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Application Note

July 02, 2016

Impact of Pressure Release and Multiple Pressure Fluctuations on Virus Retention Performance of Virosart® CPV Virus Retentive Filters

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Introduction

All biopharmaceutical products derived from human or animal origins must have a proven virus safety concept to ensure the final patients' health before the product is released to commercial manufacturing or even clinical trials. The concept must cover known and unknown potential viral contaminants. Contaminating viruses can come from external sources or be present in cell lines. Endogenous retrovirus-like particles, for example, are present in most CHO cell lines. Regulators require that manufacturers perform a risk analysis and have a strategy to remove contaminating viruses during downstream processing. The industry considers filtration to be a robust method of virus removal and hence it is a widely used method. Filtration relies on the principle of size-exclusion and can remove all types of viruses. Investigators have recently discovered that under specific conditions with some virus removal membranes, virus breakthrough can occur. Scientists believe that breakthrough is more likely to occur when the membrane is operated at high capacities, during flow-decay, under conditions of high or low process pressure or when the pressure is held and released.

Virus filtration with Virosart® virus retentive filters is an integral part of the orthogonal virus clearance technology platform of Sartorius. This orthogonal technology platform features virus clearance by filtration (size exclusion), inactivation and adsorption. The Virosart® product range includes four different virus retentive membranes, in order to provide the best solution for every application.

Find out more: www.sartorius.com/virus-filtration

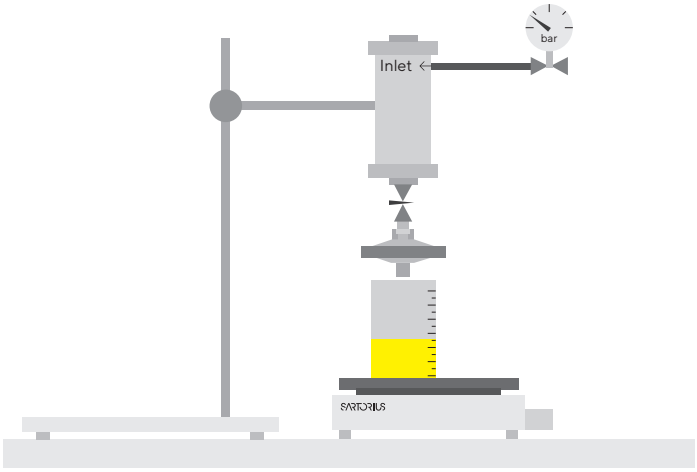
Objective

The objective of this study is to determine the impact of pressure fluctuation on the retention of parvovirus using bacteriopharge PP7, an established model system for parvoviruses. Parvoviruses are renowned for being small viruses that are typically difficult to remove.

The well established Virosart® CPV virus retentive filter membrane, from Sartorius, was tested. Virosart® CPV is a 20 nm double layer PES membrane, which provides high flow rates and superior capacity. This filter can be steam sterilized and is ideal for the application within stainless steel systems.

Materials

- Compressed nitrogen together with the Sartorius constant pressure test system with stainless steel sample reservoir
- Virosart® CPV Minisart® 5.0 cm^2
- Sartorius Balances
- Virosart® Max Minisart®, 5 cm^2
- Buffer: 50 mM Tris, 10 mM NaCl, pH 8.1
- Product: mAb; c = 5 g/L



Picture 1: Set-up of Virosart® CPV Minisart®

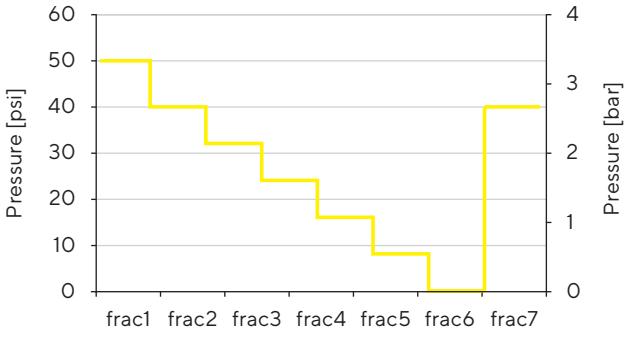
Method

A virus spike of PP7 with infectivity titer >7 log/mL was added to a monoclonal antibody solution. The mAb solution, c= 5 g/L, had been pre-filtered offline using a 5 cm^2 Virosart® Max, a 0.1 µm adsorptive pre-filter for Virosart® virus retentive filters, prior to being spiked. A pressure control device for monitoring and varying the transmembrane pressure during the filtration experiments was connected to the pressure vessel containing the spiked feed solution.

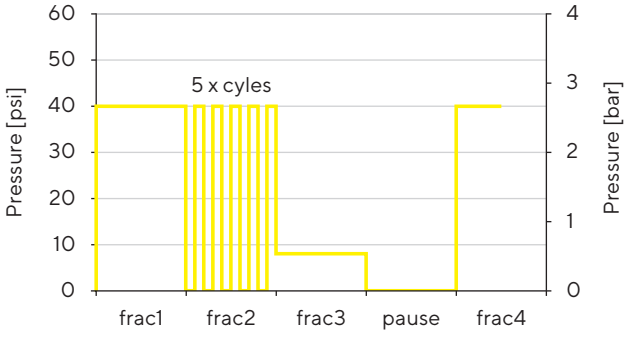
The Virosart® CPV Minisart® was loaded to a capacity of 140 L/m^2 that was equivalent to 70 ml of mAb solution. The filtrate volume was collected in a container and measured automatically using a balance connected to data recording software.

Three studies were performed as follows:

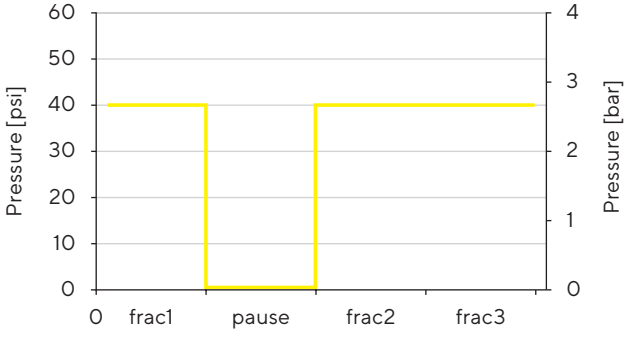
1. Multi-step pressure reduction



2. Rapid pressure changes, low pressure and pressure hold



3. Pressure release study



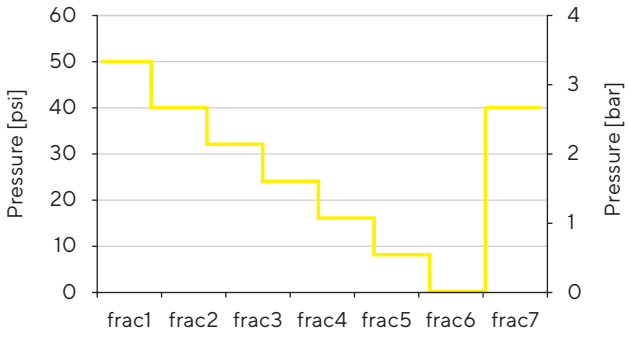
Results

Study 1: Multi-step pressure reduction

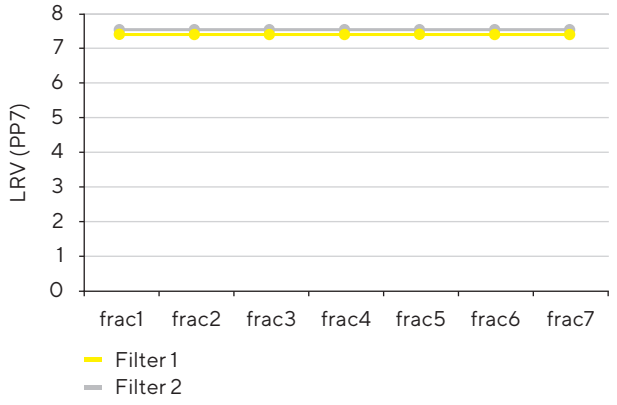
Duplicate filtration runs were performed with two 5 cm², Virosart® CPV Minisart®. Flow was started by pressurizing the reservoirs to 50 psi | 3.44 bar and an initial 10 ml fraction taken.

10 ml fractions were collected at each of the following pressure steps (40 psi | 2.75 bar, 32 psi | 2.2 bar, 24 psi | 1.65 bar, 16 psi | 1.1 bar and 8 psi | 0.55 bar). The filtration was then paused for 10 minutes and the pressure returned to 40 psi | 2.75 bar before the final fraction was collected. Graph 1 shows the pressure profile during the experiment. Graph 2 shows the corresponding LRV profile of the fractions.

	Pressure [psi]	LRV (PP7) Virosart® CPV Filter 1	LRV (PP7) Virosart® CPV Filter 2
Load		7.67	7.67
Fraction 1	50	≥ 7.67	≥ 7.67
Fraction 2	40	≥ 7.67	≥ 7.67
Fraction 3	32	≥ 7.67	≥ 7.67
Fraction 4	24	≥ 7.67	≥ 7.67
Fraction 5	16	7.37	≥ 7.67
Fraction 6	8	≥ 7.67	≥ 7.67
10 minute pause	0		
Fraction 7	40	≥ 7.67	≥ 7.67



Graph 1: Pressure course during multi-step pressure reduction



Graph 2: Multi-step pressure reduction onto Virosart® CPV showing constant independent retention performance.

Summary of Study 1:

All fractions showed stable retention above >7 log, allowing the conclusion that multistep pressure reduction had no effect on the retention of Virosart® CPV Minisart®.

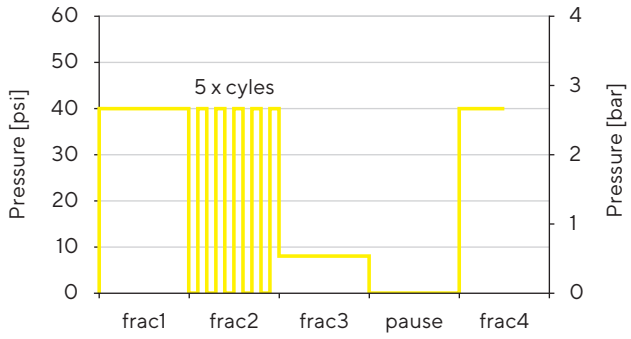


Study 2: Rapid pressure change, low pressure and pressure release

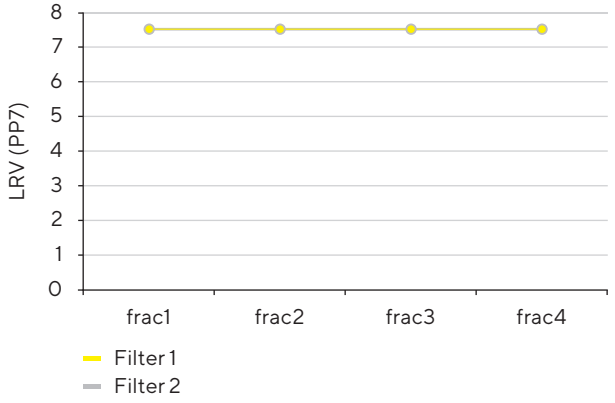
Duplicate filtration runs were performed with two 5 cm², Virosart® CPV Minisart®. The reservoirs were pressurized to 40 psi | 2.75 bar and the first fraction (20 ml) was taken. While the second 20 ml fraction was collected the pressure was rapidly switched off and on 5 times (fraction 2). Afterwards the filtration was allowed to proceed at a low pressure of only 8 psi | 0.55 bar during which the third 20 ml fraction was collected.

The filtration was then paused for 10 minutes and the pressure returned to 40 psi | 2.75 bar before the final fraction was collected. Graph 3 shows the pressure profile during the experiment. Graph 4 shows the corresponding LRV profile of the fractions.

	Pressure [psi]	LRV (PP7) Virosart® CPV Filter 1	LRV (PP7) Virosart® CPV Filter 2
Load		7.52	7.52
Fraction 1	40	≥ 7.52	≥ 7.52
Fraction 2	0 – 40 cycle 5 ×	≥ 7.52	≥ 7.52
Fraction 3	8	≥ 7.52	≥ 7.52
10 minute pause	0		
Fraction 4	40	≥ 7.52	≥ 7.52



Graph 3: Presure course during rapid pressure change, low pressure and pressure release



Graph 4: Rapid pressure change, low pressure and pressure release onto Virosart® CPV showing complete retention performance.

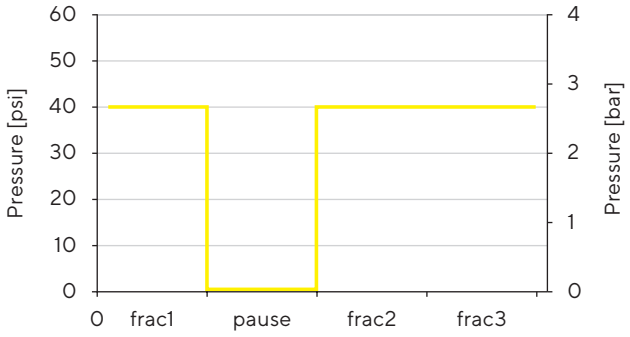
Summary of Study 2:

No fractions of the filtrate showed any virus breakthrough allowing the conclusion that the rapid pressure changes had no effect on the retention of Virosart® CPV Minisart®.

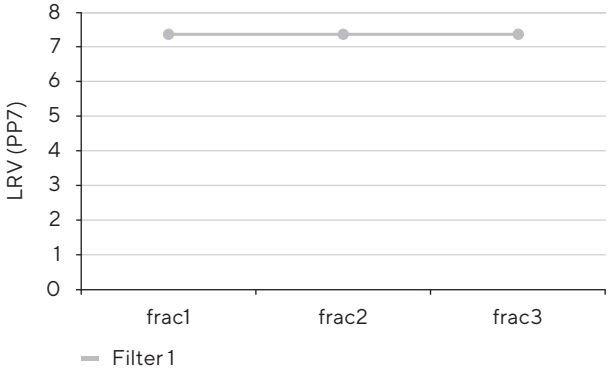
Study 3: Pressure release study

Duplicate filtration runs were performed with a 5 cm², Virosart® CPV Minisart®. Flow was started by pressurizing the reservoir to 40 psi | 2.75 bar. Upon reaching 50% of the total loading (fraction1: 70 L/m²) the system was de-pressurized and held a 0 psi | 0 bar for 10 minutes. Following the de-pressurized hold, the system was re-pressurized to 40 psi | 2.75 bar and the remaining material collected as fraction 2 (total loading capacity 140 L/m²). Finally the system was de-pressurized, wash buffer added to the reservoir, and the wash fraction was collected. Graph 5 shows the pressure profile during the experiment. Graph 6 shows the corresponding LRV profile of the fractions.

	Pressure [psi]	LRV (PP7) Virosart® CPV Filter 1	LRV (PP7) Virosart® CPV Filter 2
Load		7.38	7.38
Fraction 1 (Load 0 – 70 L/m²)	40	≥ 7.38	≥ 7.38
10 minute pause	0		
Fraction 2 (Load 70 – 140 L/m²)	40	≥ 7.38	≥ 7.38
Fraction 3: Buffer flush	40	≥ 7.38	≥ 7.38



Graph 5: Presure course during presure release study.



Graph 6: Pressure release onto Virosart® CPV showing complete retention performance.

Summary of Study 3:

No fractions of the filtrate showed any virus breakthrough allowing the conclusion that the perfromed pressure release during virus filtration had no effect on the retention of Virosart® CPV Minisart®.

Conclusions

This application note summarizes three filtration studies investigating the effect of pressure variations on the retention of PP7 bacteriophage by Virosart® CPV Minisart®.

The virus retention of the Virosart® CPV Minisart® was unaffected by pressure changes in any of the studies.

It can be concluded that the Virosart® CPV Minisart®, provide robust retention under the conditions studied in the experiments described in this application note.

Although this membrane offers high virus retention under a wide range of processing conditions, users are recommended to evaluate the impact of pressure excursions onto membrane performance under their specific process conditions.



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Publication No.: SPK4111-e | Order No.: 85037-552-81 | Status: 07 | 22 | 2021