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Application Note

November 07, 2017

Keywords or phrases:
Risk mitigation upstream, virus retention, chemically defined cell culture media, virus filtration, FBS, hydrolysate, polaxamer

Influence of Cell Culture Media Components on the Filtration Characteristics of Virosart® Media

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Abstract

Bioreactors can be contaminated with adventitious agents such as bacteria, mycoplasma, and viruses. Viruses have been the cause of multiple bioreactor contamination events in recent years. A number of biopharmaceutical companies have reported production-scale bioreactor contamination events by small non-enveloped viruses such as minute virus of mice (MVM) or vesivirus[1]. The consequences of such an event may be severe and result in GMP facility contaminations, along with drug shortages and financial losses. Therefore, several large biopharmaceutical operations are evaluating risk mitigation strategies for the minimization of contaminations by adventitious agents. Classical sterilizing-grade filters and even 0.1 µm-rated filter membranes cannot prevent contamination by small non-enveloped viruses[2].

Size exclusion-based filtration is the preferred technology for virus clearance, as it is robust and non-invasive. The Virosart® Media filter mitigates virus contamination risks which may arise from the addition of nutrients and other additives into the bioreactor system.

Find out more: www.sartorius.com/virosart-media

Introduction

The Virosart® Media filter has been developed specifically for chemically defined cell culture media. The filter is an asymmetric polyethersulfone hollow fiber membrane with 20 nm nominal pore size rating that exhibits high capacity (1000 L/m² at 2 bar in 4 hour filtration time) for filtration of chemically defined cell culture media while providing ≥ 4 LRV (log_{10} reduction value) for small non-enveloped viruses and ≥ 6 LRV for large enveloped viruses[3].

This study reports on the influence of cell culture media components on filtration characteristics of Virosart® Media filter. In total the following three studies were performed:

Study 1: Impact of Poloxamers

Study 2: Impact of Hydrolysates

Study 3: Impact of Fetal Bovine Serum

Study 1: Impact of Poloxamers

Polaxamer is a shear protectant for cells since it is used for reducing surface tension as well as cell bubble attachments. Further it can preserve cell growth and viability from decreasing compared to cases without poloxamers[5-7]. Polaxamers are known to have an impact on the filtration performance. This study is intended to evaluate the impact of different concentrations of poloxamer on the performance of the Virosart® Media filter.

Material and Methods

The filterability characteristics of five representative commercially available chemically defined cell culture media containing different concentrations of poloxamer (0% up to 0.2%) were evaluated (table 1). Whereas a concentration of 0.2% is the standard concentration used in the majority of cell culture media. The concentration of poloxamers was determined according to Ghebeh at. al. using a colorimetric assay[8]. Filtration trials were performed with Virosart® Media lab modules (5 cm², part number: 3V2--28-BVGML--V) from different production lots.

Media	Supplier	Poloxamer concentration	Order number
VP-SFM AGT™	Thermo Fischer	0%	12559019
Gibco CHO CD Efficient Feed™ B AGT™	Thermo Fischer	0.1%	A1245605
ProPer 1	Sartorius	0.2%	BE02-028Q
Ex-Cell® CD CHO 3	Merck	0.2%	C1490-1L
Express Five® SFM	Thermo Fischer	0.2%	10486025

Table 1: *Cell Culture Media Used to Evaluate the Impact of Poloxamers.[9-10]*

In addition various concentrations of poloxamers were tested in deionized (DI) water to exclude effects of other media components on the performance of Virosart® Media filter (table 2).

Poloxamers	Supplier	Poloxamer concentration	Order number
Pluronic® F-127 in DI water	Sigma Aldrich	0.1%	P2443-500g
Pluronic® F-127 in DI water	Sigma Aldrich	0.5%	P2443-500g
Pluronic® F-127 in DI water	Sigma Aldrich	1.0%	P2443-500g

Table 2: *Poloxamer Concentration Used to Evaluate the Impact of Poloxamers in DI Water[10]*

The dehydrated media was reconstituted in DI water according to manufacturer's instructions and the poloxamers were added at the according concentration as described in table 1-2. Before each run, the filters were flushed for 15 minutes with DI water at 2.0 bar | 30 psi using compressed air and the water flux was recorded. The DI water in the reservoir was exchanged to according cell culture media and the filtration at constant pressure of 2.0 bar | 30 psi was started at room temperature (20–22 °C | 68–71.5 °F). The filtration was stopped after 240 min or when the total volume was processed through the filter. The filtrate was collected and the weight recorded at specific time points in order to calculate the flow rate, flux and flux decay.

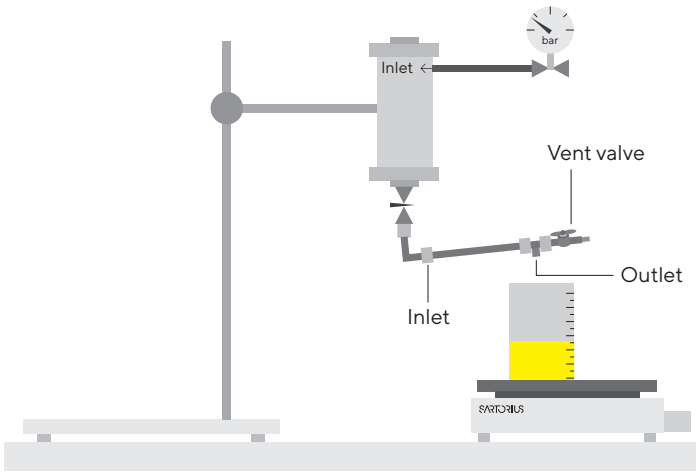

Figure 1: *Experimental Set-up of Small-Scale Filtration Using Virosart® Media Lab Module 5 cm²*

Results and Discussion

Capacity [L/m²] and flux [LMH/bar] data for media containing different concentrations of poloxamers filtered through the Virosart® Media filter are presented in figure 2. Media composition is key to obtaining optimal utilization of a media viral filter. Figure 2 shows that the initial flux correlates well to the poloxamer concentration: Increased concentration leads to lower flux. Nevertheless the further progression of the filtration performance does not correlate to the poloxamer concentration and is probably governed by other media components.

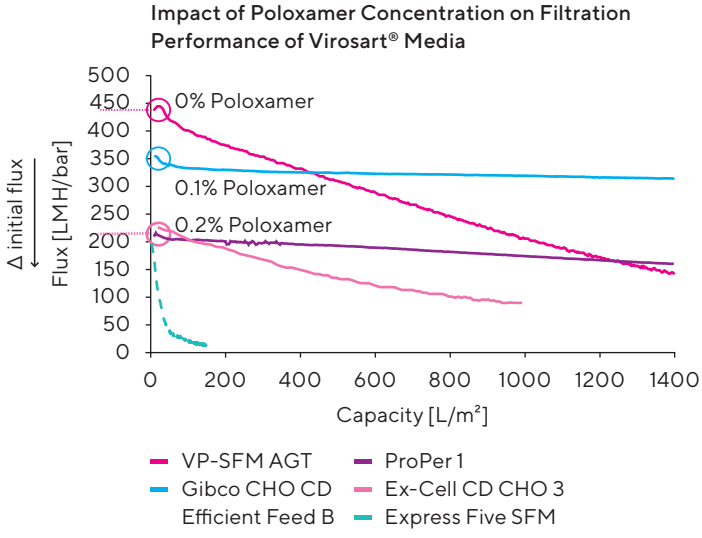

Figure 2: *Impact of Poloxamer Concentration (0–0.2%) With Five Different Media on Filtration Characteristics of Virosart® Media at 2.0 bar | 30 psi Constant Pressure During 4 Hour Filtration*

Figure 2 shows the effect of the presence of poloxamers in DI water. Higher flux decay and less throughput were observed with higher poloxamer concentrations of 0.1–1.0%. However, a complete flow decay can be observed for all three concentrations within the first minute of filtration.

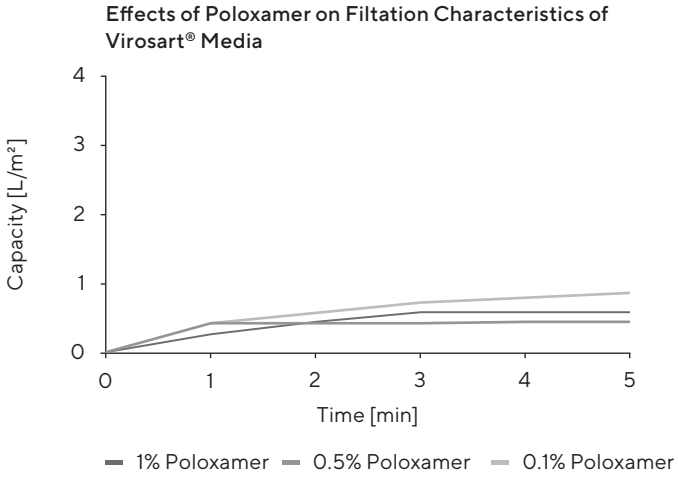

Figure 3: *Effect of Poloxamers on Filtration Characteristics Through the Virosart® Media Filter in DI Water*

Several commercially available media are formulated with poloxamers from concentration of 0.1–0.3%. The results indicate that reducing the poloxamer concentration or filtering it separately can increase significantly the capacity of the Virosart® Media filter. The flux rate during the remaining filtration is determined by other media components. Additional research could be performed to understand fully the influence that different media components can have on the blockage of the media filter. This may allow the design of media with improved filterability characteristics without affecting its performance during cultivation.

Study 2: Impact of Hydrolysates

Animal-derived hydrolysates have been used in mammalian cell cultures as companies have sought to move away from serum-containing media. They have subsequently been replaced by hydrolysates derived from animal, plants or synthetic origins to reduce the risk of adventitious agents from raw materials entering the bioreactor[11]. The industry is now moving towards chemically defined media because of the lot-to-lot variability in raw materials such as plant-derived hydrolysates[12]. Hydrolysates consist of peptides, amino acids, carbohydrates and lipids and some other non-defined components[13] which are known to have an impact on filtration performance. This study is intended to evaluate the impact of six different hydrolysates, still used in commercial processes, derived from soy, wheat, whey and synthetic origin on the filtration characteristics of Virosart® Media.

Material and Methods

The filterability characteristics of six representative hydrolysates derived from soy, wheat, whey as well as from synthetic origin were evaluated (table 3). Filtration trials were performed on Virosart® Media lab modules (5 cm², part number: 3V2--28-BVGML--V) from different production lots with (w) and without (w/o) Sartopore® 2 XLM Sartoscale 25 0.1 µm (4.5 cm², part number: 5445358MV--LX--C) as a pre-filter. Sartopore® 2 XLM is a 0.2 | 0.1 µm pleated polyethersulfone filter, that works based on the principle of size-exclusion.

The hydrolysates were reconstituted in DMEM media at a concentration of 4 g/L. Before each run, the filters were flushed for 15 minutes with DI water at 2.0 bar | 30 psi using compressed air and the water flux was recorded. The DI water in the reservoir was exchanged to according cell culture media and the filtration at constant pressure of 2.0 bar | 30 psi was started at room temperature (20 – 22 °C | 68 – 71.5 °F). The filtration was stopped after 240 min or when the total volume was processed through the filter. The filtrate was collected and the weight recorded at specific time points in order to calculate the flow rate, flux and flux decay. The same experimental set-up was used as in the first study (figure 1).

Media	Hydrolysate	Origin	Form	Concentration [g/L]	Supplier	Order number	Filter tested
DMEM	Soy Protein acid hydrolysate	Soy	Powder	4	Sigma-Aldrich	S1674-100G	Virosart® Media w/& w/o XLM
DMEM	HyPep® 4601 Protein hydrolysate from wheat gluten	Wheat	Powder	4	Sigma-Aldrich	H6784-100G	Virosart® Media w/& w/o XLM
DMEM	Lactalbumin enzymatic hydrolysate	Whey	Powder	4	Sigma-Aldrich	L9010-500G	Virosart® Media w/& w/o XLM
DMEM	Peptone Hy-Soy® T	Soy	Powder	4	Sigma-Aldrich	P6463-250G	Virosart® Media w/& w/o XLM
DMEM	Ex-Cell®CD hydrolysate fusion	Synthetic	Liquid	4	Sigma-Aldrich	14700C-500 ml	Virosart® Media w/& w/o XLM
DMEM	Soy hydrolysate UF solution 50×	Soy	Liquid	4	Sigma-Aldrich	58903C-100 ml	Virosart® Media w/& w/o XLM

Table 3: *Hydrolysates Used to Evaluate the Filterability Characteristics of Virosart® Media With and Without Sartopore® 2 XLM (XLM)*

Results and Discussion

The data presented in figure 4 show the capacity [L/m²] vs. time [min] course of the six different hydrolysate sources (concentration 4 g/L) in DMEM media. The processes were run for 240 min at 2.0 bar | 30 psi or until the total volume had been filtered as was the case for Soy Protein acid, HyPep® Protein, Ex-Cell® CD and Soy UF hydrolysates. The filtration of these hydrolysates has been extrapolated using V_{final} up to a filtration time of 240 min (shown in dotted lines) to allow a comparison with the other filtration runs. The following equation describing V_{final} was applied:

$$\frac{t}{V} = \frac{1}{V_{final}} \times t + \frac{1}{Q_0}$$

with:

T	Time [min]
V	Capacity filtered [L/m²]
V_{final}	Maximum capacity [L/m²]
Q_0	Initial flux [LMH/bar]

In all trials Soy Protein acid, HyPep® Protein, Ex-Cell® CD and Soy UF hydrolysates showed very good capacity and tended to block the filter relatively slowly (figure 4). Extrapolated to 240 min filtration time, capacities of 1300 L/m² up to 3000 L/m² can be achieved for four of the hydrolysates. Lactalbumin (orange line) and Peptone Hy-Soy® (yellow line) showed lower capacities and tended to block the filter more rapidly. Lactalbmumin reached a capacity of 400 L/m² in 240 min filtration time with an overall blocking of approximately 80%.

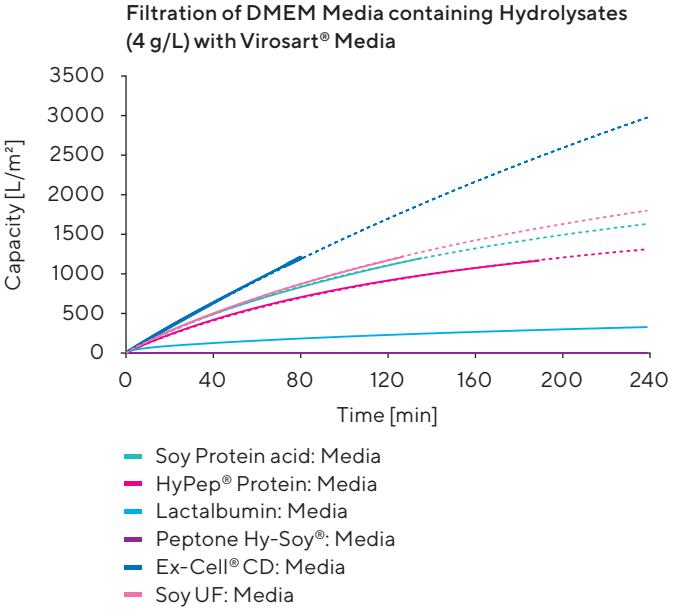

Figure 4: *Filtration of Six Different Hydrolysates in Dmem Media With Virosart® Media at Constant Pressure of 2.0 bar | 30 psi for 240 Min Filtration*

Filtration trials were also performed to determining the effect of pre-filtration of the six hydrolysates with Sartopore® 2 XLM under the same experimental conditions (figure 5). Sartopore® 2 XLM marginally increased the filtration performance with the Soy Protein acid (< 10%) and Ex-Cell® CD hydrolysates. Using Sartopore® 2 XLM as a pre-filter, a 30% increase in total capacity of Lactalbumin can be achieved. V_{final} extrapolation shows that the total capacity of HyPep® Protein and Soy UF hydrolysates can be increased by 45%. The greatest improvement due to adding a pre-filter to the system was observed with the Peptone Hy-Soy® hydrolysate.

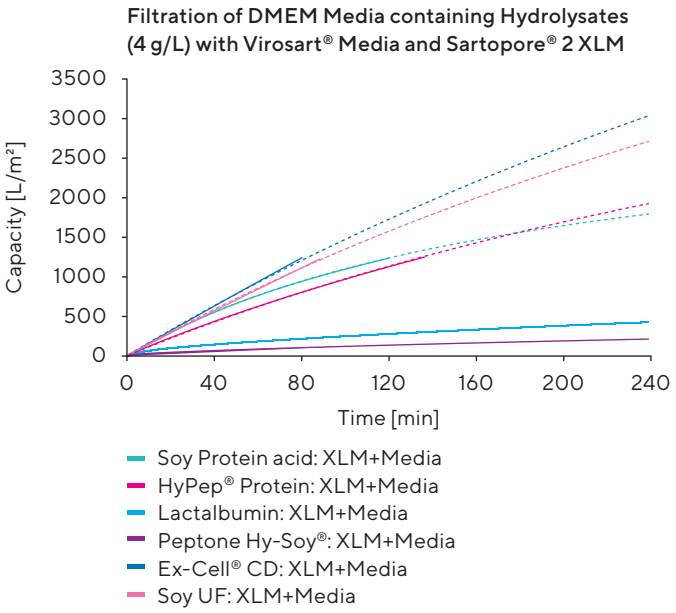

Figure 5: *Filtration of Six Different Hydrolysates in Dmem Media With Virosart® Media and Sartopore® 2 XLM at Constant Pressure of 2.0 bar | 30 psi for 240 Min Filtration.*

Figure 6 shows the total capacity after 240 min filtration time with and w/o the use of Sartopore® 2 XLM as a pre-filter. A correlation between hydrolysate origin and total capacity cannot be concluded. The overall capacity is dependent on the type of hydrolysate with total capacity of above 1500 L/m² for most of the tested hydrolysates. Ultrafiltered (Soy UF) and synthetic hydrolysates (Ex-Cell® CD) showed very good filtration performance of 3000 L/m². The Sartopore® 2 XLM increased capacity by 30 – 45% in four of the six hydrolysates tested. Prediction of filterability of hydrolysates is not possible as shown in figure 4 and 5. Most of the hydrolysates are compatible with virus retentive filtration. One possibility based on the trials performed up to date is the purification and synthetic source of the hydrolysates that might be beneficial for filterability as those hydrolysates were the easiest to filter.

We recommend to perform growth promotion studies to ensure that the various hydrolysates have not been stripped of nutritional components thereby impact the cell cultures. Whereas we have not seen impact on the cell growth, when comparing to normal 0.1 µm filtration[14].

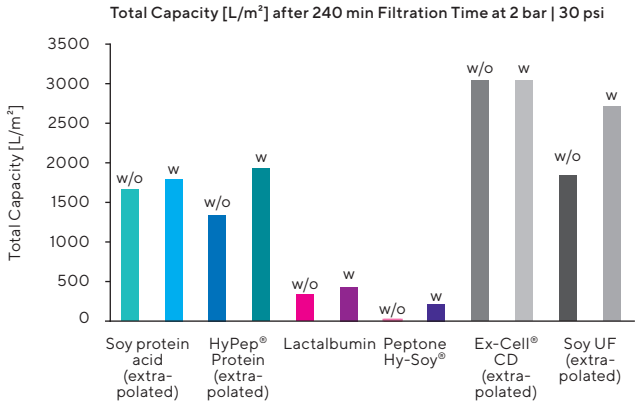

Figure 6: *Total Capacity After 240 Min Filtration Time at Constant Pressure of 2.0 bar | 30 psi in Duplicate Runs w/ And w/o Pre-filter*

Study 3: Impact of Fetal Bovine Serum

Fetal Bovine Serum was often used as a supplement in cell culture media as it contains lots of proteins, trace elements, hormones and growth factors supporting the cell growth[15]. Due to the animal origin FBS is replaced by chemically defined supplements. In this study, various concentrations of FBS in 1X PBS buffer were tested to examine the effects on Virosart® Media.

Material and Methods

FBS were reconstituted in PBS buffer. Before each run, the filters were flushed for 15 minutes with DI water at 2.0 bar | 30 psi using compressed air and the water flux was recorded. The DI water in the reservoir was exchanged to according media and the filtration at constant pressure of 2.0 bar | 30 psi was started at room temperature (20 – 22 °C | 68 – 71.5 °F). The filtration was stopped after 240 min or when the total volume was processed through the filter. The filtrate was collected and the weight recorded at specific time points in order to calculate the flow rate, flux and flux decay. The same experimental set-up of small-scale filtration was used as in the first study (figure 1).

Media	Supplier	FBS concentration	Order number	Filter tested
Fetal Bovine Serum in PBS buffer	Sigma-Aldrich	0.5%	F2442-100 mL	Virosart® Media
Fetal Bovine Serum in PBS buffer	Sigma-Aldrich	1.0%	F2442-100 mL	Virosart® Media

Table 4: *Concentration Used to Evaluate the Impact of FBS on Virosart® Media in PBS buffer*

Results and Discussion

Figure 6 shows the effects of presence of fetal bovine serum on filtration through the Virosart® Media filter. Not surprisingly, considering that the Virosart® Media filter is designed for filtration of chemically-defined media, even at concentrations as low as 0.5% FBS, minimal capacity was observed (~ 50 L/m²) at maximum flow decay (> 95%). Similar results would be expected with other complex protein-containing media. As a consequence we do not recommend Virosart® Media for serum containing media.

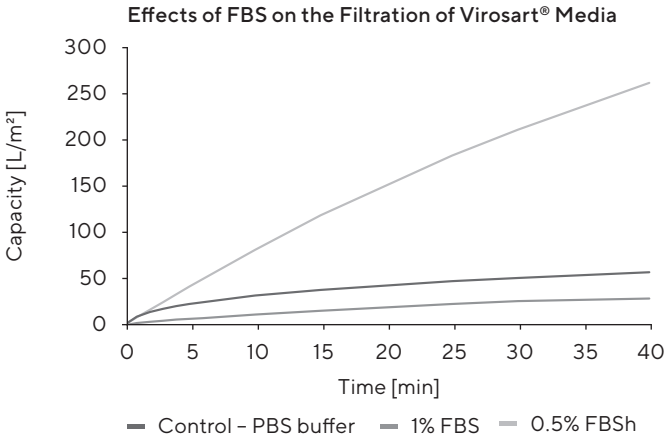

Figure 7: *Filtration of FBS Through the Virosart® Media Filter*

Summary and Conclusion

The results demonstrate that Virosart® Media is the filter of choice for upstream applications where high capacities combined with good process economics are required. Not surprisingly, presence of poloxamers and hydrolysates shown in the media adversely affected the performance of the Virosart® Media filter. Increasing the concentration of poloxamer reduces the initial flux rate.

A correlation between hydrolysate origin and total capacity cannot be concluded. The overall capacity is dependent on the presence or absence of specific constituents in the hydrolysate itself. The filter capacity of ultrafiltered and synthetic hydrolysates reached 3000 L/m².

The effects of fetal bovine serum on filtration through the Virosart® Media show minimal capacities as Virosart® Media is designed for filtration of chemically-defined media.

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Publication No.: SPK4118-e | Order No.: 85037-560-94 | Status: 06 | 08 | 2022