sartorius stedim biotech

Application Note
Sartobind® Membrane Adsorbers for Capture and Polishing

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.

The capsules and cassettes with a 4 mm bed height are typically used for flow-through applications due to their high flow rate, clear scale 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

Table 1: Comparison of bed and void volumes of optimized 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

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.

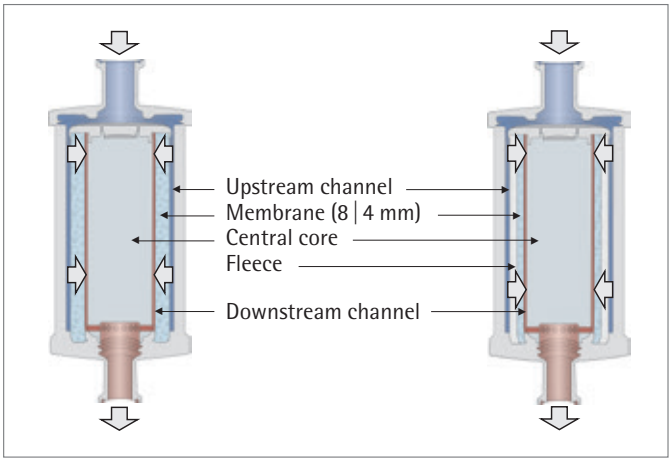


Fig. 2: Cutaway of 150 mL (left) and 75 mL (right) capsule.
The direction of flow is indicated by arrows.

Bind and Elute 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 (5.6 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.

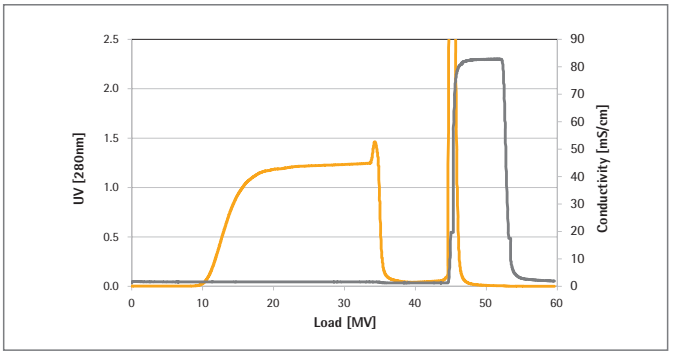


Fig. 4: Breakthrough curve of Sartobind Q 1.2 L

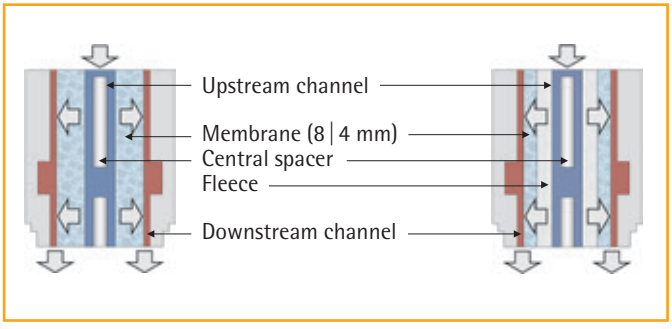


Fig. 3a: Construction and flow paths inside the 8 mm (left) and 4 mm (right) Sartobind cassettes

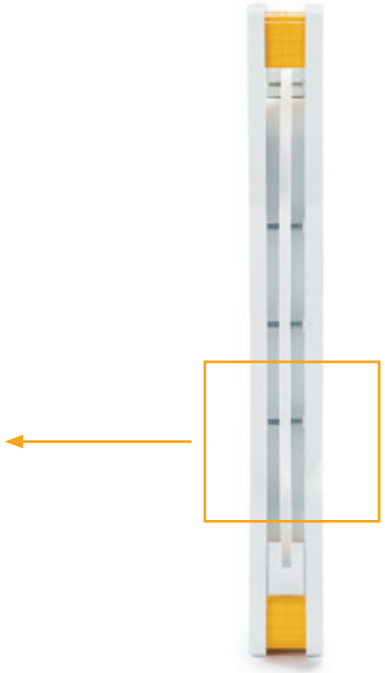


Fig. 3b: Side view of a Sartobind cassette

Scale-Up Performance

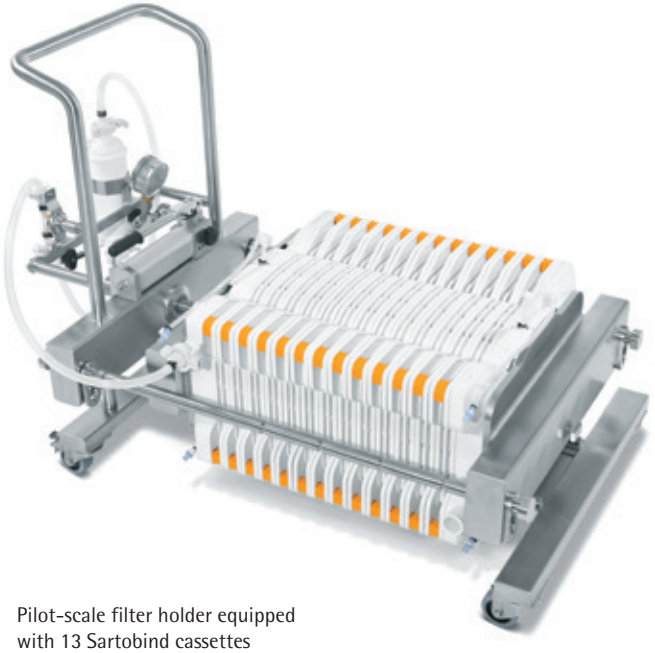
Bovine serum albumin (BSA), 2g/L, in 20 mM NaCl at a pH of 7.2 was loaded at a flow rate of 5 MV/min each of the Sartobind membrane adsorbers representing the entire scale-up range: Q nano 3 mL, 150 mL, 1.2 L, Jumbo 5 L and 10 × 1.6 L cassettes. The breakthrough curves were normalized to bed per liter 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 are 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 ▶ FT 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

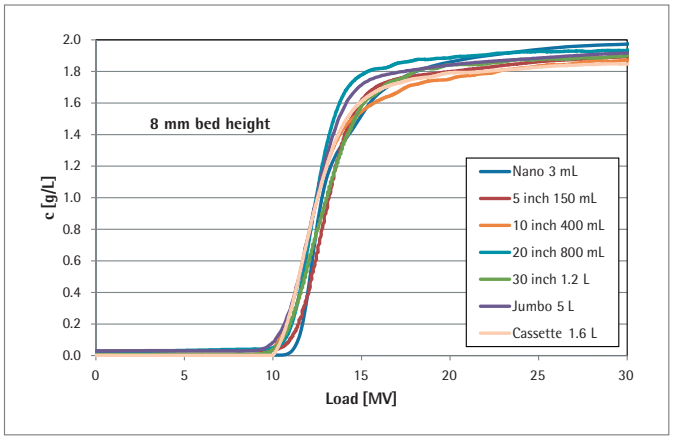


Fig. 5a

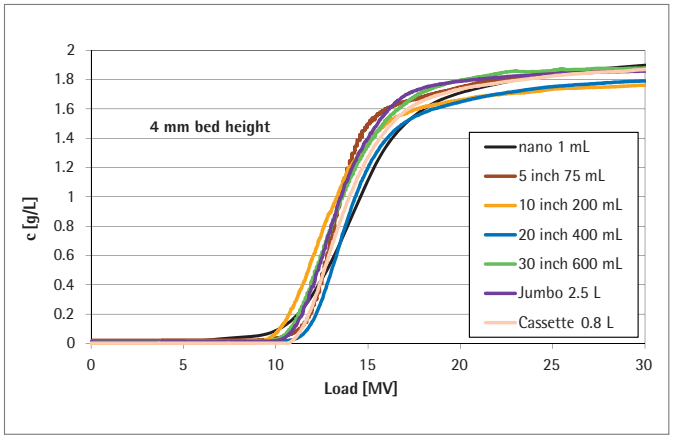


Fig. 5b

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

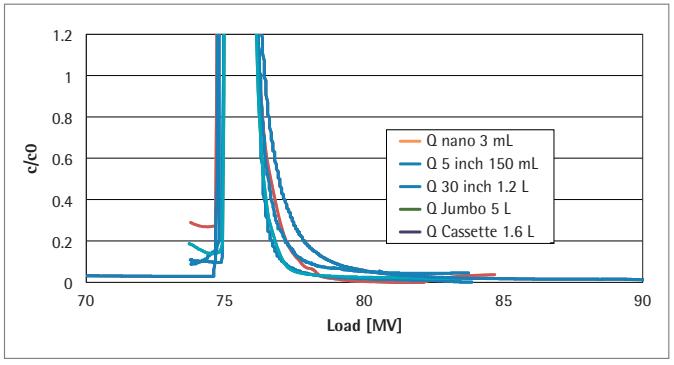


Fig. 6: Elution peak bases of void-volume-optimized Sartobind Q 8 mm capsules and cassettes.

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 µL
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

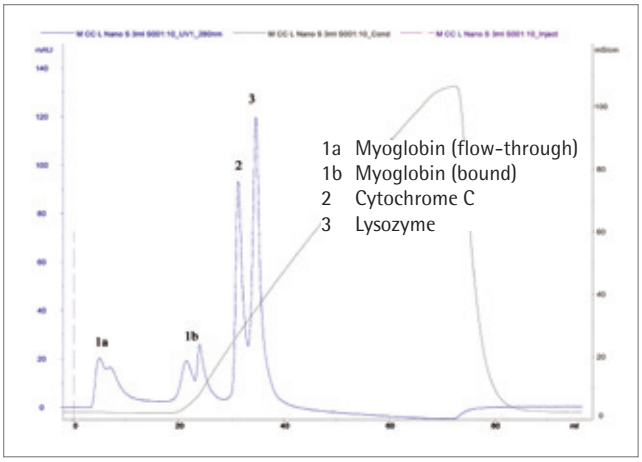


Fig. 7: Separation of myoglobin, cytochrome C and lysozyme with Sartobind S nano, 3 mL

Separation of Alpha-chymotrypsinogen A, Ribonuclease and Lysozyme with Sartobind S nano, 3 mL

The second example (Fig. 8) also shows high resolution of three sample proteins purified 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 proteins 1, 2 and 3	500 µL
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

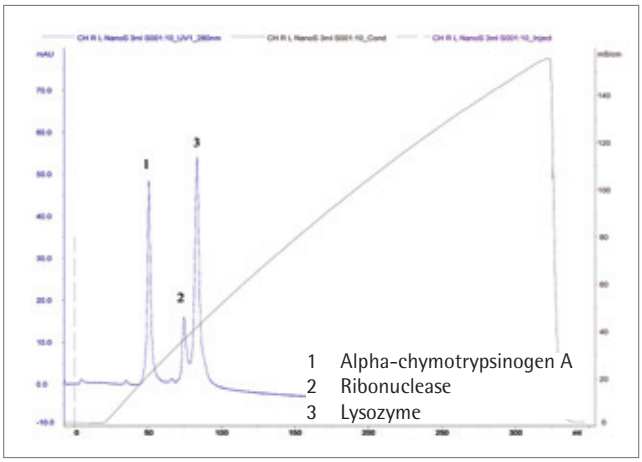


Fig. 8: Separation of alpha-chymotrypsinogen A, ribonuclease and lysozyme with Sartobind S nano, 3 mL

Summary

The complete portfolio of Sartobind capsules and cassettes with 4 mm and 8 mm bed height has been void volume optimized. Theses membrane adsorbers show comparable and scalable breakthrough behavior and elution volumes within each of the 4 mm and 8 mm bed height product lines.

Sartobind membrane adsorbers with an 8 mm bed height are versatile as they can be used in flow-through as well as in bind and elute separations. The 4 mm line is preferable for flow-through polishing.

Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen, Germany

Phone +49.551.308.0
www.sartorius-stedim.com

USA Toll-Free +1.800.368.7178
Argentina +54.11.4721.0505
Brazil +55.11.4362.8900
Mexico +52.55.5562.1102
UK +44.1372.737159
France +33.442.845600
Italy +39.055.63.40.41
Spain +34.913.586.098
Russian Federation +7.812.327.53.27
Japan +81.3.4331.4300
China +86.21.6878.2300

Publication No.: SL-4052-e180504 · Order No.: 85034-536-64 · Ver. 05 | 2018