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Application Note

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1000 Cycles Constant Binding Capacity

Sartobind® Q Anion Exchanger

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Introduction

Sartobind® Q membrane carries conventional quaternary ammonium groups linked to the cellulose backbone. A test for cycle stability has been performed to examine the number of possible chromatographic cycles with each 100th cycle a NaOH regeneration step was performed. The aim of this study was the supply of evidence that membrane chromatography can be used over 1000 cycles. An SDS gel proved the function of Sartobind® Q in the last cycles.

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 with 0.05 M Tris-HCl pH 8.8 + 10 mM NaCl 1 : 20 and filtered through 0.2 µm Minisart®.

Only the amount for 1 day was prepared every morning. Directly in front of the Q 75 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, the second the BSA. 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. From this fraction only the BSA peak was collected and stored.

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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 1 N NaOH for 30 min incubation time, then washed with buffer to start conditions. 1000 cycles were performed in about 8 working days.

The eluates were mixed thoroughly and measured against elution buffer at 280 nm in a photometer after dilution 1 : 5 with elution buffer. The amount of eluted protein was plotted against the run number to generate the figure 1. Aliquots of the eluates were diluted 1 : 50 with Laemmli buffer. 10 and 20 µL respectively were applied to a 12% Tris SDS gel (see figure 2).

Equilibration | wash buffer	0.05 M Tris-HCl pH 8.8, 10 mM NaCl
Elution Buffer	0.05 M Tris-HCl pH 8.8, 1 M NaCl
Regeneration	1 N NaOH 30 min
Flow rate	10 mL/min

Results

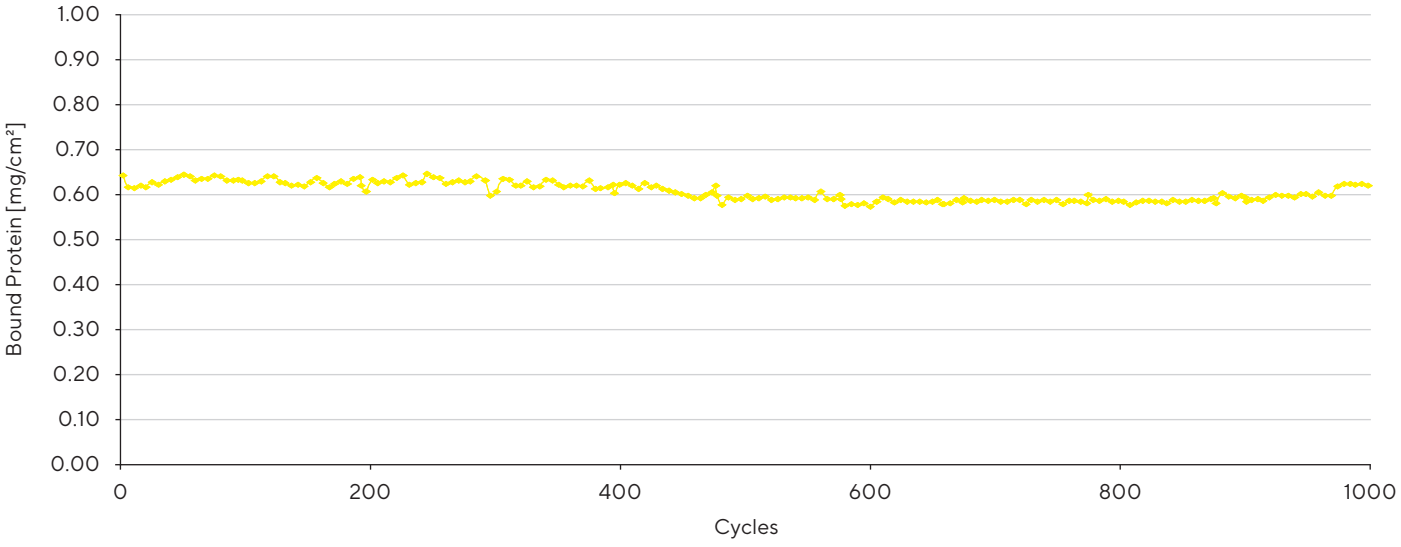
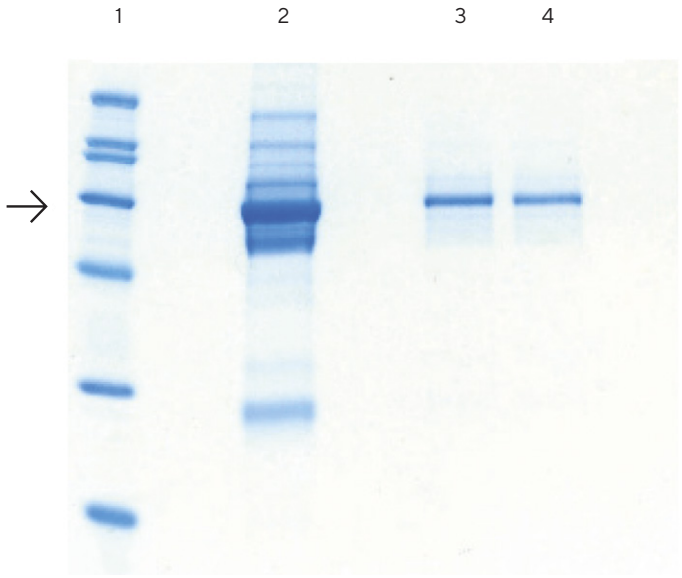


Fig. 1: Constant capacity of bovine serum albumin over 1000 cycles



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

Line 2 Serum

Line 3 cycle 999 elution

Line 4 cycle 1001 elution

Fig. 2: SDS gel

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