

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



Application
Note

#04

#05

#06

#07

#08

Roger Alsop, Carl Breuning, and Hazel Aranha, PhD, RAC Sartorius Stedim Biotech, Bohemia, NY

Abstract

Calcium is an essential supplement for CHO cells grown in serum-free media and is often supplied in the form of calcium chloride (CaCl₂). 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.

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. The Virosart® Media filter 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 (CaCl₂) and compare results of the new Virosart® Media filter 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 CaCl₂ 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 CaCl₂ 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

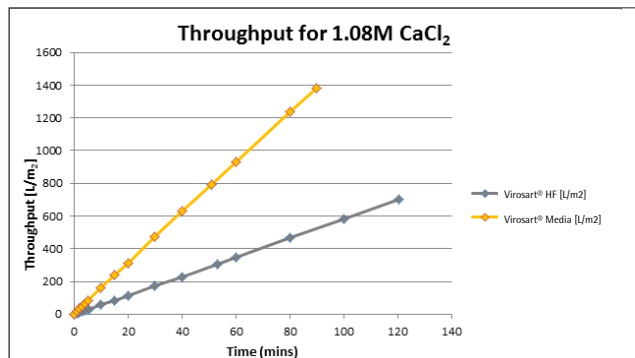


Figure 2: Flux for the Virosart® Media and the Virosart® HF filters during filtration of 1.08M CaCl₂ at 2 bar | 30 psi

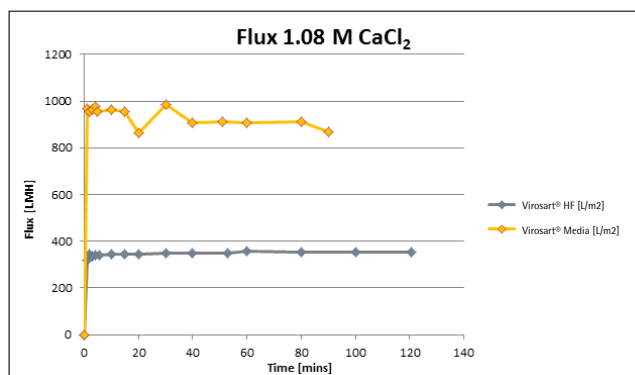


Figure 3: Relative flux decay profiles for the Virosart® Media and the Virosart® HF filters during filtration of 1.08M CaCl₂

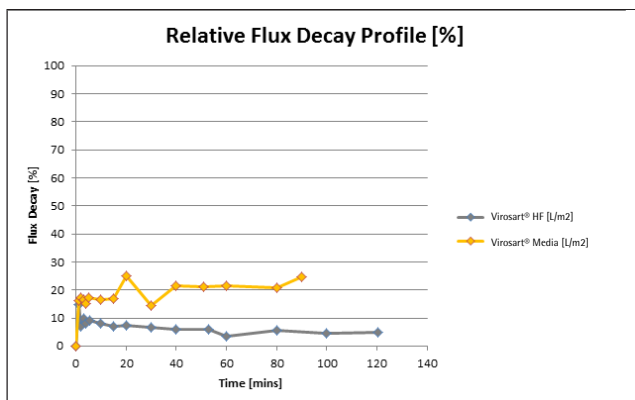


Figure 3 shows that the flux decay for both filters, Virosart® Media and Virosart® HF, rapidly plateaus and then remains relatively constant throughout the run. For the remaining part of the filtration step, both filters show only minor flow decay up to a total load of 700 L/m² for Virosart® HF and 1,380 L/m² for Virosart® Media. The initial flow decay could partly be attributed to the increased viscosity of the CaCl₂ 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 filter is the best option for a 1.08 M CaCl₂ 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/m ²]	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. The Virosart® Media filter is effective in viral safety risk mitigation, with high capacities and higher flux rates compared with established downstream virus filters such as Virosart® HF. Data demonstrate that the Virosart® Media Filter 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>

Sales and Service Contacts

For further contacts, visit www.sartorius-stedim.com

Europe

Germany

Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen

Phone +49.551.308.0
Fax +49.551.308.3289

Sartorius Stedim Systems GmbH
Robert-Bosch-Strasse 5 – 7
34302 Guxhagen

Phone +49.5665.407.0
Fax +49.5665.407.2200

France

Sartorius Stedim FMT S.A.S.
ZI des Paluds
Avenue de Jouques – CS 91051
13781 Aubagne Cedex

Phone +33.442.845600
Fax +33.442.845619

Sartorius Stedim France SAS
ZI des Paluds
Avenue de Jouques – CS 71058
13781 Aubagne Cedex

Phone +33.442.845600
Fax +33.442.846545

Austria

Sartorius Stedim Austria GmbH
Modecenterstrasse 22
1030 Vienna

Phone +43.1.7965763.18
Fax +43.1.796576344

Belgium

Sartorius Stedim Belgium N.V.
Rue Colonel Bourg 105
1030 Bruxelles

Phone +32.2.756.06.80
Fax +32.2.756.06.81

Hungary

Sartorius Stedim Hungária Kft.
Kagyló u. 5
2092 Budakeszi

Phone +36.23.457.227
Fax +36.23.457.147

Italy

Sartorius Stedim Italy S.p.A.
Via dell'Antella, 76/A
50012 Antella-Bagno a Ripoli (FI)

Phone +39.055.63.40.41
Fax +39.055.63.40.526

Netherlands

Sartorius Stedim Netherlands B.V.

Phone +31.30.60.25.080
Fax +31.30.60.25.099

filtratie.nederland@sartorius-stedim.com

Poland

Sartorius Stedim Poland Sp. z o.o.
ul. Wrzesinska 70
62-025 Kostrzyn

Phone +48.61.647.38.40
Fax +48.61.879.25.04

Russian Federation

LLC "Sartorius Stedim RUS"
Uralskaya str. 4, Lit. B
199155 St. Petersburg

Phone +7.812.327.53.27
Fax +7.812.327.53.23

Spain

Sartorius Stedim Spain, S.A.U.
Avda. de la Industria, 32
Edificio PAYMA
28108 Alcobendas (Madrid)

Phone +34.902.110.935
Fax +34.91.358.96.23

Switzerland

Sartorius Stedim Switzerland AG
Ringstrasse 24 a
8317 Tagelswangen

Phone +41.52.354.36.36
Fax +41.52.354.36.46

U.K.

Sartorius Stedim UK Ltd.
Longmead Business Centre
Blenheim Road, Epsom
Surrey KT19 9 QQ

Phone +44.1372.737159
Fax +44.1372.726171

Ukraine

LLC "Biohit"
Post Box 440 "B"
01001 Kiev, Ukraine

Phone +380.44.411.4918
Fax +380.50.623.3162

Americas

USA

Sartorius Stedim North America Inc.
5 Orville Drive, Suite 200
Bohemia, NY 11716

Toll-Free +1.800.368.7178
Fax +1.631.254.4253

Argentina

Sartorius Argentina S.A.
Int. A. Ávalos 4251
B1605ECS Munro
Buenos Aires

Phone +54.11.4721.0505
Fax +54.11.4762.2333

Brazil

Sartorius do Brasil Ltda
Avenida Senador Vergueiro 2962
São Bernardo do Campo
CEP 09600-000 - SP- Brasil

Phone +55.11.4362.8900
Fax +55.11.4362.8901

Mexico

Sartorius de México S.A. de C.V.
Circuito Circunvalación Poniente
No. 149
Ciudad Satélite
53100, Estado de México
México

Phone +52.5555.62.1102
Fax +52.5555.62.2942

Peru

Sartorius Peru S.A.C.
Av. Emilio Cavenecia 264 San Isidro
15073 Lima, Perú

Phone +51.1.441 0158
Fax +51.1.422 6100

Asia | Pacific

Australia

Sartorius Stedim Australia Pty. Ltd.
Unit 5, 7-11 Rodeo Drive
Dandenong South Vic 3175

Phone +61.3.8762.1800
Fax +61.3.8762.1828

China

Sartorius Stedim Biotech (Beijing) Co. Ltd.
No. 33 Yu'an Road
Airport Industrial Park Zone B
Shunyi District, Beijing 101300

Phone +86.10.80426516
Fax +86.10.80426580

Sartorius Stedim (Shanghai)
Trading Co., Ltd.
3rd Floor, North Wing, Tower 1
No. 4560 Jinke Road
Zhangjiang Hi-Tech Park
Pudong District
Shanghai 201210, P.R. China

Phone +86.21.6878.2300
Fax +86.21.6878.2882

Sartorius Stedim Biotech (Beijing) Co. Ltd.
Guangzhou Representative Office
Unit K, Building 23
Huihua Commerce & Trade Building
No. 80 Xianlie Middle Road
Guangzhou 510070

Phone +86.20.37618687 | 37618651
Fax +86.20.37619051

India

Sartorius Stedim India Pvt. Ltd.
#69/2-69/3, NH 48, Jakkasandra
Nelamangala Tq
562 123 Bangalore, India

Phone +91.80.4350.5250
Fax +91.80.4350.5253

Japan

Sartorius Stedim Japan K.K.
4th Fl., Daiwa Shinagawa North Bldg.
8-11, Kita-Shinagawa 1-chome
Shinagawa-ku, Tokyo, 140-0001 Japan

Phone +81.3.4331.4300
Fax +81.3.4331.4301

Malaysia

Sartorius Stedim Malaysia Sdn. Bhd.
Lot L3-E-3B, Enterprise 4
Technology Park Malaysia
Bukit Jalil
57000 Kuala Lumpur, Malaysia

Phone +60.3.8996.0622
Fax +60.3.8996.0755

Singapore

Sartorius Stedim Singapore Pte. Ltd.
1 Science Park Road,
The Capricorn, #05-08A,
Singapore Science Park II
Singapore 117528

Phone +65.6872.3966
Fax +65.6778.2494

South Korea

Sartorius Korea Biotech Co., Ltd.
8th Floor, Solid Space B/D,
PanGyoYeok-Ro 220, BunDang-Gu
SeongNam-Si, GyeongGi-Do, 463-400

Phone +82.31.622.5700
Fax +82.31.622.5799



▶ www.sartorius-stedim.com