Using the Sartobind® pico
Optimizing use of micro-scale membrane adsorbers on liquid chromatography systems

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Abstract
While adsorbers and columns share many general rules for chromatographic operation, specific differences in device preparation require attention in order to ensure optimal and trouble-free use of the membrane adsorbers. This is particularly true when using the smallest device sizes, in effect when running membrane micro-scale chromatography. Sartobind pico capsules, with only 0.08 mL membrane volume, were developed to reduce sample consumption during screening and early process development experiments. These micro devices operate at the same high flow rates as larger capsules – typically 30-fold higher than chromatography columns – and can model the same contaminant clearance at high load (i.e. flow through polishing applications). However, the impact of improper preparation and handling are magnified in the micro-scale devices. This application note details 8 steps for the optimal use of Sartobind pico.

Steps for the successful use of Sartobind pico
1. Start with a new | unused pico device
2. Carefully remove air
3. Optional: For virus spiking studies perform integrity test
4. Place an inline pre-filter in front of the adsorber
5. Set the maximum operating pressure to 6 bar
6. Perform a Cleaning In Place (CIP) step with NaOH
7. Pay close attention to proper equilibration
8. Precisely determine void volume of the liquid chromatography system for bind & elute calculations

Keywords or phrases:
membrane chromatography, micro-scale membrane adsorbers, Sartobind pico, optimal use of Sartobind pico, Sartobind nano

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Introduction

Adsorbers and columns share many general rules for operation, as the basics of sample preparation are the same. However, since chromatography membranes are typically run at flow rates up to 30-fold higher than chromatography columns, there are specific differences in preparation that should be noted to assure success when utilizing membrane adsorbers.

First, pre-filtration is of high importance when implementing membrane chromatography. Aggregate formation in the sample can occur even if it has been freshly batched filtered. Pumps, mixers, and valves may also cause aggregation of sample proteins, and therefore it is recommended that a 0.2 μm or a 0.45 μm in-line filter is placed upstream of the adsorber.

Another difference is related to the high loading capacity of membrane adsorbers. For example, during flow-through polishing of monoclonal antibody preparations, up to 20 kg protein can be loaded per liter adsorber. This high loading capacity means that a significantly large amount of sample passes through the adsorber without binding to the membrane while removing contaminating proteins, DNA, endotoxin and viruses.

To reduce the sample consumption for laboratory experiments, Sartorius has introduced the Sartobind pico capsule with 0.08 mL membrane volume (i.e. 2.9 cm² membrane area). This device can be used to model contaminant removal while using a relatively small amount of sample. For example, 20 kg/liter adsorber is equivalent to 1.6 g mAb sample loaded onto Sartobind pico.

As shown below, problems are most likely to arise with the smallest devices. If sample is not a limiting factor, one should consider using a Sartobind nano device instead of Sartobind pico. While each step is still important for larger devices, the impact of improper preparation is magnified with pico. It is also recommended that for scale-up verification, one moves from pico to nano, and then onto the larger devices.

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Air removal

- Pico 0.08 mL
- Nano 1 mL | 3 mL
- Other adsorbers above 3 mL

Fig. 1: Sartobind pico installed on a liquid chromatography system.

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Fig. 2: Cutaway of pico 0.08 mL (4 mm bed height)

1. Start with a new | unused device
Sartobind capsules can often be reused, but it is recommended that initial screening should be performed with a new, unused device. A new device is best for establishing parameters. In the laboratory, re-use is a common practice and is also possible for most membranes dependent on ligands. Reusability is the nature of the sample, its preparation, as well as the membrane ligand chemistry. If after regeneration the binding capacity and flow rate are restored, then there should not be any issues with reuse. If flow rate and binding capacity are not restored, it is recommended to use a new, unused device each time. Keep in mind that unacceptable results may due to a micro-bubble or debris that were not removed during cleaning and regeneration. These issues can carry over from the very first cleaning cycle and for these reasons it is recommended that Sartobind capsules should be implemented as single-use products whenever possible, as this minimizes validation steps. Sartobind STIC® should always be considered a single-use product, as it is difficult to regenerate the ligand and restore the flow rate and binding capacity to original levels. For an example of reuse for Sartobind Q, please see our Application note “1000 cycles constant binding capacity” (order no. 85034-535-70).
2. Carefully remove air
Just as with column chromatography, failing to implement an inline pre-filter and improper air removal are major sources of increased pressure and poor binding capacity. The difference is that air can be removed from the adsorber very simply. If even a small amount of air remains in the device it will operate, but binding capacity may be reduced by 90% and pressure will likely increase.

To remove air from the pico use a syringe to push buffer in the direction of flow (marked with an arrow) until a liquid begins to emerge from the outlet. Uncouple the syringe from the device and attach it to the other end. Push buffer in the opposite direction until liquid emerges from that outlet. Using this technique, air should be completely removed from the device.

3. Optional: For virus spiking studies perform integrity test
Membrane chromatography is attractive as virus removal step because the high loading capacity allows very rapid processing. The open porous structure allows virus particles easy access to binding sites. This high loading capacity also makes it very expensive to perform virus spiking studies because of the large amount of material needed to simulate the process. The smaller sample volume makes the test much less expensive to run, yet the results are still comparable to larger devices. Due to the cost and critical nature of virus spiking studies, it is important to assure that the device is functioning properly. Sartorius recommends to perform an integrity test for confirming proper function before use (Operating Instruction Diffusion test Sartobind pico order no.: 85032-542-96).

The pico device has a membrane stack (see Fig. 2), which differs from the nano and all other larger devices that utilize a membrane “roll” (Fig. 3). This stack is sensitive to pressure pulses that exceed 0.6 MPa (6 bar, 87 psi). The probability of exceeding the maximum pressure on a liquid chromatography system is relatively high and a pressure spike can damage the device. Therefore, a diffusion test that can detect a bypass of the membrane of even <0.1% can be utilized post use (above mentioned operation instruction, Diffusion test Sartobind pico). This is the same test recommended for large scale adsorbers at manufacturing scale.

4. Place an inline pre-filter in front of the adsorber
A major difference between adsorbers and columns is the high flow rate achievable with membranes during operation. Any aggregate or particulate matter in a sample must be removed when using adsorbers, as particles will be forced into the pores of the first membrane layer resulting in a quick rise in pressure. Users often filter the protein sample before use with either a 0.2 or 0.45 micron filter (for virus spikes). However, even in the few minutes between filtration and beginning the membrane chromatography step protein aggregation may begin and cause a quick pressure increase seen in Fig. 4.

![](image)

**Fig. 4:** Monoclonal antibody (mAb) up to 10 g IgG per mL membrane loaded on a pico with and without an inline filter. Flow rate 12.5 MV (membrane volumes)/min, c = 10 g/L mAb, pH 7.0, ~8 mS/cm, mAb solutions were both pre-filtered before the experiment with 0.2 μm filters, Adsorber: Sartobind STIC PA pico.

One reason pressure can increase is that aggregates of protein or virus in the feed material can form, due to the shear force from the pumps, valves, and mixers of the LC system. To prevent membrane blockage by aggregates, an NaOH stable inline pre-filter can be used with a liquid chromatography system (Fig. 5). If an increase in pressure becomes evident during use, the pre-filter should be replaced with a fresh filter. The pressure should then return to starting values.

![](image)

**Fig. 5:** Liquid chromatography system with two pumps for gradient elution followed by a mixer. The pre-filter is fit directly to the pico unit.
5. Set the maximum operation pressure to 6 bar
To avoid membrane damage due to high pressure, set the pressure within the limit value for pico (0.6 MPa, 6 bar, 87 psi). A reason of pressure increase can be aggregates (see Chapter 4).

Another cause of pressure increase can be a flow restrictor: The flow restrictor prevents bubble formation due to outgassing when the LC system is used with conventional columns. Bubble formation is not an issue when using adsorbers and so the flow restrictor should be removed to prevent unacceptable pressure levels when running the device at a high flow rate. Removal of the flow restrictor is described in the operator’s manual for the LC system.

Figure 6 demonstrates the effect of the flow restrictor on system pressure. Before starting the experiment, a solution of bovine serum albumin was saturated with air. One experiment was performed with the flow restrictor in place, and the other without it. The pressure of the system with the restrictor in place is nearly 2.5 bar above the level of the LC system without a restrictor.

Figure 6: Loading an air-saturated BSA solution on an LC system with and without a flow restrictor in place. Membrane adsorber: Sartobind STIC PA pico. Loading volume: 80 mL, (1000 membrane volumes and equivalent to 5 kg protein/liter membrane) pre-filtered at 0.2 μm, flow rate 12.5 MV/min, buffer Tris/HCl pH 7.4, ~16 mS/cm. 0.2 μm in-line filter.

A pressure increase can also be caused by low temperatures. Measurements on Sartobind S nano 3 mL have shown that the pressure drop increases about 20% when the temperature drops from room temperature (21.5 °C) to cold room temperatures (8°C).

6. Perform a Cleaning In Place (CIP) step using NaOH
Adsorbers are manufactured in a controlled, clean environment but are not supplied sterile. Membranes are dried from a water | glycerol solution to prevent the membrane from drying out before use. To remove any bio-burden and traces of glycerol, a cleaning step with sodium hydroxide is recommended. Treatment with NaOH is the standard cleaning method for both membrane adsorbers and chromatography columns. Using a pico without NaOH treatment is possible, but not recommended. If scale-up to a production process is intended, a CIP step will be required; therefore, performing a CIP step with the pico should be performed to achieve comparable and scalable results. The standard cleaning procedure is described in the manual. Before starting the cleaning step, pre-filter shall be connected directly in front of the pico device (see Chapter 5. about pre-filter).

7. Pay close attention to proper equilibration
The standard recommendation for Sartobind pico devices is to flush with a minimum of 100 membrane volumes of equilibration buffer. More buffer may be required depending on your LC system. This is due to the hold-up volume of the typical liquid chromatography system. The correct way to equilibrate the pico device is to continue flushing until the pH and conductivity of the effluent is equal to that of the equilibration buffer. When properly prepared, pressure increase correlates to flow rate as shown in Figure 7. The amount and the character of the protein can have an effect on the flow rate however. Loading up to 20 kg IgG per liter membrane in Tris buffers can be achieved as seen in Figure 8.

Figure 7: Pressure flow rate correlation with Tris buffer. Flow rate expressed in membrane volume (MV) per minute*bar.

Figure 8: A flow-through loading step of gamma globulin solution onto a Sartobind STIC pico. Protein up to 20 kg per liter membrane in 20 mM Tris/HCl ph 7.4, 10 mS/cm, inline prefiltered with 0.2 μm.
8. Precisely determine void volume of the liquid chromatography system for bind & elute calculations

The calculation of the dynamic binding capacity at 10% breakthrough (DBC10%) is accomplished using the following formula:

\[
\text{DBC10\%} \left( \frac{\text{mg/cm}^2}{\text{LV}_{10\%}} \right) = \frac{(\text{LV}_{10\%} - \text{VVd}) \times C_{\text{Load}}}{A_{\text{Membrane}}}
\]

Where \(\text{VVd}\) is the void volume of the LC system, \(C_{\text{Load}}\) is the concentration of the protein to be bound (in the case of a polishing application, this is the concentration of the contaminants to be removed), and \(\text{LV}_{10\%}\) is the volume of sample that must be loaded before achieving 10% breakthrough. \(A_{\text{Membrane}}\) is the membrane area (1 mL = 36.4 cm², 0.08 mL for the pico = 2.9 cm²).

In Figure 9 to 11, the DBC10% values according to the system void volume deviation of ± 0.5 mL are presented. As can be seen in Figure 9, when the loading volume is large, the void volume of the system plays almost no role in the calculation. This is typically the case in a flow-through polishing step where the contaminants are at a very low concentration in a comparably large volume. Even when a pico device with its 0.08 mL volume is used, the void volume of the system has little effect on the calculation of dynamic binding capacity.

It can be observed that an error in void volume determination is having a large impact in bind and elute applications. In this case, the target molecule is at a high concentration and therefore the loading volume is usually much smaller than in flow-through applications. Any error is also magnified when using pico, due to the small membrane area. This is demonstrated in Figure 10, where an error in the void volume calculation of just 0.05 mL will change the dynamic binding calculation by ± 2%.

Using a Sartobind nano 1 mL in the same experimental setup, the determination of the void volume is of much less significance. This is demonstrated in Figure 11. Void volume determination is not as critical since the combined void volume of the LC system (1.3 mL) and the nano device (5 mL) is small compared to the volume of sample applied (30 mL). The ease of calculation and direct scalability of the nano makes it a much better choice for bind and elute applications. Note that for bind and elute applications, typically the nano 3 mL is recommended as this device has undergone a void volume optimization which allows for sharp elution peaks and larger usage of dynamic binding capacity. The 3 mL nano with 8 mm bed height has the void volume of 4 mL.
In general, when the load volume is small compared to the membrane matrix volume, and the protein concentration is high, a 1 mL or 3 mL nano device is preferred to the pico. A summary of the effect of errors in determining void volume is provided in Figure 12.

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<td>Binding capacity determination capture</td>
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- ▼ Pico 0.08 mL
- ▼ Nano 1 mL | 3 mL
- ▼ Other adsorbers above 3 mL

Unexpected low binding and pressure problems when using a pico device can be avoided by proper sample preparation and LC system setup. It may also be worthwhile to switch to a nano 1 mL device, which is approximately the size of a small chromatography column and is not affected by sample preparation as much as is a pico capsule.

![Image of Sartobind nano](image.png)

Fig. 12: Sartobind nano installed on a liquid chromatography system. Nano devices can be used to confirm experimental data gathered with the pico. The larger size (1 mL for polishing or 3 mL for capture or polishing) minimizes the effects of void volume determination and handling errors due to its larger bed volume.

## Conclusion

- Start with a new device. Reuse the Sartobind pico only if regeneration returns the binding capacity and flow rate to acceptable levels.
- Complete removal of air is important for proper functioning.
- It is crucial to perform an integrity test before a virus removal.
- Cleaning in place with NaOH is recommended to remove residual glycerol and any bio-burden.
- Even if pre-filtration of the sample is performed immediately before loading onto a liquid chromatography system, aggregates can still form due to a high protein concentration or the share force of pumps and mixers. These aggregates must be removed using an inline filter upstream of the pico device to avoid clogging the membrane.
- Set the maximum operating pressure to 6 bar. Removing the flow restrictor and simplifying the flow path of the LC system will help to avoid problems with pressure.
- Equilibration of a pico device is best confirmed with pH and conductivity measurements.
- Correct determination of the system void volume is not critical. However, correctly determining the system void volume is absolutely essential when modeling a bind and elute application. In flow-through polishing applications however, small deviation of the determined void volume from the real one is not critical.
- Unexpected low binding and pressure problems can be avoided by switching to a nano device, which is not affected by sample preparation as much as a pico capsule.