Sartobind™
Membrane Adsorbers Discs and Cassettes

Reusable Sartobind Membrane
Ion Exchangers
for Adjustable Filter Holders
(293 mm and 142 mm ∅)

sartorius

Contents

1. General Information

Traditional chromatography uses porous particles packed into columns. As liquid flows through the column and around the beads, biomolecules in the liquid diffuse into the pores of the particles to binding sites on the inner surface of the pores. The rate limiting factor in low pressure column chromatography is the time required for the molecules to diffuse into and out of the pores where the binding sites are located. The various steps of equilibration, loading, washing, elution and regeneration can take hours.

Sartorius has attached various functional groups covalently to the inner surface of synthetic microporous membranes. Pressure filtration forces liquid through the pores of the membrane, bringing proteins into direct contact with the binding sites. This direct convection to the binding sites minimizes the diffusional limitation of mass transfer without sacrificing binding capacity. The Sartobind™ membrane ion exchangers, which are available as disposable and reusable units, include the following benefits:

- ○ High dynamic binding capacity at high flow rates
- ○ Ready-to-use units
- ○ Easy handling

Sartorius offers a complete range of Sartobind Membrane Adsorbers from the milligram scale all the way to the multi-scale.

For binding capacities ranging from about 1–20 g adjustable filter holders in two different sizes have been designed to accommodate single membranes (Discs) or membrane cassettes.

2. Application Areas

Application areas of Sartobind Membrane Ion Exchangers include:

1. Rapid concentration of dilute proteins
2. Removal of contaminants (e.g. DNA-Fragments, endotoxins can be drastically reduced from target substances)
3. Target substances can be separated from a mixture in two ways
   a) By choosing the appropriate conditions, the target substance is selectively bound, whereas contaminants pass through the membrane.
   b) By choosing the appropriate conditions, contaminants are retained, whereas the target substance can pass through the membrane.
4. Bound biomolecules can be separated by step elution.

3. Operation Procedure

For determination of the ion exchange behaviour of an uncharacterized target substance run test experiments with a disposable Sartobind ion exchanger to optimize the ionic strength and pH of the buffer used.
Make sure that stability of target substances is not affected by the washing and elution conditions. To bind a substance the pH of the equilibration buffer should be 1–2 units above the isoelectric point of the target protein for the Q- and D-type and 1–2 units below the isoelectric point for the S- and C-type.
All buffers should be prefiltered with 0.2 or 0.45 µm membrane filters (for example a Sartorius Sartobran capsule).

3.1. Insert the Sartobind membrane adsorber disks or cassettes (with the ordering number facing the inlet side) into the filter holder as shown in figure 1.

Fig. 1

The 293 mm diameter filter holder accepts up to 4 cassettes, whereas up to 7 cassettes fit into the 142 mm filter holder.
It is also possible to put 1–39 single membranes into the filter holder. When using single membrane layers center a silicone O-ring (280 x 4 mm; provided with the filter holder) on top of the membranes. Make sure, that the O-ring fits tightly in the corner between membranes and base plate. If not, stretch the O-ring carefully until it fits snuggly. Do not overstretch the O-ring.

Position the top plate of the filter holder with the membrane adsorbers in place and uniformly hand-tighten the locking clamps in opposite diagonal pairs or use a torque wrench at 10 Nm (Fig. 2 + 3).

Fig. 2

Fig. 3

All following operation steps can be assayed by measuring pH and conductivity.
Volumes in brackets are valid for 142 mm disks or modules (filter holder 16276-----3).

3.2. Depyrogenation (optional)
If it is necessary to depyrogenate the unit, please refer to section 5.

3.3. Equilibration
a) New Sartobind Membrane adsorber
Connect the filter holder to a peristaltic pump (e.g. Sartorius order number 16697) and a buffer reservoir (other pressure sources may also be appropriate).
Place a receiving vessel under the outlet of the unit and equilibrate the system with a low ionic strength starting buffer.
The volume needed for equilibration of the system depends on the dead volume and the adsorption area of the membrane. As a guideline, a minimum of 1.5 l (0.6 l) is necessary.
To vent the system open the valve and tilt the filter holder with the valve as the uppermost part until liquid exits. Then close the valve tightly. To vent the downstream part of the filter holder proceed as stated above.
For proper functioning of the membrane adsorber air bubbles in the upstream part of the system have to be avoided throughout the whole procedure.

b) Reuse of Sartobind membrane adsorber
To reuse Sartobind membrane adsorbers after storage equilibrate the system as stated above. Then flush the system with a minimun of 1.5 l (0.6 l) of 1 N HCl (for cation exchangers) or 1.5 l (0.6 l) of 1 N NaOH (for anion exchangers) and reequilibrate the system with starting buffer. To assay reequilibration check pH and conductivity.

3.4. Sample preparation
It is recommended to clarify the sample solution by filtering it through an 0.45 µm prefilter, for example a Sartorius Sartobran capsule.

3.5. Loading of the Sartobind Membrane Adsorber
Connect the sample reservoir to the peristaltic pump and load the Membrane Adsorber.

3.6 Flow rate
Unlike microporous beads binding capacity of membrane adsorbers is almost independent of the flow rate chosen. Therefore the system can be operated at elevated flow without loosing performance. Please note that the maximum recommended pressure is 500 kPa (5 bar).
For dependency of flow rate on the number of membrane adsorbers used please refer to figure 4.

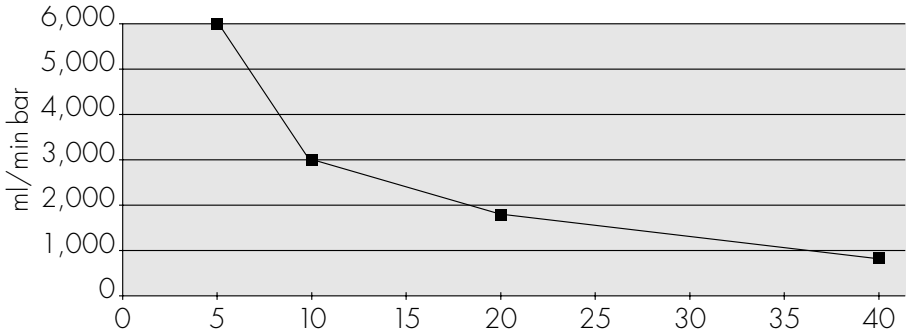

Fig. 4: **Dependency of flow rate on the number of membranes**
Operating conditions: 10 mM Sodium phosphate pH 7.0, 1.5 bar at room temperature, 293 mm filter holder

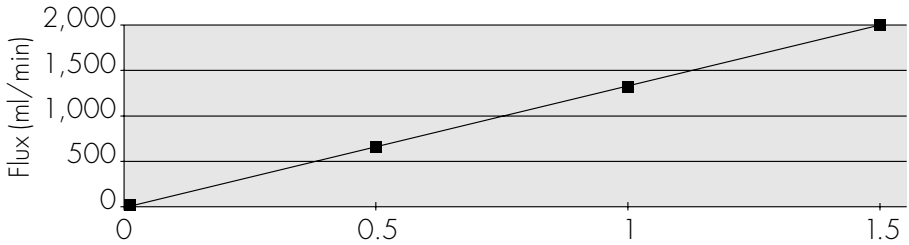

Fig. 5: **Flow rate as a function of pressure drop across the membrane layers**
Operating conditions: 20 layers of membranes, 10 mM Sodium phosphate pH 7.0 at room temperature, 293 mm filter holder

3.7. Washing
To remove unbound substances wash the system with sufficient starting buffer. Washing efficiency should be followed spectrometrically.

3.8. Elution
Elution of bound substances is accomplished by increasing ionic strength or changing pH of the buffer and can easily be followed spectrometrically. Typical elution volume is about 1.5 l (0.6 l).

3.9. Regeneration
After elution with increased salt concentrations flush the system with a minimum of 1.5 l (0.6 l) starting buffer containing 1M KCl.

After elution by pH shift wash the system with starting buffer until pH and conductivity are the same as in the starting buffer and then flush the system with 1.5 l (0.6 l) of starting buffer containing 1M KCl to remove substances still bound to the Membrane Adsorber under elution conditions.
Reequilibrate the system for the next run or disassemble the system and store the Sartobind membrane adsorber as described in section 4.

3.10. Cleaning
To remove impurities, which can not be eluted with the method described above, flush the system with ≥ 1.5 l (≥ 0.6 l) of 0.2 N NaOH. Then reequilibrate the system with starting buffer. Equilibration is completed when pH and conductivity of the effluent liquid is the same as of the starting buffer.

4. Storage of Sartobind™ Membrane Adsorbers

Store new Membrane Adsorbers dry at room temperature.
Used Membrane Adsorbers should be stored in buffer containing 1 M KCl at 4°C. To prevent microbial growth, addition of 20% Ethanol or other bacteriostatic agents, such as 0.001% chlorohexidine (Hibitane™) is recommended. Short-term storage of Membrane Adsorbers in 0.1 N NaOH at room temperature is also possible.
Do not store Membrane Adsorbers in water, always use buffer or saline solution.
It is possible to store the discs or cassettes in the package device.

5. Depyrogenation

5.1. Assemble the filter holder and insert the Membrane Adsorber disks or cassettes as described in section 3 and set up the system.
5.2. After equilibration of the system (see section 4.3.) pass through a solution of 1 N NaOH (> 1.5 l (0.6 l)). The alkaline solution should be kept in contact with the Membrane Adsorber for at least 1 hours.
5.3. Wash out the NaOH (monitored by pH-measurement) and reequilibrate the system with pyrogen free starting buffer and continue as described in section 4.4.

6. Specifications

6.1. Functional groups

Sulfonic acid (S)	$R\text{-}CH_2\text{-}SO_3^-\ Na^+$	strongly acidic cation exchanger
Quaternary ammonium (Q)	$R\text{-}CH_2\text{-}N^+\text{-}(CH_3)_3\ Cl^-$	strongly basic anion exchanger
Carboxylic acid (C)	$R\text{-}COO^-\ Na^+$	weakly acidic cation exchanger
Diethylamine (D)	$R\text{-}CH_2\text{-}N\text{-}(C_2H_5)_2$	weakly basic anion exchanger

6.2. Technical data

	142 mm ∅	293 mm ∅
Membrane material	stabilized modified cellulose	
Effective adsorption area [1]) per membrane	120 cm^2 (142 mm ∅)	550 cm^2 (293 mm ∅)
Effective adsorption area [1]) per cassettes	600 cm^2 (142 mm ∅)	5500 cm^2 (293 mm ∅)
Protein binding capacity for reference proteins		
Membran-Typ	Referenzprotein und Buffer	Capacity [mg/cm^2]
S	Lysozyme in 0.01 M Phosphat pH 7.0	> 1
Q	BSA in 0.01 M Phosphat pH 7.0	> 0.65
C	Lysozyme in 0,01 M Phosphat pH 7.0	> 0.8
D	BSA in 0.02 M Tris-Cl pH 8.3	> 0.5
pH resistance	pH 2–13	
Extractables	Membranes contain Glycerol as wetting agent	

[1]) 50 cm^2 ≅ 1 ml membrane volume

6.3. Chemical Compatibility

The chemical compatibility of all Membrane Adsorbers was determined by incubating samples of each Membrane Adsorber type with the chemicals listed below for up to **27 days** either at room temperature (20–25°C) or at 40°C as indicated in the table. After the incubation period, the membranes were rinsed with 10 mM sodium phosphate, pH 7.0, and then subjected to routine flow rate and binding capacity tests.
Conditions which were found not to affect performance are indicated by a solid square (■). Limited compatibility is indicated by an open square (□), whereas incompatible incubation conditions are represented by a bar (–).

Important:
Membrane compatibility and stability is affected by a number of factors, including temperature, concentration, mixture of substances, contact time, and Membrane Adsorber type used. The results presented in the table below should be used for guidance only. Each Membrane Adsorber should be tested for compatibility with the user's actual process stream before incorporation into a manufacturing process.

Chemical Compatibility of Membrane Adsorbers

Chemical	Membrane Adsorber Type			
Alcohols	S/18842	Q/18942	C/19142	D/19042
Methanol 98%	■	■	■	■
Ethanol 98%	■	■	■	■
Ethanol 70%	■	■	■	■
Isopropanol 100%	■	■	■	■
Glycerol 100%	■	■	■	■
Ketones				
Acetone 100%	■	■	■	■
Solvents Containing Nitrogen				
Urea (8 M)	■	■	■	■
Guanidine HCl (8M)	■	■	■	■
Acids				
Hydrochloric acid (0.1N)	■	■	■	■
Hydrochloric acid (1N)	■	□	□	□
Hydrochloric acid (1N) at 40°C	–	–	–	–
Sulfuric acid (1M)	■	■	■	■
Sulfuric acid (1M) at 40°C	■	■	■	■
Bases				
Sodium hydroxide (0.1)	■	■	■	■
Sodium hydroxide (1N)	■	■	□	■
Sodium hydroxide (1N) 40°C	■	■	□	■
Oxidative Conditions	–	–	–	–
Hypochloric acid				
Hydrogen peroxide				
DTT (10 mM)	■	■	■	■
SDS (1%)	■	–	n.t.	n.t.
Acetonitrile (100%)	■	■	n.t.	n.t.
pH Range	2–13	2–13	2–13	2–13

Conditions: 27 days at room temperature (20–25°C) unless otherwise noted.

■ Compatible
□ Limited compatibility
– Not compatible
n.t. Not tested

7. Ordering Information

	MA Discs[2])			MA Cassettes[2])		
	142 mm Ø	293 mm Ø	pieces/ pack	142 mm Ø[1])	293 mm Ø[1])	pieces/ pack
Effective membrane area	120 cm^2	550 cm^2		600 cm^2	5,500 cm^2	
Sulfonic acid	S--120 S--120B	S--550 S--550B	1 5	S--600X5	S-5500X10	1
Quarternary Ammonium	Q--120 Q--120B	Q--550 Q--550B	1 5	Q--600X5	Q-5500X10	1
Carboxylic acid	C--120 C--120B	C--550 C--550B	1 5	C--600X5	C-5500X10	1
Diethylamine	D--120 D--120B	D--550 D--550B	1 5	D--600X5	D-5500X10	1

Filter Holder

16276-----3	for discs and cassettes	142 mm Ø
16277-----7	for discs and cassettes	293 mm Ø

[1]) 142 mm cassettes contain 5 membrane layers, those with 293 mm diameter contain 10 membrane layers

[2]) Discs and cassettes require use of adjustable filter holders 16276-----3 (142 mm Ø) or 16277-----7 (293 mm Ø)

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