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Comparing Cation-Exchange Monolith and Affinity Resin for Chromatographic Capture and Purification of rAAV

Peršič Debenjak Š.*, Merkelj Koren J., Žigon R., Drmota Prebil S., Štokelj M., Nabergoj M., Peljhan S.*, Černigoj U., Leskovec M. & Štrancar A.

*Corresponding authors: Spela.p.debenjak@biaseparations.com, Sebastijan.peljhan@biaseparations.com
Sartorius BIA Separations d.o.o., Slovenia

Introduction

Using an efficient downstream process (DSP) for recombinant adeno-associated viruses (rAAVs) greatly contributes to producing high-quality gene therapy medicinal products. In addition, the rAAV purification process must be adaptable to a diverse range of rAAV serotypes. Typical rAAV DSP consists of pre-capture, capture, and polishing steps (1). In our study, the monolith CIMmultus® SO3 column was compared with two commercially available affinity resins, Affinity-Antibody (Affinity A) and Affinity-Peptide (Affinity P), as a chromatography column for the capture step. All three stationary phases are suitable for binding different rAAV serotypes. Polishing step for empty and full separation, often performed employing CIMmultus® QA high reproducibility (HR) line, is out of the scope for this study (2,3,4).
Our goal was to evaluate and compare process and step rAAV recoveries, impurity reduction, and processing time. Furthermore, we investigated the impact of various sample preparation procedures on the efficiency of chromatographic capture.

1. Study design

Two distinct sample preparation procedures were tested as shown in Figure 1. Procedure A was applied to rAAV8 (HEK293 suspension material, 2E+10 vg/mL (8.7E+10 vp/mL)), incorporating Tween-20 detergent for lysis and treatment with the DNase enzyme during the TFF step. Procedure B was applied for rAAV9 (HEK293 suspension material, 2.3E+11 vg/mL (5.2E+11 vp/mL)), using Na-deoxycholate detergent for lysis and treatment with the DNase enzyme during lysis. Each TFF retentate was divided, and separate capture steps for comparing columns were performed.

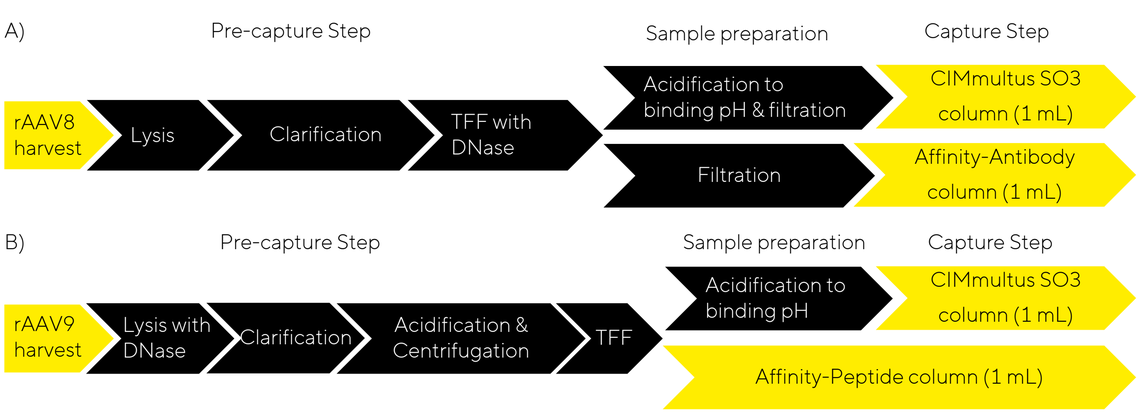

Figure 1: Schematic Diagram of the Purification Process A) Procedure A used for rAAV8 purification. B) Procedure B used for rAAV9 purification.

2. Results

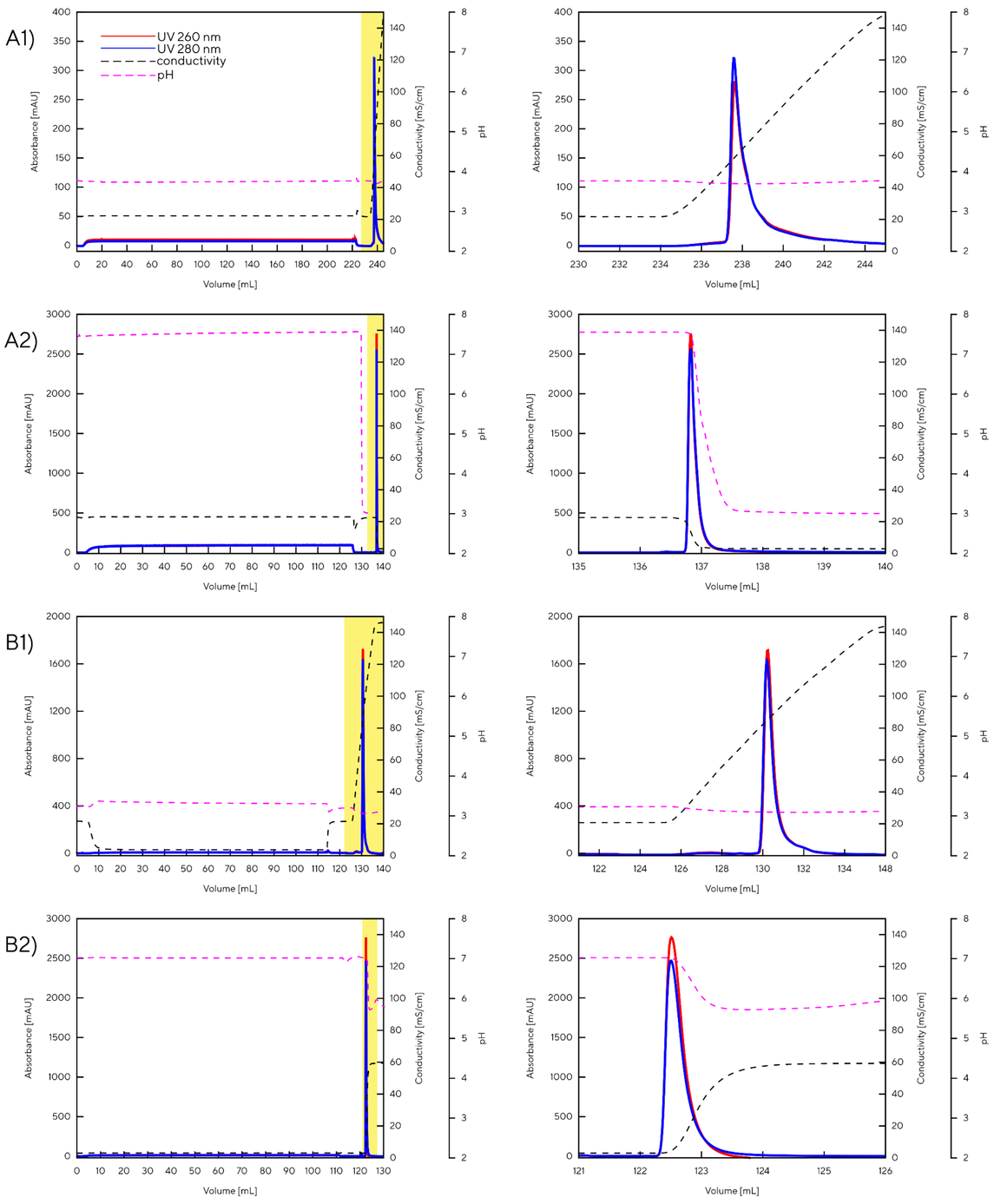

Figure 2: Chromatographic Elution Profiles of the Capture Step: on the left side complete chromatograms – sample loading, column wash and elution; on the right side – close-up of highlighted area of virus elution. A1) Purification of rAAV8 – CIMmultus SO3 column, A2) Purification of rAAV8 – Affinity-A column, B1) Purification of rAAV9 - CIMmultus SO3 column, and B2) Purification of rAAV9 - Affinity-P column.

The CIMmultus SO3 column, a strong cation exchanger, effectively binds various rAAV serotypes at low pH levels ranging from 3.5 to 5.0, in the presence of salt, maintaining binding efficacy up to 500 mM. Its elution process is straightforward, achieved by simply increasing salt concentrations while keeping the pH constant. In contrast, affinity columns bind rAAVs at neutral pH but require pH adjustments during elution. For instance, the Affinity-Antibody Column can handle binding salt concentrations up to 200 mM but requires lowering the pH to 3.0 for elution. Meanwhile, the Affinity-Peptide Column demands serotype-dependent pH conditions and can manage up to 50 mM salt for binding, necessitating changes in both pH and salt concentrations for elution, such as adjusting from pH 7.0 to pH 6.0 and increasing salt from 0 to 400 mM.

3. Process and step recoveries

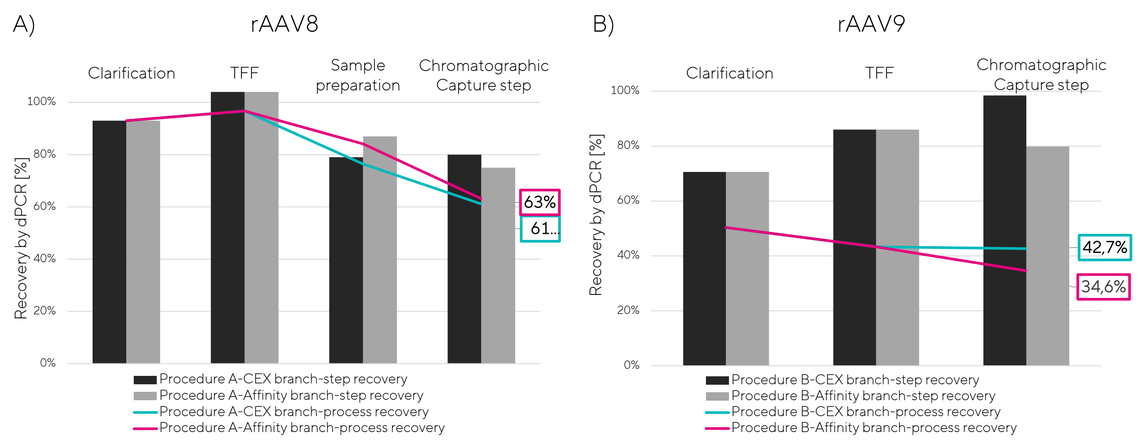

Figure 3: Process and step recoveries for both purification processes determined by dPCR. A) Purification of rAAV8, procedure A. B) Purification of rAAV9, procedure B.

Process and step recoveries were comparable for the CIMmultus SO3 column and Affinity columns across the same purification procedure.

4. Reduction of impurities

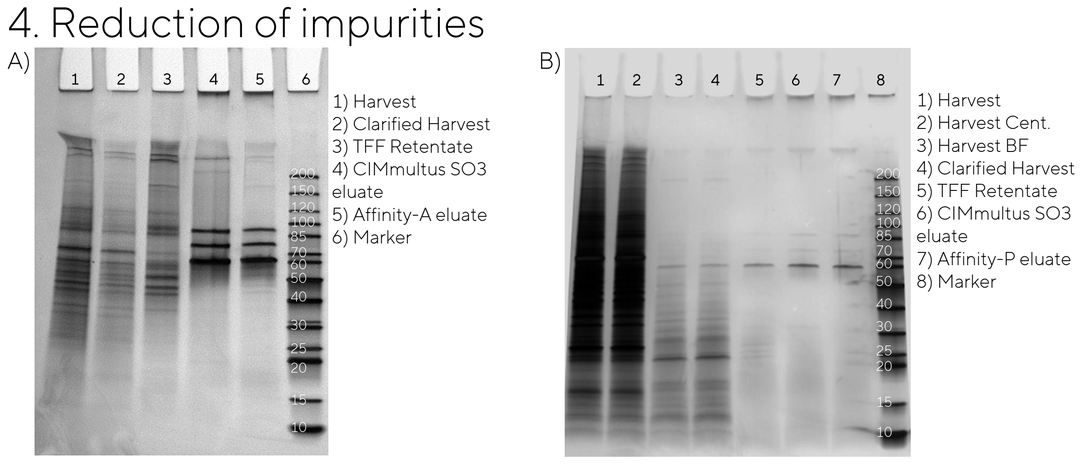

Figure 4: Silver-stained SDS-PAGE gels. A) Purification of rAAV8, procedure A. Total of 4E+9 vg was loaded per well. B) Purification of rAAV9, procedure B. Total of 4E+9 vg was loaded per well.

The impact of various pre-capture approaches is evident in the SDS-PAGE gels shown in Figure 4. Differences in protein profiles can be observed between Gel A, lane 3, and Gel B, lane 5, which correspond to TFF retentate material prepared using distinct procedures.

5. Process time comparison – Lab scale

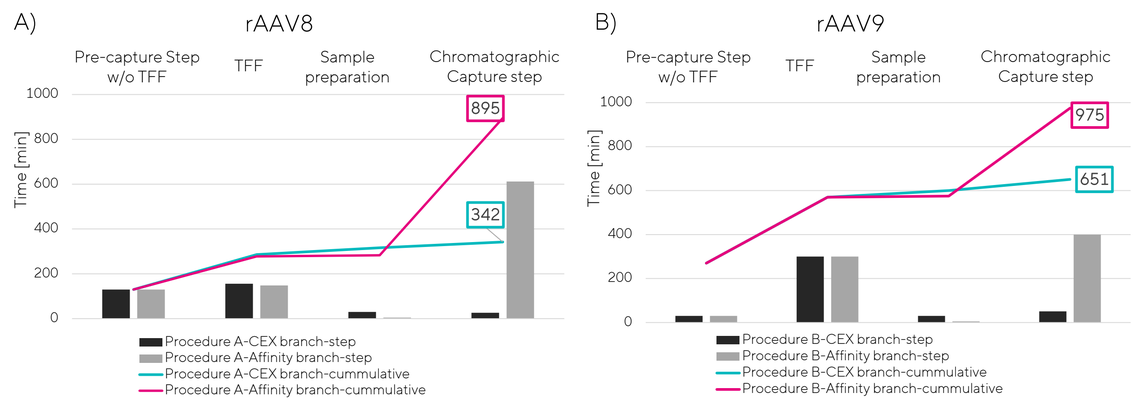

Figure 5: Comparison in process time between CIMmultus SO3 and Affinity column. A) Purification of rAAV8 and B) Purification of rAAV9.

The process time comparison data is derived from the experiments, where the same amount of virus was loaded onto the columns. In the capture step of purification process A, 1E+13 vector genomes (3.3E+13 viral particles) of rAAV8 were purified. Meanwhile, in the capture step of purification process B, 1.7E+13 vector genomes (5.3E+13 viral particles) of rAAV9 were purified. Chromatography was conducted using chromatography conditions recommended by the column manufacturer.

6. Conclusion

- The purification process using the CIMmultus SO3 monolith column significantly reduces purification time, being 2.6 times faster than the Affinity-Antibody resin-based column and 1.5 times quicker than the Affinity-Peptide column.
- In capture step similar recoveries were obtained for rAAV8 using both types of chromatographic support, while for rAAV9 the recovery was 8 % higher on CIMmultus SO3 column.
- The CIMmultus SO3 Column proves to be highly effective in the capture step, showcasing remarkable performance across diverse virus serotypes and binding conditions irrespective of pre-capture step treatments and preparations.
- CIMmultus SO3 Column is prepacked and can be CIP and SIP therefore reused many times, resulting in much lower manufacturing costs when compared with the affinity resins.

References

1. Rieser, R., et al. (2021). Comparison of different liquid chromatography-based purification strategies for adeno-associated virus vectors. *Pharmaceutics, 13*(5). 2. Žigon, R., et al. (2022). Roadmap to success in AAV purification. In-process control, high throughput & novel column modalities as necessary means for control over scalable AAV process. *Cell and Gene Therapy Insights, 08*(10), 1315–1328.
3. Bažec, K., et al. (2023). Optimization of rAAV capture step purification using SO3 monolith chromatography. *Electrophoresis, 44*(24), 1943–1952.
4. Miklavčič, R., et al. (2025). Development and Validation of AAV Capsids Separation on Specimen Columns for Reproducibility Evaluation of Large-Scale Chromatographic Monoliths. *Journal of Separation Science, 48*(3).