Simplifying Progress

Qualifying and Using Virus Filtration Integrated Into Low- and High-Flux Continuous Processes

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Introduction

Virus filtration continues to be a critical manufacturing step to enhance safety for biologics. Historically, manufacturers have performed virus filtration in batch mode, which involves a defined pooled load, a pump or pressure source to drive load material across a filter membrane, and a singular collection vessel for the new filtrate. This classic filtration method has been widely used in biopharmaceutical manufacturing for decades without complications.
Today, an industry trend toward continuous manufacturing is raising new questions regarding the performance of available virus filter products and the implications for virus clearance qualification.

We investigated the performance of current-generation virus filters across several conditions:
- Extremely low flux rates (14 LMH), long process times (71 hours), and high throughput (1,000 L/m²)
- Directly connected to a bind and elute ion exchange (IEX) chromatography column
- Operated with constant flux versus constant pressure

Materials & Methods

Table 1: *Feed Material Attributes*

Feed Material	Attributes
mAb A	NS0 cell-derived, IgG1, pH 7.2, 20 mM Tris Buffer, 10 g/L

Table 2: *Equipment*

Chromatography system
Constant pressure test system
Pressure monitoring system

Table 3: *Consumables*

Product	Part Number
Virosart® HF Lab Module (1.7 cm²)	3VI--28-TCGML--V
Virosart® HF Lab Module (5 cm²)	3VI--28-BCGML--V
Virosart® Max Lab Module (5 cm²)	54AMI---------B
Virosart® Max Lab Module (21 cm²)	54ASA---------V
Low salt buffer (20 mM sodium acetate pH 5.0)	
High salt buffer (20 mM sodium acetate, 0.5 M sodium chloride, pH 5.0)	
Ion-exchange column, 1 × 30.5 cm (23.955 mL)	
Porcine Parvo Virus (PPV), 9.5 Titer (log_{10} $TCID_{50}$/mL)	

Continuous Processing Model: Extremely Low Flux, Long Processing Time, and High Throughput Using Virosart® HF

Because scientists might integrate virus filtration unit operations into extremely low-flux column processes we developed a model to demonstrate the robustness of virus retention with extremely low-flux rates, long-duration processes, and high throughput.

Procedure

Using a peristaltic pump, we delivered the virus-spiked feedstream to a 5 cm² Virosart® HF virus filter at a constant flux rate of 14 LMH. A sensor placed upstream constantly monitored pressure. The Virosart® HF was pre-wetted with WFI at 30 psi for 10 minutes. We then reduced the flux rate to 14 LMH and loaded the virus-spiked feedstream at room temperature. The entire process used a single virus-spiked load feedstream. We loaded a total volume of 500 mL to reach a final throughput of 1000 L/m² onto the membrane in 71 hours (Figure 1). We collected fractions every day and overnight for a total of seven fractions. Fractions were analyzed for virus content using TCID50.

Figure 1: *Virosart® Filter Operated in Constant Low-Flux Mode*

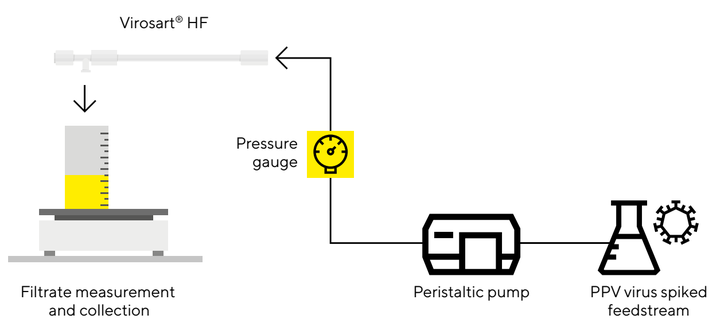


Figure 2: *Flux Rate Remained Constant at 14 LMH Throughout the Study*

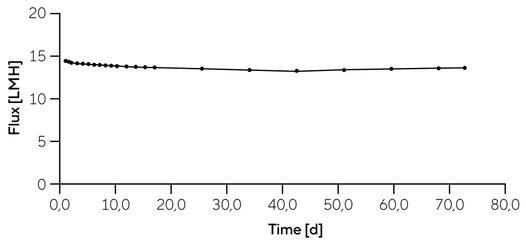


Figure 3: *The Results Show an Overall Throughput of Approximately 1,000 L/m² Was Achieved in 71 Hours of Process Time. Pressure Increased Minimally Throughout the Study, Starting With an Initial Pressure of 2 psi and Reaching 8 psi by the End of the Study.*

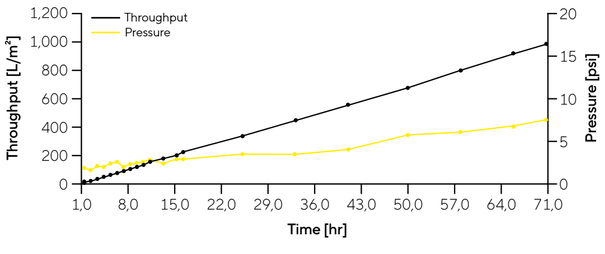


Figure 4: *The Results Show Complete Retention of PPV Virus Throughout the 71-Hour Process Operated Using Constant Low Flux*

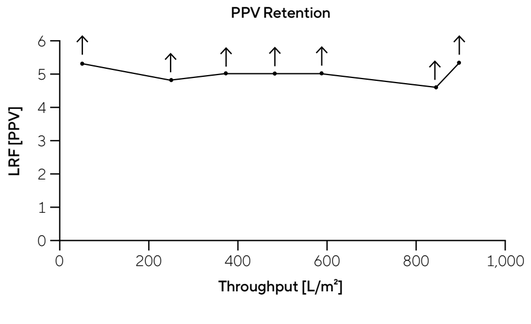


Table 4: *Throughput and LRF Results for Fractions*

Sample	Volume [mL]	Throughput [L/m²]	LRF
Fraction 1	26.0	52	>5.3
Fraction 2	99.4	250.8	>4.8
Fraction 3	60.8	372	>5.0
Fraction 4	54.9	482	>5.0
Fraction 5	52.2	586	>5.0
Fraction 6	128.6	843	>4.6
Fraction 7	26.4	895	>5.3

Continuous Processing Model: Bind/Elute IEX Chromatography Column Connected to Virosart® HF

Procedure

We performed all filtrations using Virosart® Max adsorptive prefilter technology in-line with a Virosart® HF virus filter at a ratio of 3:1. Virosart® Max filters were wetted with water using manual operation of the chromatography system before attaching and venting Virosart® HF filters. We increased flow rate to 1.1 mL/min and flushed Virosart® HF filters for 10 minutes. Sensors monitored pressure. Prior to use, we filtered feed material using a 0.2 µm bottle-top filter. For in-line virus spike runs, we minimally diluted highly purified spike with a representative column eluate material for injection with the syringe pump.

We calculated a feed volume that would target column loading of 45 g/L and achieve filter loading of >500 L/m², and ran the chromatography system method using the determined feed stream volume. After equilibration with low salt buffer, the feed was loaded onto the IEX column, washed with additional low salt buffer, and eluted with a gradient of low-to-high salt buffers. Once elution began, we opened the syringe pump to the system and began continuous spiking. At the end of elution, the syringe pump was stopped and isolated from the system. Automated software captured all mass and time data. The process setup is shown in Figure 5.

Figure 5: *Virosart® Filters Operated in Continuous Mode With a Cation Exchange Column*

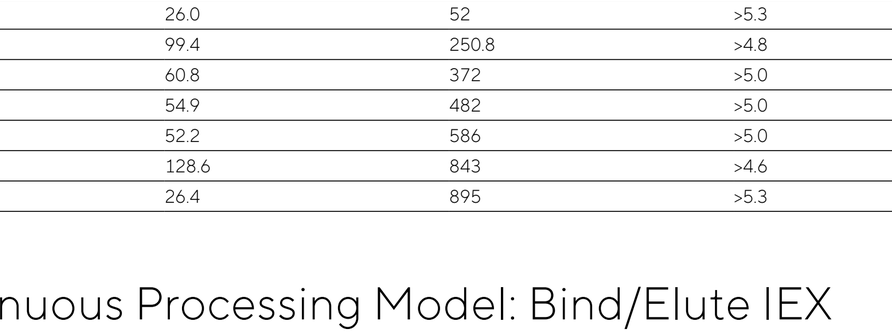


Table 7: *Throughput and LRF Results*

Run ID	Run	Throughput [L/m²]	LRF PPV [log_{10}]
9	R1	516	5.1
10	R2	501	5.7

Note. LRF = logarithmic reduction factor, PPV = porcine parvovirus.

Figure 6: *Virosart® Filter Connected In-line to IEX Column*

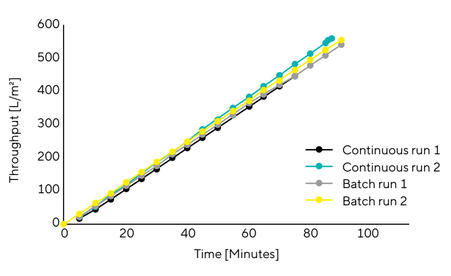
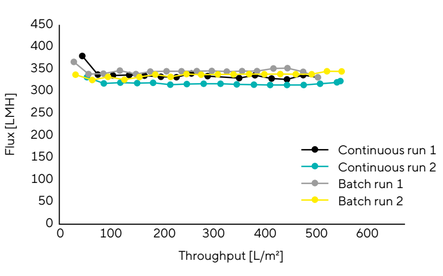


Figure 7: *Performance of Virosart® Filters (Flux vs Throughput) Operated in Continuous and Batch Mode*



The results in Figure 6 and Figure 7 show that Virosart® filters maintain consistent performance with respect to throughput and flux when operated in either continuous and batch modes.

Conclusion

In biopharmaceutical manufacturing, scientists can integrate virus filtration unit operations into continuous processes using two methods:

1. Operated in constant low flux over a long duration
2. Directly connected to a chromatography column and operated in high pressure, directing eluted product peak or flow through the product onto the virus filter

This poster evaluates Virosart® Max and Virosart® HF system performance in two different integrated continuous modes: low-flux and high-pressure. We demonstrate that combining Virosart® HF virus filter with Virosart® Max adsorptive prefilter can provide the robustness needed to effectively handle continuous virus filtration processes. Additional data showed consistent results when comparing Virosart® HF virus filtration in the batch mode and continuous mode. In conclusion, regardless of the mode of operation, Virosart® HF offers high performance with high throughput, low flux decay, and high virus retention.

Acknowledgments

The authors are deeply appreciative to Sean O'Donnell and Benjamin Walker from Eli Lilly for their unwavering support allowing us to perform this study as well as their commitment to scientific progress and collaboration.