Optimization of a Versatile Downstream Process for Multiple Novel AAV Capsids That Demonstrates Improved Recovery

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INTRODUCTION

Adeno-associated virus (AAV) is a useful gene therapy tool due to its ability to target different cell types and tissue types depending on its serotype, as well as its low pathogenicity. Capsida Biotherapeutics leverages capsid engineering technology and in-house manufacturing capability to develop robust processes for the production and purification of novel AAV capsids. Part of a robust platform process is the ability to adapt the process to any novel variant that may become a drug candidate. Within downstream process development, the focus is not only on having a versatile primary capture step for purification of AAVs from any process impurities from the upstream lysis and harvest steps, but also ensuring that any modifications or changes to primary purification are compatible with the complete workflow.

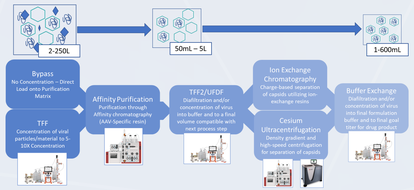

Figure 1. Overall Downstream Process and Scale - Complete downstream workflow and alternative processes for downstream steps

METHODS AND MATERIALS

Novel AAV capsid variants were brought through the process using an initial manufacturability assessment and exhibited low overall step recovery during affinity purification.
A high-throughput loose resin screen was utilized to test over 10 different formulations of elution buffers to improve overall recovery and were screened through SDS-PAGE.
Top performing buffers were then tested on chromatography systems and then scaled-up to confirm improvements in overall step recovery.
The optimized affinity purification step elution material was brought through the remainder of the downstream workflow and additional steps were optimized for an acceptable overall final process recovery.

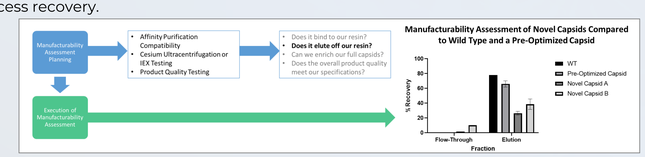

Figure 2. Standard planning for manufacturability assessment and general questions asked during the downstream portion of the assessment. Novel capsids brought into the manufacturability assessment demonstrate binding to the resin and low elution recovery, indicating the elution buffer needs optimization to increase overall step recovery.

RESULTS

Initial manufacturability assessments performed on the novel capsids are designed to show incompatibilities with the current downstream process with the primary concern being affinity purification and recent studies showed low recovery across the elution with two novel capsids (Figure 2). Formulation changes tested across 10+ elution buffers in loose resin format included pH, elution buffer type, and different salt additives. Buffer B demonstrated increased recovery when compared to other buffers (Figure 3B). Transition from loose resin to chromatography showed 4-fold increase in recovery (20% to ~80%) (Figure 4B).

Larger scale chromatography runs with the optimized conditions was performed to confirm scalability from smaller scale (≤2L) to 50L and reproducibility across multiple runs. 50L scale runs had similar chromatogram profiles (Figure 5) as well as recoveries around 80%.

Finally, the affinity elution material was processed in additional downstream process steps (TFF2, 0.2 Filter, Cesium Ultracentrifugation, and Final Buffer Exchange) to confirm compatibility with optimizations across the entire workflow.

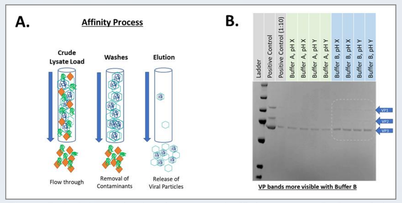

Figure 3. A. Schematic showing general affinity process. B. One of the SDS-PAGE gels from loose resin screening study where over 10 elution buffers were tested. Buffer B shows slightly more intense VP protein bands, indicating greater recovery with that buffer type.

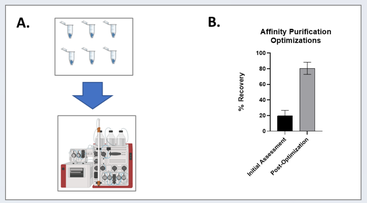

Figure 4. A. Transition from loose resin and spin based screening to column chromatography. B. Final optimizations with elution buffer showed increase from 20% to about 80% (a 4-fold increase).

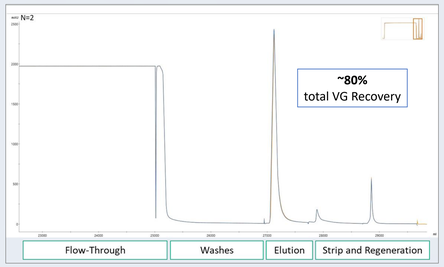

Figure 5. Scale-Up of Affinity Chromatography step to 50L shows reproducibility across two runs with recoveries at ~80% in the elution step.

DISCUSSION

Post-affinity purification optimizations, downstream steps further along in the workflow showed decreased recovery when compared to previous platform runs (TFF2 and 0.2 Filter). TFF2 (viral concentration pre-ultracentrifugation) saw improvements through pore size increase and slight changes in run parameters while the final 0.2µm step (pre-ultracentrifuge filtration) saw improvements through re-sizing (Figure 6A). The final overall 50L scale process showed improved overall recovery across multiple runs and was prepared for tech transfer to GMP manufacturing (Figure 6B).

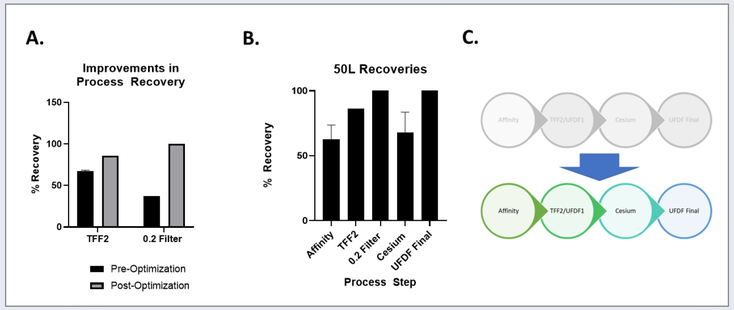

Figure 6. A. TFF2 (viral concentration pre-cesium ultracentrifugation) and 0.2µm Filter (0.2µm filter step pre-cesium ultracentrifugation) showed low overall recoveries post-optimization of affinity purification. TFF2 recoveries improved through slight modifications of parameters and consumables, while 0.2µm filter recovery improved through filter re-sizing and parameter adjustments. B. 50L Process Recoveries saw consistent and increased recoveries post-optimizations for a total process recovery higher than minimum acceptable (>20%) across multiple runs. C. Schematic showing an entirely offline process at the onset of a manufacturability assessment to a fully online process post-optimizations.

CONCLUSIONS

Through screening and small-scale optimizations, elution recoveries were improved ~4-fold during affinity purification of a novel capsid variant. Additionally, novel elution buffers are compatible with complete downstream workflow.
We have shown that the optimizations done on affinity elution demonstrate both scalability and reproducibility at a 50L scale and the downstream steps that are impacted by increased affinity recovery can be optimized through minor changes and re-sizing.

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