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[a] Sartorius Stedim North America Inc., [b] Sartorius Stedim Biotech GmbH, Goettingen, Germany, [c] Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany.
Vaccine Technology VII, Engineering Conferences International, Fairmont Tremblant, Mont Tremblant, Quebec, Canada, June 17 – 22, 2018

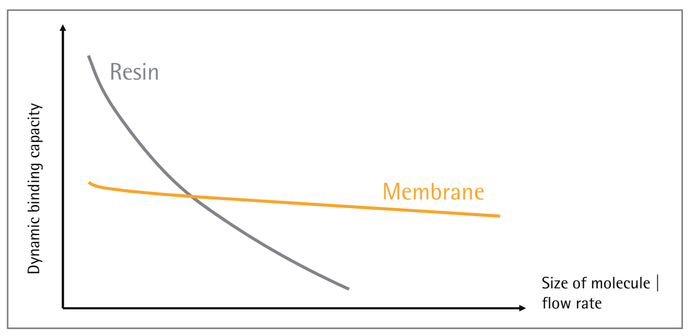
1. Abstract

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 based stationary membrane 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 optimized and efficacious purification platform process for the vaccine industry.

2. Mass transfer in membrane adsorbers

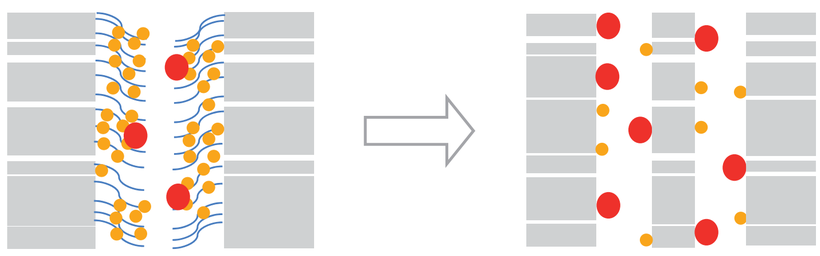
Figure 1: Schematic illustration highlighting the dependence of dynamic binding capacity on the size of the molecule and the flow rate for gel and membrane chromatography. In membrane adsorbers mass transfer is dominated by convective flow.



3. Design of the membrane adsorber stationary phase

Rationale of optimization:

I. remove the hydrogel present 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, limited surface area

increased specific surface area, higher capacity, selectivity & recovery

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

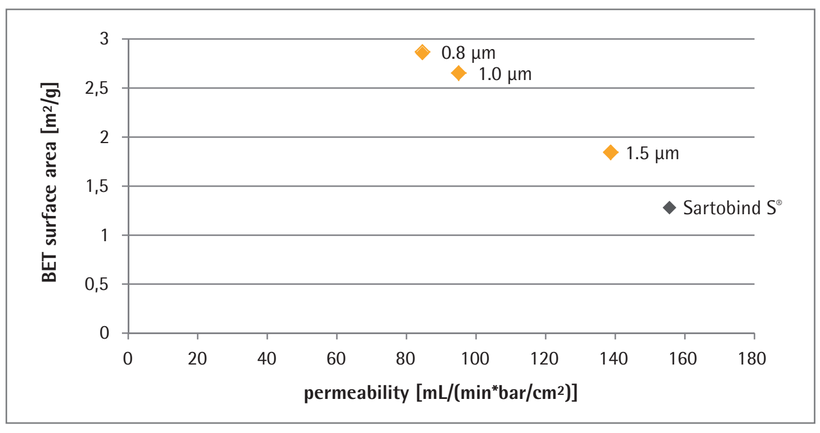


Figure 3: Tailoring the permeability and the specific surface area by pore size optimization.
Optimization of cellulose precursor membranes for virus purification.

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.
During development the prototype testing was performed with model systems:

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

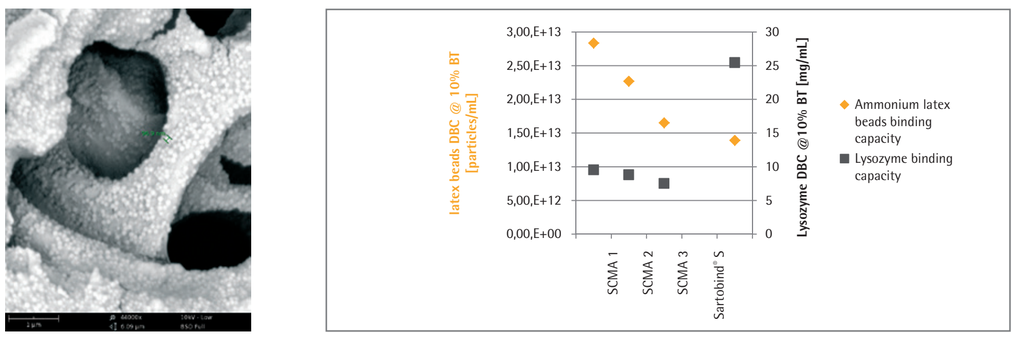
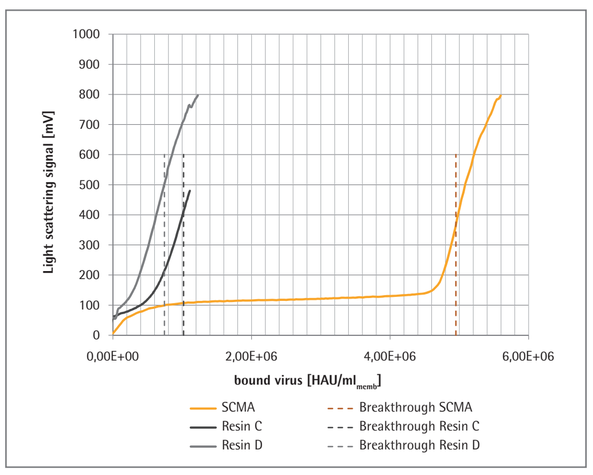


Figure 4: Prototype testing using model systems.
Left: SEM image of ammonium-functionalized latex beads bound to the surface of a SCMA prototype.
Right: Selectivity plot of SCMA prototypes in comparison to Sartobind® S. The gain in selectivity is clearly demonstrated by the increase in binding capacity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

5. Binding capacity and recovery of Influenza virus

Evaluation of the newly developed SCMA was performed in comparison to commercially available sulfated cellulose resins.



HA-activity	HAU/ml$_{memb}$	SCMA vs resin
SCMA	4,95E+06	
Resin C	1,02E+06	486%
Resin D	7,34E+05	673%

Virus particles	particles/ml$_{memb}$	SCMA vs resin
SCMA	9,94E+12	
Resin C	2,08E+12	486%
Resin D	1,50E+12	673%

Table 1: Results of DBC study

Figure 5: Binding capacity study with Influenza A/ Puerto Rico/8/1934 (H1N1).
SCMA — Resin C — Resin D —
Column volume: 0.08 mL (SCMA), 0.18 mL (Resin C, Resin D)
Flow rate: 3.75 CV/min (SCMA), 0.67 CV/min (Resin C), 1 CV/min (Resin D)
Feed: 30 kHAU/mL in 10mM TRIS, pH7.4

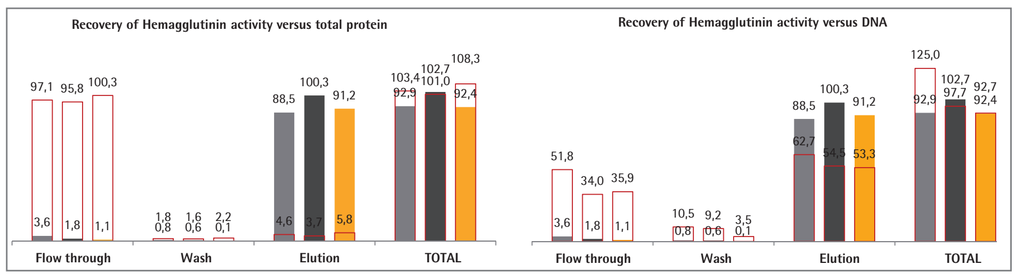


Figure 6: Recovery study with Influenza A/Puerto Rico/8/1934 (H1N1).
Legend and experimental conditions: see figure 5

6. Summary

The newly developed sulfated cellulose membrane adsorber exhibits 5 times higher binding capacity for Influenza A virus than commercially available resins while offering comparable recovery and purity.

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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sartorius stedim biotech

Stefan Fischer-Frühholz
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ECI Vaccine Technology VII Mont Tremblant, Canada, 17^{7h} -22nd June 2018

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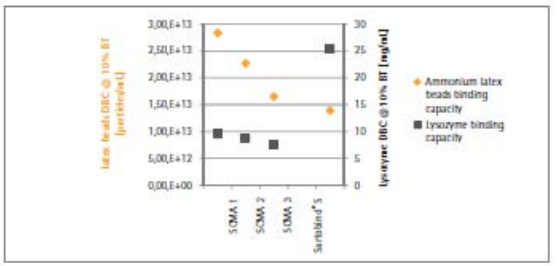

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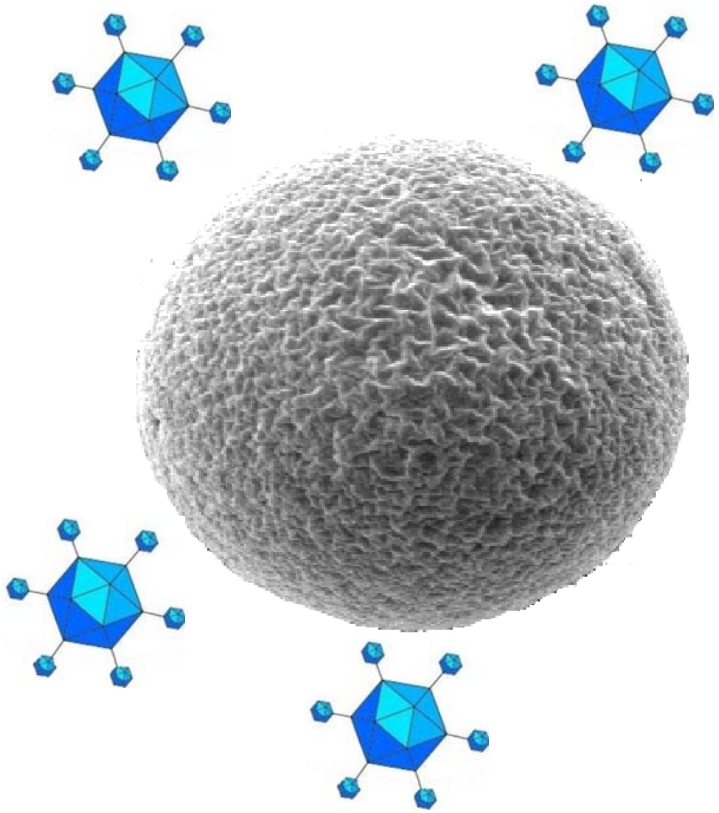
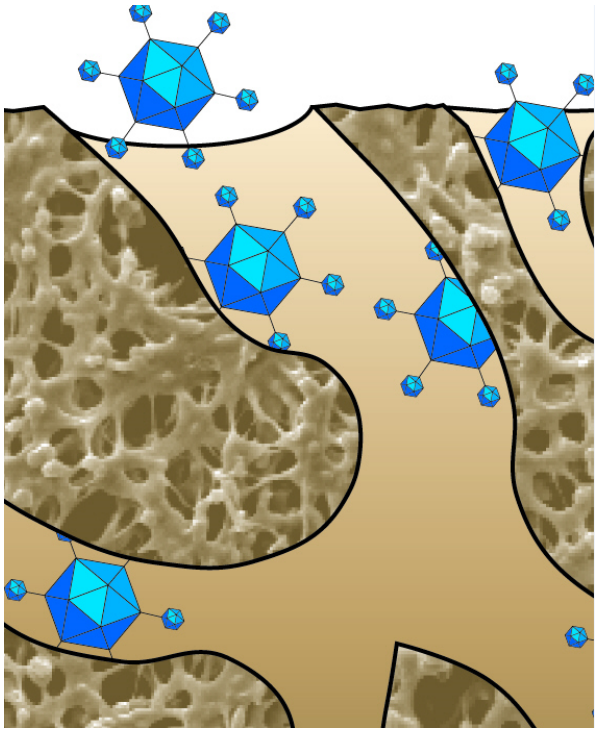
Why virus capture on membrane adsorber?



Resin Bead

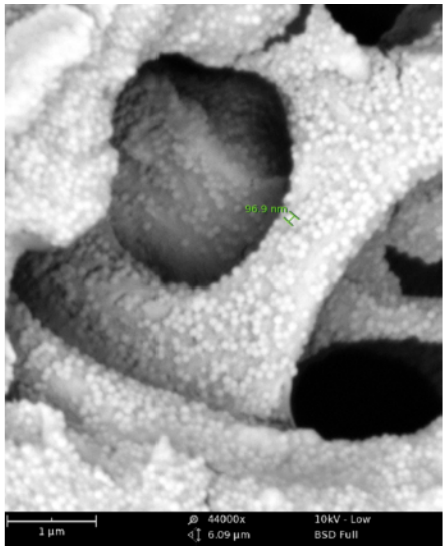


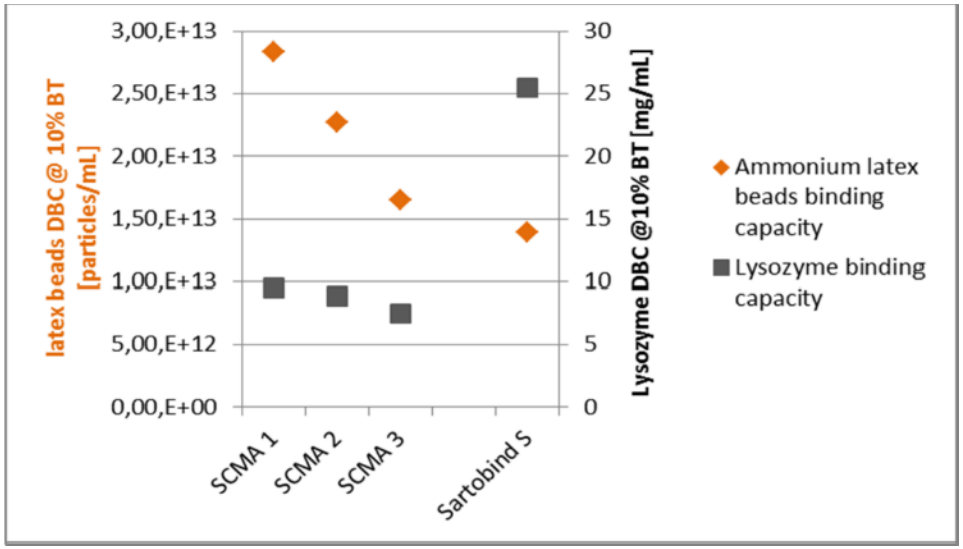
Membrane Adsorber

Membrane development - stationary phase design – higher capacity for large molecules – less capacity for small molecules

Model system for development:

- Ammonium-functionalized latex beads (100 nm) used as virus mimics
- Lysozyme used as model contaminant





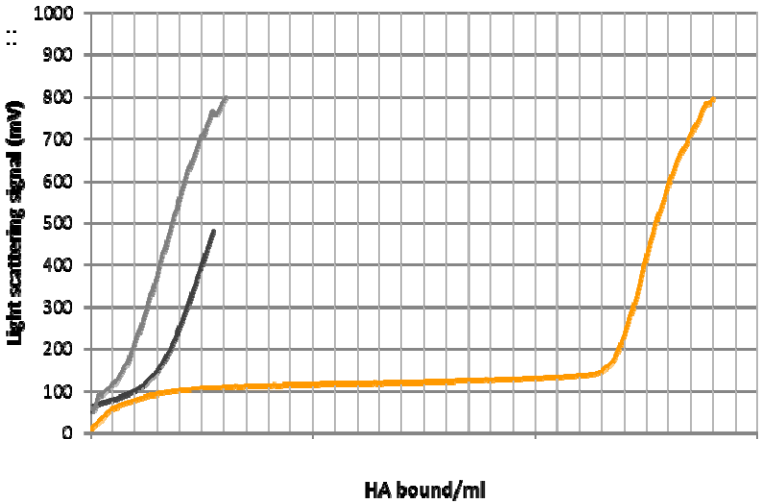
Sulfated cellulose membrane adsorber prototypes (SCMA) compared to Sartobind S

Pseudo affinity SC ligand: $\vdash OSO_3^-$

Sartobind Sulfated Cellulose - 8 to 9-fold higher dynamic binding capacity for Influenza A at ~20 times higher flow rate

- Sartobind Sulfated Cellulose (SC)
- Resin C
- Resin D

Dynamic binding capacity at 10% breakthrough, Influenza A/Puerto Rico/8/1934 (H1N1) HA-activity



Chromatography conditions

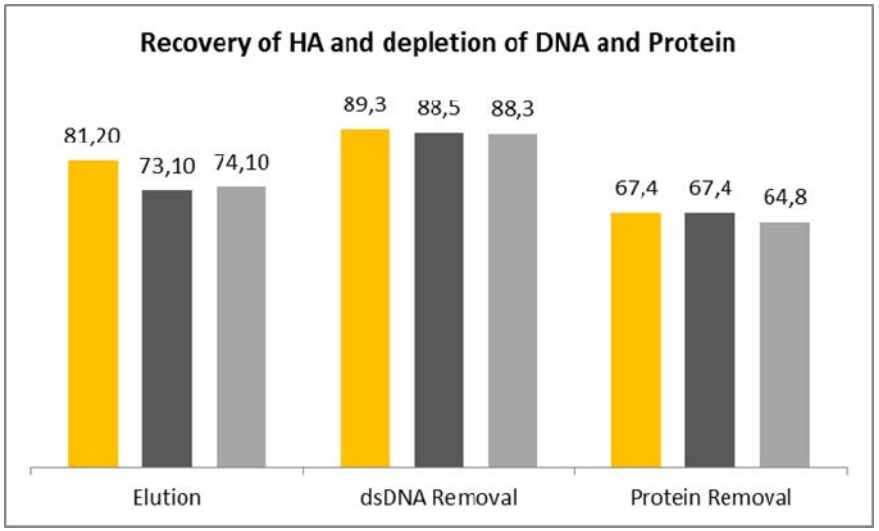
Feed:	9-14kHAU/mL, adjusted to 4mS/cm	
Flow rate:	Resin C:	0.17 CV/min
	Resin D:	0.25 CV/min
	Sartobind SC:	3.75 MV/min
Equilibration:	10mM TRIS, 50mM NaCl (pH7.4, 4mS/cm)	
Load:	Feed	
Wash:	10mM TRIS, 50mM NaCl (pH7.4, 4mS/cm)	
Elution:	10mM TRIS, 2M NaCl (pH7.4, 148mS/cm)	
Regeneration:	Resin C:	0.15M NaOH, 2M NaCl
	Resin D:	1M NaOH, 2M NaCl
	Sartobind SC:	1M NaOH, 2M NaCl

	HAU/ml_{memb}	SC vs resin
Sartobind SC	2,47E+06	
Resin C	3,31E+05	7.5x
Resin D	2,88E+05	8.6x

Sartobind Sulfated Cellulose – Recovery for Influenza – 7 to 8% higher recovery than resins – purity on same level

- Sartobind Sulfated Cellulose (SC)
- Resin C
- Resin D

Influenza A/Puerto Rico/8/1934 (H1N1) recovery / removal in %:



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

	Recovery %	SC vs resin
Sartobind SC	81.2	
Resin C	73.1	- 8.1%
Resin D	74.1	- 7.1%

Latest data show up to 23-fold binding capacity compared to resins.

DBC10% [HAU mL matrix-1]

Influenza A/Puerto Rico/8/ 1934 (H1N1)	HAU/ml_{memb}	SC vs resin
Sartobind SC	2,47E+06	
Resin C	3,31E+05	7.5x
Resin D	2,88E+05	8.6x

Influenza A/Switzerland/ 9715293/2013 (H3N2)	HAU/ml_{memb}	SC vs resin
Sartobind SC	1.64E+06	
Resin C	No binding	1.64E+06
Resin D	No binding	1.64E+06

Influenza B /Phuket/ 3073/2013	HAU/ml_{memb}	SC vs resin
Sartobind SC	1.11E+06	
Resin C	5.26E+04	21.1x
Resin D	4.79E+04	23.2x

- ✓ Seasonal vaccines bind
- ✓ DBC 10% : min.1.0 E+6 HAU/ml_{memb}

  Based on Sartobind SC pico 0.08 mL
- ✓ Can be integrated in 96well plates, capsules and cassettes (up 100 L)
- ✓ Stable to 1 N NaOH cleaning

Fortuna AR, et al., Sulfated cellulose membrane adsorbers as a platform technology for purification of cell-culture derived influenza vaccines, 11th Int Congress on Membranes and Membrane Processes, 2017 July 29 – August 4, San Francisco, USA,

Calculated productivity with continuous upstream (~4000 L per hour): 40 million doses with 54 liter Sartobind Sulfated Cellulose within 1 day

Min. Dynamic binding capacity 10%	HAU/ml memb	1.0E+06
Antigen binding capacity membrane 10%	µg/L	311.000
Percentage loaded to avoid breakthrough	%	80%
Antigen concentration bioteactor	µg/L	6730
Regeneration 1 N NaOH	MV	1,5
Membrane volume all cassettes	L	54,4
Flow rate	L/min	180
Yield	%	80%
Number of cycles per hour	n	2
Working hours in case of pandemics:	hours	24
Number of influenza doses per day	**n**	**40.341.799**

34 x cassettes x 1.6 L = 54 L

From 0.08 mL...

up to 100 L

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