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Application Note

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Effect of Phosphate on Binding to Sartobind STIC® PA

Salt Tolerant Membrane Chromatography

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Introduction

Sartobind STIC® membranes with primary amine (PA) ligands are used during the downstream processing of recombinant proteins for the removal of contaminants such as DNA, host cell proteins, endotoxins and viruses. Typically, phosphate buffers should not be used with this Membrane Adsorber as the ligand is already known for its ability to bind to polyvalent anions such as phosphate[1].

The present study, however, shows the influence of various phosphate concentrations on the binding of proteins and DNA to Sartobind STIC®. A 96 well plate Sartobind STIC® PA membrane was loaded with bovine serum albumin (BSA) or DNA and challenged with different phosphate concentrations to quantify the influence on binding. The tests for BSA binding also utilized different sodium chloride conditions.

1. BSA Binding On Sartobind STIC® PA

Materials and Methods

Membrane	Sartobind STIC® PA
Ligand	Primary amine (PA)
Membrane area (A)	0.7 cm² | well
Number of membrane layers	3
Binding buffer	20 mM Tris | HCl pH 7.6 0 – 10 mM NaH_2PO_4 0 – 300 mM NaCl
Load volume (V)	1.4 mL/cm²
BSA concentration (c)	0.8 mg/mL
BSA Load	1.2 mg/cm²

A 96 well plate (Sartorius Stedim Biotech GmbH, Goettingen, Germany) containing 0.7 cm² membrane in 3 layers per well was used in this study for the screening. The plates were built up from 12 individual 8-strip units assembled into a 96 well frame. The plates were processed by vacuum using the Vac96 vacuum manifold (Sartorius Stedim Biotech GmbH, Goettingen, Germany) supported by a robotic liquid-handling system (Zinsser Analytic GmbH, Frankfurt, Germany).

Concentration of the initial solutions (c_{Load}) and each flowthrough sample ($c_{Flowthrough}$) were measured by a Safire™ plate reader (Tecan Group Ltd., Männerdorf, Switzerland) at 280 nm. The binding capacity (BC) was calculated for each condition using the following formula:

$$BC = \frac{(C_{Load} - C_{Flowthrough}) \times V}{A}$$

Results

For each condition, four parallel runs were performed using BSA as a test protein. The results presented in Fig. 1 display the binding capacity relative to the maximal binding capacity determined at 0 mM NaH_2PO_4 and 0 mM NaCl in 20 mM Tris | HCl pH 7.6.

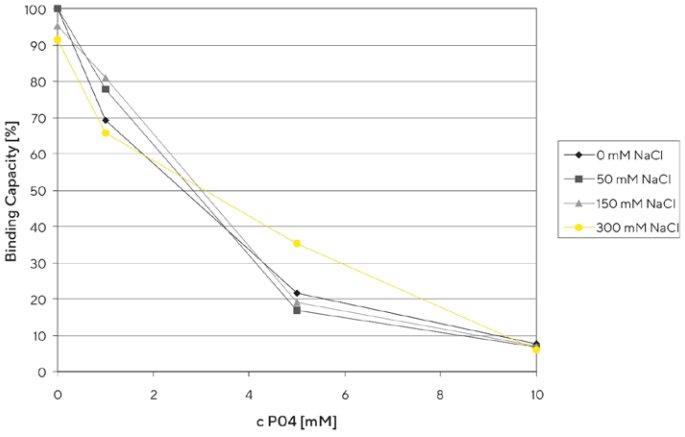

Fig. 1: Influence of phosphate on BSA binding at different NaCl concentrations. Binding capacities are indicated in relation to the maximum values.

2. Effect of Multivalent Ions on DNA Binding Using Sartobind STIC® PA²

Materials and Methods

Sartobind STIC® PA 96-well plate buffers	20 mM Bis-Tris, pH 6 and 7 20 mM Tris, pH 8 0 – 150 mM NaH_2PO_4
Equilibration volume	2.0 mL/well
Load volume	1.0 mL/well
DNA load	140 µg/cm², 98 µg/well

The plates were processed by vacuum using the Vac96 vacuum manifold (Sartorius Stedim Biotech GmbH, Goettingen, Germany) supported by a robotic liquidhandling system (Zinsser Analytic GmbH, Frankfurt, Germany). Three samples were analyzed for each condition tested. The DNA concentration was measured at 260 nm.

Results

Figure 2 shows the effect of sodium phosphate on the binding of DNA to Sartobind STIC®. Results are shown as contour plots. Gradation of the color scheme is 5 µg/cm².

The binding of DNA on STIC PA at various phosphate concentration up to 150 mM (pH between 6 and 8) was strong enough to remove DNA selectively from the protein which was collected in the flow-through effluent.

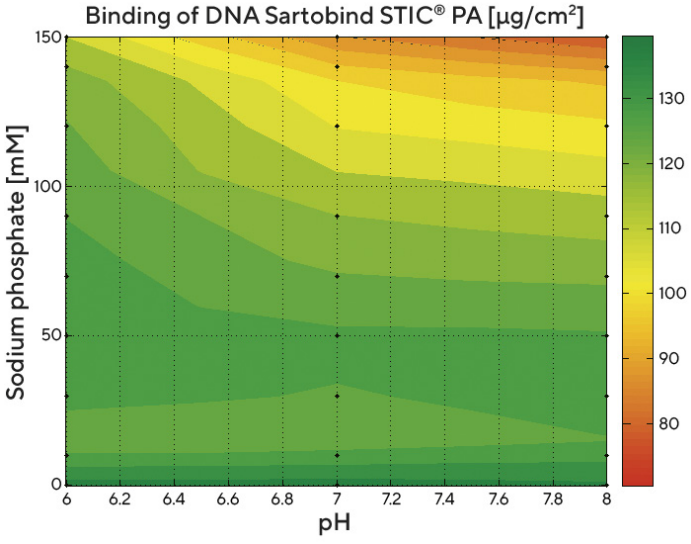

Fig. 2: Influence of phosphate and pH on DNA binding on Sartobind STIC® PA. DNA load: 140 µg/cm²

Discussion

Figure 3 explains how the use of phosphate buffers with Sartobind STIC® PA buffer opens a larger window of operation when DNA is the major contaminant. To avoid binding of target protein in flow-through mode using Sartobind® Q (for example), the buffer pH chosen would be at 1 pH unit below the isoelectric point of the target protein. When using a phosphate buffer with Sartobind STIC®, target protein binding is already reduced by the presence of phosphate and choice of buffer pH can be near or even above the isoelectric point.

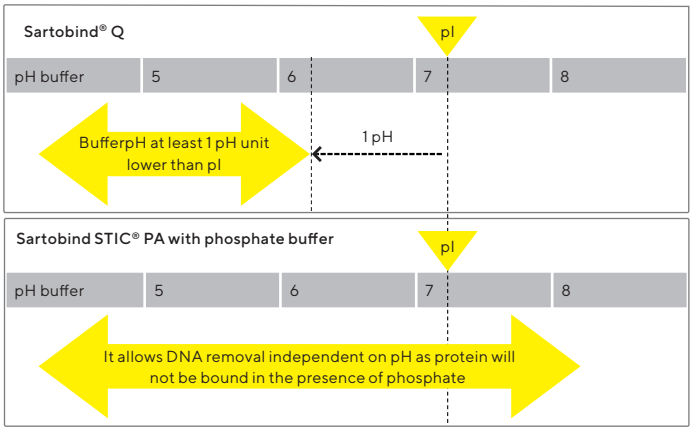

Fig. 3: Applicable pH range on Q and STIC PA anion exchanger for DNA removal in the presence of phosphate.

Summary

The presence of phosphate in the binding buffer had a strong influence on binding of protein to Sartobind STIC® PA membrane. The binding capacity decreased >90% at 10 mM NaH_2PO_4 using BSA as a model protein. At the same time, the binding capacity does not significantly change with NaCl concentration up to 300 mM. As a result, phosphate buffers should not be used when removing impurities, such as host cell proteins, but can be a useful tool when removing DNA impurities.

If DNA is the main impurity, multivalent ions can be used to selectively remove DNA from proteins in flow-through mode under pH conditions that would normally result in binding of the target protein to the membrane. Consequently, DNA can be removed at pH conditions at or even above the isoelectric point of the target molecule.

References

1. S. Randy Holmes-Farley et al. J.M.S.- Pure Appl. Chem., A36(7&8), 1085-1091, 1999

2. M. Leuthold, Characterization of Membrane Adsorbers for Contaminant Removal, Thesis, University of Hanover, 57-58, 2012

Fig. 4: Sartobind STIC® PA 96-well plate

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