Development and Optimization of a Scalable Affinity Purification of Adeno Associated Virus Serotype 6

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INTRODUCTION

The adeno-associated viral vector (AAV) provides an efficient gene therapy platform with several approved products that have marked therapeutic impact for patients. However, potency, cost, and scalability remain as bottlenecks in the manufacture and commercialization of AAV. Among all of these well-known challenges, the enrichment of high-quality AAV particles through the downstream process has been recognized as one of the most impactful. Chromatographic methods show the potential to enable the downstream process with scalability, GMP-friendly and lower cost. However, the chromatography process often requires extensive efforts of optimization to fit the specificity to each serotype. Affinity chromatography (AC) has been used in large-scale bioprocessing for almost 40 years and is considered the preferred method for primary capture in downstream processing of various types of biopharmaceuticals. The key factors for an effective AAV purification process are high overall yields and maximized impurity reduction.

In this study, a full size therapeutic AAV construct (~4.7kb) has been used to establish and further optimize the affinity chromatography process for AAV6 with improved recovery and product quality. After cell lysis and endonuclease treatment from upstream, the crude harvest is clarified and prepared for the capture step. The optimal affinity binding conditions were different for various serotypes. Here we used the design of experiment (DoE) methodology to model load adjustment and percent recovery. As data shows, the recovery of AAV6 in affinity purification step was significantly improved by load adjustment of the material to higher pH and conductivity. Changes in residence times during column loading step did not affect the recovery, however, increase of load volume (CV) was significantly improved the step recovery. Moreover, DoE study of the formulation of elution buffers demonstrated the reduction of aggregation and improvement in product quality with no effect on recovery. Overall, the recovery and vector genome titer of small scale and 2L scale runs were comparable without statistically significant difference, suggesting that the scalability of these optimized chromatography parameters.

AAV UPSTREAM AND DOWNSTREAM PROCESS OVERVIEW

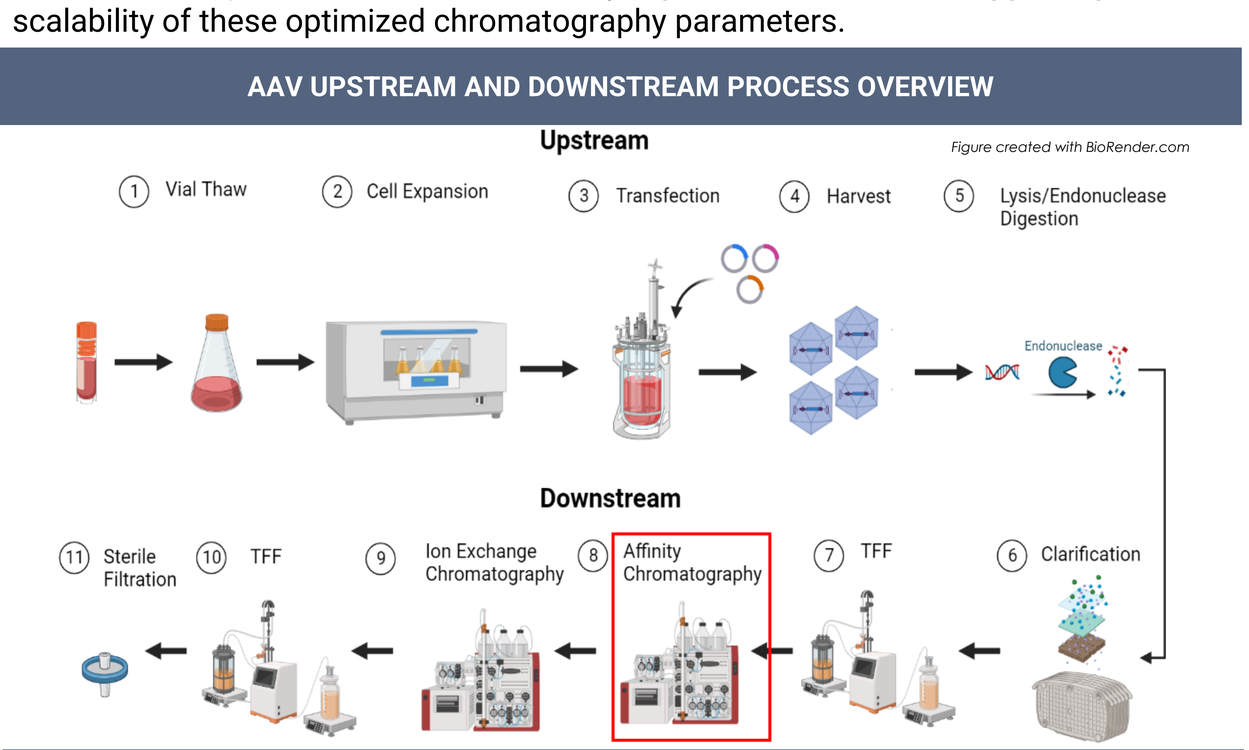
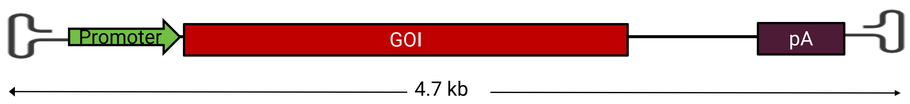


Figure created with BioRender.com

SCHEMATIC MAP of GOI PLASMID



RESULTS

DoE1: LOAD CONDITIONING

Fig 1A.

DOE Parameters
Salt (150-400 mM)
pH (7.4-8.0)

Fig 1. Load Adjustment Optimization. (A) 2 factor DoE performed in affinity batch purification looking at load adjustment conditions (B) Average of recovery percentage (C) Model from load adjustment optimization DoE. (D) The statistical analyses of control and optimized run by load conditioning showed the significant improvement (p-value 0.0009).

COLUMN DYNAMIC BINDING CAPACITY OVER RESIDENCE TIME

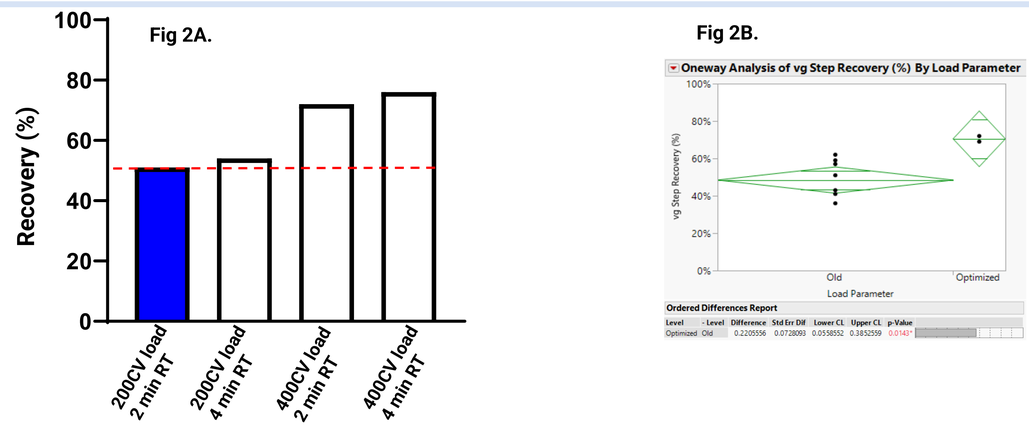


Fig 2. Column Dynamic Binding Capacity Over Residence Time Study. (A) Effect of column challenge over different residence time on recovery. (B) The statistical analyses of control and optimized run by load parameters including RT and load volume showed the significant improvement (p-value 0.0143).

DoE2: ELUTION BUFFER FORMULATION

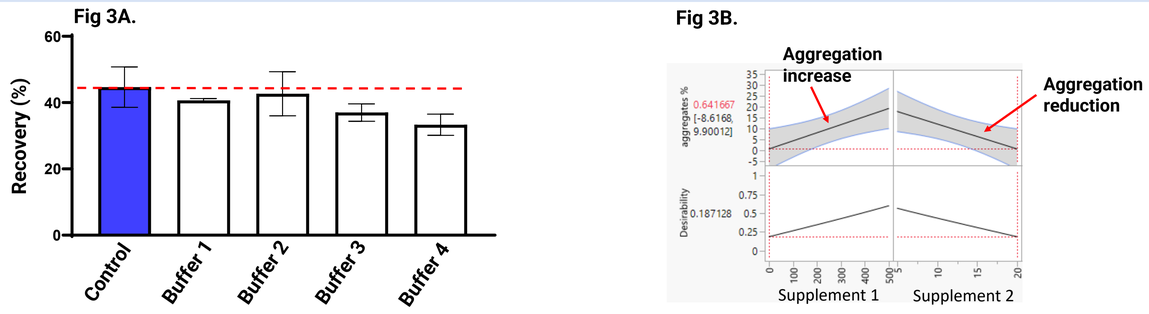


Fig 3. Elution Buffer Optimization. (A) 2 factor DoE performed in affinity batch purification looking at different elution buffers (B) The effect of addition of two different supplements in the elution buffer on eluate aggregation; data showed the increasing of supplement 1 concentration led to increase aggregation and the increasing of supplement 2 concentration led to aggregation reduction.

SCABILITY of THE OPTIMIZED AFFINITY CHROMATOGRAPHY

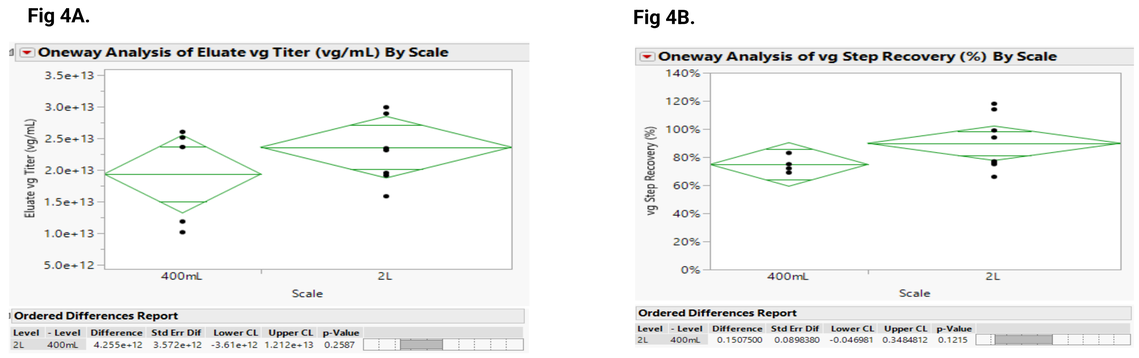
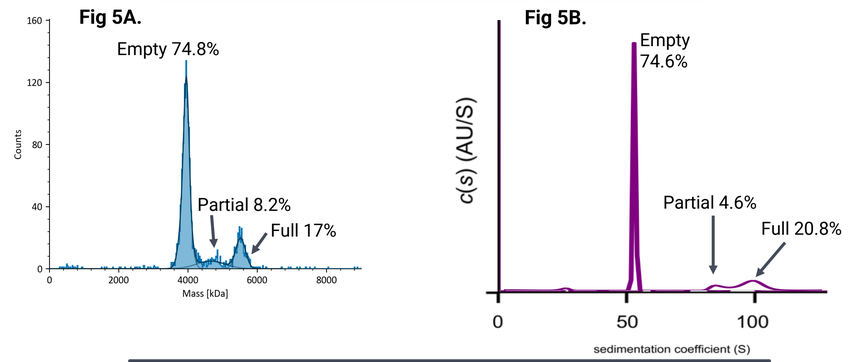


Fig 4. Scalability of the optimized affinity Chromatography. (A) The vg titer concentration of affinity eluate from 400 ml versus 2L scales showed no statistically significant difference between two scales (p-Value 0.2587) (B) Affinity step recovery data for the 400 ml small scale and 2 L scale-up runs showed no statistically significant difference between two scales (p-value 0.1215)

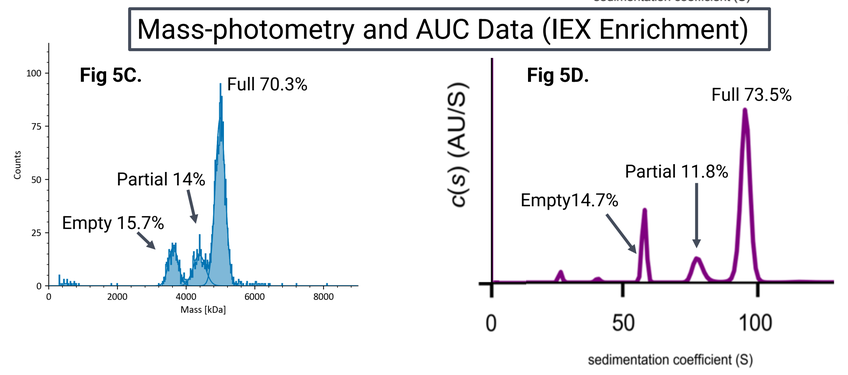
FULL AND EMPTY CAPSIDS PERCENTAGE

Mass-photometry and AUC Data (Affinity)



Full Capsids 20%

Mass-photometry and AUC Data (IEX Enrichment)



Full Capsids 73%

Fig 5. Full and empty capsids percentage. (A) Mass photometry of affinity eluate from 2L scale purification (B) AUC data of affinity eluate from 2L scale purification (C) Mass photometry of IEX eluate from 2L scale purification and (D) AUC data of IEX eluate from 2L scale purification

CONCLUSION

Our data demonstrates the optimized and scalable AAV6 affinity purification.

- The AAV recovery by affinity chromatography was improved about two times, from 15% to >35%, as a result of the DoE study evaluating the load conditioning parameters.
- The AAV recovery was improved to >70% by optimization of load volume over time.
- The amount of capsid aggregation could be reduced by optimization of buffer used for AAV elution from the affinity chromatography column.
- The optimized affinity purification showed to be scalable from shake flask to 2L reactor.
- The percent of full capsids was enriched from 20% in the affinity eluate to >70% after IEX purification.

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