AAV Capture Purification Scaling from the Bench to Clinical Manufacturing

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Introduction

Chromatographic technologies are the most important tools in downstream purification (DSP). Hence, Repligen developed its single-use (SU) KRM™ Chromatography System platform, especially for advanced therapy products such as AAV manufacturing. The systems enable increased process yield and product purities, reduce the overall process risk, and enhance user experience. Its major design features are over-molded tubing connections, compact valve manifolds, combined filter and bubble trap, and advanced pump 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 a 5-mL OPUS pre-packed column to manufacturing-scale KRM™ 10 using a 0.5-L OPUS pre-packed column.

Single-Use KRM™ Chromatography System

The KRM™ Chromatography Platform **(Figure 1)** was designed to meet the needs of CGT manufacturing. The systems can handle complex, fragile viral vectors while providing excellent process performance and robustness as well as high product recovery. Its design features enable linear scale-up from the bench to manufacturing scale.

Figure 1. KRM™ Chromatography Platform

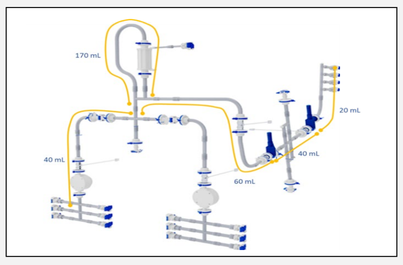

Figure 2. KRM™ 10 hold-up volume

The KRM™10 was designed with minimal hold-up volume within the flow path (**Figure 2),** being 60 mL from the mixing point to the column inlets (approx. 6% of 8x20-cm column). This minimized volume enables accurate gradient performance due to reduced back-mixing effects within the flow path, and, therefore, reduces any peak broadening during product elution.

Case Study: Scalability and Reproducibility of AAV Capture

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

The downstream **(Figure 3)** starts with capturing the AAV from the clarified lysate using POROS™ CaptureSelect™ AAV9 resin while removing cell debris, HCP, and HCDNA. Next, the process stream is filtered using UF/DF. At the second chrom step - ion exchange, the 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.

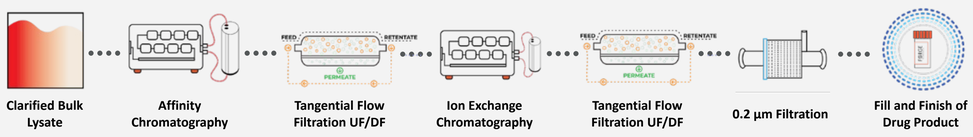

Figure 3. Downstream manufacturing platform.

The focus of this case study was only on the affinity step. A 500-L bioreactor batch was clarified and divided into three loads, which allowed three repeats of the KRM™ 10 run. For each large-scale run, at least one benchtop control was executed using approx. 1-L of feed**.** The large-scale feed was held at ambient temperature the entire time. The 1-L samples were stored at 4–7° C. Before each run, samples were taken for analysis (**Table 3** and **Table 4**).

For the KRM™10 runs, an OPUS column (8 x 10-cm) was used. As control on a benchtop chromatography system, an OPUS MiniChrom column (0.8 x 10-cm) was used. 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, UV 280 > 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, UV 280 > 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 4**, the AAV capture step was successfully scaled up. The overlaid chromatograms of the control run and the KRM™10 run 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 .

Table 2. Experimental parameters

Run #	Bench top				KRM™ 10		
	1	2	3	4	1	2	3
Load volume [L]	1			0.6	50	167	
Flow rate [mL/min]	2.09				214		
Peak volume [L]	0.009	0.009	0.01	0.008	170	198	190

All chromatograms were recorded at the UV wavelength of 280 nm. The elution seen at the bench (**Figure 4**) could be reproduced at production scale. Furthermore, the elution of two different load volumes onto the large column were compared. All three product peaks show excellent alignment with similar retention times of ca. 64.5 min.

The chromatograms overlaid in **Figure 5** verify the scalability of the capture step from the bench to the KRM™ system. All runs were executed at the same linear velocity 250 cm/h using the same feed (**Table 1 and Table 2**).

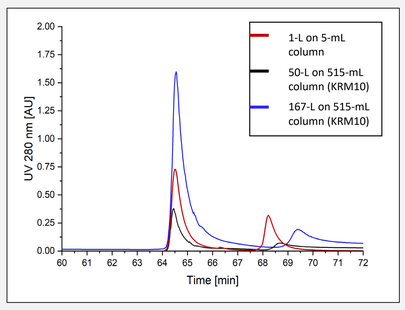

Figure 4. AAV Capture Scalability from 1 L to 167 L

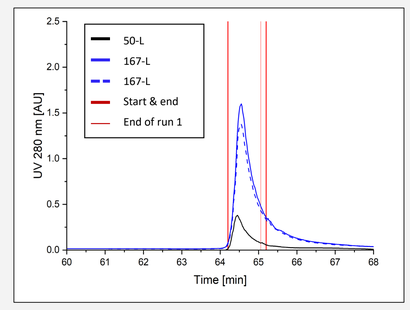

Figure 5. AAV Capture Scalability from Bench to KRM System

Table 3 and **Table 4** summarize the analytical results of all KRM™10 and benchtop runs respectively, including the sample titers, yield, and recovery % of the AAV. The values were obtained by Droplet Digital PCR (ddPCR). 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, 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
	Yield (vg)	5.1E+15	3.2E+15	1.1E+15
	Recovery (%)	100	61.3	10.6

Table 4. Analytical results of the bench-top runs

	Run/Sample	Affinity Load	Elution	Resin Strip
Overall Average	Titer (vg/mL)	3.4E+10	2.0E+12	7.8E+10
	Yield (vg)	3.4E+13	1.9E+13	2.0E+12
	Recovery (%)	100	55.8	7.1

Figure 6 and 7 display the chromatograms recorded at A254 and A280 of KRM™10 run 3. During the wash and elution steps, no differences between the UV signals of 254 and 280 nm, such as extra peaks, were observed. Difference in the A254/280 ratio can be explained by nonlinearity of UV signals and not by separation of the empty and full AAV molecules.

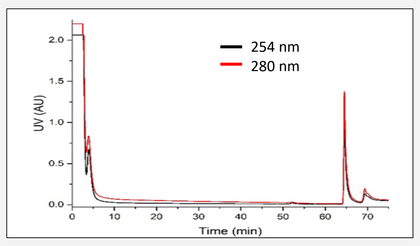

Figure 6. Example comparison of UV signals: Chromatograms starting at Wash 1

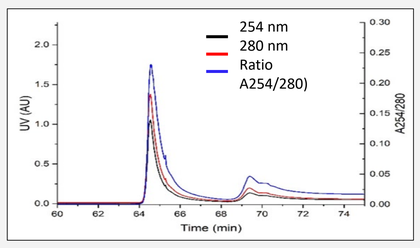

Figure 7. Example comparison of UV signals: Elution only, plus A254/280 ratio.

Study: Scaling up IEX Chromatography

In a separate study, the 10x scale up of a text-book IEX example was evaluated going from 1.1-cm to 10-cm ID column by keeping the length constant. All relevant process parameters listed (**Table 5**). The duration of the process steps and the residence time were also kept constant. **Table 6** and **Figure 8** show the results of the successful scale-up. Both chromatograms elute with almost identical retention time, leading to the same peak resolution and selectivity.

Table 5. Process steps as executed during bench-top and scale-up runs

Parameter	Bench	KRM10
Resin		Poros 50 HS (Cationic)
Column Dimensions	1.1 x 17 cm	10 x 17 cm
Column Volume	16.2 mL	1,335 mL
Residence Time	5 min	5 min
Flow Rate	3.2 mL/min	267 mL/min
Equilibration/Wash Buffer	20 mM Sodium Phosphate, 0.1 M sodium chloride, pH 6.8	
Elution Buffer	20 mM Sodium Phosphate, 0.375 M sodium chloride, pH 6.8	
Elution Gradient Length	10 column volumes	
Load Density	0.5 mb/mL_{resin} (1:1)	
Proteins Used	Cytochrome C, Chymotrypsinogen (1:1)	

Table 6. Comparing scale-up results

Test	Resolution	Selectivity
Bench	1.4	1.7
KRM10	1.4	1.9

Figure 9 displays the total flow rate and the pressure drop across the column during the gradient elution. Both parameters are well controlled, especially no pressure spikes caused by the changing elution buffer strength.

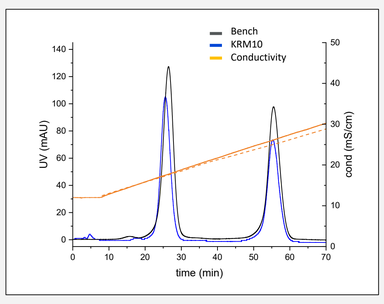

Figure 8. Comparing elution of Cytochrome C and Chymotryspinogen using bench-top and KRM10

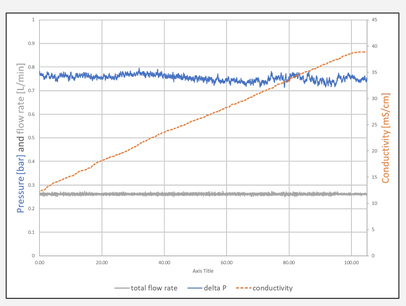

Figure 9. KRM10 profiles using conductivity and flow- controlled gradient at 0.26 L/min and using 10x17 cm OPUS column packed with Poros 50 HS

Conclusion

The case study verified the scale-up of an AAV capture 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, process robustness and reproducibility of the scale-up results were verified which are key for high productivity and cost-effective viral vector manufacturing at 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 in the process scale-up process by using KRM™ Chromatography System platform.

Looking forward, the presented IEX scale-up provided the base for upcoming empty-and-full IEX work.

References

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