Rapid Development of Ion Exchange Chromatography for Full/Empty Separation in Adeno-Associated Virus Serotype 5

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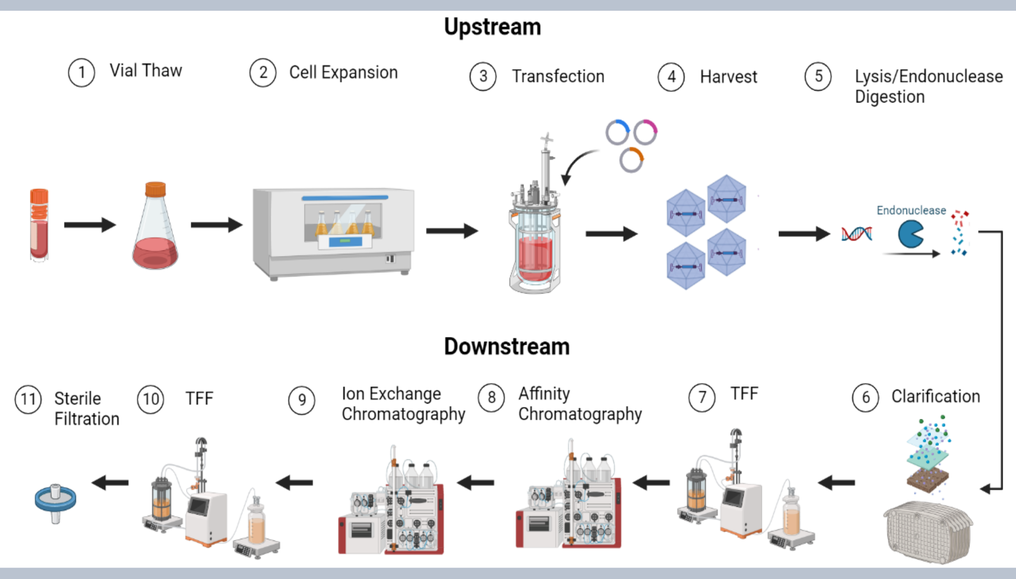
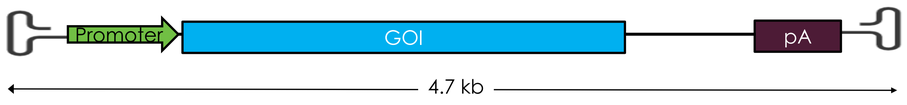
Abstract

Adeno-associated viral vector (AAV) based therapies have demonstrated substantial therapeutic benefit for the treatment and cure of genetic disorders. With promising clinical data and continued success from the hundreds of active pre-clinical and clinical studies, AAV-based gene therapies have emerged as a new class of molecular medicines. Despite the growing demand, cost and scalability remain as bottlenecks in the manufacture and commercialization of novel AAV therapeutics. Among all of these well-known challenges, the removal of empty AAV particles, as it offers no therapeutic benefit and increases the risk of immunotoxicity, and enrichment of full AAV particles through the downstream process has been recognized as one of the most impactful. Traditionally, the separation and enrichment of full AAV particles is performed using iodixanol or cesium chloride (CsCl) gradient ultracentrifugation (UC), however this method is time-consuming and not scalable. In this regard, chromatographic methods show the potential to enable the downstream process with scalability, GMP-friendly, and lower cost. Ion-exchange chromatography (IEX) has been used in large-scale bioprocessing for almost 40 years and have been shown to effectively generate concentrated purified AAV material containing high percentage of full AAV particles. However, the chromatography process often requires extensive efforts of optimization to achieve the desired empty/full separation for each serotype or gene of interest (GOI). Here we describe a 3-Design of Experiment (DoE) screening approach to streamline and quickly establish critical process parameters to achieve highly enriched AAV material. In this study, a full size therapeutic AAV construct (~4.7kb) was used to establish the IEX chromatography process for AAV5. As the data shows, changes to the load material pH and conductivity increased the step recovery by 70% from the baseline; however, overloading or underloading the column negatively impacts recovery. Moreover, selection of elution buffer conductivities for the 2-step isocratic process is critical to achieve optimal enrichment. Overall, the parameters established by the screening approach was able to enrich the starting AAV5 material by 5X, from 13.8% to ~70% full, as confirmed by analytical ultracentrifugation (AUC), at the 2L scale.

Process Overview



Schematic Map of GOI Plasmid



Results

DoE 1: Establishing Elution Buffer Formulation to Improve Full Capsid Enrichment

Fig 1A.

DOE Parameters
Base Salt
Elution pH
Supplement

Fig 1B.

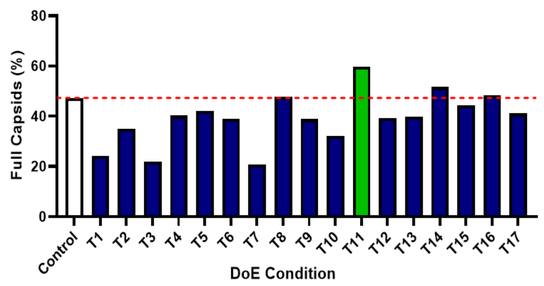


Fig 1C.

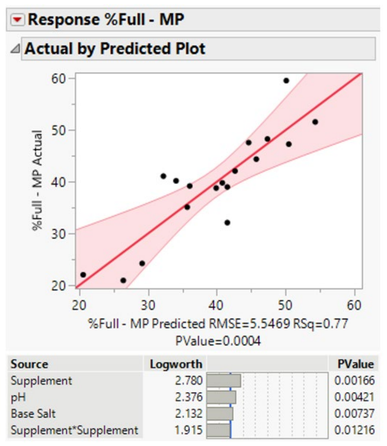


Fig 1. Elution Buffer Formulation Optimization. (A) 3-Factor DoE performed at small scale to determine base salt, pH, supplement concentration in the elution buffer (B) Percentage of full capsids in eluate as determined by mass photometry (C) Model from elution buffer formulation DoE with effect summary showing multiple factors being statistically significant (p-value < 0.05).

DoE 2: Optimization of Load pH and Conductivity to Improve Recovery

Fig 2A.

DOE Parameters
pH
Conductivity

Fig 2B.

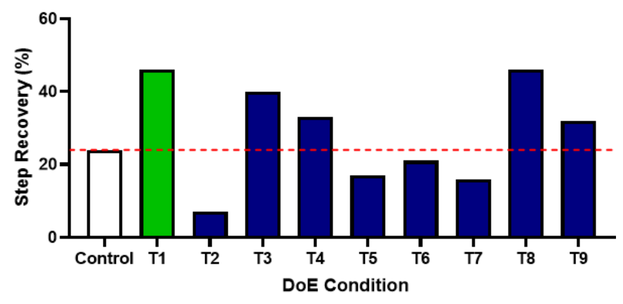


Fig 2C.

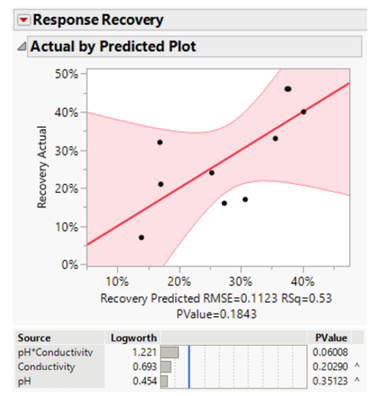


Fig 2. Load Condition Optimization. (A) 2-Factor DoE performed at 1mL scale to determine optimal pH and conductivity of the load material (B) Genomic titer recovery of eluate as determined by ddPCR (C) Model from loading condition DoE with effect summary.

DoE 3: Determination of Binding Capacity and Residence Time for Scale-Up

Fig 3A.

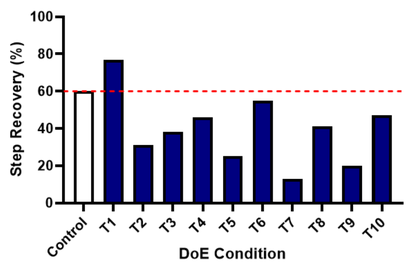


Fig 3B.

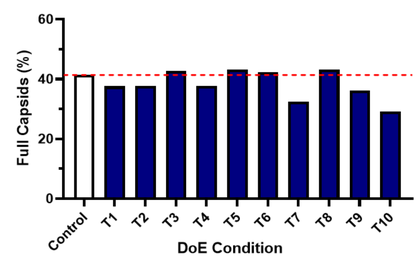


Fig 3C.

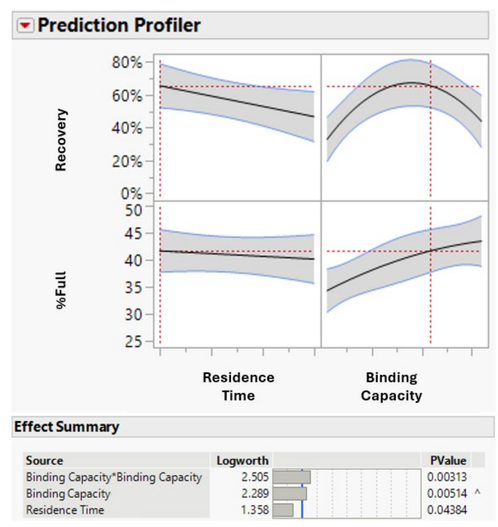


Fig 3. Binding Capacity and Residence Time Optimization. (A) Genomic titer recovery of each DoE condition as determined by ddPCR (B) Percentage of full capsids of each DoE condition as determined by mass photometry (C) The effect of binding capacity and residence time on genomic titer recovery and %full at maximum desirability, with effect summary showing multiple factors being statistically significant (p-value < 0.05).

Establishing a 2-Step Isocratic Elution Process

Fig 4A.

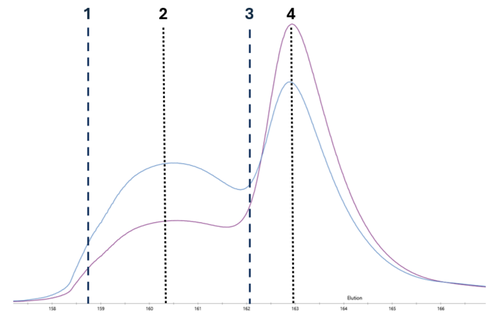


Fig 4B.

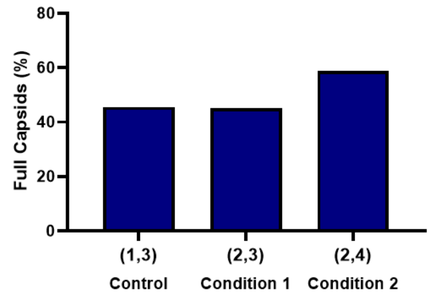


Fig 4. Conductivity Selection for 2-Step Isocratic Elution. (A) Conductivity selection for 2-step elution process follow parameter optimization (B) Percentage of full capsids in eluate for each condition as determined by mass photometry.

AUC Profiles of Starting Material vs. Optimized IEX Process Eluate

Fig 5A.

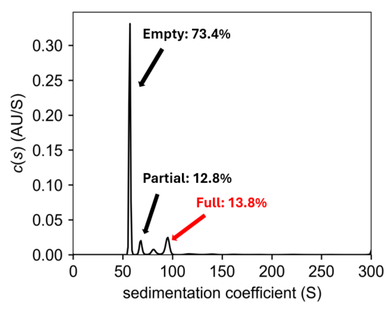


Fig 5B.

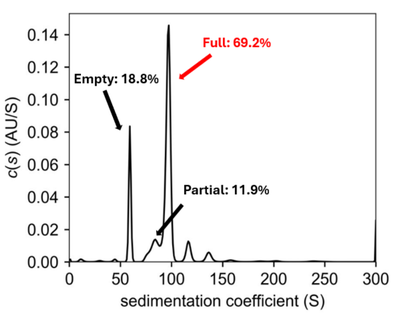


Fig 5. Full and Empty Capsids Percentage. (A) AUC data of affinity purified starting material (B) AUC data of IEX eluate from 2L scale purification using optimized parameters.

Conclusion

- Our data demonstrates that this DoE-based screening methodology can quickly establish critical process parameters and improve process of enrichment of full AAV particles through IEX.
- Optimization of the elution buffer formulation showed significant improvement of the %full enrichment in IEX unit operation.
- The percent of full capsids was enriched from 13.8% in the affinity eluate to ~70% following IEX purification at the 2L scale.

Learn More

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