SARTORIUS

Application Note

October 3, 2022

Keywords or phrases:
Regeneration, Binding Capacity, Ion Exchange Chromatography, Membrane Adsorbers, Sartobind® Lab

Sartobind® Lab Q Membrane Adsorbers Provide Constant Binding Capacities Over 1,000 Cycles

Dr. Ricarda Busse[1], Dr. Miyako Hirai[1], Dr. Carsten Voss[1], Dr. John Cashman[2]*

[1] Sartorius Stedim Biotech GmbH. August-Spindler-Straße 11, 37079 Göttingen, Germany

[2] Sartorius UK Ltd., Blenheim Road, Epsom, KT19 9QQ, United Kingdom

* Correspondence

E-Mail: john.cashman@sartorius.com

Introduction

Sartobind® Q membrane adsorbers carry conventional quaternary ammonium groups linked to a cellulose backbone. We performed a test to examine the stability of these devices over hundreds of chromatographic cycles. With each 100th cycle a NaOH regeneration step was performed. The aim of this study was to provide evidence that membrane chromatography can be used over 1,000 cycles with no loss of binding capacity. An SDS gel proved the function of Sartobind® Q in the final cycles.

Find out more: www.sartorius.com/en/products/lab-filtration-purification/membrane-chromatography

Materials and Methods

A liquid chromatography system with gradient pumps was used. All buffers were autoclaved at 121 °C for 30 min prior use. Bovine serum (prepared by Sartorius Stedim Biotech from bovine blood using 0.65 µm cross flow cassettes and stored frozen) was thawed and diluted 1:20 with 0.05 M Tris-HCl pH 8.8, 10 mM NaCl and filtered through a 0.2 µm Minisart®. Only the amount of serum needed for one day was prepared every morning.

Directly in front of the Sartobind® Lab Q 75 device, a 5 µm Minisart® filter was connected via Luer lock. This filter was replaced when pressure increase was observed. At a flow rate of 10 mL/min, 52.5 mL of sample was applied to the Q 75 so that a two step breakthrough curve could be recorded. The first step was the unbound IgG and minor components, while the second was the BSA (data not shown). The Q 75 was washed with 15 mL buffer at a flow rate of 10 mL/min and BSA was eluted with 10 mL of 1 M NaCl in 0.05 M Tris-HCl pH 8.8 buffer. During elution, only the BSA peak was collected and stored for subsequent analysis.

The Q 75 was washed with 10 mL of equilibration buffer and the next cycle started. After every 100 cycles the unit was regenerated by applying 50 mL of 1 M NaOH, incubating for 30 minutes, then washing with buffer to the starting conditions. 1,000 cycles were performed in about 8 working days.

The eluates were diluted 1:5 with elution buffer, mixed thoroughly and protein content determined by UV-vis spectrophotometry, measuring absorbance at 280 nm against elution buffer. Selected eluates were also assessed for purity by PAGE. Samples were diluted 1:50 with Laemmli buffer prior to loading 10-20 µL aliquots onto the gels. Proteins were visualized by coomassie staining after electrophoresis.

Results

The amount of eluted protein was plotted against the cycle number to generate Figure 1. These data demonstrate a highly consistent binding capacity of around 0.6 mg BSA per square centimeter of membrane area, over 1,000 cycles. Coomassie staining of polyacrylamide gels showed high purity of BSA could be achieved even after hundreds of cycles (Figure 2).

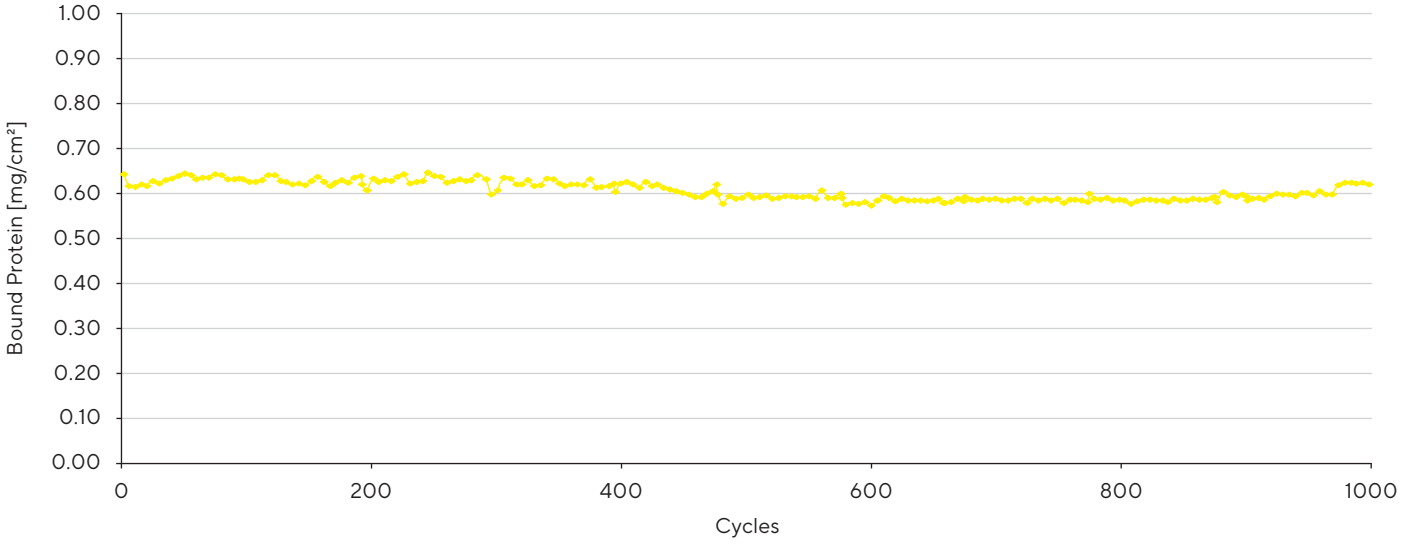
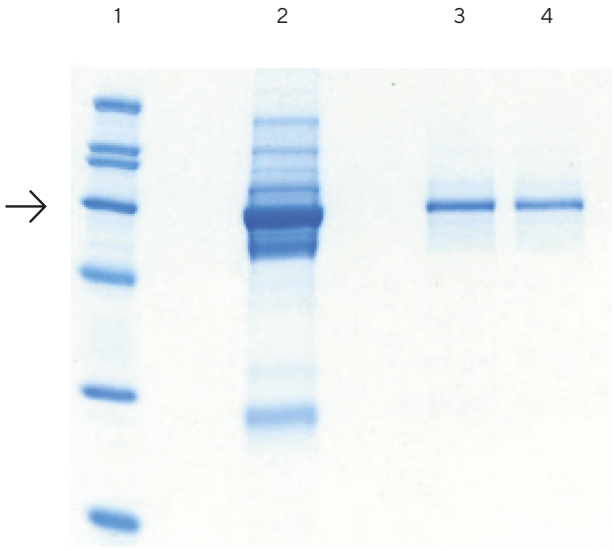


Figure 1: Constant binding capacity for bovine serum albumin over 1,000 cycles with a single Sartobind® Lab Q 75 membrane adsorber



Lane 1: Molecular weight marker:
200, 116, 97, 66 (arrow),
45, 31 and 21.5 kDa

Lane 2: Initial serum sample

Lane 3: Cycle 999 eluate

Lane 4: Cycle 1,001 eluate

Figure 1: PAGE analysis of BSA purified following 999 and 1,001 cycles of a Sartobind® Lab Q 75 membrane adsorber

Conclusion

In this study, we have presented the reusability of Sartobind® Lab Q 75 membrane adsorbers. Our results show that these devices exhibit highly consistent binding capacities while yielding high purity eluates, even after 1,000 cycles. This makes Sartobind® Lab an economical choice for any research laboratory to perform frequent, reproducible, and reliable protein purifications.

Note

Literature published up to c.2022 may reference the use of Sartobind® MA, which is a name previously used for the Sartobind® Lab membrane adsorbers. When these devices were renamed, there was no change made to fit, form or function. Therefore, results collected and methods established using Sartobind® MA devices remain valid also for Sartobind® Lab.

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Straße 11
37079 Göttingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For further contacts, visit
www.sartorius.com

Publication No.: SL-1069-e | Order No.: 85034-535-70 | Status: 10 | 03 |2022