June 7, 2006

Sartobind® Membrane Adsorbers
Overview and News

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Product Manager Membrane Adsorbers
Purification Technologies
Overview

1. Membrane Adsorber technology
2. Membrane products for laboratory and production
3. Applications production
Sartobind Ion Exchange Membrane Chromatography

- Cellulose backbone
- Pores: >3 µm
- Binding layer
- Conventional IEX ligand Q or S 29 mg/ml (36 cm²)
Conventional bead

Convective flow

Pore diffusion

Film diffusion

Membrane Adsorber

sartorius
High flow rate, large frontal surface, small bed volume, no change of binding capacity

E.g.: breakthrough curve at different flow rates on a Sartobind S module 35 ml, 4 mm bed height

Size exclusion effects on a gel*, example thyroglobulin 660 kDa

Q Gel | Sartobind Q
--- | ---
Pore size | 30–50 nm | >3 µm
Binding capacity at 150 cm/h | 2.5 mg/ml | 18 mg/ml
Binding capacity at 1200 cm/h | - | 15 mg/ml

Adenovirus purification with membrane adsorber

- 10-20 x faster – 2 h. vs. 36 h
- 2-times higher virus titer; conc. Up $10^{13}$ VP/ml
- Non toxic, no centrifuge, simple
2. Sartobind Membrane Adsorbers

<table>
<thead>
<tr>
<th>Ion exchange</th>
<th>Strong: S, Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak: C, D</td>
<td></td>
</tr>
<tr>
<td>Metal chelate</td>
<td>Iminodiacetic acid (IDA)</td>
</tr>
<tr>
<td>Coupling</td>
<td>Epoxy</td>
</tr>
<tr>
<td>Coupling</td>
<td>Aldehyde</td>
</tr>
<tr>
<td>Affinity</td>
<td>Protein A plus</td>
</tr>
</tbody>
</table>

- For production 10-30 mg/ml binding capacity
- For analytical less than 10 mg/ml
## Ion exchange Membrane Adsorbers

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Description</th>
<th>Min. static binding capacity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfonylic Acid (S)</td>
<td>Strong acidic cation exchanger</td>
<td>0.8 mg/cm² 29 mg/ml</td>
</tr>
<tr>
<td>Quarternary ammonium (Q)</td>
<td>Strong basic anion exchanger</td>
<td>0.8 mg/cm² 29 mg/ml</td>
</tr>
<tr>
<td>Carboxylic acid (C)</td>
<td>Weak acidic cation exchanger</td>
<td>0.6 mg/cm² 22 mg/ml</td>
</tr>
<tr>
<td>Diethylamine (D)</td>
<td>Weak basic anion exchanger</td>
<td>0.6 mg/cm² 22 mg/ml</td>
</tr>
</tbody>
</table>

*standard proteins: BSA / lysozyme. Membrane area: 36.4 cm² = 1 ml volume
Features of Sartobind ion exchange membrane

<table>
<thead>
<tr>
<th>Feature</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nominal pore size</td>
<td>&gt;3 µm</td>
</tr>
<tr>
<td>membrane thickness:</td>
<td>275 µm</td>
</tr>
<tr>
<td>Internal area per m²</td>
<td>~100 m²/m²</td>
</tr>
<tr>
<td>membrane (BET)</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>1.25 m²/g</td>
</tr>
<tr>
<td>Chemical resistance:</td>
<td>1 N NaOH (regeneration), 1 N HCl, 8 M urea, alcohols, ketones, unstable in peroxides</td>
</tr>
<tr>
<td>Recovery</td>
<td>&gt;90-95 %</td>
</tr>
</tbody>
</table>
### Features of Sartobind ion exchange membrane

<table>
<thead>
<tr>
<th>Feature</th>
<th>Feature Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surfactant</td>
<td>Glycerol (dried from 20 % solution)</td>
</tr>
<tr>
<td>Channeling, breakage of bed</td>
<td>Impossible</td>
</tr>
<tr>
<td>Thermal resistance</td>
<td>Autoclaveable 121°C</td>
</tr>
<tr>
<td>Shelf life</td>
<td>Minimum is 4 years</td>
</tr>
<tr>
<td>Robust handling</td>
<td>Like a filter</td>
</tr>
<tr>
<td>Storage</td>
<td>20% ethanol in equilibration buffer or 10 mM NaOH</td>
</tr>
</tbody>
</table>
Lineare pressure flow relation over a wide range: E.g.: 15 membrane layers 4 mm bed height

Flow vs. pressure of Q 75 with 10 mM KPi pH 7

Flow [ml/min]

Pressure [bar]
Sartobind Formats: large frontal surface + small bed height

IEX: 0.275 mm

Stacks:
Up to 16 mm

Bed height:
4, 8, 16 mm

For production cylindric design

Serial and parallel mode

Tangential

1 ml = 36.4 cm² ion exchange (IEX) membranes
Principle of Sartobind Direct
3. Membrane Adsorber format overview
Sartobind MA
Work horse in membrane chromatography lab scale: Sartobind MA 75

- 75 cm² total membrane area
- 2.1 ml bed volume
- Scaleable: 4 mm membrane bed (15 layers)
- Same bed height as production scale units
- Flow rates IEX and metal chelate 25 ml/min x 1 bar
- Can be used by hand (syringe)
- Housing polypropylene
LAB: Sartobind MA 75 with 4 mm bed height (15 layers), 2.1 ml bed

Sartobind Q 75
Sartobind S 75
Sartobind C 75
Sartobind D 75
Sartobind Epoxy 75
Sartobind Protein A 75
Sartobind IDA 75
Sartobind pABA 75*
Sartobind Blue 75*
Sartobind Heparin 75*
Sartobind Protein A plus*

*Sartorius Flexible Factory
1001 chromatographic cycles with IEX membranes possible

Purification of bovine serum albumin from native bovine serum on Sartobind Q 75 with 5 min. cycle time, operation of 10 working days (8 hours daily)
Sartobind Protein A plus 75

- **Equilibration buffer** PBS, 20 ml
- **Sample** 1 mg/ml polyclonal antibody, prefiltered with 0.2 µm, 100 ml
- **Washing buffer** PBS, 20 ml
- **Elution buffer** Glycine 0.1 M, pH 3.5, 20 ml
- **Regeneration buffer** 50 mM NaOH, 1 M NaCl, 20 ml
- **Flow** 10 ml/min (193 cm/h)
Sartobind pABA 75 ./ conventional benzamidine gel matrix

Dynamic Capacity of different Chromatography Matrices

- p-ABA Membrane Adsorber 2 ml Bed Vol.
- p-ABA Gel Column 4 ml Bed Vol.
Summary MA 75

High flow
- 25 ml/min x 1 bar: IEX, metal chelate
- 10 ml/min x 1 bar: Protein A, Epoxy

Robust
- No breakage of bed, channeling
- Reusable, IEX up to 1000 cycles, protein A >100 cycles
- Can be used by hand or at LC system

Scaleable
- Capsule and modules with same bed height

Application
- Protein and virus purification
- Downscale, principal tests
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Sartobind SingleSep Capsules
PRODUCTION: Single use chromatography Sartobind SingleSep

- Cylindric construction
- Strong ion exchangers only (S, Q)
- Bed volumes 1 ml (nano) up to 1.6 l (Mega)
- Flow rate e.g 10": 2.2 l/bar, Mega: 50 l
- Flow direction (from outside to inside)
- FOR CONTAMINANT REMOVAL

Optical test for evenness of binding with Ponceau S

1. Layer
2. Layer
3. Layer

sartorius
POLISHING: Single use chromatography
Sartobind Q SingleSep

Binding capacity (g)

Membrane Volume (ml)

MA 75
2
0

nano
mini
5"
10"
20"
30"

Mega 3’ x 30”
1620
Sartobind costs of goods model

No packing  ➡️ Labor
Single use  ➡️ Validation
Buffer consumption, Consumables
No hardware investment
High flow rates  ➡️ Process time
Handling and scale-up  ➡️ Speed

Cost of Goods Calculator: Andrew Sinclair
The Future of Contaminant Clearance

Membrane Chromatography as a Robust Purification System for Large-Scale Antibody Production

Joe X. Zhou and Tim Tressel

Recombinant monoclonal antibodies (Mabs) have continued to increase in importance as therapeutics for the manufacturing-scale unit operations are therefore required. In this review, the authors discuss one such example, the membrane adsorber (MA). They also introduce some basic concepts, briefly summarize the history of MA and describe the use of this technology in a late-stage monoclonal antibody purification process.

Downstream Antibody Purification

A typical large-scale purification process is often designed around the use of immobilized Protein A as the primary capture and purification step, in combination with other column operations (Figure 1). Protein A chromatography, in general, delivers a product-related purity of more than 99% — with
### Manufacturing Cost Estimate Comparison between Q-packed Bed Chromatography and Q Membrane Chromatography at AMGEN

<table>
<thead>
<tr>
<th>Items</th>
<th>Q-Resin</th>
<th>Q-MA</th>
<th>Justification/comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Development</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assay development</td>
<td>$200,000</td>
<td>$0</td>
<td>Development activities not required for Membrane Chromatography</td>
</tr>
<tr>
<td>Column storage evaluation</td>
<td>$0</td>
<td>$0</td>
<td></td>
</tr>
<tr>
<td>Column packing studies</td>
<td>$300,000</td>
<td>$0</td>
<td></td>
</tr>
<tr>
<td><strong>Manufacturing costs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hardware cost-Column and Column packing</td>
<td>$400,000</td>
<td>$0</td>
<td>No column required</td>
</tr>
<tr>
<td>Media cost-resin/filter</td>
<td>* $440,000</td>
<td>** $4,320,000</td>
<td>4.5m2 filters per cycle and 180m2 per year. 1800m2 for 10-year operation. Cost for Q membrane is estimated as $2400/m2</td>
</tr>
<tr>
<td>Process labor costs</td>
<td>$800,000</td>
<td>$280,000</td>
<td>Less time required to process; setup is comparable to column. $700 per batch</td>
</tr>
<tr>
<td>Buffer / labor costs</td>
<td>$3,459,200</td>
<td>$842,134</td>
<td>Worst case assumption, since volume requirements will be much lower, and tank size / labor costs would likely decrease as well. $2100 per batch</td>
</tr>
<tr>
<td>Cleaning validation &amp; Lifetime validation</td>
<td>$310,000</td>
<td>$0</td>
<td>No validation requirement for the disposables</td>
</tr>
<tr>
<td>10 year operation cost comparison</td>
<td>$6,109,200</td>
<td>$5,442,134</td>
<td></td>
</tr>
</tbody>
</table>

*Resin cost is calculated based on the most favorite price due to its large scale volume.

**Q membrane is calculated based on the most un-favorite price due to its small scale volume.

Summary: Capsules for contaminant Removal

Efficient:  High throughput (> 500l/hr) for trace impurity removal

Economical:  No hardware investment & maintenance
No column regeneration
No CIP/SIP & validation
No column packing or testing
Less unspecific binding
Less buffers and labor

Easy to use:  Disposable capsule
Faster development
No re-use validation
Sartobind System (reusable modules)
PRODUCTION: Sartobind System

- Cylindric 4; 8; 16 mm bed height
- Binding capacities 1 – 60 g
- Bed volumes 35 to 2130 ml
- Flow rate: 10 liters at 1 bar
- Scale-up: serial / parallel connection
Pilot plant 5 – 10 l bed: productivity 1 – 2 kg protein/ hour

1. Stage: two modules parallel
   2.13 + 2.13 L (A und B)

2. Stage: in serie 1,1 L (B)

3. Stage: in serie 0,3 L (B)

Flow rate at 2-3 bar = 3.5 liter/minute
## Features of Sartobind System reusable modules

<table>
<thead>
<tr>
<th>Set-up</th>
<th>Ready to use, short set-up time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemistries</td>
<td>IEX, Metal chelate, Epoxy, Blue, Protein A (all existing and upcoming membranes)</td>
</tr>
<tr>
<td>Flow rates</td>
<td>1200 cm/h</td>
</tr>
<tr>
<td>Fluidic behavior</td>
<td>Modules have same fluidic (= same flow rate at constant pressure) when doubling length and bed height</td>
</tr>
<tr>
<td>Mode of operation</td>
<td>Step gradients (typical), gradient</td>
</tr>
<tr>
<td>Application</td>
<td>Capture of large proteins, coagulation factors, oligonucleotides (antisense), viruses, plasmids</td>
</tr>
</tbody>
</table>
Summary: Sartobind System modules for capturing

Efficient: High throughput
0.5 l bed: 10 liters/min x 1 bar

Economical: Productivity in kg/h range
for a 5.8 liter MA plant
No column packing or testing
dyn. Binding capacity = static binding capacity Q or S 29 g/l

Easy to use: Advantage in handling

Application fields Capturing:
Proteins, large molecules, viruses, plasmids, oligonucleotides
diluted proteins, coagulation factors
## Sartobind Jumbo

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bed volume (l)</td>
<td>5 l</td>
</tr>
<tr>
<td>Dyn. binding capacity 10% (BSA)</td>
<td>110 g</td>
</tr>
<tr>
<td>Bed height (mm)</td>
<td>8 mm (30 layers)</td>
</tr>
<tr>
<td>Rec flow rate</td>
<td>25 liter/min</td>
</tr>
<tr>
<td>Ligand</td>
<td>Q</td>
</tr>
</tbody>
</table>

![Diagram](image.png)
Biotechnology Division

Sartobind Direct
Skip - Simplify – Save in Downstream Processing

- Tank
- Centrifuge / Depth filtration
- Tank
- UF
- Capture
- Tank

- Tank
- Sartobind Direct Capture
- Tank
Direct Capture of proteins without filtration

- **Inlet**
- **Distributor**
- **Solid Central Core**
- **Membrane Ads.**
- **Spacer Channel**
- **Collector**
- **Outlet**

Flow direction indicated by arrows.
Capturing without prefiltration

- Cell culture broth
- Yeast suspension
- Milk
- Whey
- Albumen
- Phages
- Virus suspensions
Loading mode for Sartobind Direct

Single-pass mode

Recycling Mode (preference)
Sartobind Direct scale up: keep residence time constant

- Residence time ~0.03 min
- BSA load: 22 mg/ml
## Sartobind Direct Scale up

<table>
<thead>
<tr>
<th></th>
<th>Membrane volume [ml]</th>
<th>Membrane area [cm²]</th>
<th>Flow l/min</th>
<th>Residence time [min]</th>
<th>Protein loaded [mg]</th>
<th>Batch volume [l]</th>
<th>Protein [mg]</th>
<th>Protein eluted [mg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5</td>
<td>120</td>
<td>0.077</td>
<td>0.032</td>
<td>70.8</td>
<td>0.062</td>
<td>71</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>950</td>
<td>0.63</td>
<td>0.039</td>
<td>567</td>
<td>0.50</td>
<td>567</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>175</td>
<td>6500</td>
<td>5.2</td>
<td>0.034</td>
<td>2924</td>
<td>4.16</td>
<td>3924</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>13400</td>
<td>10.2</td>
<td>0.034</td>
<td>8018</td>
<td>8.13</td>
<td>8018</td>
<td>0.99</td>
</tr>
</tbody>
</table>
Summary: Sartobind Direct for capturing

Skip
  - Centrifuge, prefiltration and UF
  - Intermediate tank storage

Simplify:
  - Capture directly from cell culture
  - Virus and phage binding from broth
  - Purification from high viscous media such as yeast suspension, milk, whey and albumen

Save:
  - Labor, hardware, buffer, storage space, time,

Application fields: Capturing
Endotoxins can be removed 5 LRV* with Sartobind Q

Figure: Courtesy of Prof. F.B. Anspach, Hamburg University of Applied Sciences, Department of Natural Science Technology
*A. Clutterbuck, Avecia, Membrane Adsorption within cGMP Manufacture a Case Study, Downstream Forum Goettingen, May 2006
Flow chart of monoclonal antibody production

- Cell culture
- Clarification, diafiltration
- Protein A chromatography
- Q anion exchange
- S cation exchange
- Virus ultrafiltration
- Ultrafiltration / formulation

: Capture: purification
: Polishing: removal of contaminants
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Removal of contaminants during monoclonal antibody production

- **Purification according to charge:**
  - Positively (+) charged ligand of Q adsorbers binds negatively (-) charged contaminant
  - Neutral or positively (+) charged product runs through (negative or Flow Through (FT) chromatography)

**Diagram:**
- **Isoelectric points**
- **Impurities:**
  - Endotoxin
  - DNA
  - Viruses

- **Product:**
  - Polishing: contaminants are bound
  - MABs

- **Flow Through**
- **pH buffer**
Oversizing

Column based system

Production
Ø 50 cm
30 l bed volume low flow
1000 l/h
B. capac. 1500 g oversized

Membrane based systems

0.5 l bed volume high flow
1000 l/h
B. capac. 15 g sufficient for Contaminant removal

DNA removal from a therapeutic antibody. Campath 1-H

Average of three 12,500 liter batches with 15–50 module

- clears the DNA below detection limit
- „superior pressure/flow relation
- the process time is reduced 23-fold, excluding the benefit in handling for set-up.
- diminish a loss of product
- installed as in-line filters and can be disposed after use*


Boehringer Ingelheim

FDA approval March 2001

Polishing Step: hMAB after Capturing at Abgenix Inc.

Impurity Levels after Protein A (12k run ~12,000 g product):

- 1.5% aggregate 180g
- 1000 ppm CHOP 12g
- 200 ppm DNA 2.4 g
- 30 ppm Leached ProA 0.3 g

Typical load value: 2 kg/L (FT)

*Membrane Chromatography for Purification of Human Antibodies in Commercial Processes. Gerardo Zapata et al. (Abgenix Inc.) IBC Antibody Meeting 2005
Polishing Step: hMAB nach Capture bei Abgenix Inc.

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Size (nm)</th>
<th>Enveloped</th>
<th>LRV Run 1</th>
<th>LRV Run 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVM: Minute Virus Mouse</td>
<td>16 - 25</td>
<td>no</td>
<td>≥6.77 ± 0.24</td>
<td>4.41 ± 0.37</td>
</tr>
<tr>
<td>Reo-3: Reovirus Type III</td>
<td>75 - 80</td>
<td>no</td>
<td>≥7.28 ± 0.30</td>
<td>≥7.53 ± 0.29</td>
</tr>
<tr>
<td>MuLV: Murine Leukemia Virus</td>
<td>80 - 110</td>
<td>yes</td>
<td>≥5.57 ± 0.25</td>
<td>6.29 ± 0.32</td>
</tr>
<tr>
<td>PRV: Pseudorabies virus</td>
<td>150 - 250</td>
<td>yes</td>
<td>≥5.67 ± 0.17</td>
<td>≥5.76 ± 0.23</td>
</tr>
</tbody>
</table>

(log_{10}) removal for DNA: >2 log, for host cell proteins: 1.9 log, endotoxin: >2.8 log

Polishing Step after Intermediate Purification at AMGEN

Impurity Levels after 2nd column:
: << 100 ppm CHOP
: << 20 ppm DNA

Typical load value: 10.9 kg/L

180 x more than a column!

Feng Li et al. J. BioProcessing, 09/10, 23-30, 2005
**Virus removal study for MAb**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Enveloped</th>
<th>LRV Run 1</th>
<th>LRV Run 2</th>
<th>Virus Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVM: Minute Virus of Mice</td>
<td>no</td>
<td>6.03 ± 0.21</td>
<td>6.03 ± 0.20</td>
<td>100</td>
</tr>
<tr>
<td>Reo-3: Reovirus Type III</td>
<td>no</td>
<td>7.00 ± 0.31</td>
<td>6.94 ± 0.24</td>
<td>100</td>
</tr>
<tr>
<td>MuLV: Murine Leukemia Virus</td>
<td>yes</td>
<td>≥ 5.35 ± 0.23</td>
<td>≥ 5.52 ± 0.27</td>
<td>&gt; 70</td>
</tr>
<tr>
<td>PRV: Pseudorabies virus</td>
<td>yes</td>
<td>≥ 5.58 ± 0.28</td>
<td>≥ 5.58 ± 0.22</td>
<td>100</td>
</tr>
</tbody>
</table>

Zhou J and Tressel T: Basic Concepts in Q Membrane Chromatography for Large-Scale Antibody Production. Submitted for publication.
Summary

1. Differences Sartobind / conventional gels:
   - Negligible diffusion limitation, high speed, less size exclusion
   - Larger binding capacity for large proteins
   - Rapid virus purification, high yield
   - Specific format possible with adsorbers: Sartobind Direct
   - Capturing in 2 kg protein / hour feasible

2. Single use products
   - Simple to use (like a filter), disposable
   - Save up to 80 % of column costs

   - >5 log Endotoxin
   - DNA below detection limit
   - Virus removal >6 log

- Used in FDA approved production since 2001
Thank you!

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