Structural Analysis of AAV9 Derivatives Produced on Different Platforms and Comparison with Empty Capsid

#742

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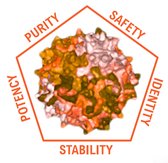
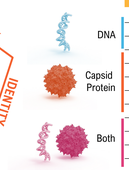
INTRODUCTION

- The goal of this study is to investigate the nature and impact of structural differences in AAV9 derivatives produced in insect and mammalian cell lines, including critical attributes stability, safety and efficacy. It may also inform the development of a purification scheme, regarding the separation and removal of product related purities such as empty capsids
- Approach 1: (AAV9 derivative 1, promoter A, B & C): examination of the effect of two different production platforms: what structural features differ and how does this affect the functional output?
- Approach 2: Direct comparison of 3 AAV9 derivatives (2, 3 & 4) with differing VP compositions and their empty equivalents: implications for functional output and chromatographic separation (%Full enrichment)



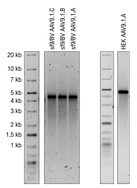


DETAILED CHARACTERIZATION	
ATTRIBUTE	**METHOD**
Vector Titer	ddPCR
Genome Integrity	Agarose Denaturing Gel
VP1,2,3 %Purity	CE-SDS (UV/LIF)
Surface Charge	CIEF
Sub –µm Aggregation	SEC/DLS
Primary Sequence/PTM	LCMS Peptide map
VP Exact Mass	LCMS Subunit analysis
Molar Mass/%Full	SEC-MALS
%Full	AUC/CsCl
Relative Potency	Protein Expression
Infectivity	TCID50

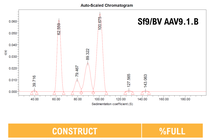
APPROACH 1

Figure 1. Genomic Integrity and Occupancy

Denaturing Gel
Efficacy

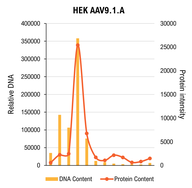


AUC (%Full)
Stability and Efficacy



CONSTRUCT	%FULL
Sf9/BV AAV9.1.A	70
Sf9/BV AAV9.1.B	71
Sf9/BV AAV9.1.C	72
HEK AAV9.1.A	79

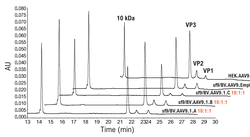
CsCl (%Full)
Stability and Efficacy



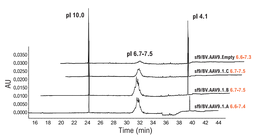
- The payload and the level of filled capsids was investigated by ddPCR, Denaturing gel, AUC and CsCl methods respectively
- Both production platforms gave a single predominant species at 4.7 kbp with comparable titers
- Similar levels %Full were obtained across all batches

Figure 2. Capsid Macrostructure

CESDS: VP Ratio and Purity
Stability, Safety & Efficacy



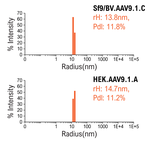
CIEF: pI Range
Stability, Safety & Efficacy



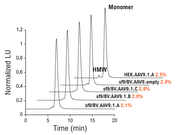
- The ratio of the viral capsid proteins was measured by CESDS, following their denaturation and separation through a gel matrix
- The pI range and number of species was determined through CIEF, where species were separated based on their differing charge states using an ampholyte gradient
- The VP ratios and pI ranges were consistent across both sets of materials, with elevated levels of VP2 observed in the HEK material

Figure 3. Aggregation State

DLS: rH, %pd & %intensity
Stability & Safety



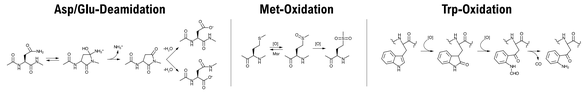
SEC-FLD-MALS
Stability, Safety & Efficacy



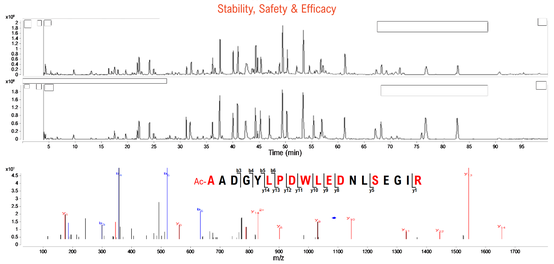
- The solution state hydrodynamic radius, polydispersity, % intensity of each species was measured through DLS
- Quantitative levels of HMW aggregates and % full were determined using SEC with online FLD, UV & MALS/RI detection
- Similar solution state profiles and comparable levels of HMW aggregates were observed for material across batches and platforms

Figure 4. LCMS Peptide Mapping

Asp/Glu-Deamidation | **Met-Oxidation** | **Trp-Oxidation**



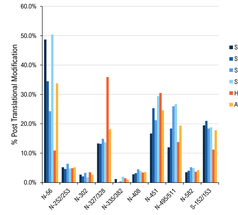
RP-HPLC-MS/MS
Stability, Safety & Efficacy



- Each AAV capsid was fully dissociated and digested using an exopeptidase. The resulting peptide fragments were separated on a RP-column and identified using MS/MS fragmentation, to give a sequence coverage and relative levels of post translational modifications (PTMs)

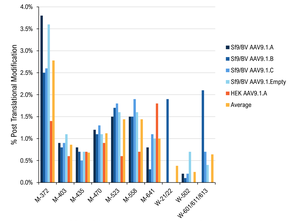
Figure 5. Post Translational Modifications

N-Deamidation, S-Phosphorylation



25 deamidation events observed, most abundant (10/25) displayed (remainder 0-2%)

M-Oxidation, W-Oxidation

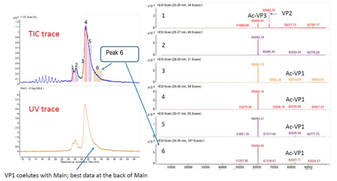


12 oxidation events observed, most abundant (remainder <1%)

- >90% sequence coverage was observed in each case, notable differences in PTMs include:
- A unique S-phosphorylation (observed on both VP 1 & VP2) that exists at higher levels in the sf9/BV material
- The levels of deamidation are similar between sf9 and HEK prep, with the exception of the N-56 residue (unique to the VP1 protein). The levels of oxidation are low (<2%) irrespective of the production method
- No evidence of glycosylation or disulfide bonds could be detected

Figure 6. LCMS Subunit Analysis

MOLECULAR WEIGHT (DA)	60463.4	60464.4	66940.7	67020.6	82021.5	82101.4
CONSTRUCT	VP3	VP3 DEAMIDATION	VP2	VP2 PHOSPHORYLATED	VP1	VP1 PHOSPHORYLATED
Sf9/BV AAV9.promoter.A	11.08	-0.33	-11.05	21.49	4.27	-2.44
Sf9/BV AAV9.promoter.B	8.43	2.48	12.70	11.34	3.90	16.69
Sf9/BV AAV9.promoter.C	7.77	39.03	14.34	10.89	11.58	2.56
Sf9/BV AAV9.Empty	8.10	20.18	9.26	32.23	0.00	14.74
HEK AAV9.promoter.A	10.58	0.99	11.50	18.35	5.36	7.92



- Methods such as CESDS can only observe protein peaks as potential VP variants, only LCMS/MS can provide identity at a high level of resolution
- The location of specific PTMs (e.g. phosphorylation) were confirmed by this method and are identical for material from both platforms

Figure 7. Approach 1 Summary

CONSTRUCT	TITER (VG/ML)	VP RATIO	%FULL	%HMW	PI RANGE	AVERAGE DEAMIDATION/ OXIDATION	RELATIVE PROTEIN EXPRESSION
Sf9/BV AAV9.promoter.A	1.23E+13	10:1:1	70	2.1	6.7-7.4	11.8%/1.1%	49%
Sf9/BV AAV9.promoter.B	1.41E+13	10:1:1	71	2.0	6.7-7.5	11.7%/1.3%	59%
Sf9/BV AAV9.promoter.C	1.55E+13	10:1:1	72	2.8	6.7-7.5	11.8%/1.1%	44%
Sf9/BV AAV9.Empty	5.71E+13[1]	7:1:1	N/A	2.0	6.6-7.3	15.3%/1.2%	N/A
HEK AAV9.promoter.A	2.98E+13	10:3:1	79[2]	2.5	ND	12.0%0.7%	100%

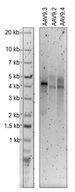
[1]Titer of empty capsid determined using MADLS; [2]%Full of HEK material measure through orthogonal CsCl separation with qPCR/dot blot analysis

- The HEK material has the highest level of relative protein expression, where selective tuning of the promoter in the sf9/BV material causes a steady increase in the protein expression
- Across the key capsid attributes, material produced from both platforms remains similar
- Notable exceptions include the average levels of VP2 protein, and specific deamidations on the VP1 subunit

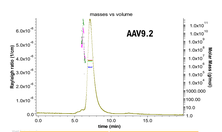
APPROACH 2

Figure 8. Genomic Integrity and Occupancy

Denaturing Gel
Efficacy



SEC-MALS (%Full)
Stability and Efficacy

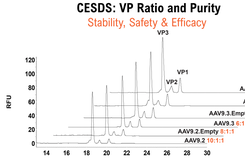


CONSTRUCT	%FULL
AAV9.2	63
AAV9.3	30
AAV9.4	49

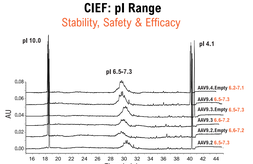
- For each variant two species of packaged material was observed (3.9 kbp corresponding to the expected TG length). In the AAV9.3 construct the secondary band was significantly smaller in size
- This may impact the packaging efficiency where this construct displayed the lowest occupancy at 30% (determined using SEC-MALS)

Figure 9. Capsid Macrostructure

CESDS: VP Ratio and Purity
Stability, Safety & Efficacy



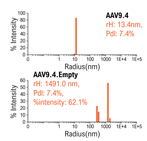
CIEF: pI Range
Stability, Safety & Efficacy



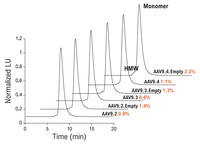
- The average VP ratios demonstrated the desired stepwise increase of VP1 & 2 subunits relative to VP3 with each change to the capsid expression cassette (10:1:1 to 4:1:1), mirrored in the empty equivalents
- Despite this no change was observed in the pI ranges, and significantly no difference is observed in their empty equivalents

Figure 10. Aggregation State

DLS: rH, %pd & %intensity
Stability & Safety



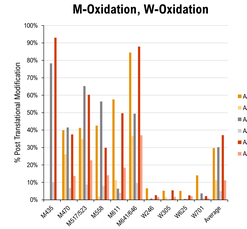
SEC-FLD-MALS
Stability, Safety & Efficacy



- The levels of HMW aggregates for each construct remained low (<5%), but the values doubled for the corresponding empty capsid
- DLS analysis showed a stark difference between the empty and full preps with the latter consisting of two species at significantly higher r_H values
- The discrepancies between the two methods may indicate the transient nature of these aggregated species

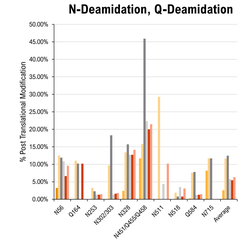
Figure 11. Post Translational Modifications

M-Oxidation, W-Oxidation



10 most abundant oxidation of either M or W were reported

N-Deamidation, Q-Deamidation



21 deamidation events observed, most abundant (10/25) displayed (remainder 0-2%)

- Each of the variants shows a greater propensity to M-Oxidation, with 6 residues that are particularly prone. In each instance the empty preparations experience this transformation to a lesser extent
- There appear to be 4 N & Q residues that are susceptible to deamidation. Residue N511 shows greater susceptibility in the empty capsids. In both the AAV9.4 preparations lower levels of deamidation is observed
- No evidence of glycosylation or disulfide bonds could be detected

Figure 12. Approach 2 Summary

CONSTRUCT	TITER (VG/ML)	VP RATIO	%FULL	%HMW	PI RANGE	AVERAGE DEAMIDATION/O XIDATION	INFECTIVITY LOGTCID50
AAV9.2	3.90E+11	10:1:1	63%	0.8	6.5-7.3	3.3%/29.7%	7.13
AAV9.2.Empty	2.20E+12[1]	8:1:1	N/A	1.4	6.6-7.2	11.8%/11.2%	N/A
AAV9.3	1.34E+12	6:1:1	30%	0.6	6.6-7.2	12.8%/30.2%	8.39
AAV9.3.Empty	6.82E+12[1]	5:1:1	N/A	1.3	6.5-7.3	5.8%/5.0%	N/A
AAV9.4	7.32E+11	4:1:1	49%	1.1	6.5-7.3	5.9%/37.1%	8.34
AAV9.4.Empty	5.30E+12[1]	4:1:1	N/A	2.2	6.2-7.1	6.9%/11.2%	N/A

[1]Titer of empty capsid determined using MADLS

- Neither the significant differences in the VP ratios or levels of deamidation across the constructs appear to influence the surface charge of the capsids. This implies separation approaches other than ion exchange chromatography will need to be investigated
- The levels of oxidation does not appear to correlate with the amount of aggregation
- From a functional perspective increasing the average number of VP1/2 components benefits the infectious behavior of the capsids when examined by the TCID50 method

CONCLUSIONS

- When examining capsid macroscale properties, the VP ratio appears to have the greatest effect on the functional output
- Examination of the residue fine structure indicates individual transformations, such as those on the VP1 protein have more of an impact than the average levels of change
- It is unlikely that a single attribute is solely responsible for determining the functional output of the products, rather a collection operating within specific ranges. Therefore, further avenues should be pursued in tandem:
  - More detailed examination of the capsid fine structure
  - Full sequencing of the transgene payload
  - Investigation of the different stages of function: transduction, transcription and functional output

Forced Degradation Studies of AAVs to Generate the Full Spectrum Aggregation Toolkit

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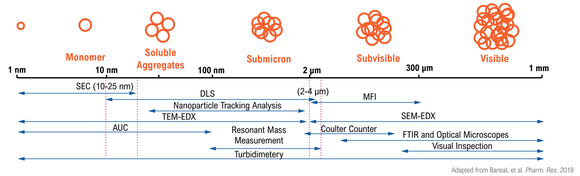
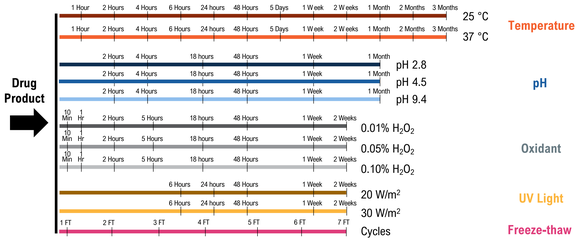
INTRODUCTION

- Aggregation remains a critical quality attribute that must be monitored due to its potential to impact any given biotherapeutic in terms of **immunogenicity** (safety), **shelf life** (stability) or **function** (efficacy)
- Forced degradation studies are essential role in determining degradation pathways and identifying stability indicating analytical methods
- No single analytical technique covers the full-size range of potential aggregates, especially larger constructs such as adeno associated viruses (AAVs)
- Handling of drug product can cause also induce changes and the impact of processes such as freeze thaw must be monitored



Adapted from Bansal, et al. *Pharm. Res.* 2019

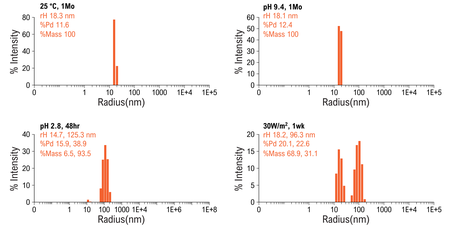
STUDY DESIGN



- The goal of this study is to determine which analytical methods are capable of observing aggregation under each stress condition
- AAV drug product was exposed to different conditions and examined using a range of analytical techniques spanning the full spectrum of particle size: SEC-FLD, DLS, MFI & visual inspection
- In the second part the impact of several freeze-thaw cycles (between -80 °C and 25 °C) will be measure, with focus on aggregation and the effect on titer/dose and function/efficacy utilizing ddPCR and relative potency methods

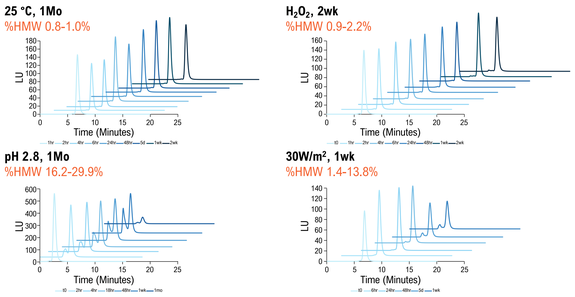
RESULTS

Figure 1. Sub-µm Aggregates: Dynamic Light Scattering



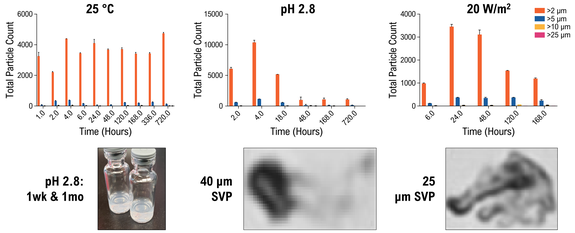
- Storage at ambient temperature, high pH or oxidizing conditions had no impact on the solution state aggregation
- Long term exposure to UV light or low pH induced significant changes to the hydrodynamic radius, polydispersity and number of species

Figure 2. Sub-µm Aggregates: Size Exclusion Chromatography



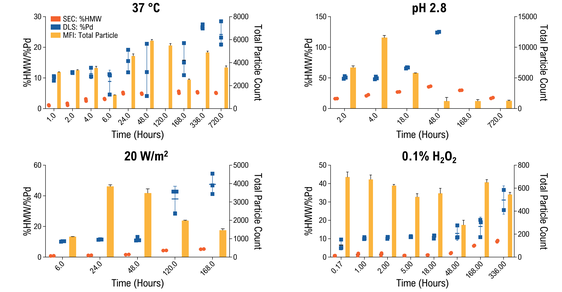
- Analogous to DLS no change in HMW aggregates was observed at 25 °C, in pH 9.4 or in the presence of 0.01% H_2O_2
- Moderate changes were observed at 37 °C or in the presence of higher levels of H_2O_2
- Rapid increases in %HMW with complete loss of material was observed on with UV light or low pH

Figure 3. µm-mm Aggregates:Micro Flow Imaging and Visual Inspection



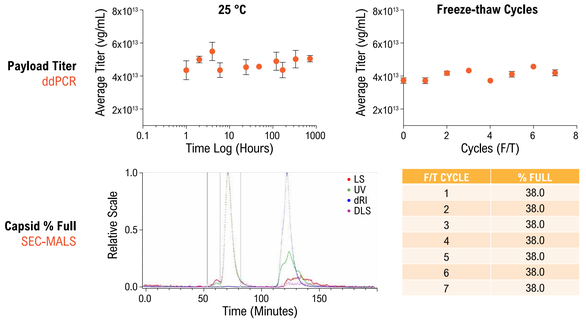
- Low pH or UV light causes an initial rise and subsequent dip with significant loss of material (markedly in the >2 µm)
- pH 2.8 induced the greatest change in sub-visible particle and exhibited the formation of visible aggregates after 48hr

Figure 4. Trends Observed Across Aggregation Methods



- Greatest impact on the overall aggregation state observed on increasing the temperature and lowering pH
- Oxidized samples follow a similar pattern with a break at the 1-week mark, dose dependent effect
- DLS output (rH, %Pd, %Intensity) correlate with other methods but show highest variability

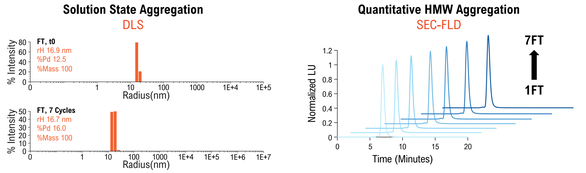
Figure 5. Impact of Freeze-thaw on Genomic Payload



F/T CYCLE	% FULL
1	38.0
2	38.0
3	38.0
4	38.0
5	38.0
6	38.0
7	38.0

- Along with storage at 25 °C, 7 freeze thaw cycles did not cause any loss in genomic payload
- %Full values also indicated no change in the occupancy of the capsids

Figure 6. Impact of Freeze-thaw on Sub-µm Aggregation

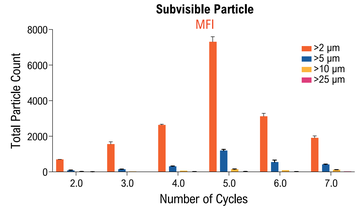


F/T CYCLE	RH (nm)	% PD	% MASS
0	16.9	12.5	100
1	17.1	16.0	100
2	17.1	15.9	100
3	17.0	15.9	100
4	17.3	16.0	100
5	16.8	15.9	100
6	16.9	16.0	100
7	16.7	16.0	100

F/T CYCLE	% HMW
0	0.78
1	0.81
2	0.84
3	0.85
4	0.84
5	0.84
6	0.84
7	0.84

- No change in the hydrodynamic radius (rH) or the number of species was observed. There was a slight increase in the polydispersity after the first F/T implying a small shift in the aggregation state
- This was not reflected in the corresponding SEC-FLD measurements with no change to the amount of HMW species

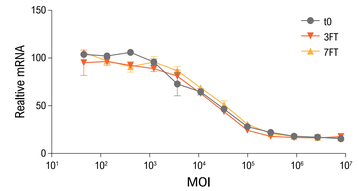
Figure 7. Impact of Freeze-thaw on µm-mm Aggregation



F/T CYCLE	AVG. PARTICLE COUNT
1	3456
2	3262
3	5937
4	9862
5	19831
6	7242
7	5414

- MFI was able to observe increases in the larger particle size range, capturing changes to the aggregation state not observed that were not observed by the other techniques DLS and SEC-FLD
- As observed under strong degrading conditions (pH 2.8, 30 W/m2) there was an initial rise and dip in the particle count suggesting a loss of material

Figure 8. Impact of Freeze-thaw on Potency

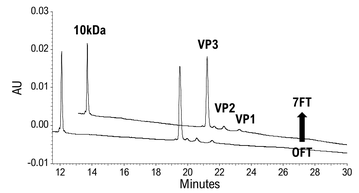


F/T CYCLE	% RELATIVE POTENCY
0	67.3
1	62.6
2	64.0
3	52.9
4	65.3
5	63.7
6	62.2
7	48.2

- The functional output involves the measure of target mRNA knock down relative to a reference material
- Relative potency retained within the range of the assay with the exception of the final freeze thaw cycle where a significant drop is observed

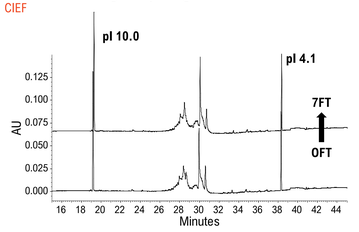
Figure 9. Impact of Freeze-thaw on Capsid Macrostructure

Capsid VP Ratios and Purity
CESDS



F/T CYCLE	VP RATIO	% PURITY	PI RANGE	PI AREA RATIO
0	18:1:1	94.8	6.4-6.7, 6.7-7.7	49:51
7	19:1:1	96.2	6.4-6.7, 6.7-7.7	45:55

Capsid Surface Charge and pI Range
CIEF



- Capsid macrostructure displayed identical characteristics following 7 freeze-thaw cycles, when examined by CESDS and CIEF
- VP ratios and % protein purity remained unchanged
- Product occupied the same pI range consisting of 2 peaks over 6.4-7.7

CONCLUSIONS

- The effects of forced degradation conditions on AAV samples was examined in terms of the impact on the aggregation state
- Low pH and UV light conditions produce the most significant changes in structure
- While the aggregation pathways differ, there is a consensus between the three techniques with SEC-FLD and MFI providing early indications of change
- The impact of 7 freeze thaw cycles induced no change in the solution state characteristics when examined in the sub-µm range using DLS and SEC-FLD
- However, MFI was able to show a change in sub-visible particles (µm-mm) with a significant increase observed between 4-5 freeze thaw cycles, indicating the necessity of studying this region
- Despite no change in the payload concentration or the capsid macrostructure, there was a drop in the relative potency after 7 cycles

Development of High Throughput Screening for DOE-Based Formulation Screening

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INTRODUCTION

The fates of recombinant viral vectors produced for use in gene therapy are intrinsically intertwined to how they are produced, stored, and handled. Much value is derived from being able to stabilize desirable drug substance properties early in the life cycle to support development as well as evolve at a pace to sustain research and CMC activities. Unless permanently tethered to one specific delivery/modality, rapidly developing a final formulation solution is required to move at a pace that supports the production of novel vectors to be used. With the potential for creation and production of numerous capsids with highly variable properties, there is a need to evaluate, test, and incorporate DOE and analysis in a data-directed, bottom-up platform approach to evaluate and rank various formulation components, test their efficacy, and predict (un)desirable characteristics. By developing a combination/matrix of screening methods fit for purpose, we successfully established a method enriching for preferred capsid attributes that was compatible with current analytical matrix testing, DOE approaches, and high throughput design.

BACKGROUND

Problems: *Know your target profiles and the measurement(s) used to compose it.*

A majority of preventative issues stem from not fully understanding what the final rAAV product should characteristically exhibit. These factors are determined through a matrix/battery of testing. Increasingly more and more, agency guidelines require a multitude of orthogonal tests to be in good agreement, and to demonstrate depth of product knowledge. Demonstrations can be reduced to two main segments showcasing producers: have a firm grasp on expectations for 1) how the rAAV product behaves prior to administration, and 2) the effects and efficacy of well characterized product after administration. These two components are intrinsically intertwined, where certain characteristics bear potential consequences for efficacy,[1] and potential toxicity (i.e., aggregation)[2,3].

In this survey, we considered the following 7 methods and others not listed for generating target profile measurements, some which* compose this matrix screen:

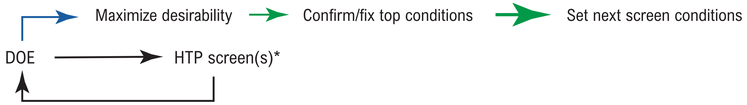
- qPCR, ddPCR, AUC, SEC, MALS, DLS, DOE/JMP analysis

We targeted 5 main product attributes (responses) to continuously refine the ending product formulation:

- A, B, C, D, E

Here, the approach was to utilize DOE testing of four (4) main inputs, and multi-factor analyze for maximum desirability response A-E, in a two step fashion. JMP customized DOE was used to generate an impartial testing matrix for initial surveys. More stringent second level testing was used to feed back into and inform the original DOE plan.

Visual Representation for DOE Feedback Path Forward

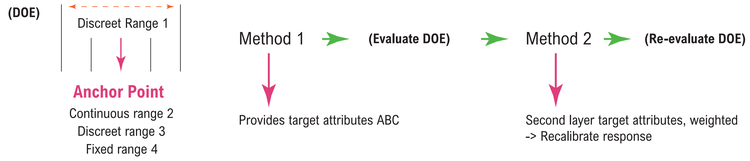


Initial Considerations

Choosing the appropriate/initial screening assay and conditions: Prior to setting initial boundaries for viral gene therapies as a whole, rAAV in its final form can be approached as a function of individual parts; a container/delivery mechanism and concomitant cargo which is delivered. Several key assumptions exist within this modality:

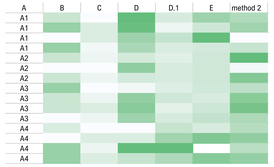
- Representative cargo (as measured) does not exist without container
- Container state dictates delivery, access, and cargo viability
- Container properties dictate initial range-finding studies

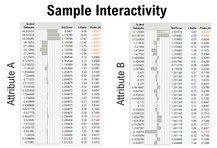
We exploit this relationship to monitor the comparative health of how the ongoing formulation either aligns with or contrasts our defined target profile. Introducing new capsids with novel properties suggests custom approaches, but with a defined selection of inert ingredients or compounds generally regarded as safe (GRAS)[4,5], secondary and tertiary response interaction exhibit (perhaps) a more significant role*. The initial range-finding studies should not be approached blindly either. Most likely there should be one or two product characterization techniques used to orient the initial survey, whether it is theoretical or empirically determined. Going into DOE, we had already established three critical parameters one empirically determined anchor point, a method to generate product attibutes ABC (method 1), and feedback response (method 2) adding a second layer into DOE interpretation.

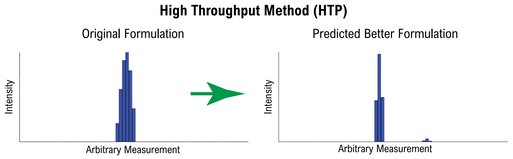


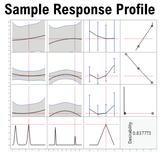
Early Assessment and DOE Examples

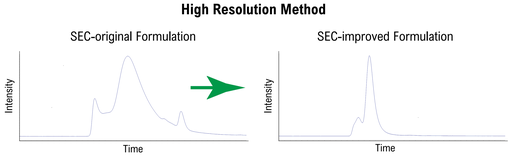
Exploring JMP generated unrestricted chemical space available compared to previous formulation forcing novel constructs to fit into the current final composition allowed for unbiased chemical sampling, and permitted our 5 main character attributes (ABCDE) to guide iterative formulation development. We found after one full initial DOE iteration, the predicted composition deviated from the original formulation. Data collected indicated primary top contributors for maximizing desirable attributes while root square mean (RSM) interactions revealed secondary interactions; synergistic and interesting negative interactivity not seen prior to compatibility testing the current process with a novel capsid. Using our matrix of Analytical techniques, we were excited to discover promising improvement concerning attributes that deviated from our target profile using the previous formulation composition.







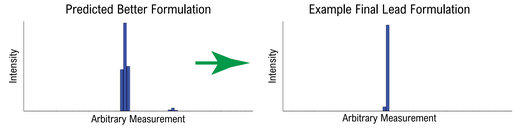




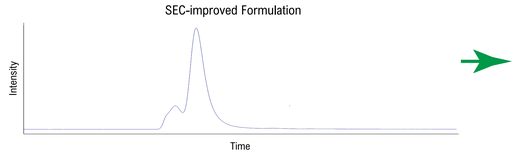
Survey Says?!

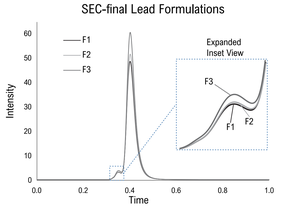
Following the initial proof of concept foray into available chemical space, our efforts were reinvested into successively constraining and optimizing key positive influencers and interactivity. Relative maximums were constrained for iterative tests, and by doing so removed variables to further refine test settings that had yet to established anchor points. Again, **method 2**, along with top candidate analysis by lower throughput methods were used to align TP and confirm a viable path forward. As an added layer of stringency, we resourced current and prior target accepted ranges and recommendations for formulation attributes as given through the active FDA accepted compounds,[4,5] considering therapeutic administration route (IIR(ref) and SCOGS(ref) lists). The final iteration of DOE yielded three (3) lead formulation to be pressure tested. These lead formulations were used to exchange starting viral material into followed by each being subjected for stability to suss out alpha and beta final formulation recipes.

Example: *Iterative formulation and further increase for desired characteristics*



Since three potential formulations developed had similar increases towards desired characteristic profiles, more rigorous analyses were required for choosing the best fit for this particular capsid type.



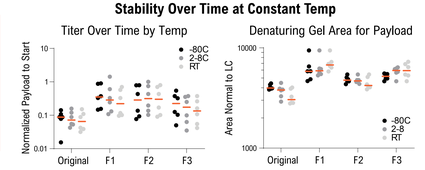


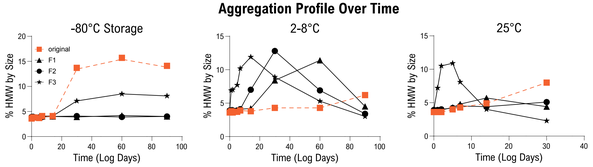
Tesing Final Formulations

Separating the best path forward from several potential formulations: With shared desirable characteristics between formulation, we decided a lead solution should stand the test of time under challenging/non-ideal conditions. Three separate temperatures consisting of -80C, 4C, and room temperature (RT, ~25C) were used to pressure test viral material behavior and resulting characteristics for an accelerated stability profile over the course of three months in addition to successive freeze/thaw analysis. The following data represents some* contrasting information that tease apart changes between both container (capsid) and payload. We interpret stability as the formulation's ability to functionally prevent changes from the starting/ideal character profile when challenged by drastic oscillation in temperature (freeze thaw) or different environmental temperatures over time (stability at temperature tested).

Stability Testing Overview: Stability and Temperature Over Time

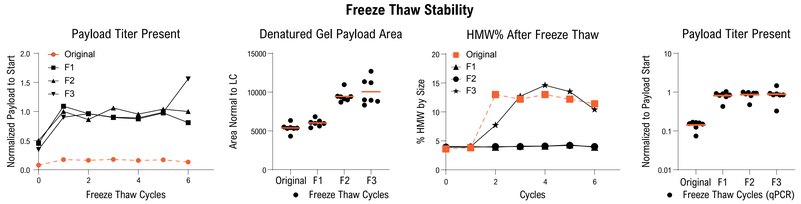
The red bars signify the average data for two months. We observe increase spread of data points and overall decrease of measurable cargo titer in a temperature storage as time progresses. At constant temperature (three graphs below), only two formulations maintain the target container profile over the course of three months (at -80C), where warmer hold temps accelerate an observable aggregation process.





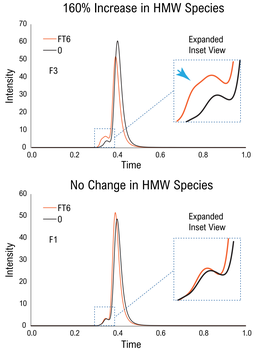
- *Increased temps increased aggregation rates*
- *Aggregation collapse seen by SEC may reach critical percentage and remain until a sustainable formulation capacity equilibrium is established*

Freeze thaw testing exacerbates extreme conditions, and sets a benchmark for resisting change by asking the formulation to withstand extreme thermal shock yet still provide a buffering stability. In our hands, after several freeze thaws, we were able to tease out our lead and potential backup formulation that met our target profile characteristics.



DATA CONCLUSIONS AND REMARKS

Being able to quickly assess compatible, custom formulations affects our current and future timelines from production start. Simultaneously, similar analytic characterizations used to define and inform on early unit process operations were harnessed. We used DOE to 1) highlight the ability in deploying formulation screens during early process stages, and 2) suggest a need for coevolution in early analytical characterization with practical formulation applications; not selectively confining formulation to exist late/post process. Conductingthese tests revealed not any singular analytical/biophysical approach produced enough data to conclusively arrive at proper formulation, but matrix-based and newly developed techniques (i.e., method 2) compatible with DOE statistical analyses were required. Interestingly, we found parallel processing in our formulation "feedback approach" DOE development also informed why various rAAV characteristics appeared during unit process operations (data not shown). An analytical lens coupled with data driven HTP approaches for this survey successfully guided formulation development during initial rapid screening for conditions and components, and progressive iterations enabled us to arrive at better final formulations that preserved desirable capsid properties.



- *Analytical techniques used in process characterization (data) directed this survey*
- *Data suggests formulation traits may also feedback into the production process*
- *HTP DOE design is viable for rapidly developing rAAV delivery vehicles*

ACKNOWLEDGEMENTS

RH Vass/M placidi incepted, incorporated, and analyzed DOE. R Bamidele, M Lohsen, D Alvarez, M Placidi, and Voyager Therapeutics Analytical staff also performed and analyzed experiments in this work. A special thanks to R Udani, P Carroll, and K Nguyen for providing various stages of viral material to be tested. This work was performed and analyzed at Voyager Therapeutics.

REFERENCES

1. Denard J, Beley C, Kotin R, Lai-Kuen R, Blot S, Leh H, Asokan A, Samulski RJ, Moullier P, Voit T, Garcia L, Svinartchouk F. Human galectin 3 binding protein interacts with recombinant adeno-associated virus type 6. *J Virol*. 2012 Jun;86(12):6620-31. doi: 10.1128/JVI.00297-12. Epub 2012 Apr 11.
2. Wright JF. Product-related impurities in clinical-grade recombinant AAV vectors: Characterization and risk assessment. *Biomedicines*. 2014 Mar; 2(1): 80–97. Published online 2014 Mar 3. doi: 10.3390/biomedicines2010080 PMCID: PMC5423478. PMID: 28548061.
3. US Department of Health Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research, Office of Regulatory Affairs Guidance for Industry: Sterile drug products produced by aseptic processing—Current good manufacturing practice. Available online: http://www.fda.gov/cberguidelines.htm.
4. IIR: https://www.fda.gov/drugs/drug-approvals-and-databases/inactive-ingredients-database-download. Downloaded 18JUL2021
5. SCOGS: https://www.fda.gov/food/generally-recognized-safe-gras/gras-substances-scogs-database. Downloaded 17SEP2021