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Viral Vector Capture Using Membrane Chromatography: Boosting Particle Recovery and Infective Yield Through Matrix Design and Optimized Binding Strength

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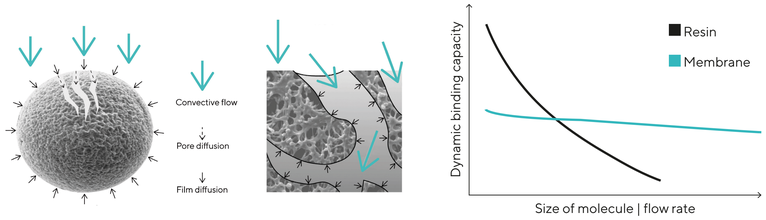
1. Abstract

Membrane chromatography is consistently used in the purification of viral particles such as adenoviruses, influenza viruses or lentiviruses. 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 anion exchange ligands for lentiviruses (Convec D) as well as highly selective pseudo-affinity ligands for influenza viruses (Convec SC) resulting in an overall increase in selectivity and product recovery. The unique capabilities of these media not only contribute to reduction of the costs associated with the bind & elute purification of viruses, but may also constitute a step forward in the development of efficient and robust purification platform processes for the viral vector industries.

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 Sartobind® Convec 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)

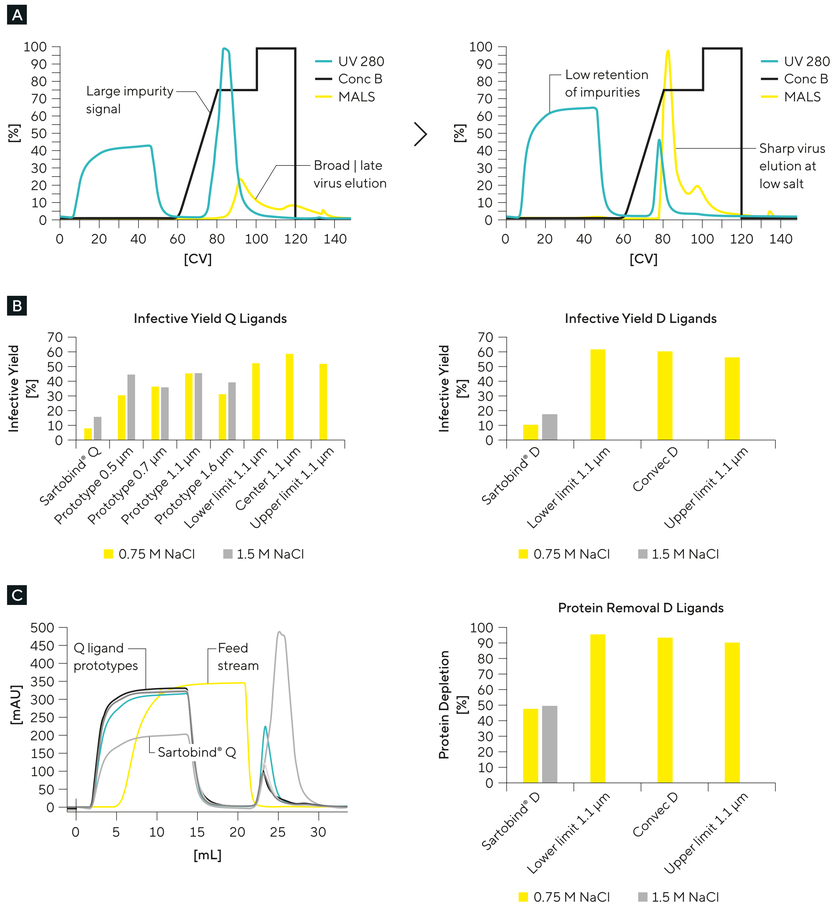
Increased specific surface area, higher capacity and selectivity for large viruses

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.*

4. Sartobind® Convec D for Lentivirus Capture

Lentiviruses are viral vectors with great potential for gene therapy applications. The high sensitivity of this class of bio-pharmaceuticals to a variety of process conditions places high demands on possible purification methods. Sartobind® Q is a widely used anion exchange membrane that is often used for the chromatographic purification of lentiviruses. Since the Sartobind® Q membrane has been optimized for binding with subsequent elution of proteins, it has a high ligand density. When purifying lentiviruses by chromatography, this leads to very strong binding and sometimes irreversible immobilization of the virus on the membrane surface[1]. In addition, high salt concentrations are required for elution, which are detrimental to viral infectivity. Optimization of the pore size, improvement of the structural homogeneity and adjustment of the ligand density resulted in significantly higher infectious yields with greatly improved protein depletion[2].

Figure 3: *Chromatographic Performance of Sartobind® Convec D in Lentivirus Purification*



Note. A) Chromatographic bind and elution purification of a lentivirus feed stream with Sartobind® Q (left) and Sartobind® Convec D (right) in a gradient of 0 – 1 M NaCl in 20 mM TRIS-HCl pH 7.2. B) Optimization of pore size and ligand chemistry onSartobind® Convec prototypes. C) Selectivity of the Sartobind® Convec prototypes towards proteinaceous contaminants.

5. Purifying Influenza Viruses Using Convec SC

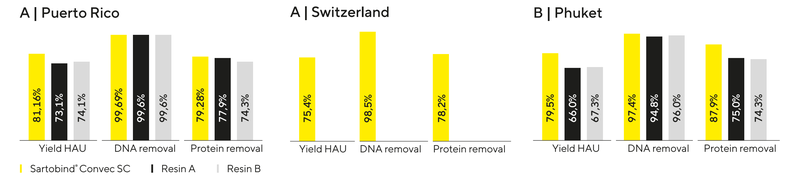
Evaluation of the new developed Sartobind® Convec SC[3] was performed with three different influenza strains in comparison to commercially available sulfated cellulose resins. Sartobind® Convec SC showed 8 to 22x higher binding capacity compared to the resins. Both resins showed an immediate breakthrough when loaded with influenza A | Switzerland virus feed[4].

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

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	2.47×10^6		1.64×10^6		1.11×10^6	
Resin A	3.31×10^5	7.5×	immediate breakthrough		5.26×10^4	22×
Resin B	2.88×10^5	8.6×			4.79×10^4	23×

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 4: *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[4].

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

The Sartobind® Convec platform addresses the optimization of the critical process parameters in the chromatographic purification of a wide range of virus-based biotherapeutics. While particularly mild process conditions with high selectivity have been achieved for lentiviruses , processes for the isolation of influenza viruses benefit from particularly high binding capacities. In all cases, the isolated physical or infectious yields and contaminant removal were equivalent to or significantly improved compared to currently available stationary phases. The obtained results also suggest that the novel stationary phase could be used for other types of 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

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