Influenza virus capture using membrane chromatography: Improving selectivity by matrix design and pseudo-affinity ligand interactions

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1. Abstract

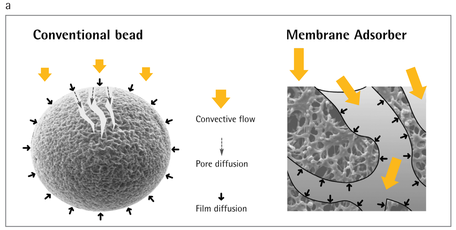
Membrane chromatography is consistently used in the purification of viral particles like adenoviruses or influenza viruses. The lack of traditional diffusion-based limitations of porous particles and increased binding capacities in a disposable format make it a viable alternative to bead chromatography.
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

In membrane adsorbers mass transfer is mainly driven by convection. As a consequence, membrane adsorbers are not affected by the diffusion limitations observed with porous bead based chromatography media for the purification of large molecules (e.g. viruses).

a



b

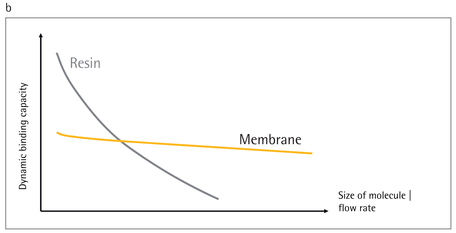
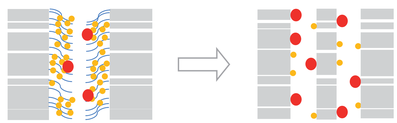


Figure 1a, b: In membrane adsorbers mass transfer is dominated by convective flow.
Schematic illustration highlighting the dependency of dynamic binding capacity on the size of the molecule and the flow rate for resin and membrane chromatography.

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 red) 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

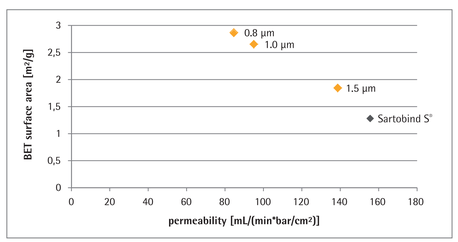
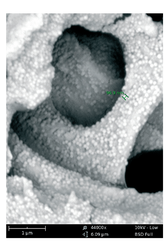


Figure 3: Tailoring the permeability and the specific surface area by pore size optimization.
Optimization of cellulose precursor membrane for virus purification.

4. Adding affinity ligands

Sulfation of the cellulose based stationary phases generated sulfated cellulose membrane adsorbers (SCMA) 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



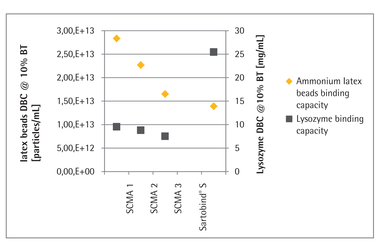


Figure 4: Prototype testing using model systems.
Left: SEM image of ammonium-functionalized latex beads bound to the surface of a SCMA prototype.
Right: Selectivity plot of SCMA prototypes in comparison to commercial CEX membranes (Sartobind® S). The gain in selectivity is demonstrated by the increase in binding capacity for large particles and the reduced binding capacity for small model contaminants.

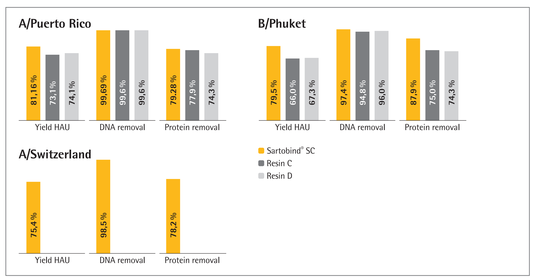
5. Binding capacity and recovery of Influenza virus

Evaluation of the new developed Sartobind® SC was performed with three different Influenza strains in comparison to commercially available sulfated cellulose resins.

dyn. binding capacity	Influenza A | Puerto Rico/8/1934 (H1N1)		Influenza A | Switzerland/9715293/2013 (H3N2)		Influenza B | Phuket/3073/2013	
	HAU/ml	Sartobind® SC vs resin	HAU/ml	Sartobind® SC vs resin	HAU/ml	Sartobind® SC vs resin
Sartobind® SC	2.47×10^6		1.64×10^6		1.11×10^6	
Resin C	3.31×10^5	**7.5x**	immediate break-through		5.26×10^4	**22x**
Resin D	2.88×10^5	**8.6x**			4.79×10^4	**23x**

Chromatography conditions
Feed: 9-14kHAU/mL, adjusted to 4mS/cm
Flow rate:
Resin C: 0.17 CV/min
Resin D: 0.25 CV/min
Sartobind SC: 3.75 MV/min
Equilibration: 10mM TRIS, 50mM NaCl (pH7.4)
Load: Feed
Wash: 10mM TRIS, 50mM NaCl (pH7.4)
Elution: 10mM TRIS, 2M NaCl (pH7.4)
Regeneration:
Resin C: 0.15M NaOH, 2M NaCl
Resin D: 1M NaOH, 2M NaCl
Sartobind SC: 1M NaOH, 2M NaCl

Table 1: Results of dynamic binding capacity (@ 10% breakthrough) studies.



Chromatography conditions
Feed: 12-14kHAU/mL, adjusted to 4mS/cm
Flow rate:
Resin C: 0.17 CV/min
Resin D: 0.25 CV/min
Sartobind SC: 3.75 MV/min
Equilibration:
10mM TRIS, 50mM NaCl (pH7.4, 4mS/cm)
Load: Feed until 70% of DBC
Wash: 10mM TRIS, 50mM NaCl (pH7.4, 4mS/cm)
Elution: 10mM TRIS, 650-850mM NaCl (pH7.4)
Regeneration:
Resin C: 0.15M NaOH, 2M NaCl
Resin D: 1M NaOH, 2M NaCl
Sartobind SC: 1M NaOH, 2M NaCl

Figure 5: Results of recovery studies.

6. Summary

Sulfated cellulose membrane adsorbers (Sartobind® 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 purification processes for viral based therapeutics.

7. References

(1) Opitz L.: Sulfated membrane adsorbers for economic pseudo-affinity capture of influenza virus particles. Biotechnol Bioeng 2009 103(6), 1144-1154.

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