CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Evaluation and performance of an AAV affinity resin: a CDMO case study

Spyridon Gerontas and Buzz Lobbezoo

Often referred to as the gold standard for *in vivo* gene therapy, the adeno-associated virus (AAV) has seen a huge increase in its use in clinical trials over the last few years, with various serotypes used depending on the target tissues and cells. There are now six AAV-based gene therapy products on the market, with many more in the R&D pipeline. This has led to an increased demand for the development of efficient and robust downstream purification processes to ensure the production of high-quality AAV vectors for clinical applications. The performance of the affinity capture step of AAV as a scalable unit operation is of particular importance to deliver high purity and recovery.

This article introduces POROS™ CaptureSelect™ AAVX affinity resins for the scalable downstream purification of a range of AAV serotypes for gene therapy applications. A case study from Pharmaron, a leading CRO/CDMO, provides an analysis of the performance of the resin under varying conditions at both small and large scales, from early development runs to assess the resin capabilities to the affinity capture of 22 L of AAV harvest material.

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INTRODUCING POROS™ CAPTURESELECT™ AFFINITY RESINS

Designed for improved process performance and productivity for a wide range of biomolecules, POROS CaptureSelect affinity resins are highly rigid and both chemically and mechanically stable, allowing for linear pressure drop versus flow curves up to very high pressures and flow rates. The POROS backbone has large through pores and a large internal pore volume for improved mass transfer, in addition to a 50 µm bead size for improved separation whilst retaining high flow rates. The pore structure of the POROS bead enables efficient purification of large molecules such

as plasmid DNA, viruses, viral vectors, and virus-like particles.

The CaptureSelect technology platform has a structure derived from the heavy chain antibodies found in *Camelidae*. The VHH domain, a small (12–15 kDa) fragment, is reverse-engineered into an animal origin-free nanobody, which is produced in yeast and is highly selective to its target—in this case, adeno-associated virus (AAV) serotypes. POROS CaptureSelect AAVX resin has an affinity to a wide range of serotypes, including both natural and chimeric vectors, with high binding capacity, purity, and recoveries.

The performance of POROS CaptureSelect AAVX resin is serotype-specific, so process development is critical to obtain optimal process performance. Despite commonalities between different serotypes, each step in the process should be optimized to improve performance, including loading, washes, elution, and clean-in-place.

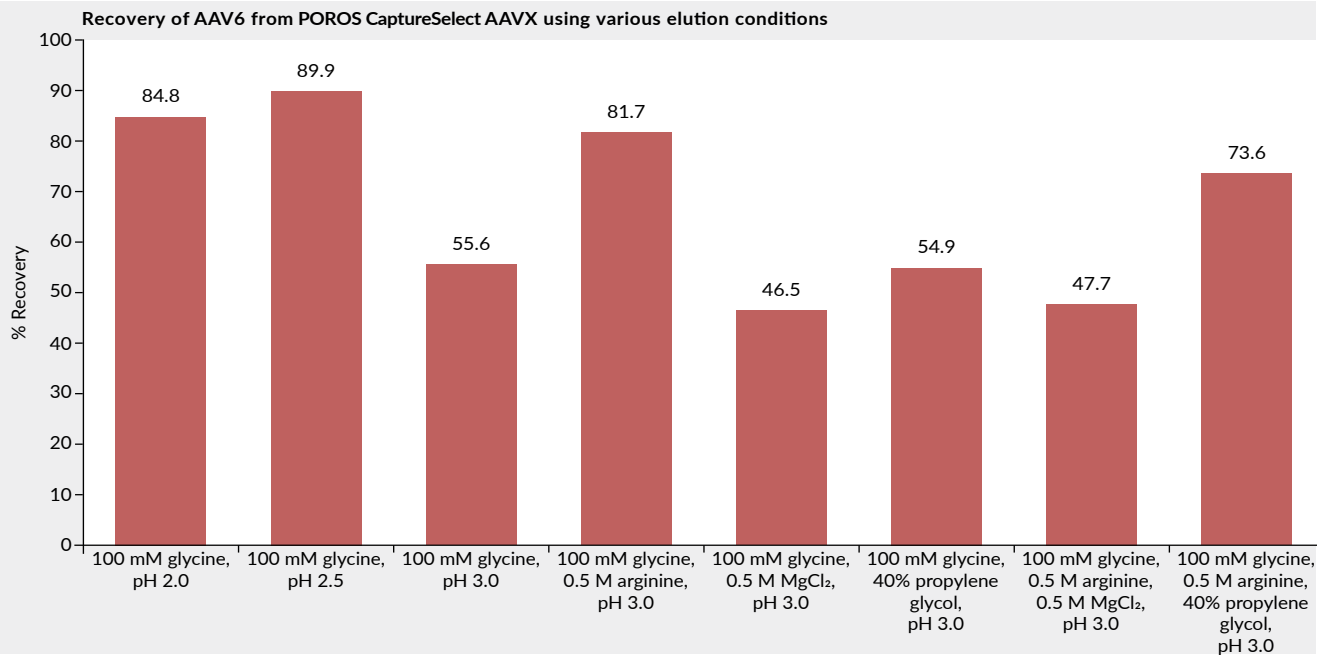
Intermediate wash optimization conducted by Thermo Fisher Scientific has been shown to improve the clearance of process-related impurities. A wash study was performed looking to remove additional non-specific DNA and host cell protein (HCP) binding employing low and high concentrations of NaCl washes with no salt and 1.5 M salt, and an increased pH wash at pH 9.0. With the washes, the levels of residual DNA and HCP were four times lower in the elution.

Optimization of elution conditions is also required to maximize AAV recovery. A study conducted by Thermo Fisher Scientific demonstrated the recovery of AAV6 was optimal at pH 2.5 yielding a recovery of around 90%. Figure 1 shows the recovery of AAV6 from POROS CaptureSelect AAVX resin using various elution conditions. This study shows that additives can be used in the elution buffer to improve recoveries at higher pH conditions.

Analytics also play an important role in ensuring accurate results. One important feature of POROS CaptureSelect AAVX is its high binding capacity for multiple serotypes at short residence times. As demonstrated in Figure 2, capacities have been shown to

FIGURE 1

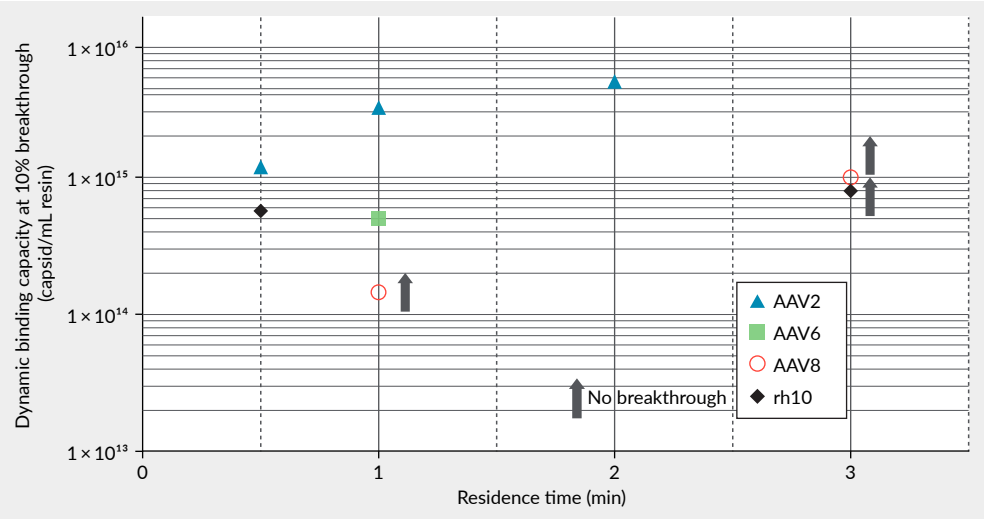
POROS CaptureSelect AAVX elution optimization study.



Data generated by Thermo Fisher Scientific.

FIGURE 2

Breakthrough as a function of capsids loaded per mL of resin.



Data generated by Thermo Fisher Scientific.

exceed 1×10^{15} viral capsids (vp)/mL of resin, achievable with no breakthrough in the case of AAV8 [1]. POROS CaptureSelect AAVX has also been shown to give consistent chromatographic performance and yield over 35 reuse cycles [2].

PHARMARON'S MULTI-SEROTYPE AAV PLATFORM PROCESS

Pharmaron is a leading fully integrated pharmaceutical R&D services platform with global operations. It has a well-established team of over 20,000 employees working in 21 different sites worldwide. Pharmaron's mission is to support their partners in discovery, development, and commercialization of innovative medicines with the vision to become the world-leading life science R&D service company.

Pharmaron Gene Therapy, Liverpool, focuses on viral vector development and clinical manufacture, delivered through our 80,000 sq ft MHRA-licensed cGMP facility. Notably, Pharmaron is embarking on an ambitious plan for the expansion of the Liverpool facility with a £151 million investment in the project, supported by a grant from the UK Government's Life Sciences Innovation Manufacturing Fund (LSIMF). This expansion will lead to a significant increase of Pharmaron' s gene therapy operations to 400,000 sq ft, facilitating the accommodation of viral vector, DNA, and RNA drug substances, along with drug product formulation.

In terms of AAV development and manufacturing, Pharmaron has established an AAV platform and purification toolbox to ensure the production of multiple AAVs, alongside a secure supply of the critical starting materials for these products. Pharmaron's upstream processing consists of a seed train and a production bioreactor, in which the AAV product is expressed following triple transfection of the human embryonic kidney 293 (HEK293) cells. During the seed train, the cells are expanded to inoculate the production single-use bioreactor. Then, they proliferate in a controlled environment to a target concentration, in preparation for the triple transfection, a step which has been optimized to maximize AAV yields and quality. Following harvest, the Pharmaron downstream processing (DSP) team uses its downstream purification toolbox to purify

the viral vector to formulated drug substance. The downstream processing is adapted to the serotype. In general, the clarified product is captured by affinity chromatography. The purification process may require an intermediate chromatography step to further reduce the impurity levels. The product is then loaded onto the polishing chromatography step to separate the genome containing or full capsids from the genome-free or empty capsids. Using ultrafiltration and diafiltration, the purified product becomes drug substance.

PHARMARON'S CASE STUDY ON EVALUATING THE POROS CAPTURESELECT AAVX AFFINITY RESIN

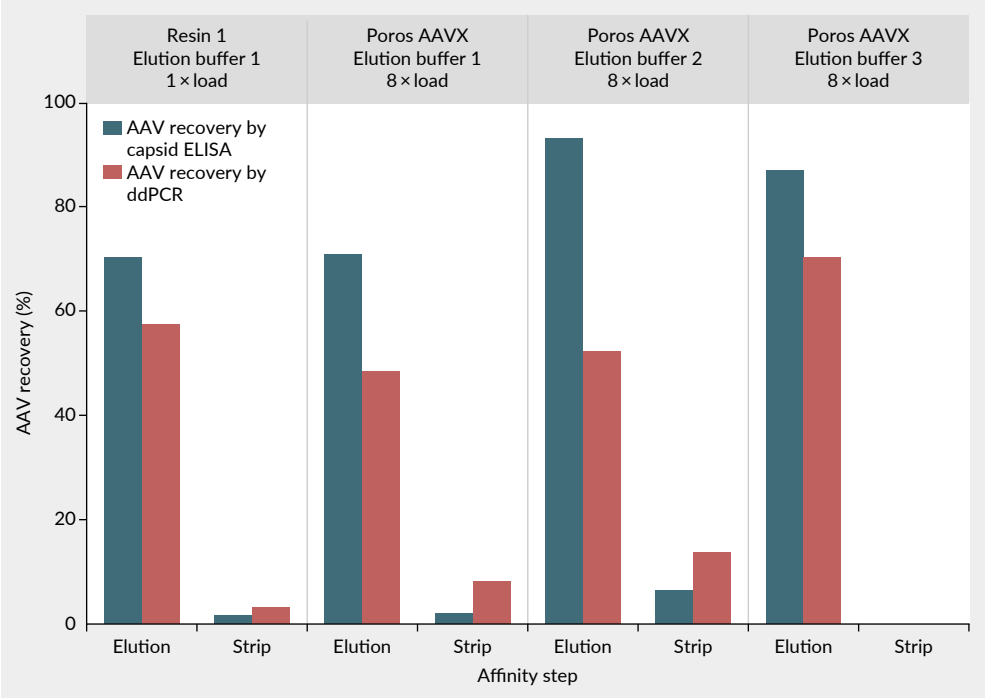
This case study focused on the capture step of the AAV platform and Pharmaron's work on evaluating the POROS CaptureSelect AAVX affinity resin. Pharmaron's assessment of the capabilities of the POROS CaptureSelect AAVX resin began through a series of milliliter-scale experiments. Subsequently, high-throughput (HTP) robotics were integrated with a design of experiments (DoE) approach to screen capture conditions using POROS™ CaptureSelect™ AAVX RoboColumns™. The focus then shifted to estimating the POROS CaptureSelect AAVX resin's dynamic binding capacity (DBC) and determining how much the resin could handle in terms of processing volume. Finally, findings were verified at larger scale by using manually packed columns filled with POROS CaptureSelect AAVX resin to capture AAV from a 22 L cell harvest.

FEASIBILITY RUNS TO ASSESS POROS CAPTURESELECT AAVX

An exploration into the capabilities of the POROS CaptureSelect AAVX resin

FIGURE 3

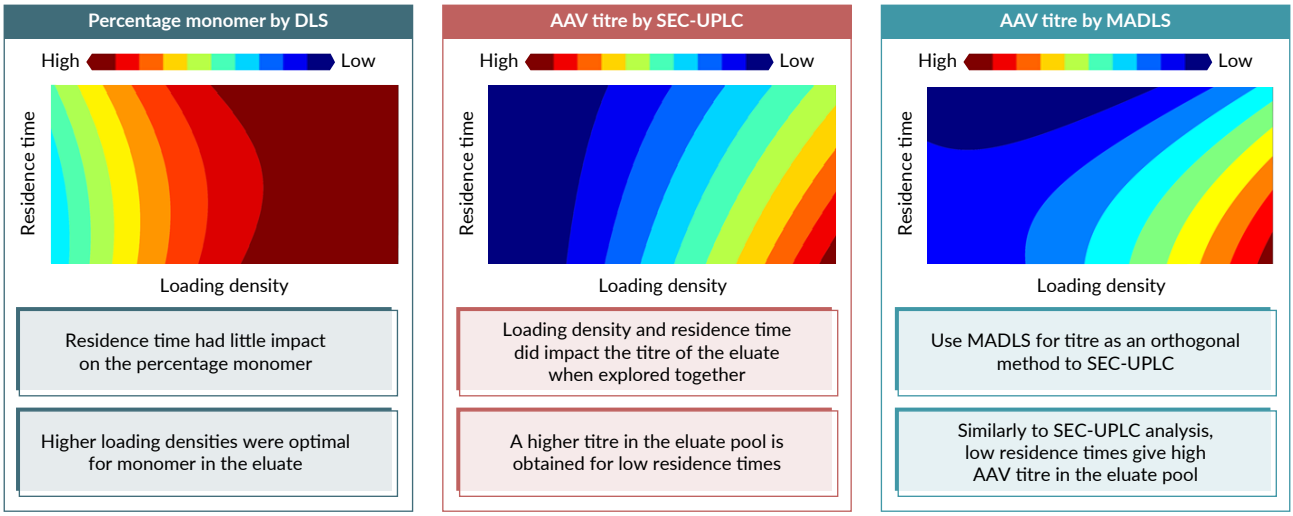
AAV recovery by capsid ELISA and ddPCR of feasibility runs to assess POROS CaptureSelect AAVX capabilities.



The chromatography runs and data analysis were performed by Pharmaron Gene Therapy.

FIGURE 4

HTP robotics/DoE contour plots showing the effect of POROS CaptureSelect AAVX process parameters on AAV monomer content and AAV elution titer.



The chromatography runs and data analysis were performed by Pharmaron Gene Therapy.

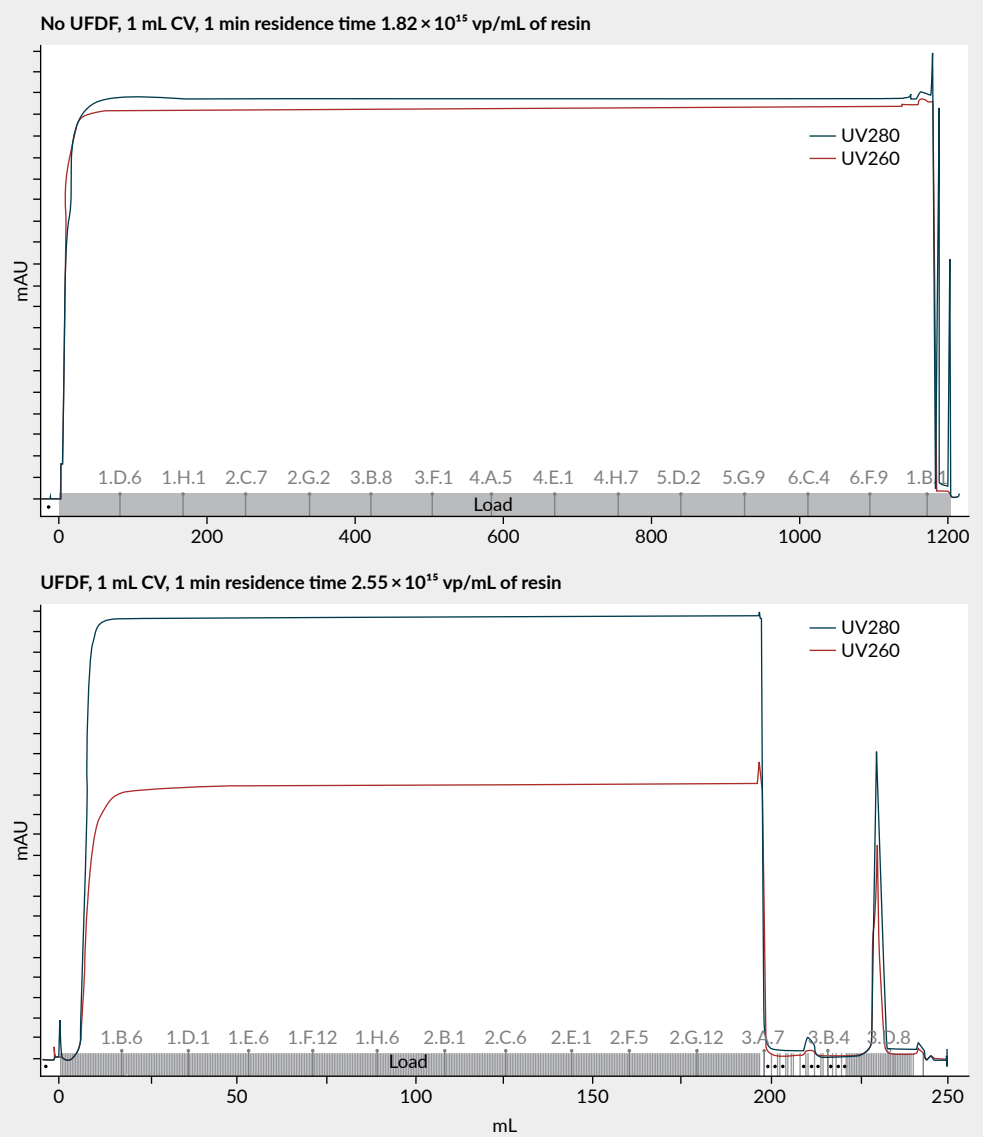
was initiated by conducting experiments on a small scale using 1 mL pre-packed columns connected to a Cytiva ÄKTA™ avant 25. The performance of the POROS CaptureSelect AAVX resin was compared in terms of recovery against a control affinity resin. Both resins were loaded at their recommended linear velocity for the same duration. The POROS CaptureSelect AAVX resin was shown to handle significantly higher linear velocities resulting in an 8-fold increase in loading volume. To ensure consistency, identical buffers were used for capturing material using both the control and POROS CaptureSelect AAVX resin. The effect of buffer composition on AAV recovery was investigated by testing two different elution buffer compositions. AAV recovery values, measured by capsid enzyme-linked immunosorbent assay (capsid ELISA) and droplet digital polymerase chain reaction (ddPCR), are shown in Figure 3. POROS CaptureSelect AAVX resin demonstrates similar recoveries with an 8-fold increase in load compared to the control resin. To enhance efficiency, the wash and elution buffers were fine-tuned to reduce the quantity of AAV in the strip.

UTILIZING HIGH-THROUGHPUT ROBOTICS AND DESIGN OF EXPERIMENTS TO OPTIMIZE POROS CAPTURESELECT AAVX FOR AAV CAPTURE

Following the feasibility runs, the use of HTP robotics and DoE was explored in the optimization of AAV capture parameters. Automation facilitated by HTP and DoE significantly streamlines product development processes. The HTP studies were conducted using POROS CaptureSelect AAVX 200 µL robocolumns in combination with a Beckman Biomek® i7 automated workstation. An experimental plan, based on the central composite design, was implemented using JMP® software to guide experiments. The focus was on optimizing titer and monomer content by exploring loading pH, loading density, and residence time. The success criteria for the HTP experiments comprised achieving a DBC>1×10^{14} vp/mL of resin, a

FIGURE 5

Loading profiles obtained by capturing AAV from post clarified material using 1 mL prepacked POROS CaptureSelect columns with and without UF/DF utilization before the affinity step.



Process parameters	No UFDF	UFDF
UV260/UV280 in loading	0.98	0.62
Loading time (min)	1180	200

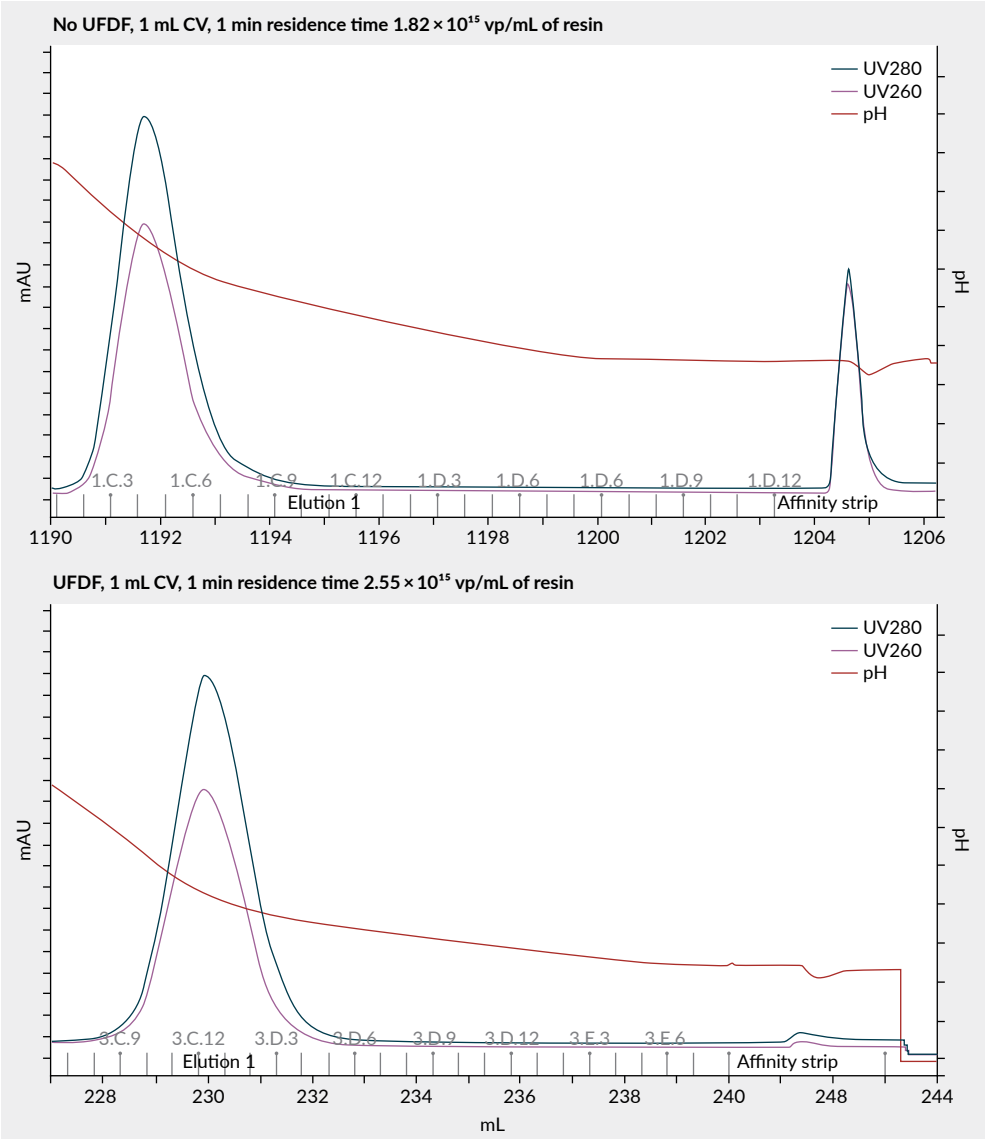
The chromatography runs and data analysis were performed by Pharmaron Gene Therapy.
UF/DF: ultrafiltration/diafiltration.

residence time <3 min, an AAV recovery rate >70%, and aggregate content <5%.

Analysis was conducted on the data produced from HTP robotics/DoE using contour plots (Figure 4). Residence time was shown to have little impact on the percentage monomer whilst higher loading densities were optimal for monomer in the eluate (left plot of Figure 4. Loading density and residence time were found to impact the titer of the

FIGURE 6

Elution profiles obtained by capturing AAV from post clarified material using 1 mL prepacked POROS CaptureSelect columns with and without UF/DF utilization before the affinity step.



POROS CS AAVX attributes	No UFDF	UFDF
Recovery by capsid ELISA (%)	107	121

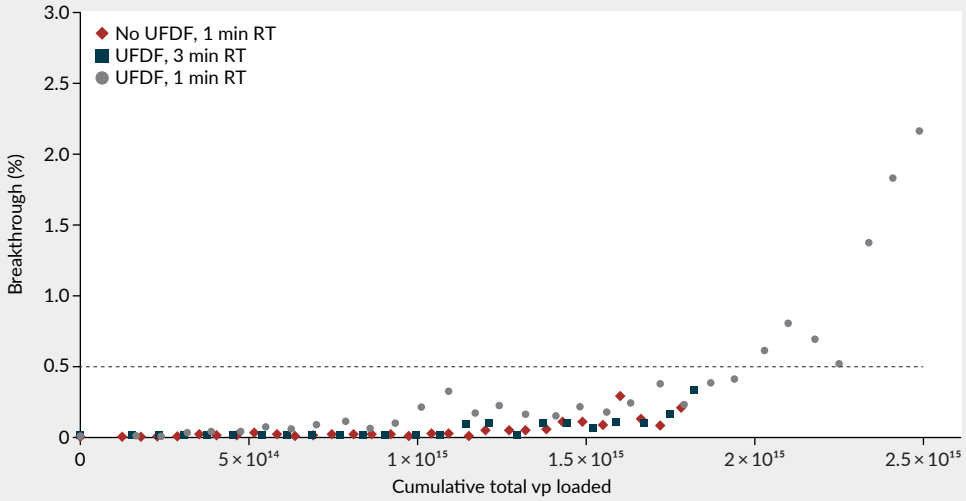
The chromatography runs and data analysis were performed by Pharmaron Gene Therapy.
UF/DF: ultrafiltration/diafiltration.

eluate when explored together, and a higher titer in the eluate pool was obtained for low residence times (center plot of Figure 4).

The AAV titer was measured by multi-angle dynamic light scattering using a Malvern Panalytical Zetasizer® Nano ZSP system in relation to loading density and residence time (right plot of Figure 4). This analytical method serves as an orthogonal method to size exclusion ultra-performance liquid chromatography (SEC-UPLC) for quantifying AAV using a Waters SEC column affixed to a Thermo Fisher Scientific Vanquish™ Horizon UHPLC. Some differences between

FIGURE 7

POROS CaptureSelect AAVX dynamic binding capacity estimation with and without UF/DF utilization before the affinity step.



POROS CS AAVX attributes	No UFDF, 1 min RT	UFDF, 3 min RT	UFDF, 1 min RT
Linear velocity (cm/h)	300	100	300
Loading time (min)	1180	100	300
Loading density (vp/mL of resin)	1.82×10^{15}	1.79×10^{15}	2.55×10^{15}
Recovery by capsid ELISA	107	99	121
HCP (ng/1 × 10^{13} vp)	<5.85	<5.43	<7.88
hcDNA (ng/1 × 10^{13} vp)	3.17	2.62	2.41

The chromatography runs and data analysis were performed by Pharmaron Gene Therapy.

the two analytical methods emerge at low loading densities, although these variations did not affect the conclusions drawn from the contour plots. As with SEC-UPLC analysis, low residence times yield higher AAV titer in the eluate pool.

To summarize the HTP results, the loading density has the greatest impact on generating monomeric AAV, and low residence times are essential in achieving high recoveries.

ESTIMATION OF POROS CAPTURESELECT AAVX DYNAMIC BINDING CAPACITY

To assess the dynamic binding capacity of the POROS CaptureSelect AAVX resin, 1 mL POROS CaptureSelect AAVX columns were loaded at a concentration of 2×10^{15} vp/mL of resin with a 1 min residence time using harvest material. Conditions in which the harvest material underwent buffer exchange and concentration using ultrafiltration/diafiltration (UF/DF) were explored to reduce loading time from 1180 to 200 min and to avoid exposing the harvest material to extended periods at ambient temperature before loading onto the resin (Figure 5). The ratio of UV260 to UV280 was also reduced indicating the removal of DNA-related impurities during the UF/DF step (Figure 5).

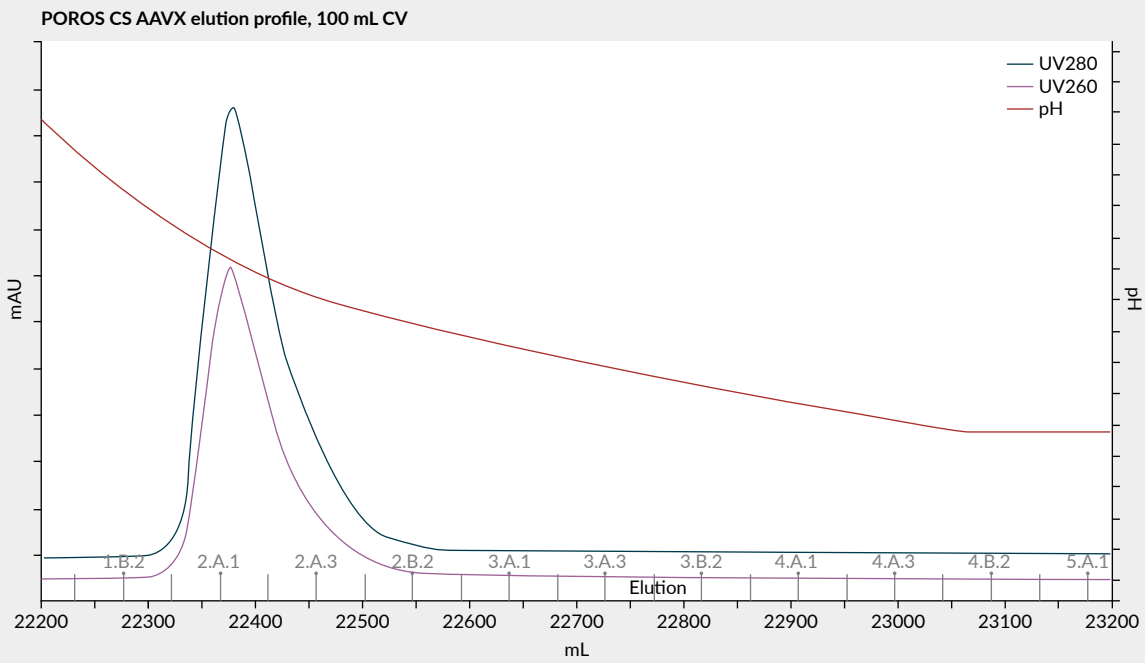
The POROS CaptureSelect AAVX elution profiles with and without UF/DF utilization before the affinity step were also analyzed.

In both cases, symmetrical AAV peaks were observed without any pre- or post-peaks, and minimal AAV loss was detected via capsid ELISA (Figure 6). Notably, in the absence of UF/DF, a peak was evident in the strip, indicating the presence of impurities during affinity load. These impurities were potentially non-specifically absorbed by the POROS CaptureSelect AAVX resin.

The percentage breakthrough of loaded capsids is shown in Figure 7, against the total viral particles loaded onto a 1 mL column for three distinct loading conditions. In the initial condition, the percentage breakthrough was assessed without using UF/DF to concentrate the material before loading, maintaining a residence time of 1 min. Even when loading POROS CaptureSelect AAVX at 1.82×10^{15} vp/mL of resin, no breakthrough was observed. Subsequently, the impact of residence time on the condition in which the cell harvest was concentrated using UF/DF was investigated. At a residence time of 1 min, the UF/DF condition showed a 0.5% breakthrough at 2×10^{15} vp/mL of resin (Figure 7). The residual host cell DNA

FIGURE 8

POROS CaptureSelect AAVX elution profile from 22 L clarified material.



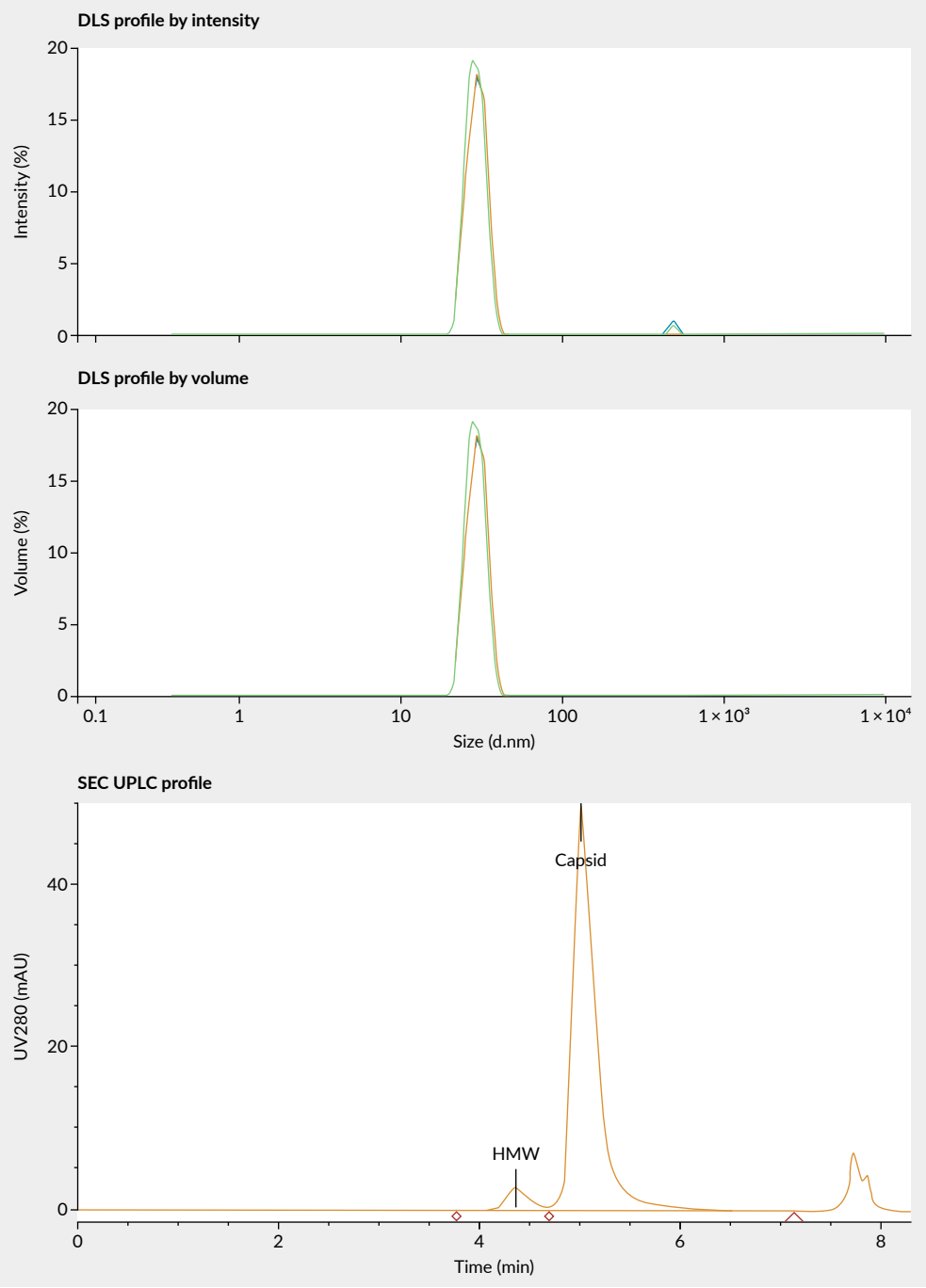
POROS CS AAVX running parameters and attributes
Processed volume=22.5 L of clarified material
Poros CS AAVX volume/height=96 mL/18.1 cm
Loading=3.90×10^{14} vp/mL
Residence time/linear velocity=~2 min; 450 cm/h
Recovery (by capsid ELISA/ddPCR)=93.4%/67.7%

The chromatography runs and data analysis were performed by Pharmaron Gene Therapy.

and HCP levels were <4 ng/1 × 10^{13} vp and <8 ng/1 × 10^{13} vp respectively for all runs (Figure 7). These experiments underscored the capability of POROS CaptureSelect AAVX resin to capture AAV at high loading densities and low residence times.

FIGURE 9

Monomer content of POROS CaptureSelect AAVX eluate from loading 22 L clarified material.



The chromatography runs and data analysis were performed by Pharmaron Gene Therapy.

AFFINITY CAPTURE OF 22 L OF AAV HARVEST MATERIAL

After small-scale runs, an evaluation of the performance of the POROS CaptureSelect AAVX resin was performed at a larger scale. The large-scale run began with manual packing of the AAVX resin following the Thermo Fisher Scientific Instructions [3].

Two columns were packed (2.6 cm diameter Cytiva HiScale™ 10/40 and 1 cm Cytiva AxiChrom™ 50) with POROS CaptureSelect AAVX resin and asymmetry results and the number of theoretical plates per meter met requirements [4].

The harvest material (22 L) was processed using a Cytiva ÄKTA avant 150 with a 2 min residence time, running at the linear velocity of 450 cm/h. Figure 8 shows the operating conditions and elution profile. the recovery rates validated via capsid ELISA and ddPCR were high. The UV elution profile showcased a single, sharp peak devoid of pre- or post-peaks, which supports the high recovery result. This result confirmed the optimized host and elution buffering conditions derived from the feasibility and DoE runs.

Additionally, analytical assessment was conducted to characterize the product quality. The findings are shown in Figure 9. The monomer content of POROS CaptureSelect AAVX eluate was estimated by dynamic light scattering (DLS) and SEC-UPLC. The DLS analysis was performed on a Malvern Panalytical Zetasizer Nano ZSP and provided an estimate of the large-size aggregates (typically, those >0.2 µm). The SEC-UPLC analysis estimated the small-size aggregates (typically AAV dimers, trimers, and tetramers) and it was performed using a Waters SEC column connected to a Thermo Fisher Scientific Vanquish Horizon UHPLC. A monomer content of 99% was measured by DLS and 93% by SEC-UPLC. The residual host cell DNA and HCP levels were <4 ng/1×10^{13} vp and <2 ng/1×10^{13} vp respectively. These large-scale run results confirmed the findings of the development work at small scale.

SUMMARY OF THE POROS CAPTURESELECT AAVX ASSESSMENT

The POROS CaptureSelect AAVX affinity resin is designed to address the high selectivity and capacity requirements for the large-scale downstream purification of a wide range of both natural and chimeric AAV serotypes. The use of Pharmaron's HTP robotics and DoE expertise in combination with POROS CaptureSelect AAVX RoboColumns delivered a rapid screening of AAV capture conditions. This helped Pharmaron to develop AAV capture conditions that enable low processing times and increased processing volumes, resulting in shorter development times for large-scale batch production. The POROS CaptureSelect AAVX rigid matrix facilitates column packing, ensuring alignment with specifications for both asymmetry factors and plates/meter, enabling Pharmaron to perform robust and repeatable large-scale affinity runs, achieving high AAV recovery and impurity clearance.

Q&A


Buzz Lobbezoo and Spyridon Gerontas

Q What is the binding capacity of the AAVX resin? Can this resin be reused?

BL: The binding capacity for the AAVX resin is variable and dependent on serotype. We have used a multitude of different serotypes and have achieved binding capacities in the region of 1×10^{15} vp. There are a few serotypes where slightly lower capacities are seen, so we recommend the completion of DBC evaluation work or breakthrough studies.

The resin can be reused. The number of times it can be reused depends on how you treat the feedstock and the resin. When the resin is treated with the right amount of cleaning material and regenerated with low pH strips, as well as a denaturant like urea guanidine, we have been able to reuse the resin for over 35 cycles.

Q What is the best approach to improving elution recovery on POROS CaptureSelect AAVX?

BL: The short answer is low pH. The best approach is to perform an optimization study looking at a range of pHs and additives. We also recommend the addition of pluronic in the elution. It is important to be confident in your analytics, so you can look at different orthogonal steps and assays to ensure that you are achieving accurate values.

Q Why does the lower residence time give a higher purification yield?

SG: AAVs can aggregate until they are loaded onto the resin. Low residence times may result in higher purification yields of AAV monomers, as they translate into shorter material hold times at ambient temperature, consequently minimizing the drop in purification yield

due to AAV aggregation. Furthermore, optimizing the buffer composition of the AAV feed solution can prolong the time AAVs remain in the monomeric state at ambient temperature, thereby achieving high purification yields of AAV monomers. Developing formulation recipes for in-process steps can be performed in-house or in collaboration with a CDMO.

BL: In our studies, we have seen that in some instances, reducing the residence time can be beneficial both from a stability standpoint and to reduce the amount of entrapment. When running at much lower flow rates, you can run the risk that some of the particles becoming trapped, leading to a reduction in recovery. It is worth investigating and optimizing residence times.

Q How does the low pH affect the infectivity of the virions?

BL: We have seen that lowering the pH, especially down to pH 2.0, can increase aggregation. This effect can be serotype-dependent. We also recommend performing the elution promptly to neutralize rapidly.

Q Can we apply cell lysate on POROS CaptureSelect AAVX resin directly without UF/DF buffer replacement?

SG: Yes, this can be done. It depends on the approach to retrieve AAVs from cell culture material. If the AAVs are retrieved through cell lysis, there can be more impurities, so a UF/DF step may be needed. The Cost of Goods (CoGs) must also be considered here, as the addition of a UF/DF step may increase DSP costs. The development team should perform a CoGs analysis to estimate whether it is better to load the AAV material directly onto the affinity resin without the UF/DF step, even though the capacities will be slightly lower.

Q What were the main challenges experienced in Pharmaron's platform process development?

SG: The main challenges were linked to the complexity of AAV vectors. The process must be looked at holistically; it is not as simple as optimizing the upstream process, and then passing the material to the downstream processing team for purification. It is crucial that the upstream and downstream processing teams work together to transform the material into a drug substance with low impurities, and to ensure a highly productive process. Achieving this goal required support from the Pharmaron analytics team, to provide high-sensitivity, low-volume analytical methods.

Q What is, in your experience, the best analytical method to detect the empty, partially full, and incomplete AAV capsids from the full capsids?

SG: The gold standard is analytical ultracentrifugation (AUC), which we use routinely at Pharmaron. We also employ mass photometry, another accurate method, to achieve higher throughput analysis with lower sample volumes for development purposes. Other methods include anion exchange-high performance liquid chromatography (AEX-HPLC) and ddPCR/capsid ELISA.

Q Why do the UF/DF versus non-UF/DF methods have different DBCs?

SG: This is due to the feed. It is key that the development team optimizes the feed composition. The preceding step always plays a huge role in what will happen, not only to the affinity step, but to the following downstream processing steps. When a team optimizes a chromatography step, for instance, they need to consider all preceding steps

BL: Different setups have different binding capacities due to how you have lysed cells and what is present in your feedstock. A high level of impurities can lead to a steric hindrance, an effect on the binding capacity, or some non-specific interaction. Washes can help with this. Potentially dirty feeds can reduce your DBC and the ability to reuse the resin. How you treat the feeders is key.

Q When evaluating purification yields, is it correct that both capsid ELISA and ddPCR methods are used, but only ddPCR measured the filled capsids?

SG: Yes, ddPCR measures the filled capsids. In the affinity step, we do not expect to have different recoveries by capsid ELISA and ddPCR, because we do not have separation of the full from empty capsids. However, ddPCR is used as an orthogonal method in this case to be sure that any buffers used do not cause rupture of the capsid surface and therefore ejection of the transgene.

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BIOGRAPHIES

BUZZ LOBBEZOO is a Senior Field Applications Scientist at Thermo Fisher Scientific supporting the CaptureSelect and POROS resin for Bioproduction group in EMEA North. He holds a Bsc (Hons) from the University of Kent in Canterbury, UK, and has over 25 years of experience in downstream processing, working for various small and medium sized biotech companies as well as the University of Cambridge. Throughout his career, he has worked on every aspect of downstream processing, working with a full gamut of techniques for the purification of a range of biomolecules.

SPYROS GERONTAS is a Senior Technical Specialist in Process Sciences at Pharmaron Gene Therapy, Liverpool. He has over 15 years of experience in developing downstream processing (DSP) platforms and performing process modelling/economic analysis for biopharmaceuticals. In his role, he leads the bioprocessing development of gene therapy products in Pharmaron's cutting-edge facilities. Furthermore, he promotes the implementation of innovative technologies in gene therapy downstream processing through strategic technology partnerships in order that Pharmaron gains/maintains a competitive edge. Moreover, he leads the scale-up and technology transfer of DSP platforms for viral vectors and recombinant proteins to Pharmaron's strategic partners. Spyros holds a PhD in Biochemical Engineering from University College London.

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AUTHORSHIP & CONFLICT OF INTEREST

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