SARTORIUS

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Ultrafiltration and Diafiltration of Adenovirus Serotype 5 With Sartocon® Slice Cassettes Installed Within a Sartoflow® Smart Benchtop Tangential Flow Filtration System

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Introduction

Viral vectors are widely used in applications such as gene therapy, vaccines or cancer treatment with oncolytic viruses. Adenoviruses are one of the most commonly used viral vectors, especially for vaccine applications. They can be efficiently produced using mammalian cells in suspension. A cell lysis step at the end of the cell culture releases viruses along with many contaminants such as HCP and DNA. Following clarification to remove cell debris, the Adenovirus suspension must be concentrated and diafiltered prior to the first capture chromatography step. This is effectively accomplished by Tangential Flow Filtration (TFF) technology using ultrafiltration (UF) cassettes.

The correct selection of UF cassettes and TFF systems ensures the highest recovery of concentrated product. The concentration and diafiltration (DF) phases can contribute to product purification and facilitates subsequent downstream processing steps.

Find out more: www.sartorius.com/tff-cassettes

The Sartoflow® Smart (Figure 1) is a modular and flexible small-scale benchtop TFF system that has been optimized for UF | DF applications within the purification of vaccines, monoclonal antibodies and recombinant proteins [1]. The system is suitable for use in laboratory settings for process development as well as for cGMP environments. Its unique compact design has an ultra-low recirculation loop volume of ~20 mL, making it possible to concentrate even small process batches.

The Sartoflow® Smart can be configured to accept either Sartocon® Slice 200 [2] or Sartocon® Slice ECO [3] UF cassettes (Figure 2). An optional holding device for 50 cm² filtration modules e.g. Sartocon® Slice 50 is available. This allows an exceptionally wide working range of membrane surface areas. Since all Sartocon® TFF cassettes have the same hydrodynamic flow path length, the Sartoflow® Smart can be used for scale-up and scale-down experiments. These cassettes are available in various cut-offs and in two membrane materials: polyethersulfone (PESU) or Hydrosart®, a stabilized cellulose based membrane that has been optimized for the biotechnological and pharmaceutical industry. The Hydrosart® membrane is a stable polymer that features a broad pH range. It is also extremely hydrophilic, making it non-protein binding, virtually non-fouling, with extremely high flux and excellent cleaning features [4].

Figure 1: Sartoflow® Smart TFF system for laboratory scale.

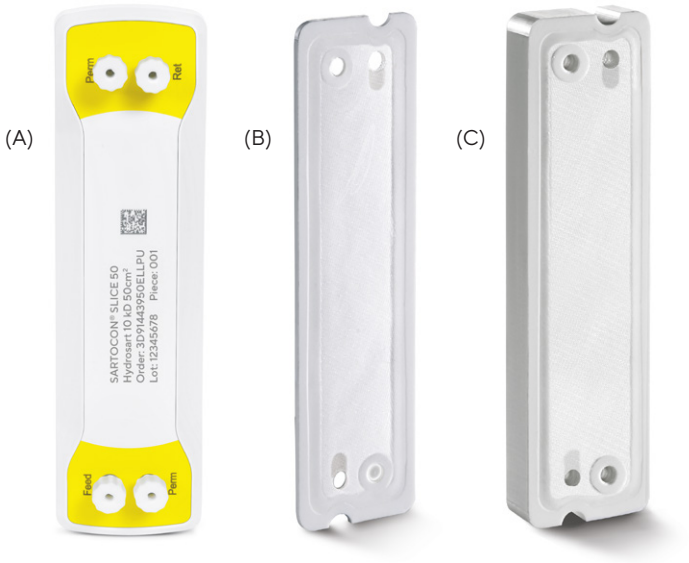

Figure 2: TFF cassettes that can be used with the Sartoflow® Smart system: (A) Sartocon® Slice 50, (B) Sartocon® Slice 200 and (C) Sartocon® Slice ECO.

Objectives

The objective of this experiment was to compare three cassettes, each with a 300kDa membrane cut-off, for the concentration and diafiltration of a clarified Adenovirus serotype 5 (Ad5) suspension. The cassettes were installed within the Sartoflow® Smart TFF system. The cassettes tested were: Sartocon® Slice 50 with PESU membrane, Sartocon® Slice 200 with PESU membrane and Sartocon® Slice 200 with Hydrosart® membrane.

Materials

1. Cassettes tested (Table 1)

Cassette	Membrane	Area [cm²]	Reference
Slice 200 PESU	PESU	200	3081467902E--SW
Slice 200 Hydrosart®	Hydrosart®	200	Test cassette
Slice 50 PESU	PESU	50	3D91467950ELLPU

Table 1: 300 kDa cassettes used in this study.

2. Adenovirus type 5 suspension:

HEK 293 cells were cultured in a commercial available serum-free media with 4mM Glutamax, in a bioreactor with a working volume of 20 L. The bioreactor inoculum was 0.5×10^6 cell/mL, the cell concentration at infection was 1×10^6 cells/mL, and a MOI (Multiplicity of Infection) of 5 was used. The bioreactor was harvested 72 hours post-infection. Prior to harvesting, the cells were lysed inside the bioreactor by adding Triton X-100 (X100, SIGMA-ALDRICH, Switzerland) to a final concentration of 0.1% (w/w) and increasing stirring to 1000 rpm for 1 minute. At the same time a nuclease treatment was performed to digest released DNA. The contents of the bioreactor were incubated for 240 minutes at 37 °C with a nuclease final concentration of 50 U/mL and with intermittent stirring. The product turbidity before filtration was 24.6 NTU. Clarification was carried out with Sartopure® PP3 0.45 µm depth filter followed by sterile filtration with Sartopore® 2 XLG [5].

3. Test system

A Sartoflow® Smart TFF system was used in this study. The system was configured with a peristaltic pump for buffer addition during diafiltration, conductivity sensor and holder for the Sartocon® Slice 50 cassette.

Methods

The filtered Adenovirus suspension was used for the UF | DF trials with three different cassettes: (i) Sartocon® Slice 50 PESU 300 kDa, (ii) Sartocon® Slice 200 PESU 300 kDa and (iii) Sartocon® Slice 200 Hydrosart® 300 kDa.

The trials were performed using the pre-defined phase "Con-Di-Con" from the Sartoflow® Smart TFF system, meaning concentration-diafiltration-concentration, keeping a constant transmembrane pressure (TMP) of 0.8-0.9 bar. Each trial started with a water flush of the cassette followed by a flush with a 50 mM Hepes pH 7.5, 200 mM NaCl diafiltration buffer. The recirculation vessel was then filled with pre-filtered Adenovirus suspension. The product was then concentrated approximately 10-fold then diafiltered with 4 – 5 × diafiltration volumes of buffer. The product was then re-concentrated to give an overall ten-fold reduction in volume from the initial volume, and harvested from the system (retentate) line. The system and cassette were flushed with diafiltration buffer and then cleaned by performing a 60 minute recirculation with 1M NaOH.

Infectious adenovirus particles were quantified using a cytopathic effect assay and the amount of DNA and HCP impurities determined using Pico Green and HEK 293 HCP assays, respectively.

Results

Before starting the trial the cassettes were flushed with purified water and the initial flux was measured (Table 2).

Cassette	TMP [bar]	Flow water [mL/min]	LMH water
Slice 200 PESU	1.25	200	660
Slice 200 Hydrosart®	1	133	399
Slice 50 PESU	1	64.3	771.6

Table 2: Clean water flux values (LMH) for the three cassettes tested.

TMP was 1.25 bar for the Slice 200 PESU and 1 bar for the other two cassettes.

The results from the three different trials are shown in Figure 3 (Slice 200 PESU), Figure 4 (Slice 200 Hydrosart®) and Figure 5 (Slice 50 PESU). Since the temperature amongst the three trials varied, permeate flow and LMH were normalized at the temperature of 25 °C. Figure 6 summarizes the results.

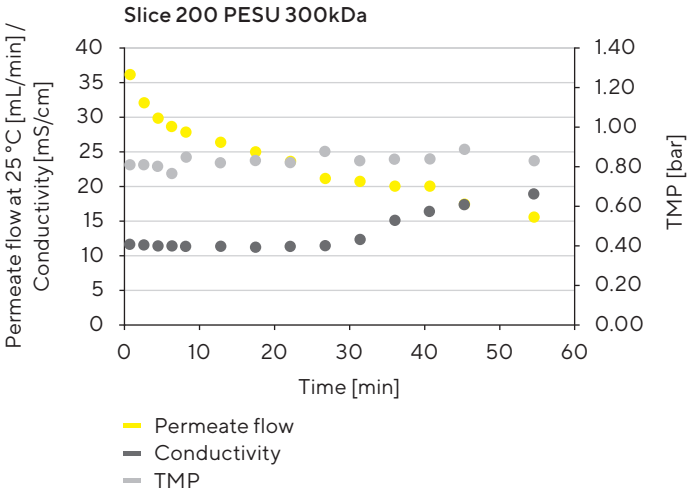

Figure 3: Adenovirus concentration and diafiltration with Sartoflow® Smart using Slice 200 PESU 300 kDa cassette.

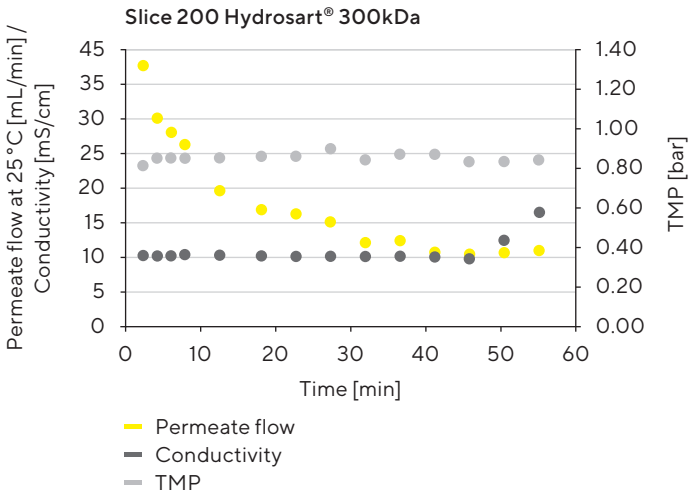

Figure 4: Adenovirus concentration and diafiltration with Sartoflow® Smart using Slice 200 Hydrosart® 300 kDa cassette.

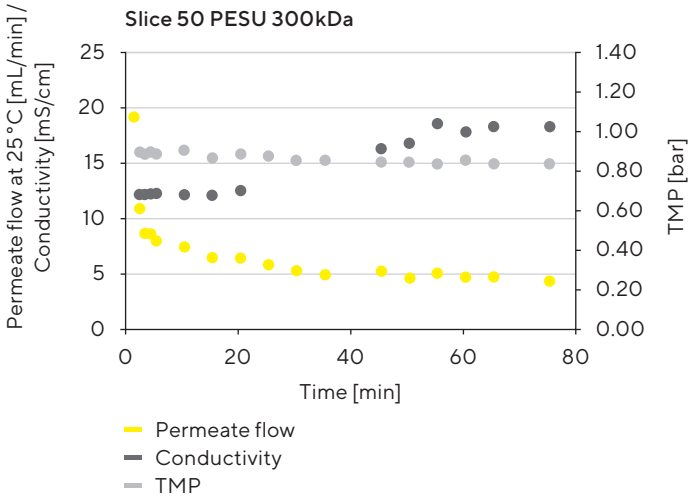

Figure 5: Adenovirus concentration and diafiltration with Sartoflow® Smart using Slice 50 PESU 300 kDa cassette.

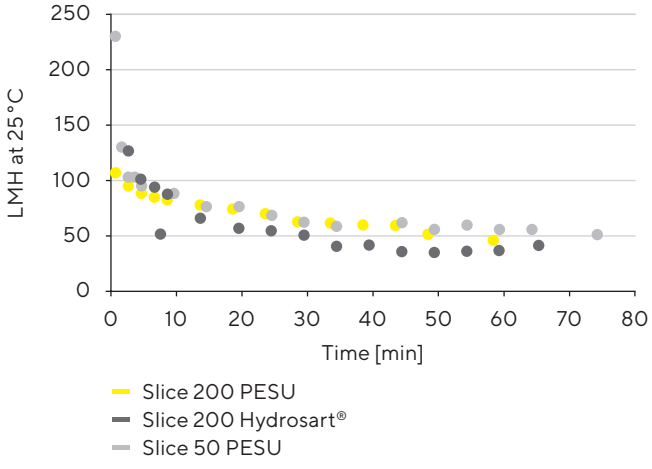

Figure 6: Permeate flow rate (LMH) normalized by temperature for the three cassettes.

Table 3 summarizes the results of Adenovirus recovery and other analytical data (DNA and protein removal).

The concentration of the clarified feedstock was performed efficiently. The TMP was maintained throughout the process during all three trials. During the concentration, a reduction in the permeate flux of 35% - 60% was observed. It was maintained in the range of 40-50 LMH depending on the cassette. This is expected because of the increasing protein concentration polarization at the membrane surface.

Cassette	Infectious particles recovery [%]	DNA removal [%]	Protein removal [%]
Slice 200 PESU	100	88	95
Slice 200 Hydrosart®	87	56	90
Slice 50 PESU	74	88	93

Table 3: Recovery of infectious particles, DNA removal and protein removal for the three cassettes tested.

Conclusion

Higher permeate flow rates were achieved with the PESU 300 kDa cassettes than with the Hydrosart® membrane. The PESU cassettes also allowed for a greater removal of contaminating DNA and protein. The variation in product recovery may be attributable to errors within the analytical assay performed to quantify the number of infectious particles. Nevertheless, Hydrosart® membranes are easier to clean compared to PESU, meaning that in the long term they are more suitable for multi-use applications.

The possibility to measure conductivity and temperature online on a Sartoflow® Smart system is a great advantage for optimization of processes with diafiltration steps.

Taking into account the results obtained with the PESU 300 kDa and Hydrosart® 300 kDa, the scale-up to 20 L (10 × concentration and diafiltration volume of 5 ×) could be performed using the parameters shown in Table 4.

Cassette	Average flow [LMH]	Process time [h]	Filtration area [m²]
PESU 300 kDa	73	1.2	0.4
Hydrosart® 300 kDa	60	1.5	0.4

Table 4: Requirements for scale-up to 20 L.

The recommended TFF system for the 20 L is Sartoflow® Advanced (Figure 7). This system is very similar to Sartoflow® Smart with the same phases concept and DCU interface but with the following additional features: (i) 10 L stainless steel recirculation tank, (ii) automatic retentate valve, (iii) flow meter in permeate line and (iv) optional Sartocon® holder with filtration area capacity of up to 2.1 m².

Sartocon® cassettes and Sartoflow® systems are part of our single-use downstream platform for adenovirus type 5 purification [5-7].

Figure 7: Sartoflow® Advanced semi-automatic TFF system.

References

[1] Datasheet “Sartoflow® Smart”, Order No.: 85037-552-73

[2] Datasheet “Sartocon® Slice 200”, Order No.: 85032-532-84

[3] Datasheet “Sartocon® Eco”, Order No.: 85032-536-67

[4] Application note “Evaluation of Feed Flow Geometry in Hydrosart® Cassettes with Protein Solutions”, Order No.: 85037-541-35

[5] Application note “Clarification of Adenovirus serotype 5 harvest bioreactor: Robust protection of downstream purification steps”, Order No.: 85037-560-72

[6] Enabling viral vaccine production, Amélie Boulais, Dr. Nick Hutchinson, Dr. Fritjof Linz, GEN, November 2016

[7] Application note “Optimizing Adenovirus Purification Processes”, Order No.: 85037-558-81

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