



High Throughput Screening using Membrane Chromatography

Membrane based 96-well plate for process development in biomanufacturing



Introduction

Sartobind® membrane adsorbers are a well accepted and powerful alternative to conventional chromatography resins for downstream processing in biopharmaceutical manufacturing. The 3 µm pores of the membrane allow high flow rates via convective flow, eliminating the diffusion limitation of traditional resins.

The road to success in downstream purification depends on selecting the optimal adsorbent, media, and operating parameters. Determining these factors is often an expensive and time consuming operation. In an effort to decrease the time and effort associated with determining the ideal parameters for purification, high throughput screening techniques have become essential tools in the development lab. The use of small scale adsorbent material is already well established for chromatography resins¹ and this application note describes a screening platform developed for membrane adsorbers.

1. Screening platform

1.1 Rapid process development

High throughput process development (HTPD) enables complex screening studies operated manually or by automated robotic systems to characterize the performance of membrane adsorbers and optimize process parameters. A typical process development is shown in Figure 1.

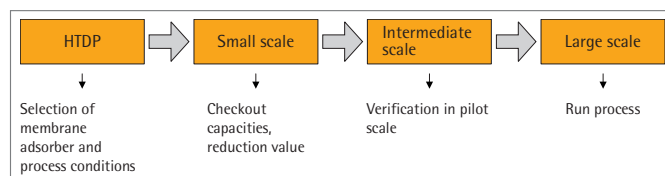


Fig. 1: Steps during process development. HTPD as a first step can help to generate a large knowledge space and improve process understanding. That advances the quality-by-design concept regarding the FDA's (Food and Drug Administration) initiative "PharmaceuticalcGMPs for the 21st century" and ICH International Conference on Harmonisation of technical requirements for the registration of pharmaceuticals for Human Use) guidelines².

1.2 Experimental setup

In the current study, an automated robotic system and 96 well devices were used to evaluate anion exchange membranes as an HTPD approach.

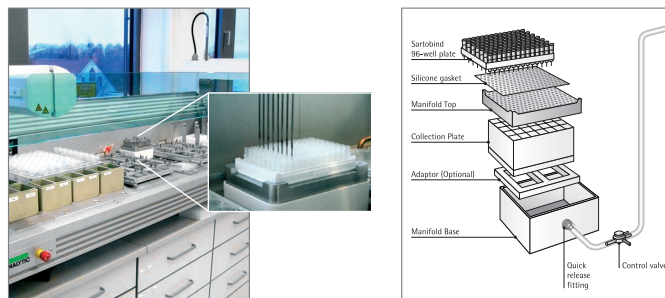


Fig. 2: Sartobind® 8-Strip 96 well plates and the Vivawell Vac96 vacuum manifold implemented on a modified robotic platform (Lissy 2002, Zinsser Analytic GmbH, Frankfurt a. M., Germany). Each well consists of 3 layers membrane with 0.7 cm² membrane. The structure of the screening device and manifold also allows manual operation with a centrifuge or pump.

1.3 Flow-through polishing

In flow-through polishing mode, the target molecule passes through the membrane while contaminants bind. The flow rate using membrane adsorbers is 10–20 times greater than that of chromatography resins, making adsorbers an especially cost-effective alternative to resins for contaminant removal where binding capacities are not crucial.

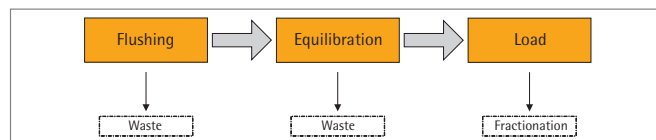


Fig. 3: All chromatography steps are processed by the liquid handling system. Flow is achieved by vacuum.

1.4 Analysis

Sample volume was 300 μL and 96 well micro plates (Greiner Bio-One International AG, Austria) were used. Deoxyribonucleic acid (DNA) concentration of the initial solutions and each fraction collected were measured by a plate reader (Tecan Safire, Tecan Group AG, Switzerland) at 260 nm. Green Fluorescent Protein (GFP) was detected by the plate reader measuring the fluorescence (475 | 509 nm). Break-through and binding are calculated by comparing the concentration in feed (c_0) and flowthrough (c). The relative selectivity was determined by plotting the break-through values of the various components. In figure 5 the selectivity relative to the maximum value is shown.

2. Model for contaminant removal

Green fluorescent protein served as a practical model system to examine contaminant removal at a low concentration of the target molecule in flow-through mode. The polishing performance of two anion exchanger membranes (Sartobind® Q, Sartobind STIC® PA) was investigated at different salt and phosphate concentrations.

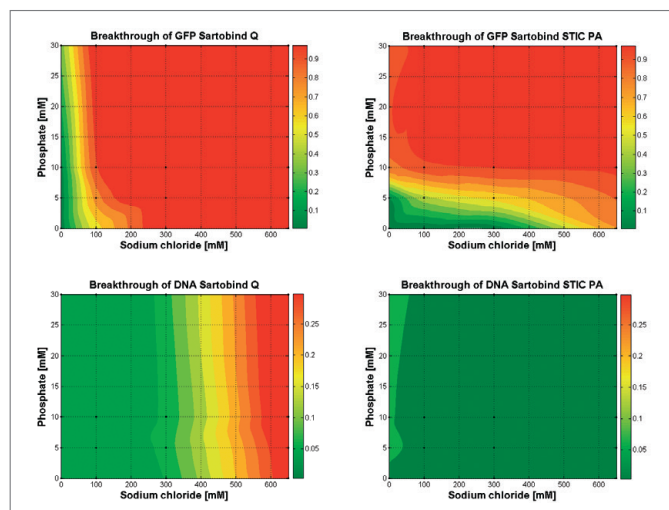


Fig. 4: The given break-through for both components of the model system. After conditioning with 0.5 mL 1 M sodium chloride in binding buffer pH 8 and equilibration with 1 mL buffer, a mixture of DNA (150 $\mu\text{g}/\text{well}$) and GFP (1.6 $\mu\text{g}/\text{well}$) were added in 2 steps of 0.5 mL each. A visual representation of the binding is provided by contour plots developed by the linear fitting of the data to a surface plot. The colour scheme differentiates results dependence on the two parameters. Red means a high breakthrough of the component.

Results show the effect of mono- and multivalent salts on the binding of Protein and DNA for both membranes. As seen in the plot, manipulating the concentration of multivalent salts is another way to separate contaminants and target molecules when using Sartobind STIC PA membrane. The data shows that under NaCl concentrations conducive to binding both the RNA and GFP, differing phosphate levels inhibit the binding of GFP while not affecting RNA removal.

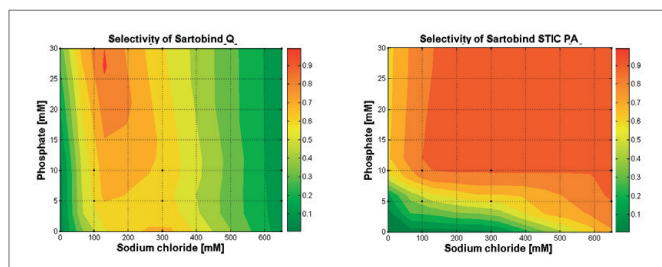


Fig. 5: The results of figure 4 help to identify conditions of high separation for further upscale studies. The next step during process development would to determine the capacity of the membrane for the contaminants which will indicate the amount of membrane necessary for the process scale chosen. Red indicates conditions of a high selectivity.

3. Summary

Screening for process conditions of high binding and selectivity show the potential of the HTPD approach to support the development of membrane adsorber-based process steps. Furthermore, the system allows for processing large sets of experiments, and accelerates the development of new membrane adsorbers.

References

- ¹ Bergander T.; Nilsson-Välimaa K.; Oberg K. and Lacki KM., High-Throughput Process Development: Determination of Dynamic Binding Capacity Using Microtiter Filter Plates Filled with Chromatography Resin. *Biotechnol. Prog.*, 2008, 24, 632-639.
- ² Yu L. X., *Pharmaceutical Quality by Design: Product and process development, Understanding, and Control*. Pharmaceutical Research, 2008, 25, 781-791.

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