SARTORIUS

Simplifying Progress

# Influenza Virus Capture Using Membrane Chromatography: Improving Selectivity by Matrix Design and Pseudo-Affinity Ligand Interactions

**David-Raphael Dauer[1]*, Florian Taft[1]**

[1]Sartorius Stedim Biotech GmbH, August-Spindler-Straße 11, 37079 Goettingen, Germany
*Corresponding author: david.dauer@sartorius.com

## 1. Abstract

Membrane chromatography is consistently used in the purification of viral particles like adenoviruses or influenza viruses. The lack of traditional diffusion-based limitations of porous particles and increased binding capacities in a disposable format make it a viable alternative to bead chromatography. Further, disposable, ready-to-use gamma-irradiated devices allow single-use chromatography unit operations and enable closed, aseptic processing. This poster presents a novel cellulose membrane based stationary phase whose specific surface area is designed for maximum virus accessibility.
The membrane also utilizes highly selective pseudo-affinity ligands for influenza viruses resulting in an overall increase in selectivity and product recovery. The unique capabilities of this media not only contribute to reduction of the costs associated with the bind & elute purification of viruses but may also constitute one step forward in the development of an efficient and robust purification platform process for the vaccine industry.

## 2. Mass Transfer in Membrane Adsorbers

Resins are diffusion limited materials, which have a high binding capacity for small molecules and when low flow rates are applied. In contrast, membranes are convective materials, which have a relatively constant binding capacity for a wide variety of molecules of different sizes and over a wide range of flow rates. Up to 20x higher flow rates can be applied to membranes compared to resins. Therefore, membranes are beneficial for capture of large molecules such as virus and for flow through polishing applications. To achieve higher flow rates with packed bed columns (resins), the diameter of the column needs to be increased which results in oversizing of the column and the required capacity.

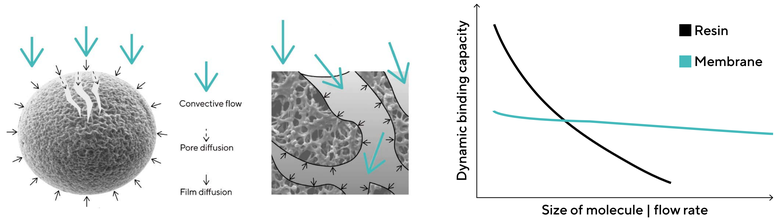


**Figure 1:** *In Resins Mass Transfer is Dominated by Diffusion (Left), whereas Convective Flow Predominates in Membrane Adsorbers (Middle). Schematic Illustration Highlighting the Dependency of Dynamic Binding Capacity on the Size of the Molecule and the Flow Rate for Resin and Membrane Chromatography (Right).*

## 3. Design of the Membrane Adsorber Stationary Phase

Rationale of optimization:

I. Remove the 3D-hydrogel coating used in membrane adsorbers for polishing applications
II. Reduce | optimize the distribution and size of the pores of the precursor membrane
III. Couple the ligand directly to the precursor membrane

hydrogel subject to swelling, reduced ligand accessibility for large viruses (in black) and high capacity for smaller contaminants (in yellow)

increased specific surface area, higher capacity and selectivity for large viruses

**Figure 2:** *Schematic Representation of the Stationary Phase Design.
Left: Conventional Membrane Adsorber With 3D-Hydrogel (e.g. Sartobind® S).
Right: Membrane Adsorber Specifically Designed for Virus Capture.*

The pore size of the membrane was optimized for the best trade-off between surface area as a measure of binding capacity and permeability, which is used as a measure of pore size. Figure 3 shows prototype membranes with different pore sizes in comparison to Sartobind® S. It was found that 0.8 µm pore size provides the best trade-off between surface area and permeability for virus capture.

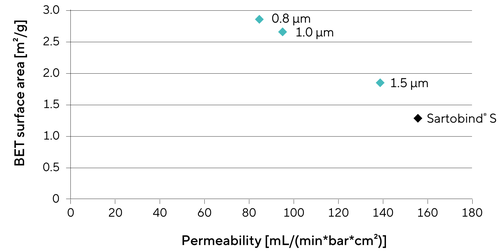


**Figure 3:** *Tailoring the Permeability and the Specific Surface Area by Pore Size Optimization. Optimization of Cellulose Precursor Membrane for Virus Purification.*

## 4. Adding Affinity Ligands

Sulfation of the cellulose based stationary phases generated sulfated cellulose membrane adsorbers (Sartobind® Convec SC) which exhibit pseudo-affinity interactions with influenza viruses[1]. During development the prototype testing was performed with model systems:

- Ammonium-functionalized latex beads (100 nm) were used as virus surrogates
- Lysozyme was used as model contaminant

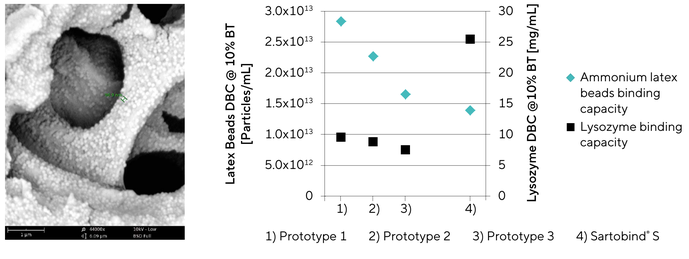


**Figure 4:** *Prototype Testing Using Model Systems.
Left: SEM Image of Ammonium-Functionalized Latex Beads Bound to the Surface of a Sartobind® Convec SC Prototype.
Right: Selectivity Plot of Sartobind® Convec SC Prototypes in Comparison to Commercial CEX Membranes (Sartobind® S). The Gain in Selectivity Is Demonstrated by the Increase in Binding Capacity for Large Particles and the Reduced Binding Capacity for Small Model Contaminants.*

## 5. Binding Capacity and Recovery of Influenza Virus

Evaluation of the new developed Sartobind® Convec SC was performed with three different influenza strains in comparison to commercially available sulfated cellulose resins. Sartobind® Convec SC showed 8 to 22x higher binding capacity compared to the resins. Both resins showed an immediate breakthrough when loaded with influenza A | Switzerland virus feed[2].

| **Dyn. Binding Capacity** | Influenza A \| Puerto Rico \| 8 \| 1934 (H1N1) | | Influenza A \| Switzerland \| 9715293 \| 2013 (H3N2) | | Influenza B \| Phuket \| 3073 \| 2013 | |
|---|---|---|---|---|---|---|
| | **HAU/mL** | **Sartobind® vs resin** | **HAU/mL** | **Sartobind® vs resin** | **HAU/mL** | **Sartobind® vs resin** |
| Sartobind® Convec SC | $2.47 \times 10^6$ | | $1.64 \times 10^6$ | | $1.11 \times 10^6$ | |
| Resin A | $3.31 \times 10^5$ | 7.5x | immediate breakthrough | | $5.26 \times 10^4$ | 22x |
| Resin B | $2.88 \times 10$ | 8.6x | | | $4.79 \times 10^4$ | 23x |

**Table 1:** *Results of Dynamic Binding Capacity (@ 10% Breakthrough) Studies.*

**Chromatography conditions**
Feed: 9-14 kHAU/mL, adjusted to 4 mS/cm
Flow rate: Resin A: 0.17 CV/min, Resin B: 0.25 CV/min;
Sartobind® Convec SC: 3.75 MV/min
Equilibration: 10 mM TRIS, 50 mM NaCl (pH 7.4)

Load: Feed
Wash: 10 mM TRIS, 50 mM NaCl (pH 7.4)
Elution: 10 mM TRIS, 2 M NaCl (pH 7.4)
Regeneration: Resin A: 0.15 M NaOH, 2 M NaCl, Resin B: 1 M NaOH, 2 M NaCl, Sartobind® Convec SC: 1 M NaOH, 2 M NaCl

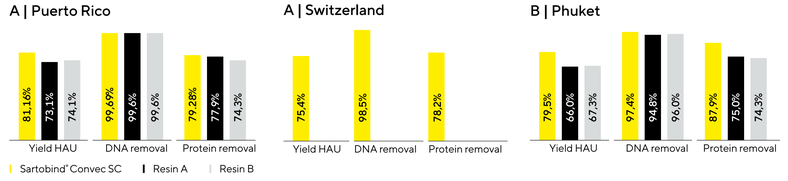


**Figure 5:** *Results of Recovery Studies.*

Three matrices (Sartobind® Convec SC membrane and two resins) were tested for binding capacity, recovery and contaminant removal with three different influenca strains. Results showed clearly that Sartobind® Convec SC exhibits a higher DBC10% and was able to capture all three influenca strains. Virus recovery in the product fraction (> 66%) and contaminant removal (> 74% and > 96% for total protein and DNA, respectively) were comparable[2].

**Chromatography conditions**
Feed: 12-14 kHAU/mL, adjusted to 4 mS/cm
Flow rate: Resin A: 0.17 CV/min, Resin B: 0.25 CV/min,
Sartobind® Convec SC: 3.75 MV/min
Equilibration: 10 mM TRIS, 50 mM NaCl (pH 7.4, 4 mS/cm)

Load: Feed until 70% of DBC
Wash: 10 mM TRIS, 50 mM NaCl (pH 7.4, 4 mS/cm)
Elution: 10 mM TRIS, 650-850 mM NaCl (pH 7.4)
Regeneration: Resin A: 0.15 M NaOH, 2 M NaCl, Resin B: 1 M NaOH, 2 M NaCl, Sartobind® Convec SC: 1 M NaOH, 2 M NaCl

## 6. Summary

Sulfated cellulose membrane adsorbers (Sartobind® Convec SC) based on the newly developed stationary phase exhibit a significant higher binding capacity and lower strain dependency for various influenza viruses than commercially available sulfated cellulose resins while offering comparable recovery and purity. These data demonstrate the suitability of the developed membrane for the production of seasonal and pandemic cell culture based influenza vaccines.
The obtained results also suggest that the novel stationary phase could be used for other types of affinity ligands and has the potential to enable the development of next generation highly productive and robust single-use processes by using gamma-irradiated devices for closed, aseptic processing for viral based therapeutics.

**References**

1. Opitz, L., Lehmann, S., Reichl, U., Wolff, M.: Sulfated membrane adsorbers for economic pseudo-affinity capture of influenza virus particles. Biotechnol Bioeng 2009 103(6), 1144-1154.
2. Fortuna, A. R., van Teeffelen, S., Ley, A., Fischer, L., Taft, F., Genzel, Y., Villain, L., Wolff, M., Reichl, U.: Use of sulfated cellulose membrane adsorbers for chromatographic purification of cell cultured-derived influenza A and B viruses. Separation and Purification Technology 2019 226, 350-358.