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Application Note

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Risk Mitigation for Calcium Chloride Solution as an Additive to Cell Culture Media Using High Flux Virus Filters

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Abstract

Calcium is an essential supplement for CHO cells grown in serum-free media and is often supplied in the form of calcium chloride ($CaCl_2$). Due to the potential for introduction of adventitious agents into the bioreactor through raw materials, these additives are subjected to methods such as heat inactivation and filtration to decrease viable contaminant load. This study describes the filtration performance of two parvovirus retentive hollow fiber filters, the Virosart® HF and the Virosart® Media filter. Virosart® HF is a high flux virus filter intended for applications in the monoclonal antibody market and the latter is specifically designed for upstream risk mitigation to minimize contamination from viruses. Data demonstrate that both filters provide necessary throughput and flux; however, the Virosart® Media filter is the filter for upstream applications when high throughputs and process economics are desired.

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

Introduction

Calcium plays a pivotal role in cell metabolism and is involved in several functions including cell signaling and cell attachment; it also mediates many cellular events that affect cell movement, shape and three-dimensional structure. While serum-containing cell culture medium fulfils the calcium requirement of cells grown in vitro, calcium supplementation is often required when serum-free cell culture medium is used. The concentration of calcium used in media for CHO culture typically ranges from 0.30 to 1.05 mM with 1.05 mM calcium being frequently used as a base for development of CHO media used in biomanufacturing (1).

Risk mitigation to minimize introduction of adventitious agents into the bioreactor through raw materials addition is currently advocated by regulatory and industry groups. Size exclusion-based filtration is the preferred technology for viral clearance, as it is robust and non-invasive. Virosart® Media is a risk mitigation filter developed for chemically defined cell culture media in order to reduce the risk of virus contamination prior to the addition of nutrients and other additives into the bioreactor system. The Virosart® Media filter is an asymmetric polyethersulfone hollow fiber membrane that exhibits high capacity (1000 L/m² at 2 bar in 4 hour filtration time) while providing ≥ 4 LRV (log10 reduction value) for small non-enveloped viruses and ≥ 6 LRV for large enveloped viruses.

This study was conducted to evaluate filterability of calcium chloride ($CaCl_2$) and compare results of the new Virosart® Media with that of the Virosart® HF.

Methods

A 1.08 M solution of calcium chloride was prepared and filtered using a Sartolab® 0.2 µm filter. Single units of either the Virosart® Media filter (5 cm², Part Number: 3V2--28-BVGML--V) or a Virosart® HF filter (5 cm², Part Number: 3VI-28-BCGML--V) were assembled. For each respective run, filters were flushed with approximately 20 mL of filtered deionized water (DI) at 2.0 bar | 30 psi. Following the water flush, the water permeability was measured by recording the average flow rate for 5 min. The reservoirs were then emptied of DI water, filled with 1.08 M $CaCl_2$ and pressurized to 2.0 bar | 30 psi. Filtrate weight was recorded at specific time points.

Results and Discussion

Data for throughput and flux for the Virosart® Media and the Virosart® HF filter units are presented in Figures 1 and 2. For filtration of a 1.08 M $CaCl_2$ solution, the Virosart® Media is the best option providing better throughputs and flow rates compared with the Virosart® HF.

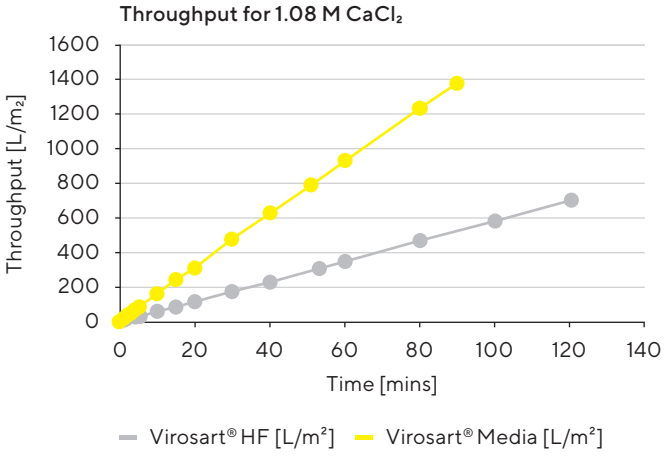

Figure 1: *Throughput for the Virosart® Media and the Virosart® HF Filters 2 bar | 30 psi*

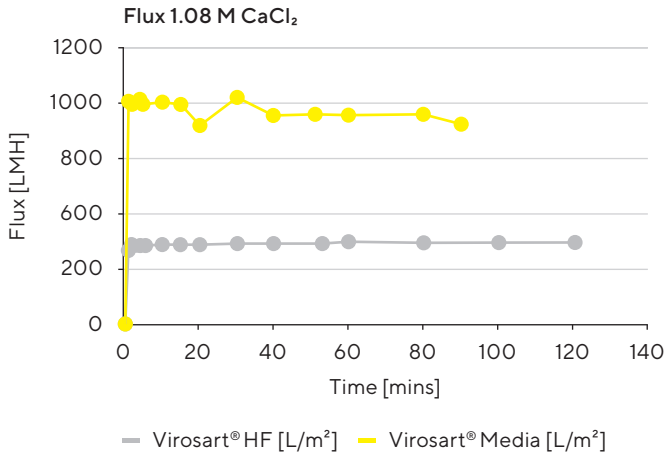

Figure 2: *Flux for the Virosart® Media and the Virosart® HF Filters During Filtration of 1.08 M $CaCl_2$ at 2 bar | 30 psi*

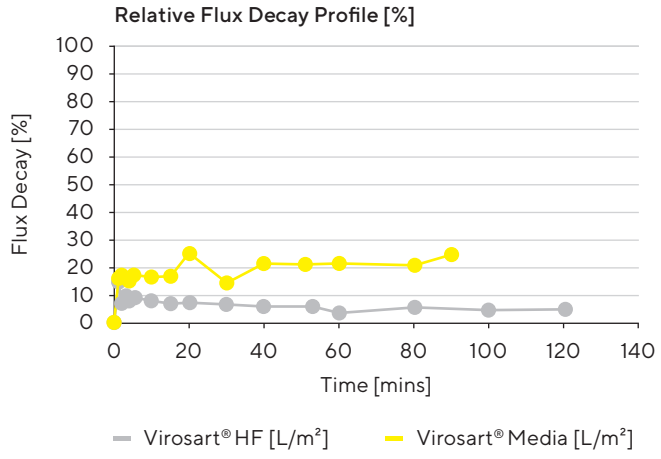

Figure 3: *Relative Flux Decay Profiles for the Virosart® Media and the Virosart® HF Filters During Filtration of 1.08 M $CaCl_2$*

Figure 3 shows that the flux decay for both filters, Virosart® Media and Virosart® HF, rapidly plateaus and then remains relatively constant throughout the run. For the remaining part of the filtration step, both filters show only minor flow decay up to a total load of 700 L/m² for Virosart® HF and 1,380 L/m² for Virosart® Media. The intial flow decay could partly be attributed to the increased viscosity of the $CaCl_2$ solution compared to water.

Table 1 provides a snapshot of the data at 60 minutes. The average flux was much faster for the Virosart® Media filter. This data demonstrates, that the Virosart® Media is the best option for a 1.08 M $CaCl_2$ solution; processing 2.7 times the volumes per hour when compared with the Virosart® HF.

Table 1: *Snapshot of Product Throughputs and Flux at 60 Minutes*

Filter type	Throughput [L/m²]	Flux [LMH]
Virosart® HF	348	359
Virosart® Media	930	905

Summary and Conclusion

The Virosart® Media filter provides high flux, minimal flux decay, as well as favorable process economics. Virosart® Media is an effective risk mitigation tool, with high capacities and higher flux rates compared with established downstream virus filters such as Virosart® HF. Data demonstrate that the Virosart® Media is the filter of choice for upstream applications where high throughputs and process economics are desired.

References

(1) Sigma Aldrich. Calcium in cell culture.
http://www.sigmaaldrich.com/life-science/cell-culture/learning-center/media-expert/calcium.html

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