

## **Application Note**

May 04, 2018

#### Keywords or phrases:

Membrane chromatography, Membrane adsorbers, Sartobind®, Capture, Polishing, Capsules, Cassettes

# Sartobind® Membrane Adsorbers for Capture and Polishing

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#### Introduction

Membrane adsorbers in a choice of 4 mm and 8 mm bed heights are designed for flow-through and bind and elute applications as their flow channels have been optimized for the smallest void volume. Such optimization is essential to achieve sharp breakthrough curves, small elution volumes of about two bed volumes (column volumes) and no back mixing of already separated solute samples.

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Table 1: Comparison of bed and void volumes of 4 mm and 8 mm capsules and cassettes. Data were measured using acetone breakthrough curves. The porosity of the membrane is ~80% and is enclosed in the void volume results.

#### 4 mm bed height: Q, S and STIC PA capsules and cassettes Average void volume: 2.8 MV

Bed volume [mL]	1	75	200	400	600	2,500	800
Void volume [mL]	3.5	200	540	1,080	1,600	7,000	2,500
Void volume [MV]	3.5	2.7	2.7	2.7	2.7	2.8	3.1

#### 8 mm bed height: Phenyl, Q and S capsules and cassettes Average void volume: 1.4 MV

Bed volume [mL]	3	150	400	800	1,200	5,000	1,600
Void volume [mL]	4	200	540	1,080	1,600	7,000	2,900
Void volume [MV]	1.3	1.3	1.4	1.4	1.3	1.4	1.8

The capsules and cassettes with a 4 mm bed height are typically used for flow-through applications due to their high flow rate, scalability and use of same materials throughout the product line.

By contrast, the 8 mm capsules and cassettes provide more membrane per adsorber unit for higher binding capacity and smaller void volumes compared with the 4 mm designs (see Table 1). They have only 1.4 membrane volumes (MV) compared with 2.8 MV for the 4 mm series and thus meet the expectations for small elution volumes and peak resolution, as shown later.



**Fig. 1:** The Sartobind® 4 mm and 8 mm portfolio. Top line: bed volume of 8 mm devices; bottom line: bed volume of 4 mm devices

One capsule accommodates two adsorber sizes (4 mm or 8 mm); these adsorbers share the same core. As a result, the space in the 4 mm capsule is filled with additional fleece (Fig. 2).

A direct comparison of the capsule and cassette designs shows same construction principles, bed height and flow scheme. To reduce the internal void volume, the capsules contain a core and the cassettes a spacer element, as shown in Figures 3a and 3b. The sample flows in from the top and across the membrane from the upstream to the downstream channel.

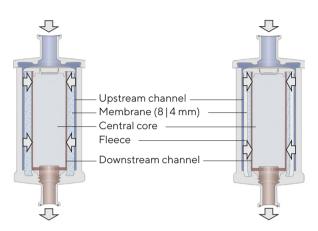


Fig. 2: Cutaway of 150 mL (left) and 75 mL (right) capsule. The direction of flow is indicated by arrows.

## Bind and Flute Performance of Sartobind® Q 1.2 L

Sartobind® Q 1.2 L was loaded with 2 g/L BSA in 10 mM potassium phosphate buffer, pH 7.4, a flow rate of 4 MV (4.8 L)/min (Fig. 4). The complete cycle of loading, washing and elution was achieved within 11 minutes. The profile shows a sharp breakthrough with a small elution volume of about 2 MV (2.4 L) and a dynamic binding capacity of ~22 g.

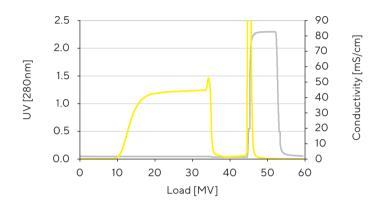


Fig. 4: Breakthrough curve of Sartobind® Q 1.2 L

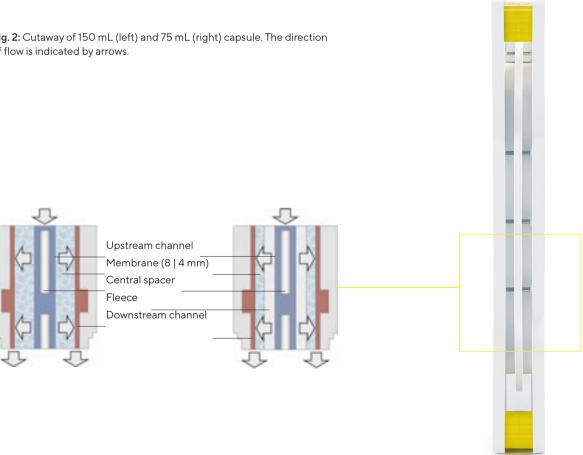


Fig. 3a: Construction and flow paths inside the 8 mm (left) and 4 mm (right)

Fig. 3b: Side view of a Sartobind® cassette

## Scale-Up Performance

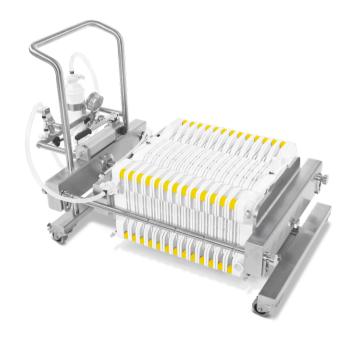
Bovine serum albumin (BSA), 2 g/L, in 20 mM NaCl at a pH of 7.2 was loaded at a flow rate of 5 MV/min each of the Sartobind® Membrane Adsorbers representing the entire scale-up range: Q Nano 3 mL, 150 mL, 1.2 L, Jumbo 5 L and 10 × 1.6 L cassettes. The breakthrough curves were normalized to bed per liter of membrane volume for comparison. The curves show an equivalent shape in their breakthrough behavior (Figs. 5a and 5b) as well as an identical shape in their elution profiles (Fig. 6). The elution volumes were about 2 MV for the 8 mm devices.

The lower void volume of the 8 mm devices enable the preferred applications to be determined:

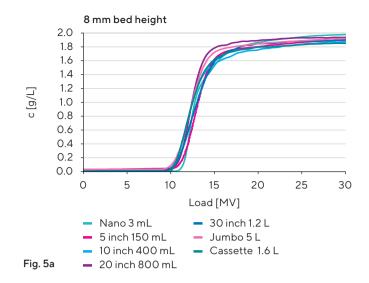
### Recommended Applications:

8 mm: Bind and elute 4 mm: Flow-through polishing

The corresponding results are shown in Figure 5a. This behavior makes the 8 mm capsules more suitable for bind and elute applications than the 4 mm ones, which are more commonly used in flow-through polishing (Fig. 5b).



Pilot-scale filter holder equipped with 13 Sartobind® cassettes



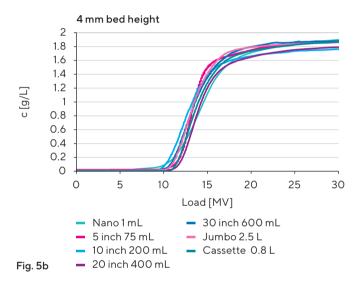


Fig. 5: Overlay of breakthrough curves of void-volume-optimized Sartobind® Q 8 mm (Fig. 5a) and 4 mm (Fig. 5b) capsules and cassettes.

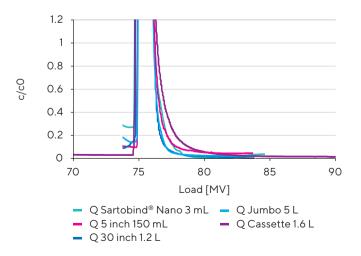


Fig. 6: Elution peak bases of void-volume-optimized Sartobind® Q 8 mm capsules and cassettes.

## Separation of Myoglobin, Cytochrome C and Lysozyme With Sartobind® S Nano 3 ML

Sartobind® Nano 3 mL has been specially designed for analytical protein purification. The flow pattern shows good resolution comparable to chromatographic column technology (Fig. 7). The main difference from columns is the high speed at which purification can be achieved. The flow rate measured in this study was 10 mL/min (3.3 MV/min).

Pre-conditioning	2 M NaCl in equilibration buffer	20 MV
Equilibration	20 mM Tris pH 7.4, 1.8 mS/cm	25 MV
Loading of protein mixture	~ 1-1.5 mg/mL for protein 1, 2 and 3	500 μΙ
Wash	20 mM Tris pH 7.4, 1.8 mS/cm	4 MV
Elution by linear gradient	2 M NaCl in equilibration buffer	16 MV

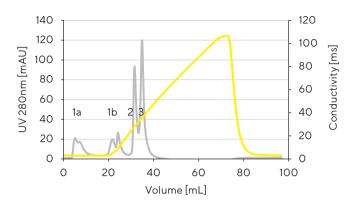


Fig. 7: Separation of myoglobin (1a and 1b), cytochrome C (2) and lysozyme (3) with Sartobind $^{\circ}$  S Nano 3 mL

## Separation of Alpha-Chymotrypsinogen A, Ribonuclease and Lysozyme With Sartobind® S Nano 3 ML

The second example (Fig. 8) displays as well high resolution of three sample proteins within 10 minutes.

Pre-conditioning	2 M NaCl in equilibration buffer	20 MV
Equilibration	20 mM NaAc pH 5.0, 1.1 mS/cm	25 MV
Loading of protein mixture	~ 1-1.5 mg/mL for protein 1, 2 and 3	500 μΙ
Wash	20 mM NaAc pH 5.0, 1.1 mS/cm	4 MV
Elution by linear gradient	2 M NaCl in equilibration buffer	100 MV

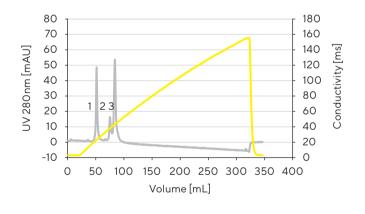


Fig. 8: Separation of alpha-chymotrypsinogen A (1), ribonuclease (2) and lysozyme (3) with Sartobind $^\circ$  S Nano 3 mL

## Summary

Complete Sartobind® capsules with 4 and 8 mm bed height are void-volume-optimized. They show comparable and scaleable breakthrough behavior and elution volumes within each 4 or 8 mm capsule line.

Sartobind® Membrane Adsorbers with 8 mm bed height are versatile as they can be used in flow-through and bind and elute separations. The 4 mm line can be used preferably for flow-through polishing.

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Publication No.: SL-4052-e | Order No.: 85034-536-64 | Status: 06 | 10 | 2021