SARTORIUS

Application Note

November 30, 2010

DNA Binding On Sartobind STIC® PA Compared to Sartobind® Q

Salt Tolerant Membrane Chromatography

Dr. Miyako Hirai, Dr. Carsten Voss
Sartorius Stedim Biotech GmbH. August-Spindler-Straße 11, 37079 Göttingen, Germany

Correspondence
E-Mail: miyako.hirai@sartorius.com, carsten.voss@sartorius.com

Introduction

One of the key applications for membrane chromatography is contaminant removal in flowthrough mode. Anion exchange by Sartobind® Q membranes is proven to remove DNA below the detection limit at process conditions[1]. The present study shows that Sartobind STIC® PA with its primary amine ligand is much less susceptible to decreasing capacities at high salt conditions than the Q (quaternary ammonium) matrix. The measurements have been performed in 96 well plates by vacuum manifold or at a liquid chromatography system in a membrane holder.

1. Screening Of Salt and pH Conditions for DNA Binding With 96 Well Plates (High DNA Concentration)

The influences of salt and pH conditions for DNA removal were evaluated using Sartobind STIC® PA and Q membranes. Due to the 96 well plate format, the sample volume and analysis time could be reduced considerably.

Materials and Methods

Device format	96 well plate
Membrane Types	Sartobind STIC® PA and Q
Membrane area	1 cm²/well
Membrane volume	0.028 ml
Membrane layers	3
Conditioning	0.5 ml/well 1 M NaCl
Equilibration buffer	Bis Tris 20 mM, pH 6 and 7 Tris 20 mM, pH 8 and 9
Equilibration volume	1 ml/well
Binding buffer	Each equilibration buffer with 0, 300, 500 and 650 mM NaCl (approx. 0.8, 6.5, 20 and 36 mS/cm respectively)
DNA sample	Salmon sperm DNA (500 - 1000 bp)
DNA concentration (c)	~145 µg/ml
DNA loading volume (V)	1.0 ml per well
Plate reader	Tecan Safire (Tecan Group Ltd. Männersdorf, Switzerland)
Robot liquid handling system	Lissy 2002 (Zinsser, Frankfurt, Germany)

In total, 32 conditions (4 different salt concentrations at 4 different pH for two types of membranes) with each 4 parallel samples were tested using automatic robot system. The amount of DNA loaded was chosen so that a breakthrough could be observed. Salmon sperm DNA concentration in the flowthrough fraction was determined at 260 nm by automatic sampling.

Results

Figure 1 shows the influence of sodium chloride concentration and pH conditions for DNA binding to anion exchanger membranes. Sartobind STIC® PA bound stable salmon sperm DNA at pH 6–9 in the entire NaCl concentration range (0 to 650 mM). Sartobind® Q shows breakthrough at 300 mM NaCl. Small working volumes, an automatic liquid handling system and high flow rates allowed the testing of up to 96 conditions in less than 1 hour.

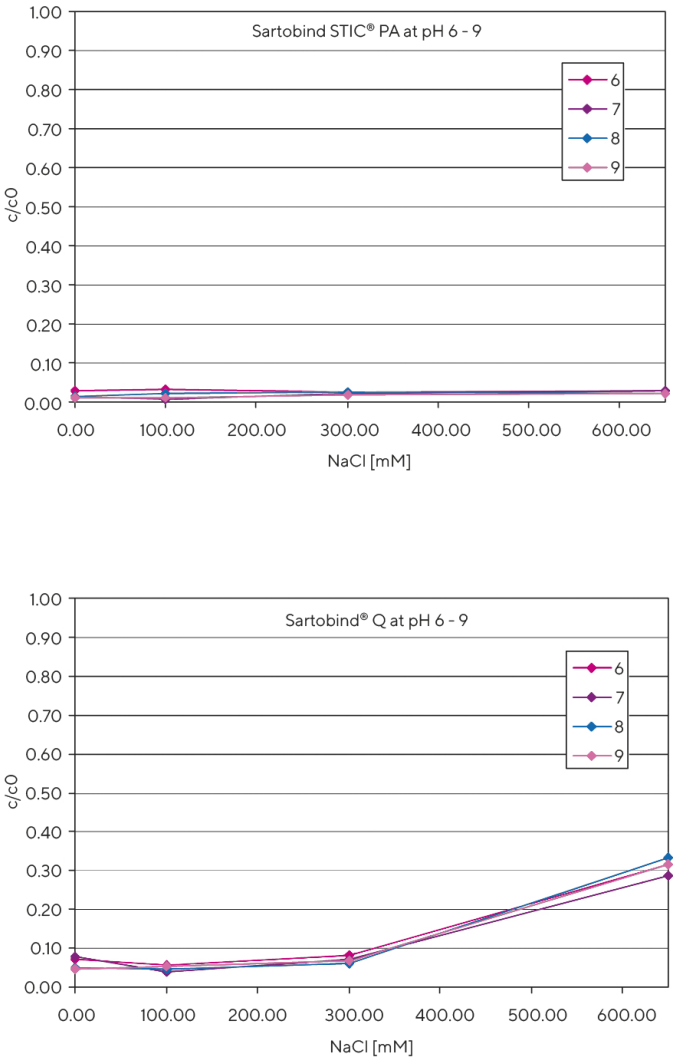

Fig. 1: Breakthrough performance of DNA of Sartobind STIC® and Q at different salt and pH conditions (high DNA concentration)

2. Screening Of Salt and pH Conditions for DNA Binding With 96 Well Plates (Low DNA Concentration)

In this experiment, large DNA fragments in low concentration were used at different pH and NaCl concentrations in comparison to the first experiment.

Materials and Methods

Equilibration buffer	20 mM NaAc pH 4.5 and 5.5 20 mM Bis Tris 6.5; 20 mM Tris 7.5 and 8.5 20 mM CHES pH 9.5
Binding buffer	Each equilibration buffer with 0, 300, 500 and 1000 mM (approx. 1.1, 26, 41 and 75 mS/cm)
DNA sample	Calf thymus DNA (up to 24000 bp)
DNA concentration (c)	~100 ng/ml

Refer to 1.2 for other materials.

Because of a very low DNA concentration, PicoGreen standard assay (PicoGreen dsDNA Quant-iT P7581 Reagent, Life Technologies, Carlsbad, USA) was used for DNA analysis. The sampling was performed manually and for the assay, 96 well micro plates (Greiner Bio-One International AG, Austria) were used. Breakthrough was calculated by comparing the PicoGreen signal of each stock solution and flowthrough. Detection limit of the PicoGreen Assay was ~1 ng/ml.

Results

As in the first trial with smaller DNA in higher concentration, no breakthrough was found on Sartobind STIC® PA in the whole range of pH (4.5–9.5) and NaCl concentration (0–1000 mM). On Sartobind® Q, breakthrough was seen at 500 mM NaCl compared to the first trial.

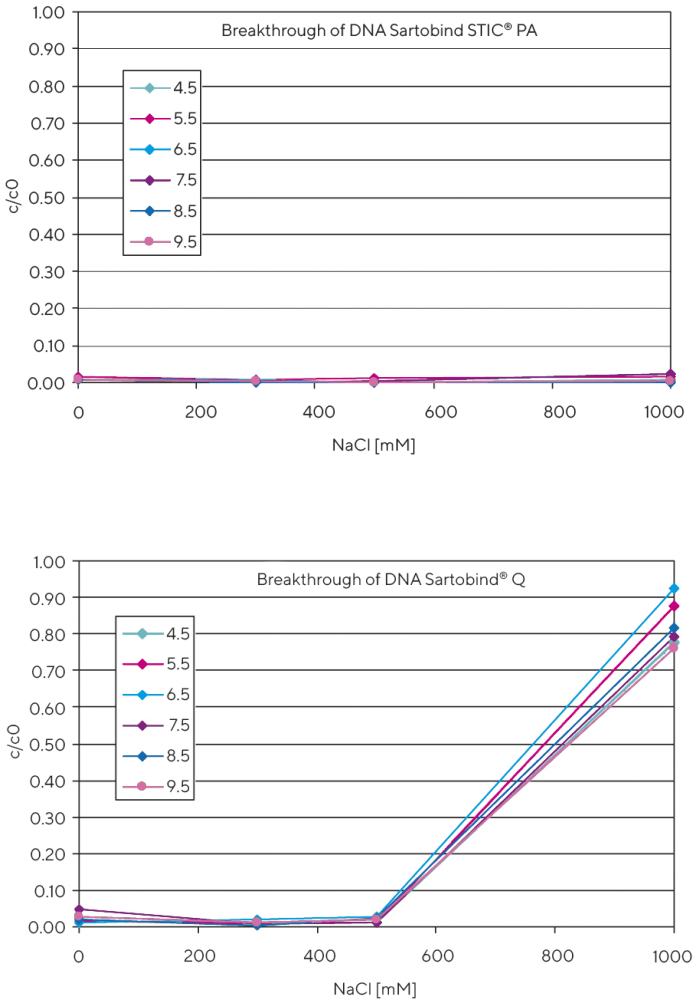

Fig. 2: Breakthrough performance of DNA of Sartobind STIC® and Q at different salt and pH conditions (low DNA concentration)

3. DNA Binding Capacity Measurement at LC System

The DNA binding capacity at 10% breakthrough for both Sartobind STIC® PA and Q membranes was measured using a LC system.

Materials and Methods

Device format	Membrane discs in a holder
Membrane area	15 cm² (5 cm² each layer)
Membrane layers	3
Binding buffer	20 mM Tris/HCl pH 7.2, 150 mM NaCl
DNA sample	Salmon sperm DNA (500 - 1000 bp)
LC system	Akta explorer (GE Healthcare)

The flow rate was 10 ml/min. After equilibration with 10 ml buffer, salmon sperm DNA binding capacity at 10% breakthrough was determined at 260 nm.

Result

The binding capacity for Sartobind STIC® PA was 10.9 and for Q 7.3 mg/ml in presence of 150 mM NaCl.

Summary

Sartobind 96 well plate membrane devices are effective tools for rapid process development. DNA removal using Sartobind STIC® PA membrane is almost independent of NaCl concentration up to 1 M NaCl.

Reference

[1] Joachim K. Walter, Boehringer Ingelheim Pharma KG, Strategies and Considerations for Advanced Economy in Downstream Processing of Biopharmaceutical Proteins. In: Bioseparation and Bioprocessing; G. Subramanian, (Ed.), Processing, Quality and Characterization, Economics, Safety and Hygiene, Wiley VCH, 1998, vol. II, pp.447-460

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

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For further contacts, visit
www.sartorius.com

Specifications subject to change without notice.
© 2021 Sartorius Stedim Biotech GmbH, August-Spindler-Strasse 11, 37079 Goettingen, Germany

Publication No.: SL-4058-e | Order No.: 85032-538-43 | Status: 05 | 27 | 2021