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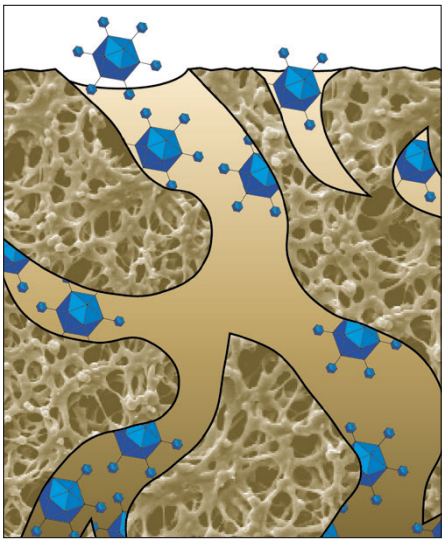
Virus Purification and Removal

Ion Exchange Chromatography with Sartobind® Membrane Adsorbers

Application Note



Introduction

Sartobind Membrane Adsorbers are chromatographic membranes utilized in purification of bioparticles and for impurity removal. The macroporous structure of the membrane allows even larger viruses to enter it and to bind to the inner pore surface easily. The Membrane Adsorber (MA) technology offers a number of benefits compared to conventional chromatography such as easy handling, high flow rates, minimal mass transfer effects, high capacities, low unspecific adsorption, less buffer consumption, and easy up-scaling. Five publications about virus purification and virus removal with Sartobind ion exchangers are summarized here.

Abbreviations

PrV	Pseudorabies virus
PrV-Ka	PrV strain Kaplan
PrV-gD⁻ Pass	PrV lacks glycoprotein gD
BHV-1	Bovine herpesvirus 1
AeDNV	Aedes aegypti densonucleosis virus
MuLV	Murine leukemia virus
SV-40	Simian virus-40
Reo-3	Reovirus type III
pfu	Plaque forming units
LRV	Log reduction values (log10)
MAb	Monoclonal antibodies
MVM	Minute virus of mice
CHO	Chinese hamster ovary cell
HCP	Host cell proteins

1. Purification of alphaherpesviruses with Sartobind S cation exchanger

A. Karger et al.[1] published the first report of an ion exchange purification of herpesviruses.

Test conditions

Virus	PrV and BHV-1
Sample	Infected cell culture supernatant
Adsorber	Sartobind S 100 cation exchanger
Purification steps	– Equilibration with 20 mM 2-morpholinoethane sulfonic acid, pH 6.2 (MES) – Application of diluted supernatant 1 : 2 in MES with a 50 ml-syringe – Washing with 25 mL MES – Elution with each 10 mL of 200, 400, 600 and 1000 mM KCl in MES – Sedimentation of particulate materials by centrifugation (25,000 rev./min, 1 h, 4°C) – Resuspension in 100 µL of TBSal (200 mM NaCl, 2.6 mM KCl, 10 mM Tris-HCl, pH 7.5, 20 mM $MgCl_2$, 1.8 mM $CaCl_2$)
Flow rate	10 mL/min

Results

Most of infectious virus is found after elution with 400 mM KCl in MES.
Over 85 % of the virus was eluted within a single fraction.

Virus yields after cation exchange purification

	PrV-Ka	PrV-gD⁻ Pass	BHV-1
pfu applied (100 %)	$1.4 \times 10^{10} \pm 8 \times 10^{9}$	8.1×10^{6}	1.5×10^{9}
pfu % eluted in 400 mM KCl in MES	85.6 ± 10.7 (mean ± SD of 3 tests)	99	93

Remarks

Cation exchange purification has several advantages over sucrose density gradient centrifugation:
1. Virus yields and purity are high.
2. Specific infectivity is ca. 10-fold higher compared to gradient purified virions.
3. The procedure is rapid and can be completed within 3 h.

2. Densonucleosis virus purification by ion exchange membranes

Specht et al.[2] purified AeDNV particles with Sartobind MA 75 (bed height 4 mm, bed volume 2.1 ml) anion and cation exchange units. Both exchangers may be used to adsorb the viral particles depending on feed pH. The results indicate that adsorptive membranes may be ideally suited for virus capture as no size exclusion effects can be detected.

Results of viral binding capacity

Unit type	pH	Virus charge	Membrane charge	Capacity Protein (mg)	Capacity Virus in water	Capacity Virus in medium
Q 75	7.0	negative	positive	60 BSA	$> 2.79 \times 10^{10}$	$> 1.36 \times 10^{10}$
D 75	7.0	negative	positive	45 BSA	$> 1.54 \times 10^{9}$	$> 1.36 \times 10^{10}$
S 75	3.5	positive	negative	60 Lysozyme	$> 3.91 \times 10^{8}$	0

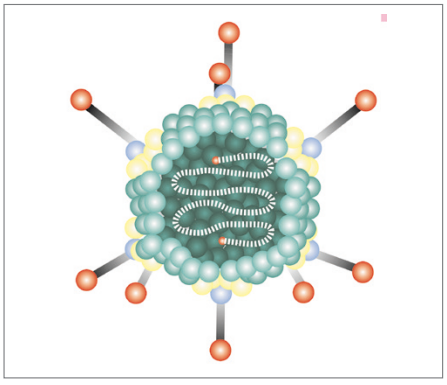
3. Viral removal validation in CAMPATH®-1H manufacturing process

P. Galliher and E. Fowler[3] (Millennium Pharmaceuticals Inc.) presented the results of the validation of virus removal for Campath-1H with Sartobind Q anion exchanger. The Q adsorber was used to bind negatively charged contaminants such as DNA, host cell proteins, endotoxins and viruses in flow through mode. Campath was approved by FDA in March 2001.

Campath-1H is a monoclonal antibody that recognizes human CD52 glycoprotein on T and B lymphocytes and induces lysis by antibody dependent cellular cytotoxicity used for treatment of chronic lymphocytic leukemia.

Viral validation condition

Sample	Campath-1H
Purification steps	– Protein A – Cation exchange – Sartobind Q – nanofiltration – Tween 80
Load volume on Sarobind	43.3 L/m² membrane area
Flow rate	2.6 L/min/m²



Production condition

Sample	Campath-1H
Sample concentration	~ 80 mg/L secreted into broth
Batch volume	12,000 L
Load volume on Sartobind	42.0 L/m² membrane area
Flow rate of Sartobind	2.6 L/min/m²
Purification steps	– Cell removal by micro- and nanofiltration – Concentration by ultra- and diafiltration – Product capture by Protein A – Purification by S-Sepharose – DNA removal by Sartobind Q – Viral removal by nanofiltration – Concentration by ultra- and diafiltration – Final purification by size exclusion chromatography – Formulation by dilution and addition of Tween 80 – Sterile filtration into bulk containers

Feature of viruses

Virus	Genome	Enveloped	Size (nm)	Resistance
SV-40	ds-DNA	no	45	very high
Reo-3	ds-RNA	no	75–80	high
MuLV	ss-RNA	yes	80–110	low
PrV	ds-DNA	yes	150–250	medium

Results of viral clearance

Virus	LRV Run 1	Run 2	Total LRV over all purification steps
SV-40	1.25 ± 0.46	1.34 ± 0.43	≥ 10.7–11.1
Reo-3	4.07 ± 0.50	3.62 ± 0.42	≥ 10.0–10.4
MuLV	3.80 ± 0.39	4.40 ± 0.56	≥ 14.7–15.5
PrV	3.97 ± 0.44	3.88 ± 0.38	≥ 21.2–21.3

Summary

The porous structure of the membrane allows viruses of various sizes (30–200 nm) to be purified. Therefore, application of Sartobind Membrane Adsorbers is advantageous especially in purification and removal of viruses for biopharmaceutical processes. The linear scale-up is supported by various dimensions of Sartobind devices.

4. Viral removal study for human antibody after the capturing step

R. Zhang et al.[4] (Abgenix Inc., CA, USA) presented the results of the virus removal study for human MAb with Sartobind Q anion exchanger. These studies were designed and performed to measure removal of CHO proteins, DNA, leached protein A and model viruses by Sartobind Q Membrane Adsorbers.

Methods

The human MAb production process with a 2000 L harvested cell culture fluid volume was expected to yield approx. 90 L of antibody material at the end of the affinity chromatography step. Based on the capacity of Sartobind Q membrane obtained from HCP breakthrough studies, two Sartobind Q SingleSep 10" Capsules were be used for the purification of this affinity chromatography pool. A scale down model was designed for the virus removal study (**Tab. Process scale-down**).

500 ml starting sample were spiked with model viruses. Spiked samples represented conditions similar to real process conditions.
A duplicate run was performed with each 50 ml of real process material (**Tab. The scope of the viral clearance study**)

Process scale-down

	Clinical production scale	Scale down to bench scale	Scale down ratio
Sartobind Q used	2 × SingleSep 10"	MA 75	n/a
Total volume of Sartobind Q (ml)	360	2	180
MAb loading volume (L)	90	0.5	180
Load MAb (L/ml membrane)	0.25	0.25	n/a

The scope of the viral clearance study

Virus	Genome	Enveloped	Size	Load sample pH 7, < 3 mS/cm 500 ml	50 ml
MVM	ss-DNA	no	18–26	Protein	Protein
Reo-3	ds-RNA	no	60–80	Protein	Protein
MuLV	ss-RNA	yes	80–120	10 mM Tris buffer	Protein
PrV	ds-DNA	yes	120–200	10 mM Tris buffer	Protein

Results of viral clearance

Virus	LRV of virus spiked sample 500 ml Protein/buffer	50 ml protein
MVM	4.41 ± 0.37	≥ 6.77 ± 0.24
Reo-3	≥ 7.53 ± 0.29	≥ 7.28 ± 0.30
MuLV	6.29 ± 0.32	≥ 5.57 ± 0.25
PrV	≥ 5.76 ± 0.23	≥ 5.67 ± 0.17

Performance comparisons between Q Sepharose® FF column and Sartobind MA 75

	Level in the antibody solution loaded	QSFF column	Sartobind Q
Device		1.6 × 20 cm	MA 75*
Device volume (ml)		40.2	2
Flow rate (ml/min)		13.4	20
Loading velocity (cm/h)		400	240
MAb load (g)		3.6	3.5
Capacity (mg/ml)		90	1750
HCP (ppm)	368.5	12.9	35.3
DNA (ppm)	0.058	< 0.007	< 0.007
Leached protein A (ppm)	3.26	1.54	2.82
Yield (%)		95	92

Buffer: 10 mM Tris buffer, pH 7, 2.5 mS/cm
* 5 cm^2 surface area × 0.4 cm bed height

Summary

Results from these studies confirmed that process sized MA removed the aforementioned contaminants. In addition to good contaminant removal and viral clearance data, MA proved to be an economical method for processing human MAb. Data from this study were used to support a technology shift from column to MA chromatography.

5. Viral removal study for MAb after intermediate purification

J. Zhou and T. Tressel[5] (Amgen Inc., CA, USA) were successful with the virus clearance of MAb. The material after second column was the feed stock for the polishing step with Sartobind Q. They were able to test four viruses (MVM, Reo-3, MuLV and PRV) in duplicate at their proposed loading conditions (3.0 kg MAb/m^2 at 450 cm/h). In addition to this, they were able to test MuLV at 3.6 kg MAb/m^2 at 600 cm/h.

Process conditions

Parameter	Run 1 and 2	Run 3
pH and conductivity	pH 7.2, 4 mS/cm (all runs)	
MAb concentration	4.3 mg/ml (all runs)	
Load MAb (per membrane area)	3.0 kg/m^2	3.6 kg/m^2
Load MAb (per membrane volume)	10.9 kg/L	13.1 kg/L
Flow rate	450 cm/h	600 cm/h

Results of viral clearance

Virus	LRV Run 1	Run 2	Run 3	Viral Recovery (%)
MVM	6.03 ± 0.21	6.03 ± 0.20	–	100
Reo-3	7.00 ± 0.31	6.94 ± 0.24	–	100
MuLV	≥ 5.35 ± 0.23	≥ 5.52 ± 0.27	≥ 5.59 ± 0.31	> 70
PrV	≥ 5.58 ± 0.28	≥ 5.58 ± 0.22	–	100

Summary

A team from Amgen Inc. and Sartorius Stedim Biotech GmbH redesigned a Q membrane scale-down model. The Sartobind Q mini with 7 ml (250 cm^2) was reduced by 50 % of membrane volume to 3.5 ml (125 cm^2). This new scale-down model mimics the liquid flow path found in the large-scale modules. The newly designed 125 cm^2 capsule achieved performance parameters comparable to the larger Sartobind Q units. With the new scale-down model, the Amgen Inc. team was able to obtain excellent viral clearance at a process capacity of >3600 g/m^2 (total membrane surface) or >13.2 kg/L (membrane volume) and a maximal flow rate of 600 cm/h with an operational backpressure of less than 0.12 MPa (1.2 bar, 18 psi).

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