Simplifying Progress

Integrating Upstream and Downstream Processes for Enhanced Purity and Yield of Adenovirus 5

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Introduction

Optimizing adenovirus (AdV) production requires a comprehensive approach that addresses both the upstream and downstream processes, while ensuring process robustness. We developed a bioprocess for improved AdV yield and purity, with simplification of operations in mind. An industry-relevant production strategy was chosen for suspension HEK cells, along with a scalable downstream process featuring a single chromatographic step on CIMmultus QA monolithic column (1). The integration of multi-detector analytical chromatography with common analytical methods, such as dPCR and infectivity assays, highlights the critical role of process analytical technology. This approach forms a comprehensive understanding of AdV production and effectively bridges upstream and downstream processes. These findings demonstrate that adopting advanced analytical tools and innovative bioprocessing strategies can significantly improve the quality and scalability of adenovirus production, meeting the demands of both research and commercial applications Optimized process results in enhanced purification efficiency and improved adenovirus yield, making it suitable for applications in gene therapy and vaccine development.

Production of Adenovirus

An outline of Adenovirus production process is shown in Figure 1. Suspension HEK293 were seeded in Biostat B-DCU (Sartorius) 2 L vessel. The cells were infected with Adenovirus serotype 5 (Ad5) with one (Ad5-GFP) or two expression cassettes (Ad5-S-GFP) for GFP and SARS-CoV2 protein S. The production lasted for 2 days post infection in batch mode. The upstream strategies were selected based on previous experience to achieve the highest virus titer and low impurity profile (1). The concentration of viral seed differed between the productions, with low MOI tested in H-1, high MOI in H-2 and a middle concentration in H-3 (Table 1). The lowest physical titer was measured in H-1 with MOI 3 in Ad5-S-GFP. Considering the excess viral seed used in H-2, selected conditions for H-3 likely represent a more optimal balance between efficiency and cost.

Table 1: Conditions used in upstream processes and their effect on viral titer.VCD – viable cell density, expressed as viable cells per mL (vc/mL, MOI – multiplicity of infection, based on functional titer, expressed as infectious units per viable cell (IFU/vc). IFU – infectious unit. vg – vector genome. *Different cell line was used for infectivity assay of Ad5-S-GFP

Production process	Construct	VCD at production start (vc/mL)	MOI (IFU/vc)	VCD at harvest (vc/mL)	Cell viability at harvest (%)	Physical titer in lysate (vg/mL ± SD)	Functional titer in lysate (IFU/mL ± SD)
H-1	Ad5-S-GFP	1 E+06	3	1 E+06	62	3.1 ± 0.2 E+10	9.2 ± 0.6 E ± 07*
H-2	Ad5-GFP	7 E+05	140	1 E+06	89	1.2 ± 0.1 E+11	1.4 ± 0.1 E+09
H-3	Ad5-GFP	9 E+05	10	1 E+06	87	8.6 ± 0.4 E+10	1.6 ± 0.0 E+09

Lysis and clarification of Ad5 harvest

At production end, saccharose was added to the bioreactor in the final concentration of 5%. The harvest was lysed in the bioreactor vessel by the addition of salt and detergent to a final concentration of 0.5 M NaCl and 0.25% Tween 20. The lysate was clarified through a series of in-line stacked coarse and fine filters: 3 µm Sartopure PP3 3 (Sartorius), 0.65 µm Sartopure GF+ (Sartorius) and 0.8/0.45 µm Sartopore 2 300 (Sartorius) using a peristaltic pump.

Filtrate pre-treatment and purification of Adenovirus using CIMmultus QA

Before chromatography, the clarified lysate was pre-treated with TFF (Sartocon slice PES 300 kDa, E-screen, Sartorius) where it was concentrated and exchanged to conditions for nuclease treatment containing Tris buffer pH 8.0, 500 mM NaCl, 2.5% saccharose, 0.1% Poloxamer and 5 mM MgCl2. The retentate was treated with nuclease Saltonase (Blirt) for 2 hours, then buffer exchanged to loading conditions containing Tris buffer pH 8.0, 300 mM NaCl, 2.5% saccharose and 0.1% Poloxamer 188. Following the pH adjustment to pH 8.5 and 0.45 µm filtration, the retentate was loaded to a CIMmultus QA-8 mL (2 µm) column (Sartorius) and eluted using a linear salt gradient to buffer containing 2 M NaCl (Figure 2). The Adenovirus peak was collected as shown (Figure 2B) and analysed for physical titer with digital PCR (dPCR), functional titer with infectivity assays, and impurity profile using Bradford total protein and Picogreen assay. The protein concentration was reduced from 6.6E+5 µg in lysate to 3.0 µg per 1E+10 vg in the purified fraction, and the dsDNA content was reduced from 2.1 E+05 ng in lysate to 2.4 ng per 1E+10 vg in the purified fraction resulting in 4-5 log bioburden reduction in H-2. Two more harvests were purified using this process and the yields were evaluated in Table 2.

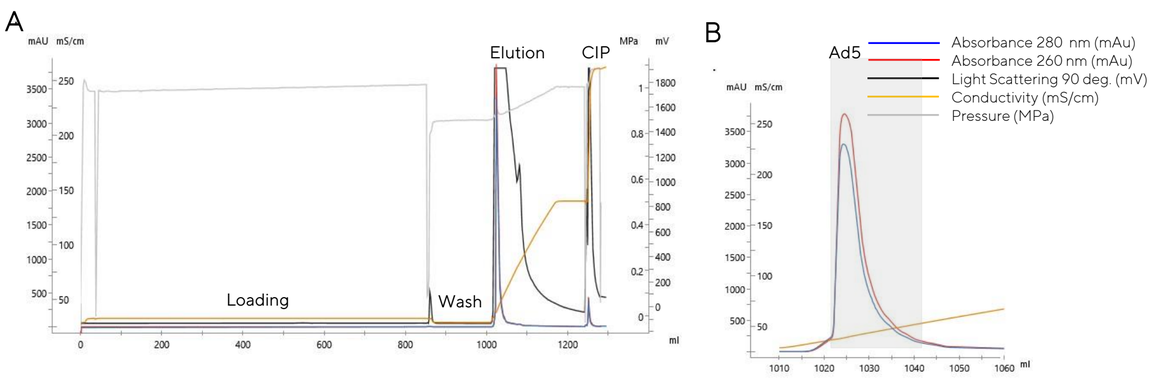

Figure 2: Preparative chromatogram of Ad5 purification using CIMmultus QA monolithic column – loading, wash, elution and cleaning-in-place (CIP) (A), Zoomed Ad5 peak in elution (B).

Table 2: Downstream process evaluation based on physical and functional titer. 3 different harvests were processes using the same downstream process with a chromatographic purification on CIMmultus QA column. Physical titer was measured with dPCR and functional titer was measured with infectivity assays. Resulting process and column recoveries were expressed in percentages.

	dPCR			Infectivity		
Process	Physical titer in elution (vg/mL)	Process recovery (%)	Column recovery (%)	Functional titer in elution* (IFU/ml)	Process recovery (%)	Column recovery (%)
H-1	1.4 E+12	71%	77%	2.32E+09	79%	97%
H-2	9.1 E+12	56%	81%	1.20E+11	69%	65%
H-3	7.5 E+12	80%	97%	1.55E+11	89%	107%

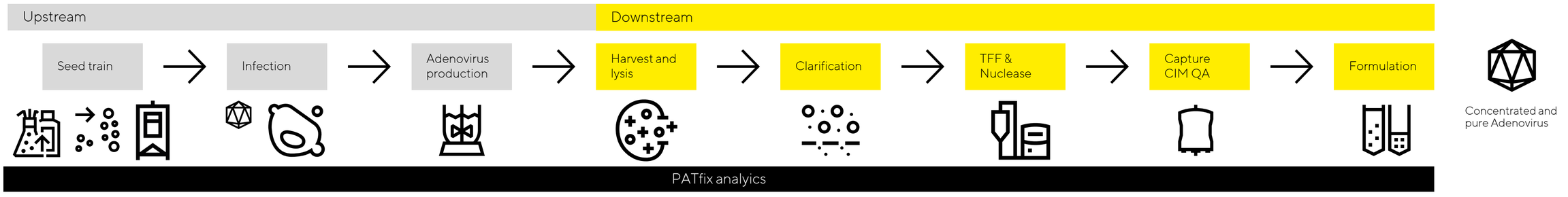

Figure 1: Scheme of Adenovirus manufacturing process. Upstream process consists of cell cultivation, infection, and production of Adenovirus. Cells are lysed in the bioreactor and the lysate is collected for further downstream steps consisting of clarification, tangential flow filtration (TFF) with integrated nuclease treatment, followed by a chromatographic purification on CIMmultus QA and formulation.

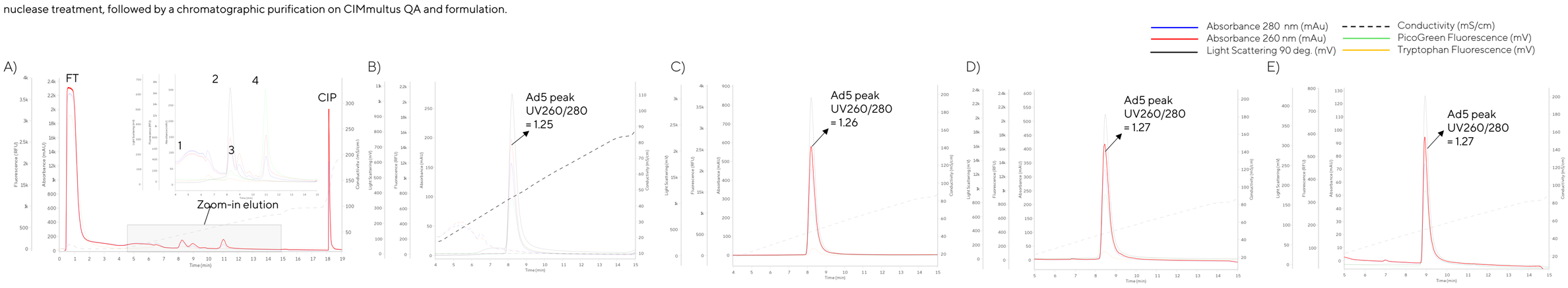

Figure 3: PATfix Adeno analytical chromatograms of in-process samples and final purified Adenoviral fractions: A) clarified lysate of Ad5-GFP (H-3). Excluded impurities in flow-through (FT), multiple peaks in the elution profile 1- protein-related, 2- Ad5 peak indicated by LS, 3- uncharacterised impurities, 4 – dsDNA indicated by PicoGreen staining, cleaning-in-place (CIP) containing complex impurities including chromatin B) TFF process overlay with retentate (full lines) and permeate (dashed lines) from H-3 process C) CIM-QA purified Ad5 elution from H-3 (A260/280=1.25), D) CIM-QA purified elution from H-2 and E) CIM-QA purified elution from H-1.

PATfix analytics of in-process samples

PATfix analytical chromatography system enables at-line monitoring of sample composition during upstream and downstream processing. Multiple detector technology simultaneously detects absorbance, light scattering and fluorescence, providing sample composition information during crude, partially-purified, and final process stages. In this study we used the analytical method based on bind-elute anion exchange chromatography using CIMac Adeno column. The absorbance was measured at 260 nm and 280 nm, providing the profile of low-molecular weight proteins and nucleic acid components of the sample. In samples with high impurity content, such as lysates, light scattering is used to identify Ad5 peak (shown monitored at 90 angle). Additionally, samples were spiked with PicoGreen dye (Invitrogen) localizing dsDNA components. Picogreen fluorescence was excited at 485 nm and measured at 520 nm wavelength. Intrinsic tryptophan fluorescence was excited at 280 nm and measured at 348 nm, providing a more sensitive protein detection method in purified samples. Ad5 elution samples exhibit a sharp peak on UV and LS detector with UV260/280 ratio indicating purified Adenovirus (Figure 3).

Conclusions

- A scalable Ad5 production process includes cell lysis directly in the bioreactor, followed by clarification, tangential flow filtration (TFF), and chromatographic purification using CIMmultus QA column.
- The downstream process enables ~100-fold concentration of Ad5 and achieves 4-5 logs removal of host cell protein and DNA.
- The PATfix system provided real-time insight into sample composition and was instrumental in assessing both intermediate and final purified Ad5 fractions.
- The analytical profile of purified Ad5 fractions, analyzed via PATfix, was consistent across different productions, demonstrating the robustness and reproducibility of the purification method.

Literature

[1] Petrović Koshmak, I., Jug, H., Vrabec, K., Mavri, A., Novak, V., Dekleva, P., Fujs, V., Leskovec, M., & Štrancar, A. (2023). Bridging upstream and downstream for improved adenovirus 5 bioprocess. *Electrophoresis*. https://doi.org/10.1002/elps.202300131