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Sartobind STIC® salt-tolerant membrane chromatography

Polishing in antibody production is usually performed on quaternary ammonium (Q) membranes in flow-through mode, since the overall speed and productivity is much higher than on traditional anion-exchange columns. However, the binding capacity of Q ligands is reduced at high conductivities, so concentrated feed streams must be diluted to adequately remove contaminants. Sartobind STIC PA (primary amine) anion-exchange membrane overcomes this limitation and can be used in high-salt conditions.

Salt-tolerant interaction chromatography (STIC)[1] with PA ligand is based on anion-exchange chromatography (AEX) principles. AEX is the established method for removing process-derived contaminants such as host-cell proteins (HCP), DNA, endotoxins, and adventitious and endogenous viruses. It is performed during the downstream processing of monoclonal antibodies (mAbs) in flow-through mode. The process is driven by throughput and not by mass, and thus membrane chromatography (MC) is advantageous over columns for contaminant removal, as membranes run up to two orders of magnitude higher flow rates. Membranes are easy to operate and can be disposed of after one use, just like filters, to save on validation cost.

Current mAb downstream processes consist of at least one initial capture and one AEX chromatography step. The limitation of AEX steps is often that the Q chemistry requires low-salt conditions. In processes with cation exchange (CEX) as the second chromatography step, the CEX pool needs to be diluted to conductivities of 4–5 mS/cm to reestablish binding conditions for the following AEX step.

The newly developed Sartobind STIC PA membrane is composed of cross-linked, regenerated macroporous cellulose. The primary amine ligand is attached to the matrix at approximately six-fold higher ligand density than that of Q membrane. Both the free amine and the high ligand density were crucial for developing its high salt tolerance[1].

HCP removal/protein-binding capacity

The protein concentrations after the first capture steps are typically between 1,000 and 10,000 p.p.m. (ng HCP per mg mAb), and after the CEX step they may drop to below 100–1,000 p.p.m. However, if increasing the pH to >8, one needs to consider the weak anion-exchange character of the Sartobind STIC matrix, as the positively charged amine ligand starts to deprotonate and may lose binding efficacy. Although Sartobind STIC can remove HCP to below 10 p.p.m. at high throughput when up to 10 kg mAb per liter membrane is loaded in flow-through mode, it can be essential to reduce the working pH 8 to 7.5 or 7 to achieve this performance after the first or second chromatography step[2].

The binding capacities of both Sartobind Q and STIC ion exchangers were determined in 20 mM Tris-HCl buffer, pH 7.5, and then with 150 mM salt (16.8 mS/cm) with BSA. At low conductivity, Sartobind STIC achieved almost double the binding capacity of Sartobind Q. At high conductivity, Sartobind STIC maintained its binding capacity at almost the same level, about 50 mg/ml, whereas the binding capacity of Sartobind Q dropped, as the salt concentration was already at the elution conditions for the Q membrane (**Fig. 1a**).

ΦX174 and virus clearance

To investigate virus removal in high-salt conditions, we chose ΦX174 phage and minute virus of mice as models. The phage concentration

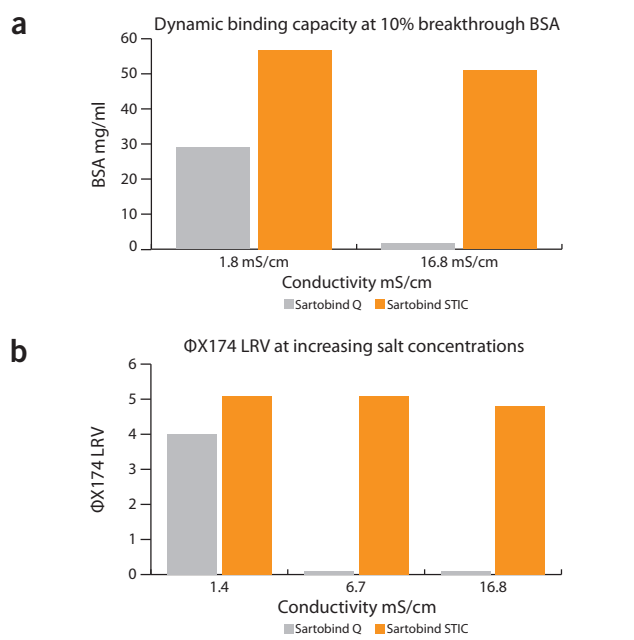

Figure 1 | (**a**) Dynamic binding capacity of Sartobind Q and STIC at 10% breakthrough determined at low (left bars) and high conductivities (right bars). (**b**) Phage ΦX174 binding on Sartobind Q and STIC.

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was 4×10^7 PFU/ml in 25 mM Tris-HCl, pH 8.0, with 50 and 150 mM NaCl (1.4, 6.7 and 16.8 mS/cm, respectively). The log reduction value (LRV = log [start concentration/filtrate concentration]) showed a drastic decrease in binding capacity for Sartobind Q even at 50 mM salt, but for Sartobind STIC, salt concentration had almost no influence (**Fig. 1b**): the LRV remained at ~5.

To compare phage results with those of actual model viruses, we performed a virus-spiking study at a conductivity of 16.8 mS/cm. Sartobind Q obtained an LRV of 1.81, whereas for STIC, no virus breakthrough could be measured and the LRV was >4.96.

DNA removal[3]

The measurements were performed in 96-well plates. Each well had three layers of AEX membrane (0.7 cm^2, 0.019 ml) per well, and the operation was done by vacuum manifold on a robotic system. Salmon sperm DNA (300–700 base pairs) was used in concentrations of 50–1,800 $\mu g/cm^2$ (66 mg/ml) to challenge the membranes with increasing NaCl concentrations up to 1.5 M. Sartobind STIC PA showed no breakthrough of DNA at up to 1.5 M NaCl (**Fig. 2**).

Endotoxin removal

In this experiment, Sartobind STIC PA was tested for the ability to remove endotoxin at high salt concentrations. Sartobind STIC PA nano 1 ml (**Fig. 3a**) was used and sanitized with 1 N NaOH for 30 min at room temperature, followed by thorough washing with 250 ml Arium® demineralized purified water to reach a conductivity of <2 mS/cm. The 20 mM Tris-HCl, 150 mM NaCl, pH 7.5 buffer with a conductivity of 16.8 mS/cm was spiked with endotoxins (Lonza N185 LPS *E. coli* 055:B5) at a concentration of 1,000 EU/ml, and 4,000 ml of this endotoxin solution was pumped at 10 ml/min through the capsule. No breakthrough could be measured here either. Given the detection limit of 0.05 EU/ml for the used gel clot LAL (*Limulus* amoebocyte lysate) test, the calculated LRV was >4.3.

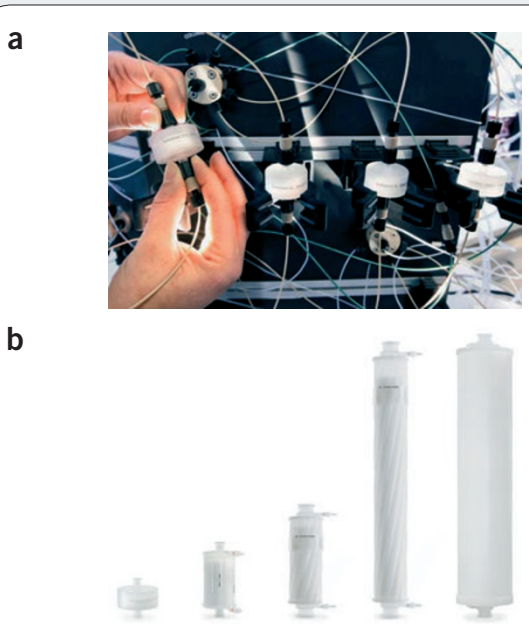

Figure 3 | (**a**) Endotoxin removal with Sartobind STIC PA nano with 1 ml membrane volume. The device can be used in conventional liquid chromatography systems or with syringe by hand. (**b**) Different capsule sizes for Sartobind STIC: nano, 5 inch, 10 inch, 30 inch and mega (1, 70, 180, 540 and 1,620 ml membrane volume).

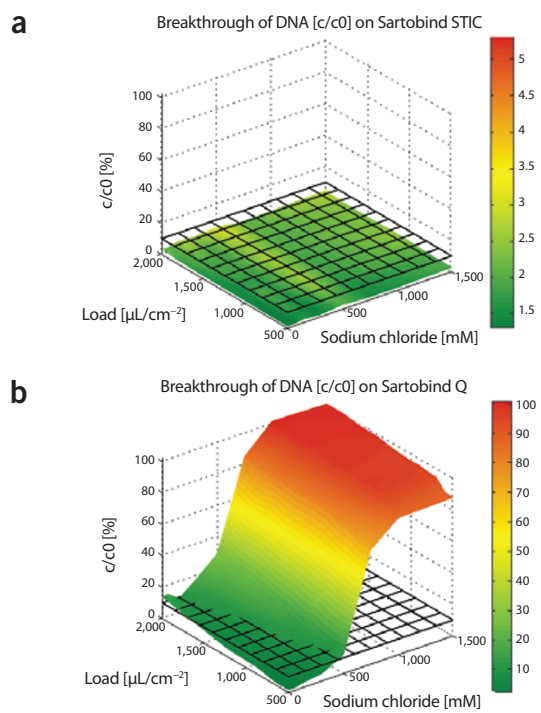

Figure 2 | Breakthrough behavior of DNA on Sartobind STIC (**a**) and Sartobind Q (**b**) measured in 96-well plates.

Summary

Sartobind membranes with Q chemistry are proven and robust platforms and have been validated in hundreds of processes. Sartobind STIC with primary amine ligand overcomes the limitation of Q chemistry and can bind contaminants at high conductivities. Higher binding capacity for impurities in high salt provides more robustness in mAb production. The salt-tolerant anion exchanger can reduce plant bottlenecks, buffer consumption and buffer tank investments.

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2. Fraud, N., Shomglin, K., Faber, R., van Reis, R., Gottschalk, U. & Mehta, A. New membrane adsorber for polishing at high salt concentration. *Recovery of Biological Products XIV Conference* (Lake Tahoe, California, USA, 1–6 August 2010).
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