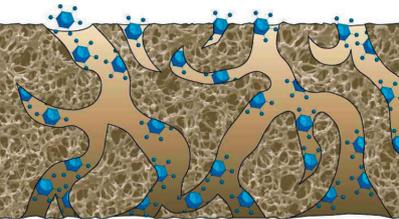


# Virus Purification with Membrane Chromatography

Miyako Hirai, Stefan Fischer-Fruehholz, Laura Chirica, Suma Ray  
Sartorius Stedim Biotech GmbH, D-37079 Goettingen  
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## Introduction

Sartobind Membrane Adsorbers are chromatographic membranes utilized in purification of viruses and virus like particles and for impurity removal from therapeutic proteins. The macroporous structure of the membrane allows large viruses to enter it and to bind to the inner pore surface easily. The Membrane Adsorbers technology offers a number of benefits compared to conventional column chromatography such as



- easy handling
- high flow rates
- minimal mass transfer effects
- high capacities
- low unspecific adsorption
- less hardware investments
- less buffer consumptions
- easy scale up

The first application of ion exchange membranes for the purification of viruses<sup>1</sup> has been made possible by avoiding size exclusion effects due to large pore sizes. The pores of Sartobind<sup>®</sup> membranes with >3 µm are two orders of magnitude above conventional chromatographic bead materials. Compared to columns such inherent membrane feature allows for:

- Higher binding capacities for virus particles in the range of 10<sup>13</sup>
- Higher throughput at >5–30 bed volume | minute flow rate to achieve shorter process times and higher yield
- Disposable | batch reuse formats for higher flexibility in production as membranes are fitted into ready-to-use capsules

Nowadays the applications for chromatographic ion exchange membranes (Sartobind Q and S) as well as HIC membranes (Sartobind Phenyl) can be subdivided into two operational modes.

## 1. Capture of viruses and virus like particles

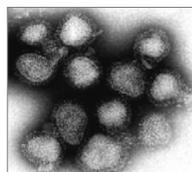


- Influenza virus<sup>2,3</sup>
- Adenovirus<sup>4,5,6</sup>
- Lentivirus<sup>7</sup>
- Baculovirus<sup>8</sup>
- Densonucleosis virus<sup>9</sup>
- Pseudorabies virus<sup>1,10</sup>
- Bovine herpesvirus<sup>1</sup>
- Foot and Mouse disease virus<sup>11</sup>
- Rotavirus like particles<sup>12</sup>
- Bacteriophages<sup>13</sup>

Binding of the viruses on ion exchange membranes depends on charge distribution on the virus and can be purified either on cation or anion exchange.

### 1.1 Influenza A virus<sup>2</sup>

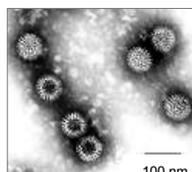
- Human and equine influenza A virus in cell culture supernatant (serum-free and serum-containing cultivation) was directly adsorbed to Sartobind Q and D 75 anion-exchangers.
- Elution of adsorbed virus from Sartobind Q by displacement with sodium chloride (up to 1.5 M, pH 7.0) resulted in average yields of 86% (based on HA activity).
- Due to their high productivity, ease of operation and acceptable yields Sartobind Q anion-exchangers can be considered promising candidates for the large-scale purification of cell culture derived influenza virus.



Influenza virus

### 1.2 Rota virus like particles<sup>12</sup>

- Sartobind D membrane adsorber from Sartorius was used and thoroughly studied for the adsorption of VLPs.
- Anion-exchange membrane chromatography was finally used in a larger scale downstream process, confirming that rotavirus VLPs can be reproductively purified to (overall outcome):
  - clinical grade at 46% global recovery yield
  - nearly 100% removal of host bulk DNA and
  - approximately 98% of host cell proteins are removed.



Rotavirus

## 2. Removal of contaminants in flow through operation



- Host cell proteins
- DNA
- Endotoxins

Membrane Adsorbers have initially been used for polishing of therapeutic proteins.

### 2.1 HCP<sup>14</sup>

- In general, the charged membrane is used as a polishing step to remove impurities such as Host Cell Proteins (HCP), DNA, endotoxin and even product-related impurities such as aggregates.
- It was found that a charged-membrane was shown to be more effective than Q-ligand resin in terms of yield and HCP clearance. The yield and HCP clearance by a charged membrane was 97% and 21-fold respectively compared with 93% and 5-fold for the resin.

### 2.2 DNA<sup>15</sup>

- The DNA removal capability by Q resins and membranes is extremely high.
- The loading capacity for Q resins is limited by flow rate and process time.
- Sartobind Q membrane has a significantly better loading capacity profile (g/ml matrix) and shorter processing time than resin.

### 2.3 Endotoxins<sup>16</sup>

- Two 100 l GMP batches were performed with Sartobind Q SingleSep 20" capsules. Endotoxins were removed from GST-protease from 26.900 EU/mg to 0.13 EU/mg.
- Membrane chromatography technology offers significant advantages with regards to product recovery, time saving and equipment requirements during manufacturing.

## Summary

Membrane Adsorbers can be successfully used in bind and elute applications, especially for large bio-particles as viruses. Flow through polishing applications for protein manufacture are already established and suggest the use of Membrane Adsorbers for removal of impurities in vaccine manufacturing.

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