



Phenyl Membrane Adsorber for Bioprocessing

Sartobind® Hydrophobic Interaction Membrane Chromatography

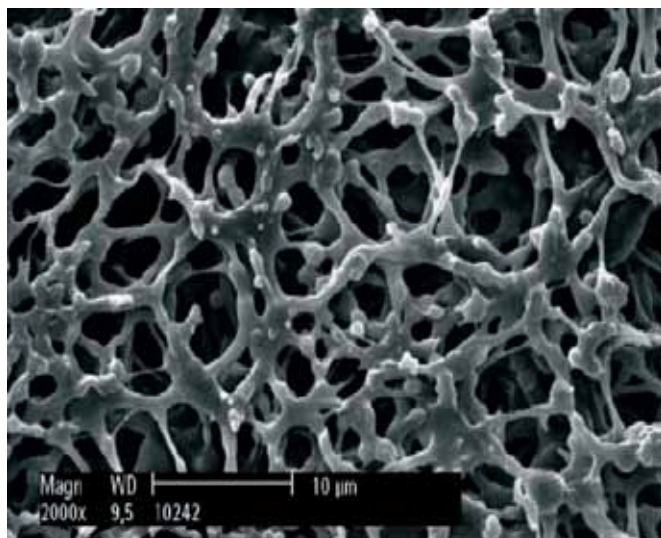


Fig. 1: Sartobind Phenyl membrane

Introduction

Hydrophobic phenyl membrane adsorbers with a macroporous structure have been developed (Fig. 1). The chromatographic membranes are rolled up to cylindrical devices and incorporated into capsules with a bed height of 8 mm (Fig. 2). The capsules are of the same design and housing materials as Sartobind capsules used in downstream processes for the removal of charged contaminants or for purification of large biomolecules or viruses.



Fig. 2:
Sartobind Phenyl capsules

Hydrophobic interaction membrane chromatography separates and purifies biomolecules based on differences in their hydrophobicity. On average 50 % of a protein or peptide surface is accessible for such interaction. The strength of the interaction depends on the sufficient number of exposed hydrophobic groups of the sample and on membrane ligand type and density. Sample properties, temperature, type and concentration of salt and pH as well as additives influence the binding process as well.

The low substitution of the phenyl ligand on the membrane allows for mild elution of biomolecules such as peptides, proteins, viruses, phages or other biomolecules. The biological functions of the molecules are preserved.

The use of chaotropic (salting-in) agents such as urea, guanidinium chloride or organic solvents such as ethylene glycol, dimethyl sulphoxide, dimethyl formamide or propanol will compete very effectively with bound proteins and displace them. Such additives can help during membrane cleaning as the phenyl membrane has been developed not only for single use flow through but also for bind and elute and reuse.

Mab binding on Sartobind Phenyl and on a conventional phenyl column

Materials

Load samples

Mab1 and Mab2: each IgG 1 mg/ml and 0.8 M $(\text{NH}_4)_2\text{SO}_4$ in 50 mM potassium phosphate pH 7.0

BSA, lysozyme: each 2 mg/ml and 2 M $(\text{NH}_4)_2\text{SO}_4$ in 50 mM potassium phosphate pH 7.0

Elution buffer: 50 mM potassium phosphate pH 7.0

Method

Two monoclonal antibodies (Mab1 and 2) were loaded onto a membrane device and on a column at specified flow rates. The bed height and bed volume were 30 mm/1 ml for the column and 4 mm/2 ml for the membrane.

Results

Dynamic binding measurements showed that the phenyl membrane had comparable binding capacity to a conventional resin (Tab.1, Fig. 3). A major difference is the reduced process time with the adsorber by a factor of 15 due to the high flow rate.

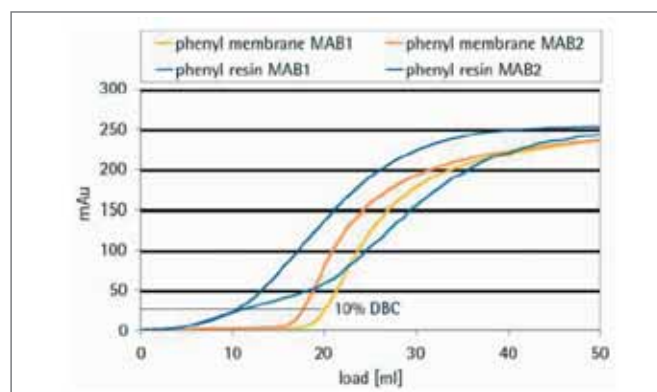


Fig. 3: Dynamic binding capacity of Mab (10% breakthrough) on membrane and resin

Tab. 1: Mab binding on phenyl membrane and resin

	Sartobind Phenyl	Phenyl resin
Number of layers	15	-
Bed height	4 mm	30 mm
Bed volume	2 ml (80 cm ²)	1 ml
Flow rate	10 ml/min	1 ml/min
Flow rate	5 BV/min	0.33 BV/min
Binding capacity 10 % with:		
Mab1	8.9 mg/ml	10.5 mg/ml
Mab2	12.5 mg/ml	10.8 mg/ml
BSA	10 mg/ml	n.d.
Lysozyme	23 mg/ml	n.d.

Lab scale screening: Binding of an antibody in 0.1 – 1 M ammonium sulphate

The membrane was incorporated into 96 well plates for high throughput screening (HTS).

400 µl of a 1 mg/ml IgG sample in 50 mM potassium phosphate buffer pH 7 with different ammonium sulphate concentrations were loaded on a phenyl 96-well plate (4 mm bed height, 0.15 ml bed volume). The amount of IgG in flow through, wash and elution was determined by measuring absorbance at 280 nm.

In the subsequent experiments, IgG was loaded at 1 M salt in the same buffer.

Result

IgG was almost completely bound at 1 M ammonium sulphate (Fig. 4a). Recoveries greater than 95 % were found in the samples loaded at a salt concentration of 1 M in binding buffer (Fig. 4b).

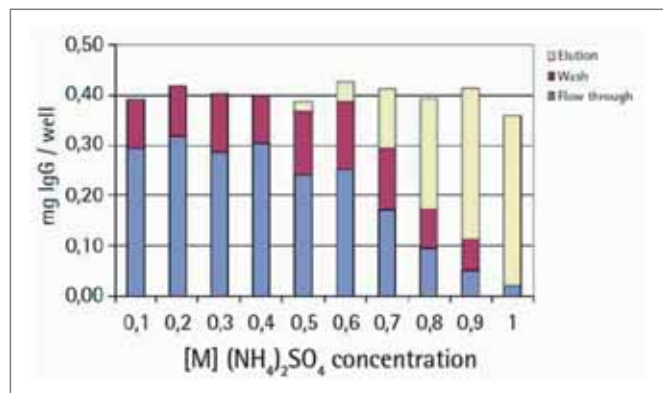


Fig. 4a: Amount of IgG bound on phenyl membrane at increasing (NH₄)₂SO₄ concentrations

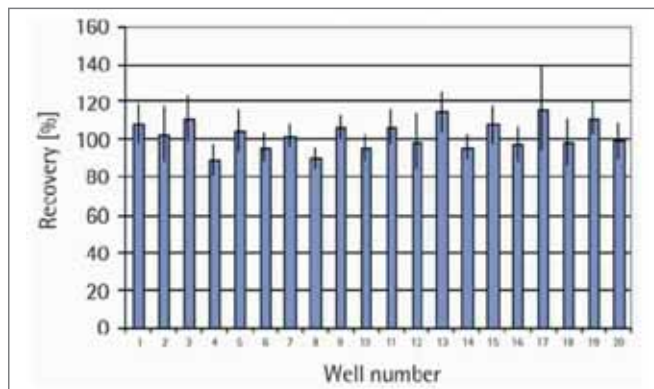


Fig. 4b: IgG recovery (%) eluted from Sartobind Phenyl

Sartobind Phenyl and a phenyl resin challenged with a mixture of cytochrome c, trypsinogen and polyclonal antibody

The proteins were eluted by linear gradient: 1.5 M (NH₄)₂SO₄ in 50 mM potassium phosphate pH 7 to 50 mM potassium phosphate.

Result

A better resolution was observed on the membrane thereby improving the purity of the proteins (Fig. 5).

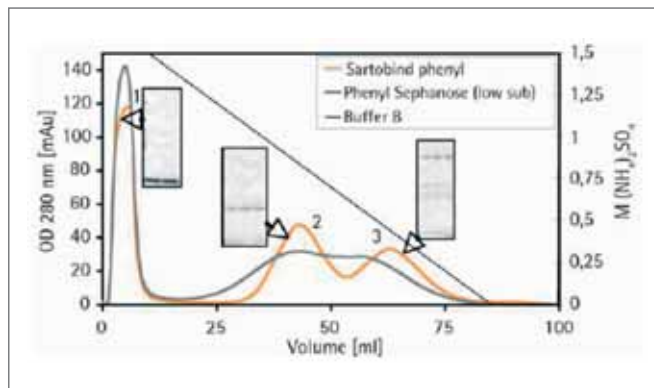


Fig. 5: Gradient elution of cytochrome c (1), trypsinogen (2) and IgG (3) bound on membrane and resin.

Separation of cytochrome c, myoglobin, lysozyme and α-chymotrypsinogen

Proteins were dissolved in 1.7 M (NH₄)₂SO₄, 50 mM potassium phosphate at pH 7.0. Elution was done by step gradient with low salt buffer in 50 mM potassium phosphate pH 7.0 at a flow rate of 10 ml/min.

Result

Proteins were separated on Sartobind Phenyl 75 with 15 membrane layers at room temperature (Fig. 6). Base line separation was achieved.

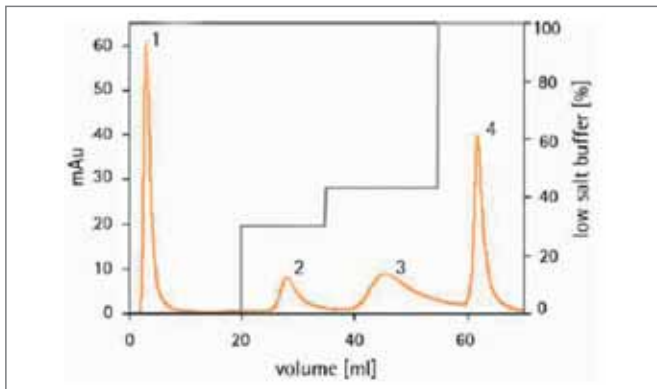


Fig. 6: Step elution of cytochrome c (1), myoglobin (2), lysozyme (3) and α -chymotrypsinogen (4) bound on the membrane

Aggregate removal

Sartobind phenyl membrane was used for aggregate removal in flow through mode in a purification process for a recombinant protein. The loading conditions were chosen to selectively retain the aggregates whereas the target protein did not bind to phenyl membrane. However, it was not optimized. Elution was done by washing the membrane with pure H_2O .

Result

Fig. 7 shows higher amount of aggregates were bound on the membrane at increased ammonium sulfate concentration in loading buffer.

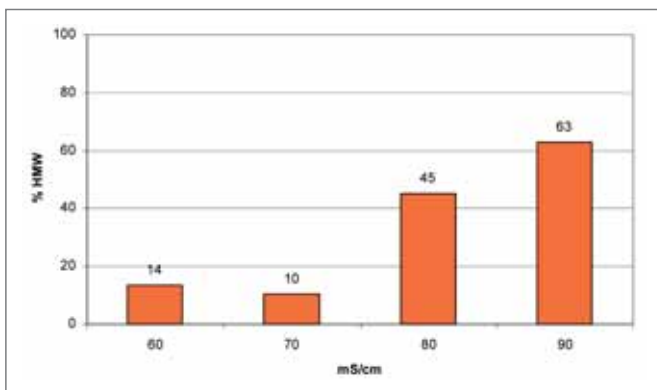


Fig. 7: Aggregates (%) eluted from Sartobind Phenyl. The conductivity represents the different salt concentrations at loading

Influence of flow rate

Result

Fig. 8 shows the breakthrough curves with Sartobind Phenyl nano (3 ml) at different flow rates of 5, 10 and 20 ml/min. The sample was a human monoclonal antibody at a concentration of 1 mg/ml.

A higher flow rate had only minor influence on breakthrough.

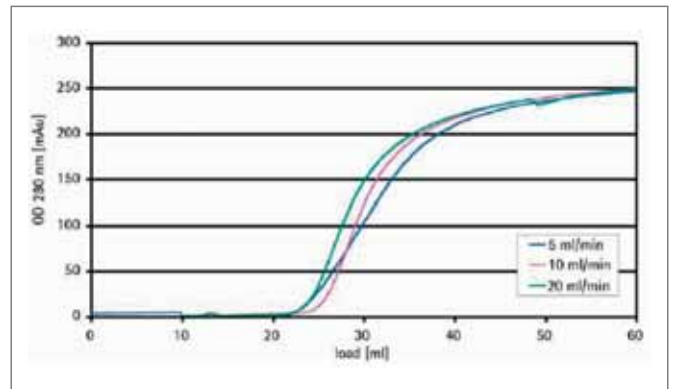


Fig. 8: Mab binding on the membrane at different flow rates

Scale up

Conventional applications from hydrophobic columns can be transmitted to hydrophobic membranes, especially applications with <1 kg biomolecules per hour due to the current limitation of capsule size. Parallel and serial connections of capsules are possible for increased capacity, although use of one module in recycling mode is preferable because it is the simplest set up. The high flow rates can allow for 4 to 6 complete chromatographic cycles per hour possible. A calculation of binding capacity per ml and hour results in a productivity of 50 to 100 mg IgG per ml per hour.

In a scale up experiment with nano and 5 inch capsules, human polyclonal IgG in 0.9 M $(NH_4)_2SO_4$, 50 mM potassium phosphate, pH 7.0 was loaded at 10 ml/min (nano: 120 cm^2 , 3 ml) and 500 ml/min (5" capsule: 5000 cm^2 , 125 ml) respectively. The amount of polyclonal IgG was normalized to membrane area.

Results

Breakthrough curves of Sartobind Phenyl nano and 5 inch capsules in Fig. 9 represent a successful 42 fold scale up.

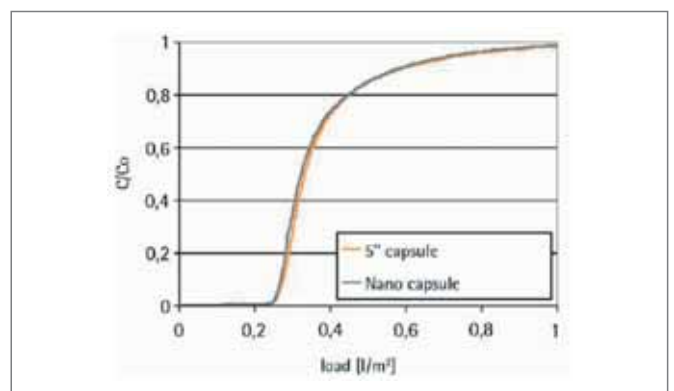


Fig. 9: Scale-up performance of Sartobind Phenyl capsules

Applications of hydrophobic interaction membrane chromatography

- Hydrophobic interaction chromatography (HIC) principle on membrane and beads is comparable. High salt concentrations in loading buffer promote the binding of the target molecule to the HIC adsorbent. By decreasing the salt concentration in a linear or step gradient the bound molecule is eluted. In all applications it is advisable to optimize by moderating temperature, pH, salt concentration and type of salt.
- One of the preferred applications of membranes is their use in flow through steps. Such conditions need certainly less binding capacity and reduce consumption of salt. The ready to use capsule format does not require packing or testing and allows for a “plug and play” approach in downstream processing
- Misfolded proteins, aggregates, dimers, trimers, tetramers of biomolecules as well as leached chromatographic ligands can display higher hydrophobicity than the protein of interest. Those impurities are specifically retained whereas the target protein can flow through.
- Directly after the protein A column, a HIC membrane flow through mode can be implemented as a polishing step to remove host cell proteins from a monoclonal antibody fragment for example. This step reduces also leached protein A and misfolded proteins.
- HIC membranes can be used for the removal of endogenous and adventitious viruses from biopharmaceutical proteins as a complementary step to virus filtration, ion exchange chromatography and inactivation.
- The HIC membrane is applicable for removal of endotoxins from target proteins produced e.g. from inclusion bodies of *Escherichia coli* when a denaturing step is required to solubilize the target molecule.
- Another field is the removal of process chemicals from proteins: Virus inactivating chemicals such as lipids with the addition of surface active agents, non ionic detergents such as Triton X 100® (trademark of Union Carbide), Tween® (trademark of ICI Americas), anti foam agents such as Pluronic® (trademark of BASF AG), and hydrophobic dyes and aromatic compounds. All hydrophobic components which have been described to be removable by conventional HIC resins are a potential target for HIC membranes.

- Hydrophobic membranes have been successfully applied in bind and elute applications. The large pore size of > 3 µm makes them especially suitable for the separation of large biomolecules above 100 kDa including vaccines, conjugated vaccines, virus particles and phages. There is almost no visible size exclusion effect detectable.
- A HIC step can be ideally used as an initial step after a precipitation or ion exchange chromatography step when the conductivity is already high and addition of salt for high binding of the proteins fits perfectly into the bioprocess scheme.
- Double stranded DNA displays higher hydrophobicity than single stranded DNA. This enables separation of such products during plasmid DNA purification. Hydrophobic membranes can also be applied to separate oligonucleotides from derivatized oligos when a difference in structure and |or chemistry results also in a difference of hydrophobicity.

Summary

The Sartobind Phenyl membrane can be considered as a replacement to columns for polishing (flow through) operations and a number of bind and elute applications as they work at much higher flow rates, reduced complexity and without size exclusion effects when purifying large biomolecules.

Cost savings can be anticipated as re-validation is not necessary when used as disposables.

www.sartorius-stedim.com/sartobind
membrane.adsorbers@sartorius-stedim.com

Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen, Germany
Phone +49.551.308.0
Fax +49.551.308.3289
www.sartorius-stedim.com

USA Toll-Free +1.800.368.7178
UK +44.1372.737159
France +33.442.845600
Italy +39.055.63.40.41
Spain +34.90.2110935
Japan +81.3.3740.5407

Sartobind® is a trademark of Sartorius Stedim Biotech GmbH
Technical data are subject to change without notice.
Printed in Germany on paper that has been bleached without any use of chlorine.

First published Nov. 20, 2008
Status January 18, 2010

Specifications subject to change without notice. Printed and copyrighted by Sartorius Stedim Biotech GmbH W - G
Publication No.: SL-4048-e10014
Order No.: 85034-536-19
Ver. 01 | 2010