Simplifying Progress

Comparability Study of an Ion Exchange Monolith and Affinity Resin for the Purification of AAV8

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Introduction

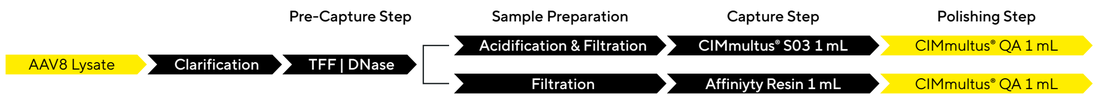
The main objective of every downstream process (DSP) for adeno-associated virus (AAV) production is to achieve high recovery, purity, and potency. The capture step for AAV purification is typically either affinity or cation exchange chromatography, both of which concentrate the product and remove impurities. Following the capture, the eluate is generally further processed to enrich for full capsids and carry out further purification. CIMmultus® QA, a monolith-based anion exchange chromatography column, is widely used for the enrichment and polishing of full AAV capsids[1].

Since the polishing step relies on only small differences in charge of the AAV capsids, any process-induced heterogeneity or charge modulation of the capture eluate will diminish the separation efficiency and affect the step's robustness. Affinity elution samples are reported to contain additional impurities[2] which influence the performance and duration of subsequent DSP steps. A side-by-side comparison was performed using a CIMmultus® SO3 – 1 mL (2 µm) column and a commercially available affinity resin that binds several AAV serotypes. Both columns were evaluated for process and step recoveries, impurity reduction, product capacity, and processing time. The results shown are based on two parallel experiments for each capture approach.

1. Experimental Design

Two AAV8 batches (HEK293 suspension material) with a vector genome titer of 2E+10 viral genomes (vg)/mL (8.7E+10 viral particles [vp]/mL) were clarified and further processed by tangential flow filtration (TFF) and DNase treatment. The TFF retentate was divided, and the two capture strategies were performed followed by a polishing step (Figure 1).

Figure 1: *Schematic Diagram of the Purification Process*



The following features were compared between the processes:

- Process and step recoveries (dPCR, PATfix® analytics)
- Product- and process-related impurity reduction: empty capsids (PATfix® analytics and mass photometry), residual host cell DNA (hcDNA; qPCR), plasmid DNA (pDNA; dPCR), host cell protein (HCP; ELISA HEK293 kit), endotoxin (ETX; Endosafe®), and protein content (SDS-PAGE)
- Capacity (determined from TFF retentate or clarified lysate)
- Processing time

2. Results

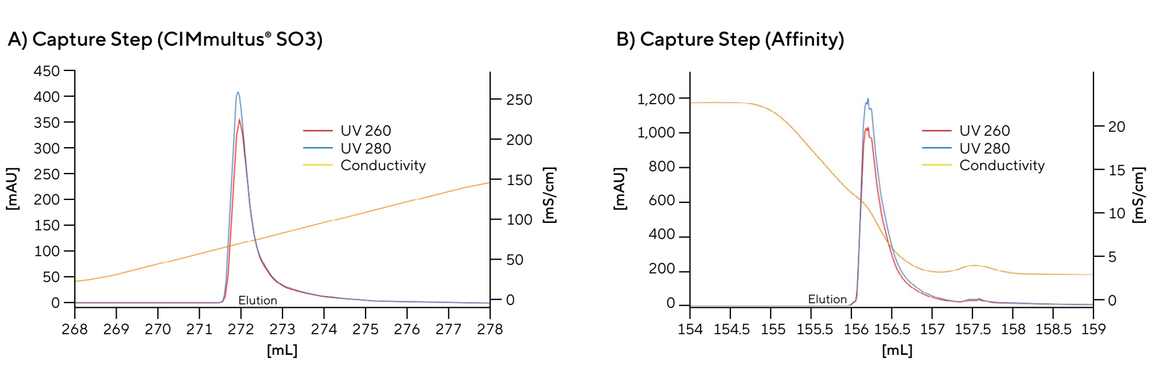


Figure 2: *Chromatographic Elution Profiles of the **(A)** and **(B)** Capture Step and **(C)** and **(D)** Their Corresponding CIMmultus® QA Run. Overlay of Absorbance at 280 nm. Ovelay of the Polishing Step Runs in the **(C)** First and **(D)** Second Repetition.*

In the first repetition of the polishing step, the ratios for empty and full peaks were consistent between samples purified by CIMmultus® SO3 and affinity resin. However, the 'damaged' peak was slightly more pronounced in the case of affinity purification (ratio 1:1.4, Figure 2C). Moreover, the full peak migrated to the left occurred in the affinity approach (17.23 mS/cm in the CIMmultus® SO3 approach and 16.34 mS/cm in the affinity process; Figure 2C). Results for the polishing step after affinity showed poor reproducibility in the second repetition, although binding conditions were met (Figure 2D). Fractions of this run could not be included in the performed analytics. The lack of robustness of the affinity process will be the subject of further research.

3. Process and Step Recoveries

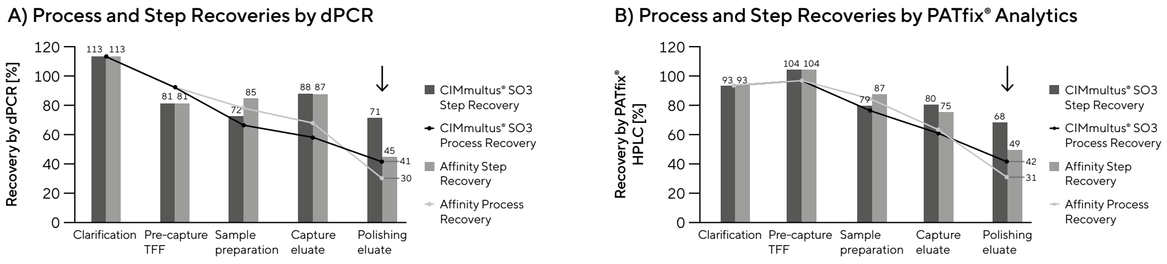


Figure 3: *Process and Step Recoveries for Both Processes. **(A)** Analyzed by dPCR. **(B)** Analyzed by Cation Exchange Chromatography Fingerprint (CEX-FP) on the PATfix® System. The Results Shown in the Step Recoveries Are the Average Values of the Two Repetitions. The Highest Discrepancies Between the Two Processes Are Seen in the Polishing Step (Arrow)*

The overall process recovery for the CIMmultus® SO3 approach was 41% or 42%, in contrast to 30% or 31% for the affinity approach, based on orthogonal dPCR and PATfix® system analytics, respectively (Figure 3). The two processes show comparable step recoveries except during the final polishing step, where the CIMmultus® SO3 approach delivered significantly better recovery (arrow).

4. Reduction of Impurities

The two processes have comparable hcDNA, pDNA, and ETX reduction efficiency. However, the CIMmultus® SO3 process achieved a statistically significant reduction in HCP compared to the affinity process (additional 0.6 log). The majority of the hcDNA and pDNA reduction in the pre-capture step was due to DNase treatment conducted during TFF (Figure 4A). Based on the mass photometry results, full capsid enrichment was better with CIMmultus® SO3 purification (from 30% full capsids in the starting material to 72.3% in the final fraction), compared to affinity purification (from 30% full capsids in the starting material to 56.5% in final fraction; Figure 4C). Comparable results were obtained with the PATfix® biochromatography system (75.9% full capsids following CIMmultus® SO3 purification vs 54.6% following affinity purification; Figure 4D).

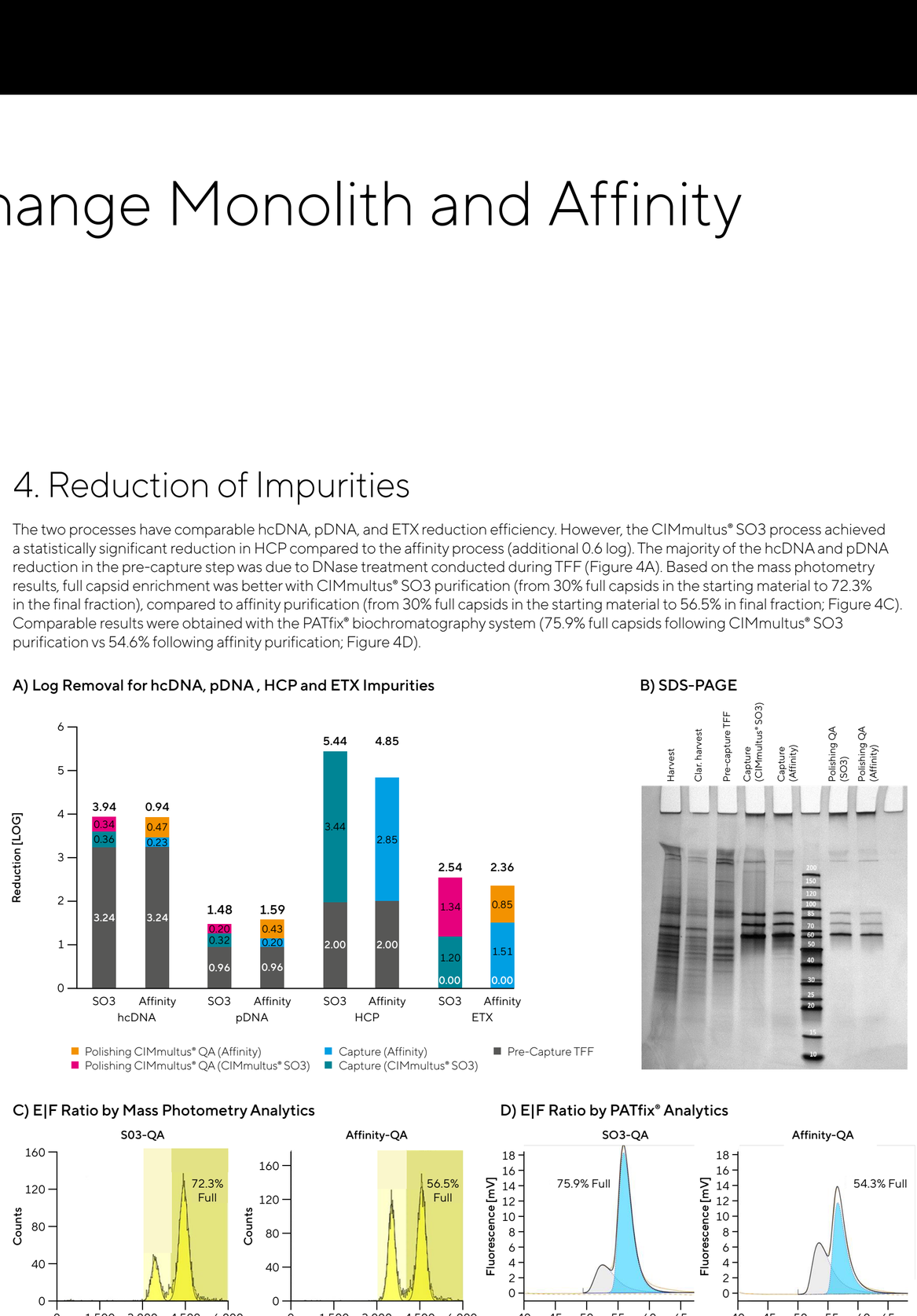


Figure 4: ***(A)** Comparison of Impurity Reduction of Both Capture Approaches. Bolded Values Represent Total Reduction (Sum of Individual Steps). **(B)** Silver-Stained SDS-Page for Both Processes at Each Purification Step. A Total of 4E+9 vg Was Loaded per Well for Capture and Polishing the Main Elution Fractions. Bands Above 200 kDa Present in Both Polishing Steps Correspond to vg DNA, Only Found in Full Capsids. Percentage of Full AAV Capsids in the Main CIMmultus® QA Elution Measured by **(C)** Mass Photometry **(D)** and PATfix® Analytics. Examples Shown Are From the First Repetition*

5. Column Capacity

The CIMmultus® SO3 column outperformed the affinity method in terms of capacity. The implementation of a TFF step increased the capacity by 17-fold compared to direct harvest loading. For the affinity column, capacity using clarified harvest was not performed due to non-process feasible loading time duration (> 100 hours).

A) Capacity Using TFF Retentate

	vg per mL Column (dPCR)	vp per mL Column (ELISA)	vp per mL Column (PATfix® AEX)
CIMmultus® SO3	5.03E+13	2.23E+14	1.52E+14
Affinity	4.64E+13	2.06E+14	1.41E+14

B) Capacity Using Clarified Lysate

	vg per mL Column (dPCR)	vp per mL Column (ELISA)	vp per mL Column (PATfix® AEX)
CIMmultus® SO3	3.14E+12	1.64E+13	8.97E+12
Affinity	Loading not performed > 100 hrs		

Table1: ***(A)** Capacity of TFF Retentate Loaded for Both Columns Calculated by Three Different Analyses. **(B)** Capacity of Clarified Harvest Loaded for Both Columns Calculated by Three Different Analyses*

6. Results – Process Time Comparison

The CIMmultus® SO3 process was faster than the affinity process with and without the TFF load preparation step. This was true for the small-scale runs and the predicted 100 L runs.

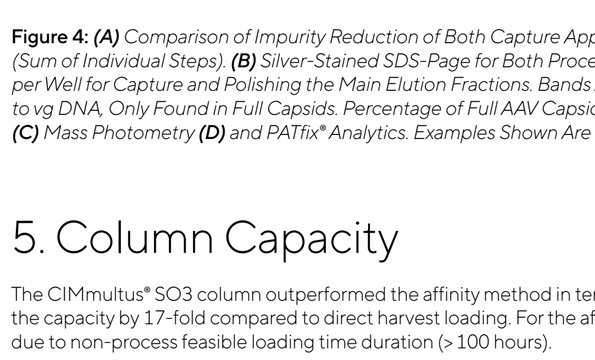


Figure 5: *Comparison of Processing Times Between CIMmultus® SO3 and Affinity Processes*

Note. Actual processing time from this study (approximately 1 L batch) and predicted processing time for the purification of a 100 L batch with titer 1E+11 vg/mL (1E+16 total vg) from the initial material up to capture step eluate are shown. Calculations for predicted comparison were based on using a 5 m^2 large TFF membrane and running TFF at 30 LMH permeate flow rate. For the CIMmultus® SO3 process, a 400 mL column* and flow rate of 2 CV/min was chosen, and for the affinity process, a 430 mL* column and 0.33 CV/min were taken into account. For the direct lysate loading, we considered a 4,000 mL SO3 column* and a flow rate of 1 CV/min, for the affinity process, the same column size and flow rate as above were selected.

* Column size was chosen with regard to experimental capacity results.
** For the affinity, column packaging time was not taken into consideration.

7. Conclusions

- The full process with the CIMmultus® SO3 capture step delivered a 30% increase in the number of doses available to the clinic compared to the full process with the affinity capture step.
- Comparable reduction of impurities was observed for the two capture steps. However, the CIMmultus® SO3 column demonstrated superior performance in the removal of HCP and empty capsids.
- Processing times were reduced by 2x with CIMmultus® columns compared to affinity columns.

References

1. Rieser, R., Koch, J., Faccioli, G., Richter, K., Menzen, T., Biel, M., ... Michalakis, S. (2021). Comparison of different liquid chromatography-based purification strategies for adeno-associated virus vectors. Pharmaceutics, 13(5). https://doi.org/10.3390/pharmaceutics13050748
2. Martin, D. (2022). Adeno-associated virus process development: optimization & development of a scalable elution for polishing chromatography. Cell and Gene Therapy Insights, 08(03), 421–429. https://doi.org/10.18609/CGTI.2022.061