Purification scalability and reproducibility of AAV9 viral vector moving into clinic

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Introduction

One of the most important tools in downstream purification (DSP) are chromatographic technologies; because of that, Repligen developed its single-use (SU) KRM™ Chromatography System platform specifically for advanced therapy medicinal products (ATMP) such as AAV production. These systems enable increased process efficiency and overall process step yield, protect potency and product integrity, reduce the overall risk of deviations due to their design, and enhance user experience. Major design features are over-molded tubing connections, compact valve manifold designs, combining filter and bubble trap, and advanced gradient control.

In a recent collaboration, Repligen and Forge Biologics, one of the leading global GT CDMOs in the field of AAV manufacturing, tested the KRM™10 Chromatography System by verifying the process performance, its scalability from the benchtop to manufacturing, and its robustness and reproducibility. The study focused on the AAV capture purification step, scaling 100x from the bench-top system using 5-mL OPUS pre-packed column to manufacturing-scale KRM™ 10 using 515-mL OPUS pre-packed column.

Single-Use KRM™ Chromatography System

The KRM™ Chromatography Platform **(Figure 1)** was designed with the needs of gene and cell therapies manufacturing in mind. The systems can handle complex, fragile viral vectors by providing improved process performance and robustness as well as high product recovery. Its design features enable the linear scale-up from the bench to the manufacturing scale.

Figure 1. KRM Chromatography Systems Platform

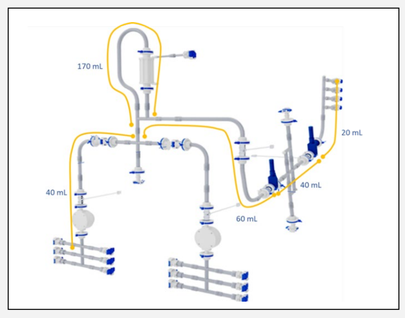

Figure 2. KRM™ 10 hold-up volume

The design of the KRM™10 allows minimization of its hold-up volume of the flow skids, as shown in **Figure 2**. A volume of 60 mL, from the mixing point to the column inlets, corresponds to approximately 6% volume of an 8x20-cm column, the smallest recommended column for this system. These minimal hold-up volumes enable the accurate gradient performance by reducing back-mixing effects in the flow path, and, therefore, reduce any peak broadening during the elution.

Case Study: Scalability and Reproducibility of AAV Capture

For this case study, the upstream and downstream platforms for the AAV manufacturing process were applied. The current upstream platform runs bioreactors up to 500 L; however, will be increased up to 5000-L scale. After the transfections, with a hold time of 4 to 5 days, the cells are lysed and filtered using a 0.2-micron filter.

The downstream process starts with loading the clarified lysate onto the affinity column, packed with POROS™ CaptureSelect™ AAV9 capturing AAV while removing further cell debris, HCP, and HCDNA. Before entering the next chromatography step, the process stream is filtered using UF/DF. At the IEX step, empty and full AAV vectors are separated using linear gradient elution. After another UF/DF step and a sterile filtration, the final drug product is filled into vials.

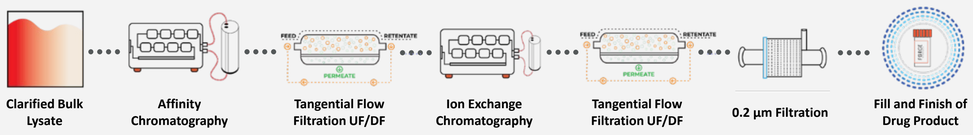

Figure 3. Downstream manufacturing process.

This case study focused only on the first AAV purification step, the capture. A 500-L bioreactor batch was clarified and then divided into three loads, allowing three large-scale runs. For each KRM™ system run, at least one benchtop control run was executed using approx. 1-L of feed (**Figure 4**)**.** The feed material for the large-scale runs was at ambient temperature during the entire run. The 1-L samples were stored at 4–7° C. Before each run, samples were taken from both feed materials for analysis (**Table 3** and **Table 4**).

Bench	Run #	1, 2	3	4
	Load (L)	1	1	0.6
Timeline →				
KRM	Run #	1	2	3
	Load (L)	55	167	167

Figure 4. Experimental design for scale-up runs: one 55 and two 167 L, with their parallel bench-top runs.

For the scale-up runs, an OPUS column (8-cm ID and 10-cm length) on the KRM™10 system was used. As a control, an OPUS MINI column (0.8-cm ID and 10-cm length) was used on a benchtop chromatography system. Both columns were packed with POROS™ CaptureSelect™ AAV9. At both scales, the same process steps were executed (see **Table 1**).

Table 1. Process steps as executed during bench-top and scale-up runs

Step #	Buffer/Solution	Linear Velocity [cm/h]	Duration [CV]	Transition / Comments
Sanitization	-	250	5	-
Equilibration	-	250	10	pH 7.2 +/- 0.2
Load	(Clarified lysate material)	250	100/200*/334	Standard: 200 to 400 CV
Wash 1	10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl	250	20	pH 7.2 +/- 0.2 > 2 AU
Wash 2	10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 500 mM NaCl, 2.7 mM KCl	250	5	pH 7.2 +/- 0.2 > 2 AU
Elution	40 mM citric acid, 10 mM sodium citrate, and 0.001% (w/v) Pluronic F-68, pH 3.0	250	4	Start collecting peak @ UV 280 at 1 AU, stop after 1.5 CV or 1 AU
Strip	100mM phosphoric acid	250	9	< 5 mAU

Results and Discussion

As shown in **Figure 5**, the AAV capture step was successfully scaled up. The overlaid chromatograms of the control and the KRM™10 have excellent alignment, even by the column-scaling factor of 100x. **Table 2** summarizes the actual experimental parameters for loads and flow rates. It also lists the collected peak volumes as well as the ratio of the main- to the late-eluting peak.

All chromatograms were recorded at the UV wavelength of 280 nm; however, flow cells with different pathlengths were used. The elution seen at the bench (**Figure 5**) could be reproduced at production scale. Furthermore, the elution of two different load volumes onto the large column are compared. All three product peaks show excellent alignment with similar retention times of ca. 64.5 min.

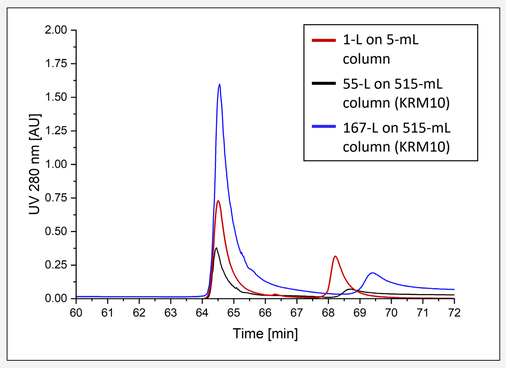

Figure 5. AAV Capture Scalability from 1 L to 167 L

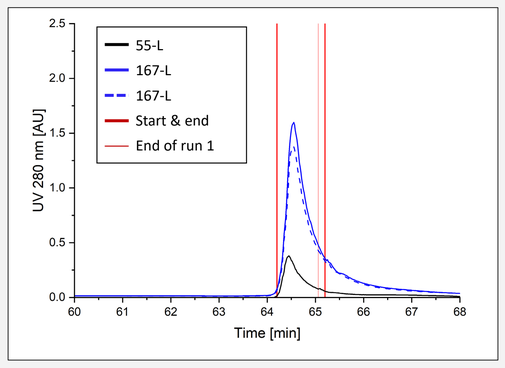

Figure 6. AAV Capture Scalability from Bench to KRM System

The chromatograms overlaid in **Figure 6** verify the scalability of the capture step from the bench to the KRM™ system. All runs were executed at the same linear velocity of 250 cm/h using the same feed material, and thus should have the same concentration.

Table 2. Experimental parameters

Run #	Bench top				KRM™ 10		
	1	2	3	4	1	2	3
Load volume [L]	1			0.6	55	167	
Flow rate [mL/min]	2.09				214		
Peak volume [mL]	3	3	3.2	2	170	198	190

Table 3 and **Table 4** summarize the analytical results of the KRM™10 and benchtop runs respectively, including the sample titers, yield, and recovery % of the AAV. The values were obtained by following the procedure Droplet Digital PCR (ddPCR) as described earlier. The tables list the results for the load, elution, resin regeneration, and flowthrough (FT). The recovery % of the wash steps were minimal (less than 2%); thus, they are not listed here.

Table 3. Analytical results of the scale-up runs

	Run/Sample	Affinity Load	Elution	Resin Strip
Overall Average	Titer (vg/mL)	3.8E+10	1.2E+13	1.6E+11
	Recovery (%)	-	77.3	9.0

Table 4. Analytical results of the bench-top runs

	Run/Sample	Affinity Load	Elution	Resin Strip
Overall Average	Titer (vg/mL)	3.4E+10	6.8E+12	7.8E+10
	Recovery (%)	-	63.9	6.0

In **Figure 7 and 8**, the chromatograms recorded at the two wavelengths of the KRM™10 run 3 are displayed as an example. During the wash and elution steps, no significant differences between the UV signals of 254 and 280 nm, such as extra peaks, were observed. The difference in the A254/280 ratio can be explained by the nonlinearity of the UV signals and not by separation of the empty and full AAV molecules.

In **Figure 9**, The average amount of AAV9 vg recovered by the benchtop chromatography system was 2.1E+13, once scaling up from 1 L to 167 L and loaded to KRM large scale system, the average was 5.04E+15 vg of AAV9

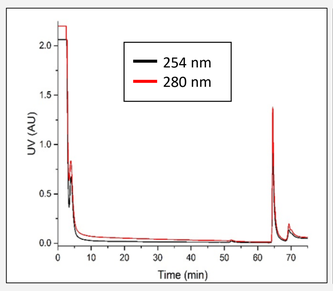

Figure 7. Comparison of UV signals: Complete chromatograms starting at Wash 1

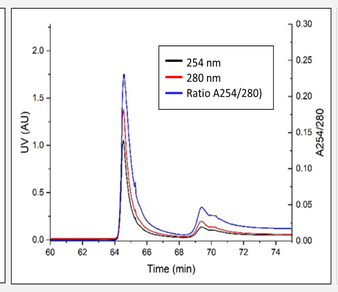

Figure 8. Comparison of UV signals: Elution profiles only plus A254/280 ratio.

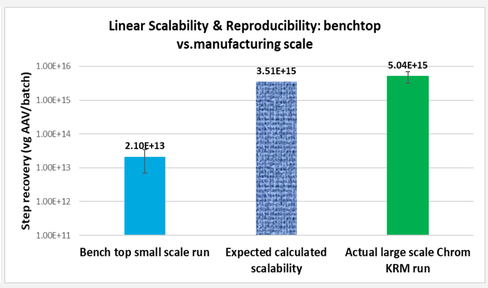

Figure 9. Linear scalability and reproducibility of purification step

Conclusion

In this case study, we verified the scale-up of an AAV capture purification step from the benchtop to the manufacturing scale by maintaining quality attributes, such as purity, and even improving process recovery while maintaining process parameters. Furthermore, the scale-up results were verified by a robustness evaluation that shows the required reproducibility of the manufacturing scale. Using the KRM™10 Chromatography System with its gentle fluid management, low hold-up volume, and accurate pump performance led to higher recovery and high consistency between the control benchtop and large-scale runs.

Process robustness and reproducibility are key for high productivity and cost-effective viral vector manufacturing at scale. We have demonstrated that process performance and consistency of the scale up process can be achieved by using KRM™ Chromatography System platform.

References

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Learn more at repligen.com/products/chromatography/krm-systems