal stress (reduction of full capsids) (AUC data not shown)
  → pattern of retained capsids after 4 weeks at 40 °C (stabilizing effect of the formulations) matched the pattern obtained with DSF (T_{m2}), nanoDSF, and DSC at t=0w
- SEC-MALS showed similar genome load as MADLS and qPCR-ELISA and additionally allowed aggregation analysis
- In AEX-HPLC, peaks of full & empty capsid were not separable after 4 weeks at 40°C, probably because of affected and changed net charge of capsids

A **Full capsids (AAV-2) quantified by SE-HPLC/UV/MALS**



B **Size distribution analysis of AAV-2 (SE-HPLC/UV)**



C **Determination of genome load (AAV-5)**

Analytical method	Determined genome load [% full]		Expected genome load [% full]
	Mean	SD	
MADLS	2.3**	n.a.	3.3 (*qPCR+ELISA)
SEC-MALS	3.9	0.1	
AUC	6.0	n.a.	

* measurement performed by AAV vendor
** calculated based on qPCR results

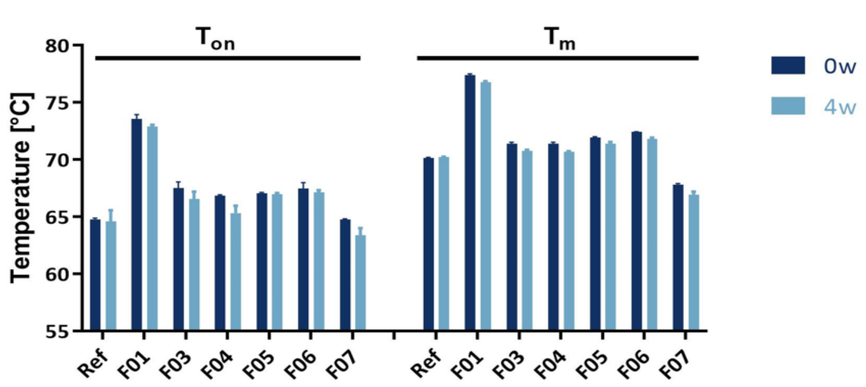
➢ **SEC-MALS & AEX-HPLC successfully assessed full/empty capsids.**
➢ **SE-HPLC /UV /MALS was stability indicating at accelerated aging condition, thereby discriminating between different formulations, and might replace AUC in formulation screening. SE-HPLC / UV / MALS combines genome load and aggregation analysis in one method.**

Capsid protein unfolding and genome release: Analysis by nanoDSF, DSF, and DSC as a standard

Analysis of intrinsic tryptophane fluorescence by nanoDSF, before and after thermal stress application (4 weeks at 40 °C):

- Stability pattern (T_{on}, T_m) matched expectations based on experience with formulations
- No relevant impact of thermal stress observed by nanoDSF & DSC performance
- nanoDSF matched DSC results very well (tested on AAV-5)

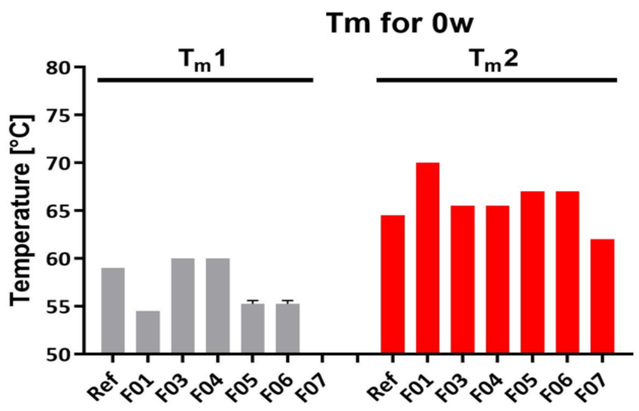
nanoDSF (AAV-2)



Analysis by DSF / extrinsic dye-mediated DNA fluorescence (DSF / SYBR Gold)

- T_{m2} pattern matched expectations & nanoDSF results on protein unfolding
- T_{m1} indicated genome release prior to capsid protein unfolding; despite high capsid protein stability, the pattern differed from T_{m2} results

DSF / SYBR Gold (AAV-2)



■ **T_{m1}: DNA leakage** prior to potein unfolding

■ **T_{m2}: DNA release** related to protein unfolding

➢ **All methods (NanoDSF, DSF, DSC) gave comparable results, could discriminate formulations, & predicted stability (t=0w vs SEC-MALS t=4w_40°C).**
➢ **But these methods did not show relevant effects of thermal stress (t=0w vs t=4w_40°C, not stability indicating), which were detected by SEC-MALS.**
➢ **DSF additionally provided information on DNA release prior protein unfolding detection, however, requires a fluorescent dye.**

Summary

	AAV attributes	Analytical method	Stability indicating	Stability predicting	Low material	High throughput	Comment
Predict stability	Capsid protein unfolding & genome release	DSC	x	✓	x	x	
		NanoDSF	x	✓	✓	✓	
		DSF/SYBR Gold	x	✓	✓	✓	Potential interference
	Infectious titer determination	Laser Force Cytology	x	x	x	✓	Stability indicating for replication competent viruses
Indicate stability	Particle size distribution	NTA	-	-	-	-	Only virus > 35 nm
		DLS	x	x	x	✓	
		MADLS[1]	x	x	x	x	
		SE-HPLC	✓	x	✓	✓	Only virus < 100 nm
	Capsid quantification & genome load determination	**SEC-MALS**	✓	x	✓	✓	Only virus < 100 nm
		AEX-HPLC	✓*	x	✓	✓	Only virus < 100 nm
		ELISA	✓	x	✓	✓	Genome load: + qPCR
		AUC	✓	x	x	x	
	Genome quantification	**ddPCR**	✓	x	✓	✓	
		qPCR	✓	x	✓	✓	

[1] also applicable for capsid quantification
* After 40 °C stress application: no separation of empty & full capsids possible

Conclusion

For each relevant formulation parameter at least one suitable method could be identified that fulfilled our requirements. These were defined to be stability indicating or predictive.

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- MADLS

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