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Application Note

December 04, 2017

Optimizing Adenovirus Purification Processes

Using Sartobind® Q and STIC PA Anion Exchange Membranes

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Introduction

Adenovirus vectors are finding increasing application within the vaccine and gene therapy industries. Companies developing adenovirus-based biopharmaceuticals will benefit from single-use process platforms that are quick and easy to install and have been demonstrated previously to produce purified adenovirus[1]. The platform can be adapted to new adenovirus products with a minimum investment of time and resource in process development activities.

Various purification methods have been described that could be considered for inclusion into an adenovirus platform process. Gradient ultracentrifugation was the traditional method for virus purification, however, more recently, anion exchange chromatography has been reported as a superior technique[2, 3]. The macroporous structure of ion exchange membrane adsorbers enables higher binding capacity for large molecules compared to resins. The virus has access to all of the ligands because of the absence of a size exclusion effect and so the technology shows promise for virus purification[4].

The aim of this study was to optimize an adenovirus downstream production process using membrane chromatography. This application note focuses on the development and verification of two purification steps. Sartobind® Q is a strong anion exchange membrane and was used in this study in bind-and-elute mode for the initial virus capture. Sartobind STIC® PA is a salt-tolerant membrane adsorber that can remove contaminants at high salt concentrations to give further purification and was used in this study in flow-through mode for polishing. Purification process development was performed with either Sartobind® Nano (1 mL) or Pico (0.08 mL) devices and then verified with a full adenovirus purification run from a 20 L bioreactor using either 150 mL or 1 mL Sartobind® adsorbers.

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Analytical Assays and Requirement

The yields across each step in the process were calculated using once adenovirus had been quantified by real-time PCR. DNA clearance was measured using the Pico Green assay and HCP clearance using the HEK 293 assay.

Final Impurity Targets

The following targets for impurities in the final product were established. Host cell DNA < 10 ng/dose, HCP < 20 µg/mL, endonuclease < 5 ng/mL (specifications | guidelines for pre-clinical and phase I studies) where 1 dose = 10^{10} virus particles (VP).

1. Adenovirus Purification With Q and STIC 2 L scale

Upstream process

HEK 293 cells were cultured at the 2 L scale in serum-free media. The temperature was controlled at 37 °C. The bioreactor inoculum was 0.5×10^6 cells/mL, the cell concentration at infection was 1×10^6 cells/mL, and a multiplicity of infection of five was used. The bioreactor was harvested 72 hours postinfection, a cell lysis step with 0.1 % Triton was used to maximize the yield of virus particles from the bioreactor but led to a release of large quantities of DNA so an endonuclease digestion (Denarase®) 50 U/mL was performed prior to the harvest being clarified by filtration. The filtrate was then concentrated and diafiltered by tangential flow filtration and used to develop the Sartobind® Q capture step.

1.1 Development of an Adenovirus Capture Step With Sartobind® Q

An adenovirus capture step was developed with the Sartobind® Q membrane adsorber operated in a bind-and-elute mode.

Summary of Sartobind® Q purification conditions

Sample	Virus sample after UF | DF* step
Adsorber	Sartobind® Q nano 1 mL (96IEXQ42DN-11) Bed height 4 mm
Chromatography mode	Bind and elute (capture)
Flow rate	15 mL/min (15 MV**/min)
Equilibration	50 mM HEPES, 200 mM NaCl, pH 7.5
Load	158 mL
Elution	20 mL 50 mM HEPES, 600 mM NaCl, pH 7.5

* Ultrafiltration | Diafiltration
** Membrane volume

Results

The binding capacity at 10 % breakthrough was determined to be 6.26×10^{12} VP/mL of membrane. The virus recovery was 75 % and the DNA level reduced more than 15-fold from 176 to 10.5 ng/dose. However, the level was still slightly higher than the target of 10 ng DNA/dose. The capture step was performed within 30 minutes. The chromatogram for the capture step is shown in Fig. 1.

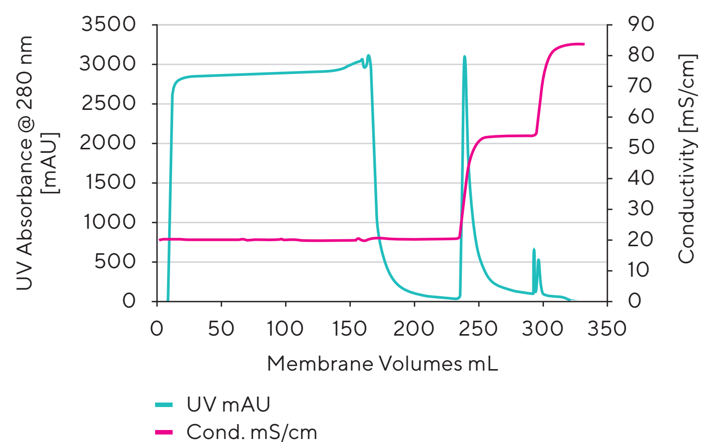

Fig. 1: Chromatogram for the capture of adenovirus using a Sartobind® Q nano 1 mL membrane adsorber

1.2 Development of an Adenovirus Polishing Step With Sartobind STIC® PA

Residual nucleic acids can be removed from the Sartobind® Q elution pool with a subsequent Sartobind STIC® PA polishing step. STIC PA is a weak anion exchanger with primary amine ligands. If a polyvalent buffer, such as one containing phosphates, is used ions in the buffer compete with virus particles and prevent them from binding, however, they do not prevent DNA from binding[5]. Virus particles can be separated, therefore, from DNA if the optimum buffer concentration is selected.

1.2.1 Selection of the optimum phosphate buffer concentration for the flow-through polishing step

An initial experiment was performed to find the optimal phosphate concentration to capture and separate DNA from virus in the flow-through. First, the Sartobind® Q eluate was loaded onto the STIC PA pico 0.08 mL device in the absence of phosphate ions and then a gradient equilibration performed starting with 0 M phosphate and ending at 1 M phosphate. The results indicated that a buffer containing 50 mM HEPES, 250 mM sodium phosphate, 200 mM NaCl should be used to achieve recoveries of almost 100 % of the loaded virus (Fig. 2). This step reduced the DNA concentration of the feed stream to 0.7 ng DNA/dose, far below our target of 10 ng DNA/dose. The Sartobind STIC® eluate can be concentrated, if required, and diafiltrated with the formulation buffer during a final ultrafiltration step.

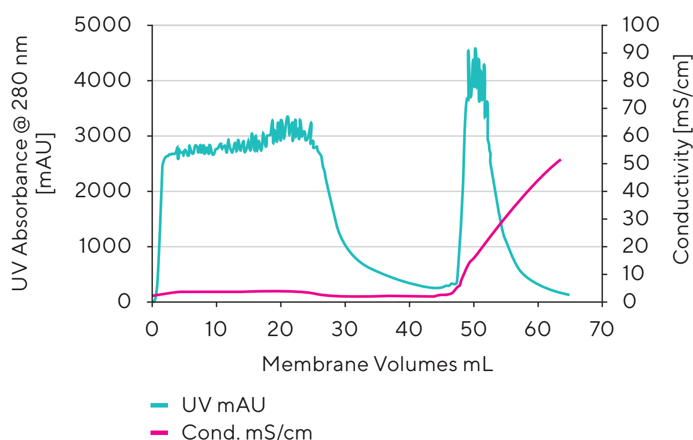

Fig. 2: Chromatogram for the polishing of adenovirus using a Sartobind STIC® PA pico 0.08 mL membrane adsorber; Load without phosphate buffer and elution by linear gradient up to 1 M phosphate.

1.2.2 Capacity determination for flow-through polishing step

Breakthrough curves for total protein and DNA were determined to optimize the Sartobind STIC® PA polishing step.

Summary of Sartobind STIC® PA purification conditions

Sample	eluted sample from Sartobind® Q nano 1 mL
Adsorber	Sartobind STIC® PA pico 0.08 mL (92STPA42DD-11--D) Bed height: 4 mm
Chromatography mode	Flow-through polishing
Flow rate	1.6 mL/min (20 MV/min)
Equilibration	50 mM HEPES, 200 mM NaCl, 250 mM phosphate, pH 7.5

Results

The breakthrough point to 10 % of total protein was reached after 2.8 mL (35 MV) of sample loading (Fig. 3a). The total protein concentration at the outlet started decreasing after 13 mL (162.5 MV) injection of the sample. The breakthrough point to 10 % of the feed DNA concentration was reached after 1.7 mL (21.3 MV) of sample loading (Fig. 3b). The DNA was reduced from 10.5 to 1.2 ng/dose equivalent to an 8-fold reduction. The recovery of virus was 81 %. These results were used to scale-up the process for the purification of a 20 L cell culture.

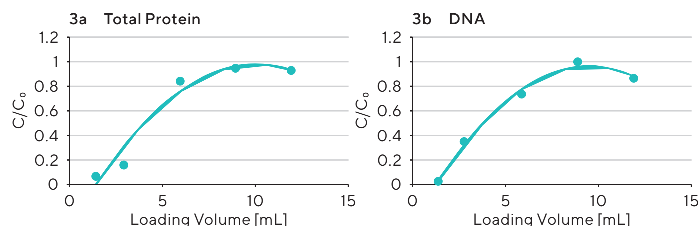

Fig. 3a and 3b: Sartobind STIC® PA pico 0.08 mL Breakthrough curves for total protein and DNA

2. Purification of a 20 L Bioreactor With Sartobind® Q and STIC PA Membrane Adsorbers

Process overview

The complete process flow is shown in the Fig. 4. The cell culture conditions were the same as those used at the 2 L scale. Purification conditions determined at the 2 L scale were used to purify virus at the 20 L scale. Following the polishing step with Sartobind STIC® a final ultrafiltration step can be performed prior to filtration with a sterilizing-grade filter.

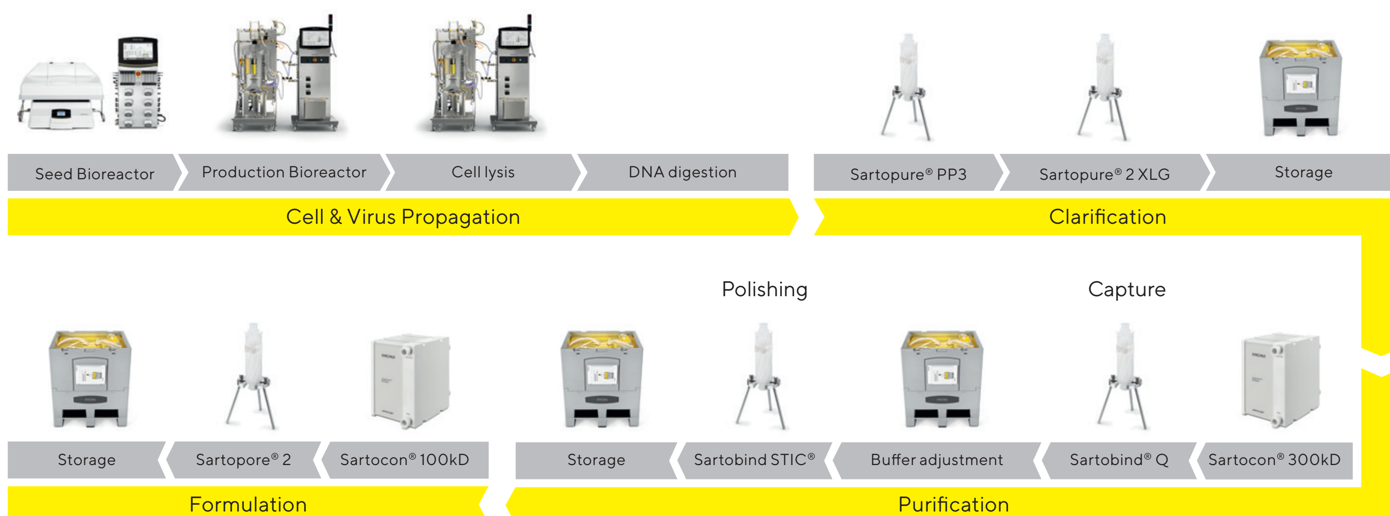

Fig. 4: Adenovirus 20 L process flow using Sartobind® Q for initial capture followed by Sartobind STIC® PA flow-through polishing

Summary of purification conditions at the 20 L scale

The adenovirus 20 L batch process was performed on an ÄKTA® avant 150 based on the results obtained at the 2 L scale.

Sample	Virus sample after UF | DF step	eluted sample from Sartobind® Q 150 mL
Adsorber	Sartobind® Q 150 mL (96IEXQ42E9BFF) Bed height 8 mm	Sartobind STIC® 1 mL (11x) (96STPA42DN-11- -A) Bed height 4 mm
Chromatography mode	Bind and elute (capture)	Flow-through polishing
Flow rate	150 mL/min (1 MV/min)	11 mL/min (1 MV/min)
Equilibration	1.5 L (10 MV) of 50 mM HEPES, 200 mM NaCl, pH 7.5	50 mM HEPES, 200 mM NaCl, 250 mM sodium phosphate
Load	approx. 1.6 L	265 mL
Wash*	50 mM HEPES, 200 mM NaCl, pH 7.5	n.r.
Elution	50 mM HEPES, 650 mM NaCl, pH 7.5	n.r.
Strip**	50 mM HEPES, pH 7.5, 1 M NaCl	n.r.

* Until a stable baseline at 280 nm was achieved (approx. 4 MV)
** This step was performed because of high salt concentration (1 M NaCl) for removing more negative adsorbed species and trying to close the mass balance of the experiment
n.r.: not relevant

Results

The Sartobind® Q 150 mL chromatogram is shown in Fig. 5. The elution profile is comparable with that of the 2 L scale. The conductivity of the elution buffer was increased from 56 to 65 mS/cm to maximize virus recovery. The data showed good DNA and HCP (> 95 %) clearance with an average virus recovery of 78 %. The HCP level in the eluted virus sample was 0.15 ppm. Furthermore, the virus sample concentration was increased by a factor of 6 – 8.

In the larger scale DNA was reduced to 1.25 ng/dose (within the requirement) compared to 10.5 ng/dose at the 2 L scale trial. This result raises the possibility that the polishing step with Sartobind STIC® could be removed entirely or that the Sartobind® Q capacity could be reduced. Table 1 shows the virus particle recoveries and increase in purity achieved through each purification step in the process.

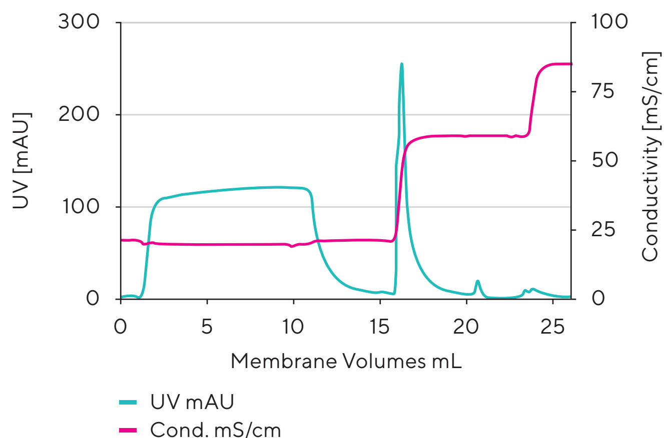

Fig. 5: Sartobind® Q 150 mL chromatogram for adenovirus capture

Table 1: Total VP, impurity removal, virus recovery and duration at 20 L scale

Operation unit	Process volume* [L]	Total VP (yield)	DNA [ng/dose]	Step DNA removal [%]	HCP [ppm]	Denarase [ng/dose]	Step VP recovery [%]	Duration [min]
Initial bulk after cell lysis	20	9.5×10^{14}	1260	–	240	8	100	–
Clarification	20	9.3×10^{14}	149	88	17	–	98	80
UF | DF	1.6	9.2×10^{14}	8.3	94	3	0.11	99	120
Sartobind® Q	0.265	7.2×10^{14}	1.25	85	0.15	-	78	46
Sartobind STIC®	0.265	6.1×10^{14}	0.7	44	0.14	-	85	26
Final formulation | sterile filtration	0.265	5.2×10^{14}	0.4	38	–	0.018	86	40
Total				99.97			55	

* Volume at the end of each step

Discussion

Process parameters determined at the small-scale allowed a purification process to be developed, scaled-up and verified at the 20 L scale. The Sartobind® Q step not only purified the adenovirus but also allowed the sample to be concentrated by a factor of eight. The Sartobind STIC® PA flow-through step used a phosphate buffer to remove DNA without binding adenovirus. This approach has previously been reported as an effective polishing method for influenza virus[6].

Table 2 shows that membrane adsorbers have a higher binding capacity and allow greater recovery compared to reported anion-exchange resin chromatography data[7,8].

The flow rate through the membrane was limited by the chromatography system to 1 MV/min, however the process step required less than one hour.
The recommended flow rate for the device is 5 MV/min. In comparison, chromatography resins with working flow rates of 300 – 500 cm/h[9] require much longer to perform the same separation.

Fast, simple and economical processing with membrane adsorbers could be applied to the purification of a variety of virus-products.

Table 2: Adenovirus purification results of Sartobind® Q and STIC PA and the reference values of Q Sepharose XL

Device	Total VP/device	VP/mL device	Virus recovery [%]
Sartobind® Q nano 1 mL	6.26×10^{12}	6.26×10^{12} (Section 1)	75
Sartobind® Q 150 mL	7.2×10^{14}	4.8×10^{12} (Section 2)	78
Sartobind STIC® PA (FT*)	6.1×10^{14}	5.5×10^{13} (Section 2)	85
Q Sepharose XL 20 mL[7]	1.4×10^{13}	7.0×10^{11} mL	74
Q Sepharose XL 20 mL[8]	6.7×10^{12}	3.4×10^{11} mL	–

* Flow-through

Conclusion

A purification process for adenovirus has been successfully developed and then verified at the 20 L scale. The specified limits for the final bulk purified virus of Host Cell DNA < 10 ng/dose, HCP < 20 µg/mL, endonuclease < 5 ng/mL (10^{10} VP/dose) have been achieved. The data obtained confirm that Sartobind® Q and STIC PA capsules are valuable technologies for the purification of adenovirus.

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The study in this application note originate from a cooperation project led by Dr. Cristina Peixoto, iBET, Portugal.

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Publication No.: SL-4079-e | Order No.: 85037-558-81 | Status: 06 | 17 | 2021