Scalability and reproducibility of the AAV9 capture step using KRM™ Chromatography System

Case Study

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Introduction

Advancing the disease indications of cell and gene therapies (GT) beyond rare diseases, as well as developing more complex, yet fragile viral vectors, requires not only expanding existing manufacturing facilities, but also developing new approaches. Special requirements in viral vector manufacturing processes, such as low shear stress and low residence times, create scale-up challenges that cannot be addressed by traditional platform approaches. Advanced technologies are needed to provide the required reliability and robustness for manufacturing to enable gene therapies to meet their full potential.

Chromatography technologies play a key role in the productivity of downstream operations, prompting Repligen to develop its single-use (SU) KRM™ Chromatography System platform specifically for advanced therapy medicinal products (ATMP), such as adeno-associated virus (AAV). These systems increased process efficiency and overall process step yield, protect potency and product integrity, reduce the overall risk of deviations through design, and enhance user experience.

Major design features of the systems include:

- Over-molded tubing connections
- Compact XO® valve designs
- Combined filter and bubble trap
- Advanced gradient control

In a recent collaboration, Repligen and Forge Biologics, one of the leading global GT contract development manufacturing organizations (CDMOs) in the field of AAV and plasmid DNA manufacturing, evaluated the KRM™ 10 Chromatography System. The AAV capture purification step was scaled 100X from a bench-top system with a 5 mL OPUS® Pre-packed Column to a manufacturing scale KRM 10 System with a 515 mL OPUS Pre-packed Column. Clarified harvest from a single 500 L bioreactor was used to compare the process performance and product quality at both scales.

Single-use KRM Chromatography System

The KRM chromatography platform was specifically designed to address the needs of cell and gene therapy manufacturing. The systems can handle complex, fragile viral vectors by providing improved process performance and robustness as well as high product recovery.

Design features enable the linear scale-up from the bench to the manufacturing scale. The KRM Chromatography System platform (Figure 1) is designed for linear scale-up from bench to manufacturing. Four standard sizes cover flow rate ranges from 1 to 3600 L/h and support columns with internal diameters (ID) from 8 cm to >1 m.0.5 M NaOH

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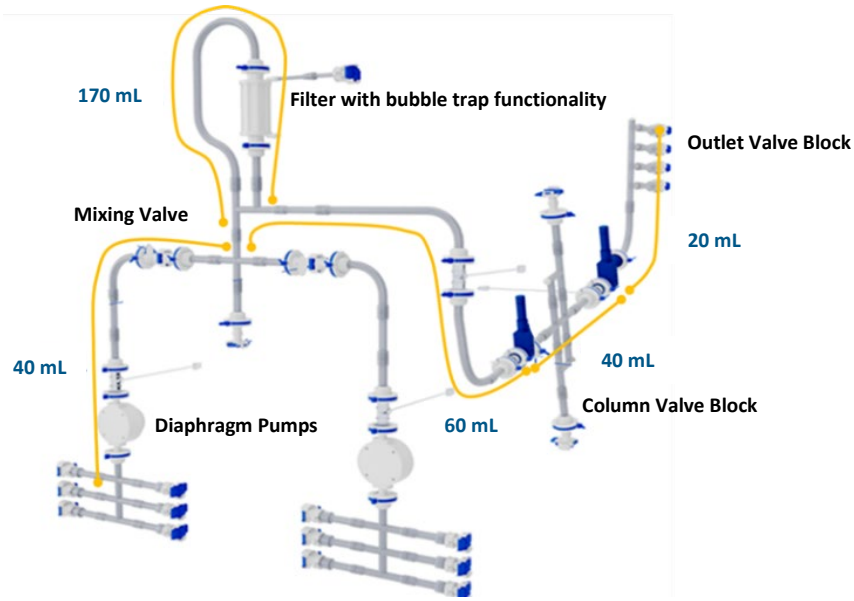
Figure 1. KRM Chromatography Systems

Process flow is the nearly same across all four sizes of the KRM System platform, using standard, pre-assembled single-use flow kits (Figure 2) that include diaphragm pumps and pre-calibrated process analytics to monitor and control the chromatographic processes. The systems are equipped with single-use flow meters for each pump, pressure sensors, a post-column pH/conductivity meter, a dual wavelength UV detector, and an optional pre-column pH/conductivity meter.

Each pump has 6 automated inlet valves for feed and buffer supplies. The systems come standard with 4 outlet valves for waste and product fractions with the option of expanding up to 7 or 8.

Figure 2. Pre-assembled flow paths of the KRM 10 System with corresponding hold-up volumes

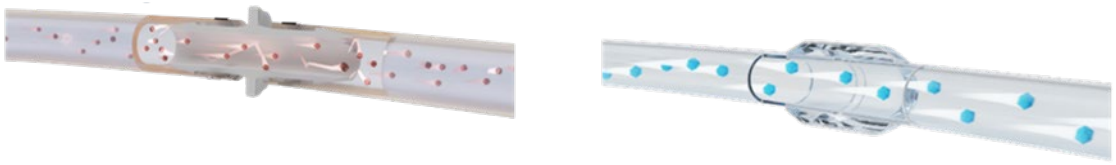


An important design feature of KRM Systems is the minimized hold-up volume of the flow kits (Figure 2). A volume of 60 mL, from the mixing point to the column inlets of the KRM 10 Chromatography System, corresponds to 6% of the volume of an 8 x 20 cm column, the smallest recommended column ID for the KRM 10 System. These minimal hold-up volumes enable accurate gradient performance by reducing back-mixing effects in the flow path, thereby reducing peak broadening during the elution.

KRM Chromatography Systems were designed with the following features:

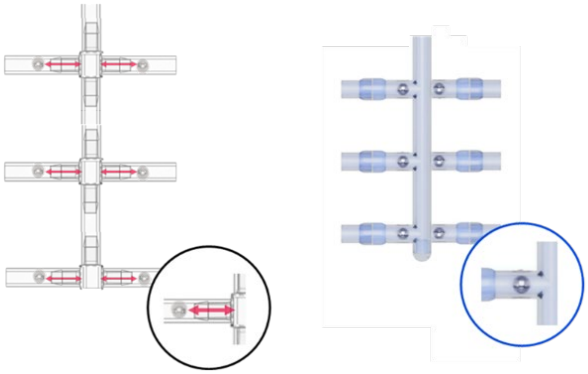
1. Over-molded tubing connections: Traditional hose barbs were replaced with seamless inner tubing, closer connection points, and a lack of crevasses to reduce turbulence, hold-up volumes, and degradation (Figure 3).

Figure 3. Flow pattern comparison of traditional barb fittings (left) and over-molded connections (right)



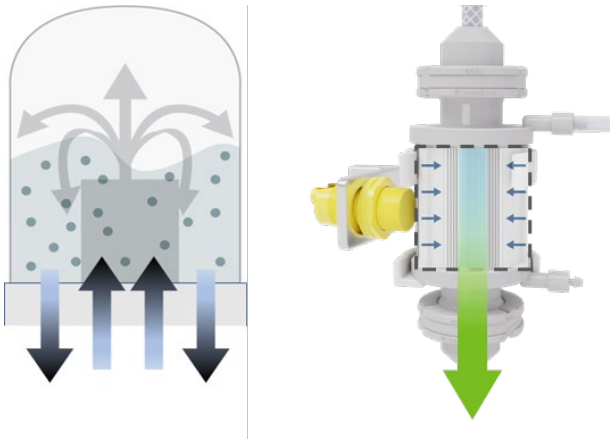
2. Compact XO valves: Traditional pinch valves were replaced with XO valves not only to reduce hold-up volumes, but also to eliminate dead-legs, thereby avoiding cross-contaminations during processing (Figure 4).

Figure 4. Comparison of traditional pinch valve design (left) and the XO valve design (right)



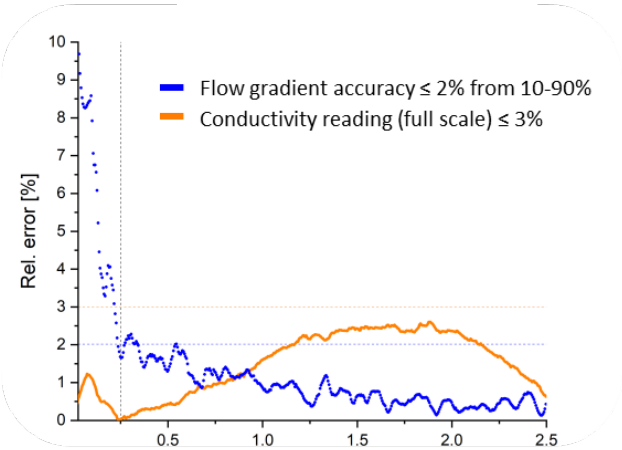
3. Combined filter and bubble trap: By combining the bubble trap with a filter, hold-up volumes are minimized, and the potential for back-mixing is decreased (Figure 5).

Figure 5. Comparison of traditional bubble trap (left) and combination of filter/bubble trap (right)



4. Advanced gradient control: Control loops between each pump and its corresponding flow meter, as well as the conductivity and pH meters ensure accurate pump performance and buffer delivery, resulting in reproducible and robust product elution. The highly accurate gradient control enables separation of empty and full capsids at manufacturing scale, eliminating the need for ultracentrifugation. The flow accuracy for 10 - 90% salt gradient is within 2%, and the observed conductivity reading of the gradient is within 3% when under flow control (Figure 6).

Figure 6. Gradient accuracy and conductivity monitoring

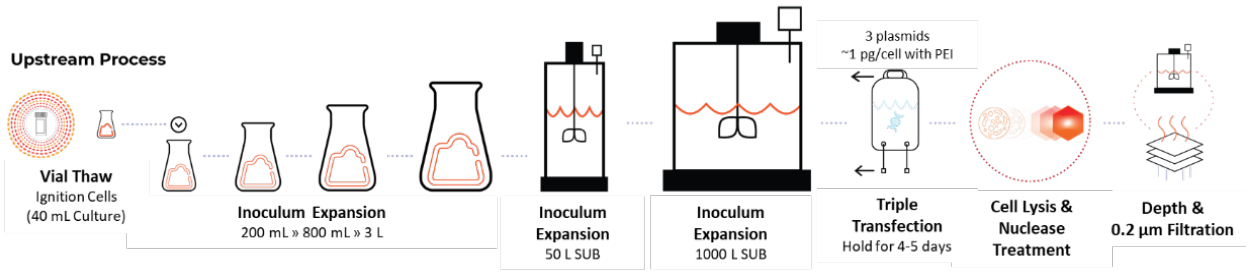


Study was conducted on KRM 10 in flow range 0.25 – 2.5 L/min

Case Study: Scalability and reproducibility of AAV capture step

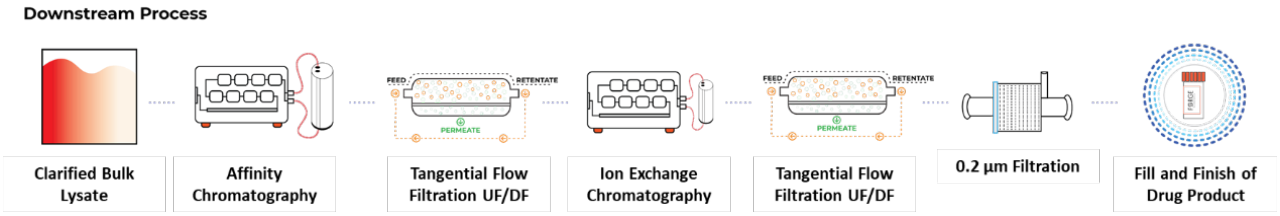
The upstream and downstream platforms for the AAV manufacturing process were performed (Figure 7, Figure 8). The current upstream platform runs bioreactors up to 500 L; however, the platform will be increased up to 5000 L scale. The upstream process (Figure 7) begins with the cell thaw and seed train. Following expansion to 500 L, transfection is performed and held for 4 - 5 days. Finally, the cells are lysed, and the lysate is 0.2 µm filtered.

Figure 7. Upstream platform process steps



The downstream process (Figure 8) starts with loading the clarified lysate onto an equilibrated POROS™ CaptureSelect™ AAV9 affinity column, which captures AAV capsids while removing host cell protein (HCP) and DNA (HCDNA) impurities. Before entering the next chromatographic step, the elution pool is filtered. At the ion exchange (IEX) step, empty and full AAV vectors are separated using a linear salt gradient elution. After the final ultrafiltration/diafiltration (UF/DF) formulation step and a sterile filtration, the final drug product is filled into vials.

Figure 8. Downstream platform process steps



Experimental design

This case study focused on the AAV affinity purification step. A clarified 500 L bioreactor batch was divided into smaller load batches, allowing three large-scale runs and four bench-scale runs (Figure 9). All runs were executed following the standard platform capture process (Table 1). The same process parameters were applied at both scales. The recipes used the same linear velocities, column bed heights, and buffers (Table 1).

The KRM System has a dual wavelength UV detector measuring at 254 and 280 nm using a 2.5 mm pathlength. The bench-top system has a single wavelength UV detector measuring at 280 nm using a 2 mm pathlength. Sample collection started at the inflection of the elution peak and continued for 1.5 CV.

A pre-packed OPUS Column (8 cm ID and 10 cm length) was used on the KRM 10 System. A pre-packed 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 resin.

The lysate of a 500 L bioreactor was clarified using depth/sterile filtration. For each KRM System run, at least one benchtop run was executed. The feed material for the large-scale runs was kept at ambient temperature for the duration of the study. The feed material for the bench-scale runs were stored at 4 - 7°C in three 1 L and one 0.6 L aliquots. Before each run, samples were taken from feed material for analysis.

Figure 9. Experimental setup

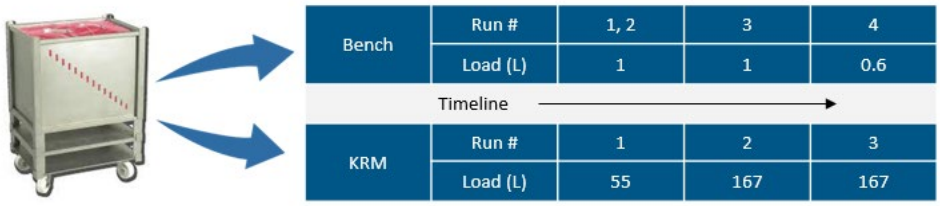


Table 1. Purification process

Step #	Buffer/Solution	Linear Velocity	Duration	Comments
Sanitization	100mM phosphoric acid	250 cm/h	5 CV	
Equilibration	10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl	250 cm/h	10 CV	pH 7.2 +/- 0.2
Load	Clarified lysate material	250 cm/h	100 - 400 CV	100 and 334 CV (KRM10) 120 and 200 CV (benchtop)
Wash 1	10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl	250 cm/h	20 CV	pH 7.2 +/- 0.2
Wash 2	10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 500 mM NaCl, 2.7 mM KCl	250 cm/h	5 CV	pH 7.2 +/- 0.2
Elution	40 mM citric acid, 10 mM Sodium citrate, and 0.001% (w/v) Pluronic F-68	250 cm/h	4 CV	pH 3.0 Peak collection: beginning at peak 1 AU, ending after 1.5 CV
Strip	100 mM phosphoric acid	250 cm/h	9 CV	UV < 5 mAU

Analysis by droplet digital polymerase chain reaction (ddPCR) was performed to quantify AAV genome copies. AAV samples were prepared by treating them with DNase I to remove non-encapsulated DNA. DNase-treated samples were further diluted to the linear working range of the assay. Dilutions were added to prepared PCR reaction mixtures containing master mix, primers, and a Taqman™ hydrolysis probe. The combined sample and PCR reaction solution were partitioned into approximately 20,000 individual droplets. Due to the partitioning, some droplets contained AAV, while others did not. All droplets underwent end-point PCR amplification.

Upon completion of the end-point PCR amplification, each droplet was read to determine either positive or negative fluorescence intensity. Analysis of ddPCR results was performed using Poisson statistical modeling through Bio-Rad QX Manager Software, Regulatory Edition, whereby the absolute quantification of a sample was determined.

Results and discussion

Data indicate that the AAV capture step scale-up resulted in comparable process performance (Table 2). Bench-scale runs were performed in parallel with manufacturing-scale runs. For the first manufacturing-scale run, two bench-scale runs were executed sequentially. Subsequently, one bench-scale run was performed in parallel with each KRM System run.

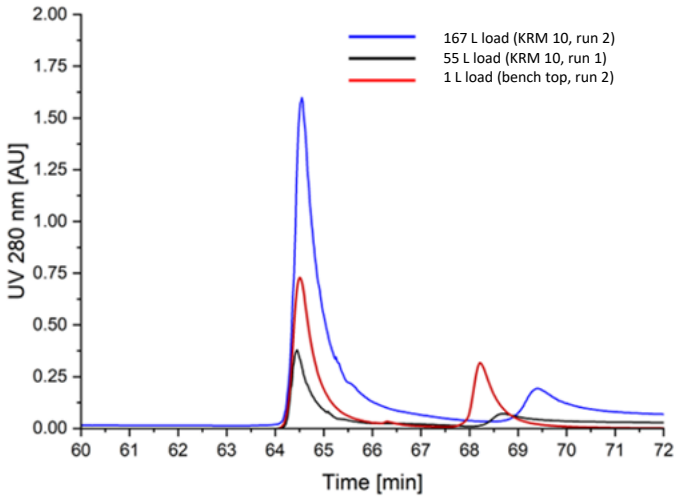
Table 2. Load and peak volumes

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

A sample of elution profiles overlaid from both bench- and manufacturing-scale show the scalability of the capture step (Figure 10). Runs at both scales were executed at 250 cm/h using material from the same bioreactor. All chromatograms were recorded at the UV wavelength of 280 nm. The elution peaks of the bench-scale run, the 55 L scale run, and the 167 L scale run have similar retention times. Variations in peak area are attributed to differences in load volumes, but similarities in peak shape and retention times suggest that the bench-scale process was reproduced at the manufacturing scale.

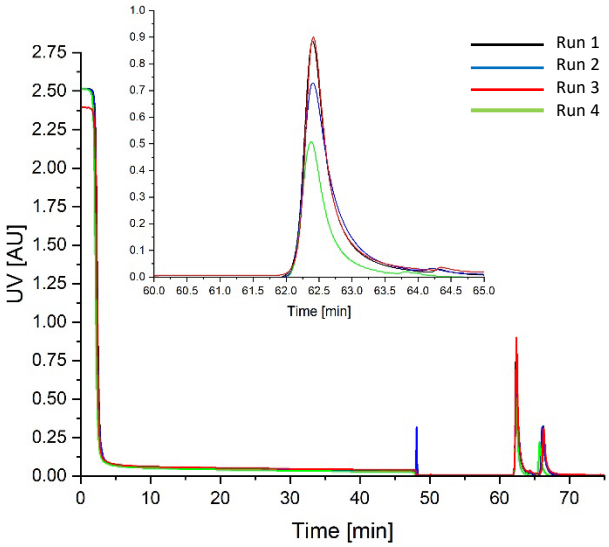
The strip/regeneration peaks contain impurities, degradations, and non-eluted product. The differences in peak area, shape, and retention time might be caused by when the regeneration step started, which is defined by the completion of the elution step (Figure 10) There may also be slight differences in the ratios of the strip and elution peaks between scales. Slightly larger regeneration peaks in the bench-scale runs may indicate that not all product was eluted, leading to lower process recoveries.

Figure 10. Benchtop and KRM System – overlayed elution and strip profiles



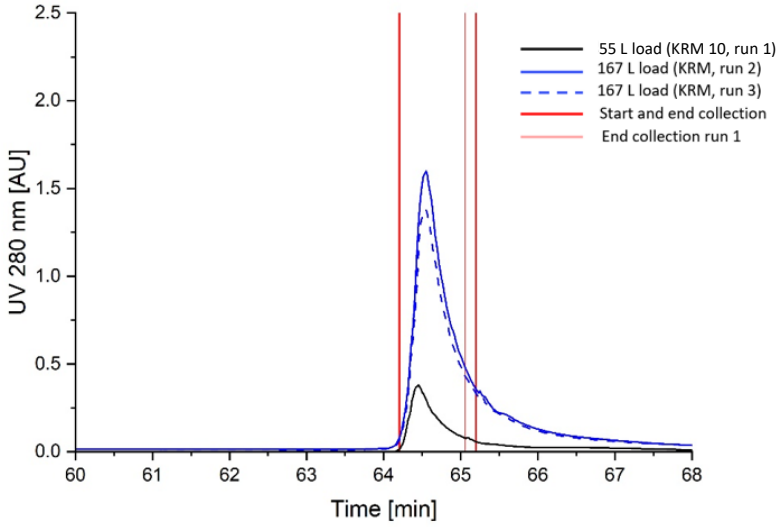
The elution peaks of the bench-scale runs are similar, indicating reproducibility and robustness within the process (Figure 11). The peak for run 4 is smaller due to the lower load. The slight difference in peak height in run 2 versus runs 1 and 3 may be attributable to an unexpected loss of material during a wash step.

Figure 11. Overlay of chromatograms at bench-scale



The elution profiles of the KRM 10 runs are also similar, indicating reproducibility and robustness (Figure 12). The peak area of run 1 is smaller than that of runs 2 and 3 because of the lower load.

Figure 12. Overlay of elution chromatograms at manufacturing scale



Analytical results, including the titers, yield, and recovery of AAV were obtained from ddPCR (Table 3, Table 4). Data fall within acceptable ranges of the assay. Load, elution, resin strip, and flowthrough (FT) are included. The recovery values of the wash steps were minimal (<2%) and are not included.

Table 3. Analytical results: benchtop runs

Run/Sample		Affinity Load	Elution	Resin Strip	FT
1	Titer (vg/mL)	2.8E+10	4.5E+12	6.6E+10	2.0E+08
	Yield (vg)	2.8E+13	1.4E+13	1.6E+12	2.0E+11
	Recovery (%)	—	48.6	5.9	0.73
2	Titer (vg/mL)	3.8E+10	6.7E+12	9.2E+10	2.3E+09
	Yield (vg)	3.8E+13	2.0E+13	2.3E+12	2.3E+12
	Recovery (%)	—	52.5	6.0	6.1
3	Titer (vg/mL)	4.0E+10	9.1E+12	8.0E+10	2.3E+08
	Yield (vg)	4.0E+13	2.9E+13	2.0E+12	2.3E+11
	Recovery (%)	—	72.5	4.96	0.57
4	Titer (vg/mL)	2.9E+10	6.8E+12	7.4E+10	3.0E+08
	Yield (vg)	1.7E+13	1.4E+13	2.0E+12	1.8E+11
	Recovery (%)	—	77.6	11.7	1.04
Average	Titer (vg/mL)	3.4E+10	6.8E+12	7.8E+10	7.6E+08
	Recovery (%)	—	63.9	6.0	0.9

The manufacturing-scale run with the 55 L load was underloaded, causing a lower yield than the other runs. The highest recovery was obtained with the first 167 L load. During the second 167 L manufacturing run, product was lost due to the degradation of the feed material, which was stored under ambient conditions. This degradation was not seen during the bench-scale runs due to cold storage.

Table 4. Analytical results: KRM 10 System runs

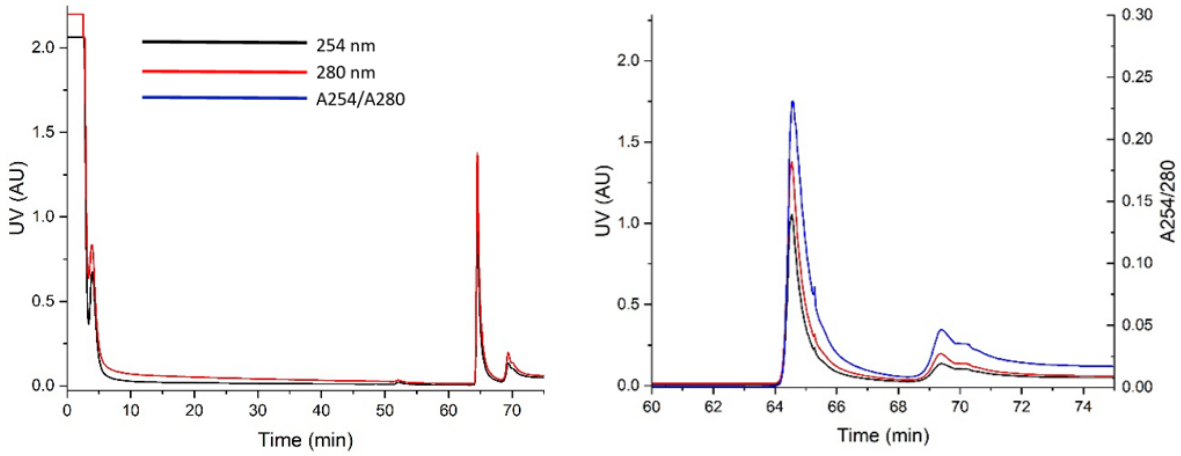
Run/Sample		Affinity Load	Elution	Resin Strip	FT
1	Titer (vg/mL)	3.8E+10	6.3E+12	7.8E+10	BLOQ
	Yield (vg)	2.1E+15	1.4E+15	2.0E+14	BLOQ
	Recovery (%)	—	69.4	9.4	BLOQ
2	Titer (vg/mL)	3.8E+10	2.0E+13	2.2E+11	2.9E+09
	Yield (vg)	6.3E+15	5.4E+15	5.4E+14	4.8E+14
	Recovery (%)	—	85.1	8.6	7.6
3	Titer (vg/mL)	3.8E+10	1.0E+13	2.0E+11	3.9E+08
	Yield (vg)	6.3E+15	2.7E+15	9.8E+14	6.5E+13
	Recovery (%)	—	45	15.52	1.03
Average	Titer (vg/mL)	3.8E+10	1.2E+13	1.6E+11	1.1E+09
	Recovery (%)	—	77.3	9.0[1]	3.8[1]

[1]Averages include only runs 1 and 2 due to hold-time issue during proof-of-concept runs.

As observed in the bench-scale chromatography capture step (runs 1 - 4) the average recovery was 63.9%. The actual average recovery from the manufacturing-scale runs (runs 1 and 2) was 77.3%, which is an average 13% increase in yield.

A comparison of the KRM 10 System chromatograms at 254 and 280 nm for run 3 indicates that no extra peaks were observed (Figure 13). The ratio of the A254/280 remained below 0.25 AU.

Figure 13. Comparison of UV signals; KRM 10 System run 3

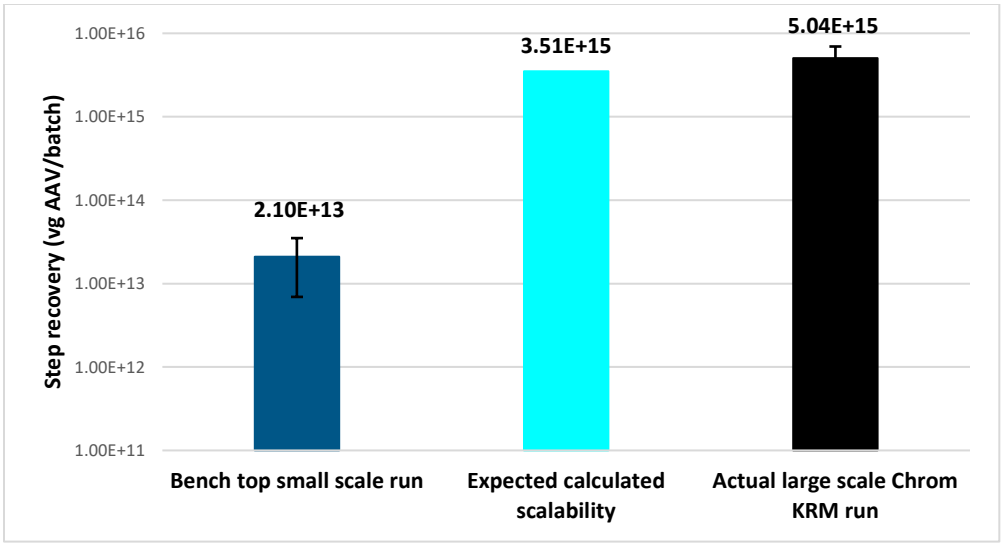


KRM 10 System run 3: Wash 1 through strip

KRM 10 System run 3: Elution through strip including A254/A289

The average amount of AAV9 recovered at small-scale was 2.1 x 10^{13} vg. Scaling up the load by 167-fold, the expected recovery was calculated to be 3.5x 10^{15} vg. Actual average recovery at large-scale was 5.04 x 10^{15} vg, demonstrating successful scalability using the KRM 10 system (**Figure 14**)

Figure 14. Linear scalability and reproducibility



Conclusions

The scalability of the AAV capture step from the benchtop to the manufacturing scale using KRM Chromatography Systems was analyzed by comparing process performance and product quality at two scales. There was no increase of endotoxins, particle aggregations, or residual non-product related impurities such as HCP (data not shown). An increase in overall recovery of ≥13% was demonstrated. The average AAV recovery for the benchtop and KRM 10 System runs were 63.9% and 77.3%, respectively.

In addition, the robustness of the KRM 10 System operation was demonstrated. The capture **process** was reproducible with similar product quality at large scale, even with different load volumes or somewhat degraded feed quality.

Repligen KRM Chromatography Systems enable robust, reproducible, and scalable processes, which are key for high productivity and cost-effective viral vector manufacturing. These systems are specifically designed with low hold-up volumes, gentle internal flow paths, and accurate pump performance that meet the needs of complex biomolecules such as AAV, lentivirus, and exosomes.

Contact Information

Forge Biologics
Forge Biologics, and its leadership team with over 200 years of combined experience, is a leading global genetic medicine CDMO, providing AAV and plasmid manufacturing solutions for gene therapy companies up to the 5000 L scale. Their 200,000 sq. ft. cGMP facility is located in Columbus, Ohio.

Repligen
Repligen is a bioprocessing-focused life sciences company bringing expertise and innovation to our customers since 1981. We inspire advances in bioprocessing through the development and commercialization of high-value products and flexible solutions that address critical steps in the production of biologic drugs.

To learn more about the Repligen Single-Use KRM Chromatography Systems and OPUS columns contact your sales representative or email customer service at regional addresses as listed below. OPUS columns are also available through the Repligen E-Store for most regions.

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