Addressing Downstream Purification Challenges: Solving AAV2 Aggregation Issue and Developing Innovative Approaches for Empty Capsid Removal

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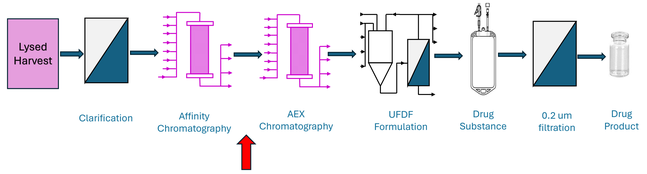
Case 1 Introduction: Solving rAAV2 Aggregation Issues

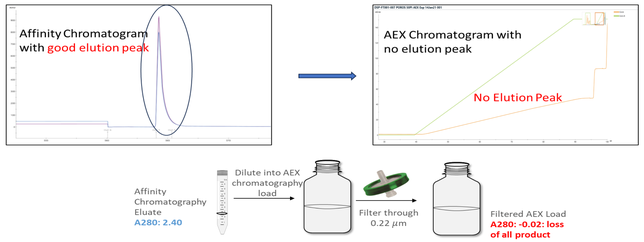
- rAAV2 is prone to aggregate, causing significant challenges during manufacturing (MFG) production, especially product loss
- Aggregation was observed during the transition from Affinity chromatography column to AEX chromatography column, almost 100% product loss before the AEX column
  - A two-step dilution method was developed to avoid product loss based on the aggregation mechanism hypothesis

Frontera Downstream Production Platform



- Scalable MFG production platform with high purification recovery
- rAAV aggregation was observed after Affinity column before transitioning to AEX column (red arrow), causing 100% product loss

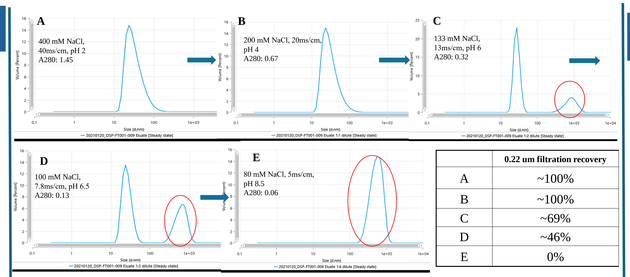
Issue: rAAV Aggregation Observed Post Affinity Chromatography



- Affinity column elution after diluting into AEX load buffer, the product was entirely lost post 0.22 μm filtration
- rAAV aggregation was identified as the underlying cause

Investigation: rAAV Aggregation when Transitioning from Affinity to AEX Chromatography

- Aggregation detected and quantified by dynamic light scattering (DLS)
- Following elution from affinity chromatography (condition A), the sample exhibited 100% monomer content. However, after buffer dilution for AEX chromatography (condition E), the sample showed complete aggregation.
- rAAV was lost post a 0.22 um filter.



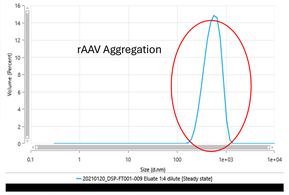
	0.22 um filtration recovery
A	~100%
B	~100%
C	~69%
D	~46%
E	0%

Hypothesis: Low Ionic Strength and High rAAV Concentration Led to rAAV Aggregation

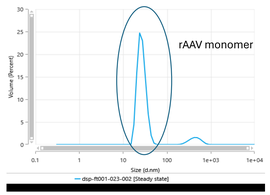
- rAAV aggregation occurred upon dilution of the purified rAAV with a low ionic strength buffer.
- Through experimental investigation, we discovered that charged species that both low ionic strength and high rAAV concentration are contributing factors to the aggregation.
- Uncharged species, such as carbohydrates and surfactants, were unable to prevent the aggregation.

Solution: Two Step Dilution to Avoid Aggregation

- To mitigate aggregation formation, a two-step dilution method was developed
  - Step 1: Dilute rAAV concentration while maintaining a high salt concentration.
  - Step 2: Reduce both salt concentration and AAV concentration simultaneously.



Original salt reduction method led to 100% rAAV aggregation



Two steps dilution avoided rAAV aggregation

Case 1: Conclusions

- rAAV aggregation observed during downstream purification process.
- Interplay of salt and rAAV concentration was found to be critical for rAAV2 aggregation
- During transition from affinity chromatography to AEX, controlling the salt concentration and rAAV concentration simultaneously was the key to prevent aggregation

Acknowledge and Reference

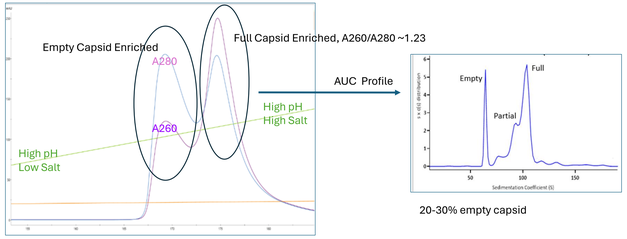
- We thank Sabrina Moisan for her contribution to rAAV aggregation work
- J.F. Wright , et al, Mol. Ther., Volume 12, Issue 1, July 2005, Pages 171-178

Case 2 Introduction: Innovative Approach for Empty Capsid Removal

- Empty capsids are categorized as impurity in rAAV gene therapy
- Empty capsid increase the total capsid dosing, potentially increase the risk of immune response
- AEX is widely used to separate empty from full capsid and salt gradient is a common approach
- A novel pH gradient method to replace salt gradient was developed to enrich the % empty capsid down to 0%

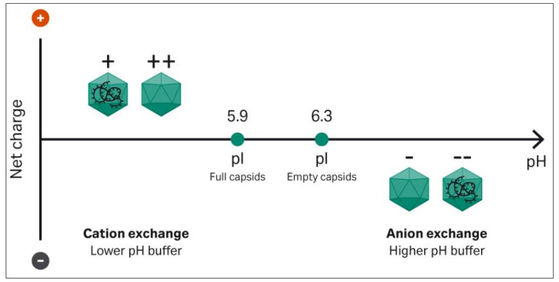
Problem: Suboptimal Empty and Full Capsid Separation Using Platform Salt Gradient

- Suboptimal separation observed from harvest with higher % empty capsid, with an A260/A280 ratio of approximately 1.23, indicating about 20-30% empty capsids following AEX chromatography
- A260/A280 ratio: (Empty Capsid) 0.6 < A260/A280 <1.4 (Full Capsid)



20-30% empty capsid

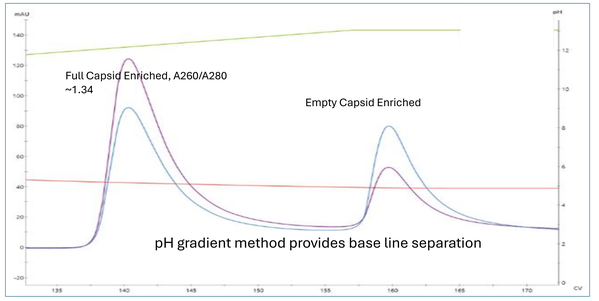
Theory: Empty and Full Capsid Separation Using IEX



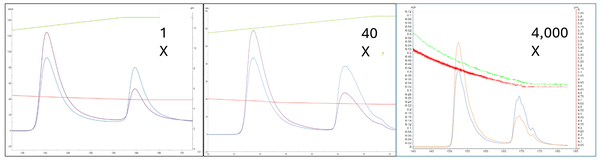
- Both salt gradient and pH gradient theoretically enable the separation of empty and full vectors through IEX chromatography, based on the vector surface charge
- Besides the net charge, the distribution of charges on the capsid surface significantly influences its binding behavior to the resin.

Solution: Empty and Full Capsid Separation Using Modified pH Gradient

- A new pH gradient process was developed using the same AEX Chromatography.
- Full capsid enriched peak eluted before empty capsid enriched peak.
- A260/A280 of the full capsid peak is around 1.34.

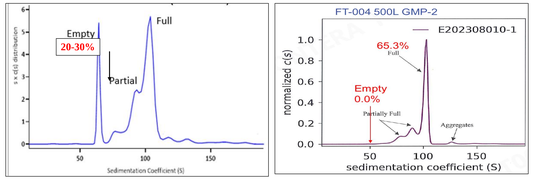


Scale up: Consistent Resolution during Scale up



- Achieved robust scalability from bench scale to GMP scale, a 4,000x scale up.

Result: Complete Removal of Empty Capsids (AUC Profile)



- Empty capsid was improved from 20-30% from salt gradient method to 0% from pH gradient method by AUC

Case 2: Conclusion

- AEX step for empty capsid removal was developed and used in Frontera downstream purification platform
- Traditional salt gradient process is powerful, however, if harvest has higher % empty capsid, , this method is only able to enrich to 70-80% full capsid
- A new novel pH gradient method was developed to improve the % empty capsid removal
  - A baseline separation between empty capsid and full capsid was achieved
  - New pH gradient was able to scale up from bench scale to GMP scale
  - New method improved % empty capsid removal from 20-30 % to 0%

Acknowledgement

- We thank Eric Tang for his contribution to this pH gradient AEX development work