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Short communication

Comparing multi-module connections in membrane chromatography scale-up

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ABSTRACT

Membrane chromatography is increasingly used for protein purification in the biopharmaceutical industry. Membrane adsorbers are often pre-assembled by manufacturers as ready-to-use modules. In large-scale protein manufacturing settings, the use of multiple membrane modules for a single batch is often required due to the large quantity of feed material. The question as to how multiple modules can be connected to achieve optimum separation and productivity has been previously approached using model proteins and mass transport theories. In this study, we compare the performance of multiple membrane modules in series and in parallel in the production of a protein antigen. Series connection was shown to provide superior separation compared to parallel connection in the context of competitive adsorption.

1. Main body

The many advantages of membrane chromatography over conventional packed-bed column chromatography have made it a prospering technology (Langer, 2013; Roper and Lightfoot, 1995; Ghosh, 2002; Teeters et al., 2002; Zhou and Tressel, 2006). Owing to the continuous macroporous structure, mass transport in membrane adsorbers occurs predominantly by convection rather than diffusion. Hence, membrane chromatography processes can be operated at a very high flow rate without compromising separation efficiency. The pressure drop across a membrane adsorber is low due to the large bed diameter to height ratio (Ghosh, 2002; Thommes and Kula, 1995). As a disposable technology, membrane chromatography also brings in process flexibility and helps manufacturers reduce cost in labor, buffers, cleaning validation and so on (Lim et al., 2007).

Modern commercial membrane adsorbers are generally manufactured as ready-to use modules for consistent quality and ease of assembly by end users (e.g. Sartorius, Pall and Natrix). Modules of different sizes, commonly described in the unit of membrane volume (MV), are available from several milliliters (for screening and process development) to several liters (for pilot scale production). Linear scale-up of membrane modules can be achieved by keeping the MV per minute flow rate constant (Satorius, 2014). Unlike large packed-columns that can contain hundreds of liters of resin, the size of a membrane module is limited by the strength of plastic housing and by flow distribution constraint within the module (vendor communications). Currently, several liters of MV are the upper limit for large membrane modules (e.g. Sartorius, Pall and Natrix). As such, for large scale purification of biomolecules, it is common that even the largest membrane module cannot provide the desired binding capacity. The use of multiple modules for a single batch is thus inevitable. It has been described that multiple membrane modules connected in series provide higher separation performance in analytical chromatography (Josic et al., 1992), and multiple modules in parallel can be used to achieve higher capacity in preparative chromatography (Demmer and Nussbaumer, 1999).

In the present study, we sought to compare and contrast the performance of multiple membrane modules in series and in parallel in the production of a recombinant protein SP1, a vaccine candidate under development at Sanofi Pasteur. A critical unit operation in SP1 purification is a cation exchange step, which exemplifies a classic application of membrane chromatography in flow-through mode with the goal to remove host cell proteins (HCPs). Currently, feed material from a 200 L fermenter consumes a 5 L membrane module, which is the largest module available. As the final fermentation scale of SP1 is set to be 2000 L, how to connect multiple membrane modules is a pertinent question to be answered.

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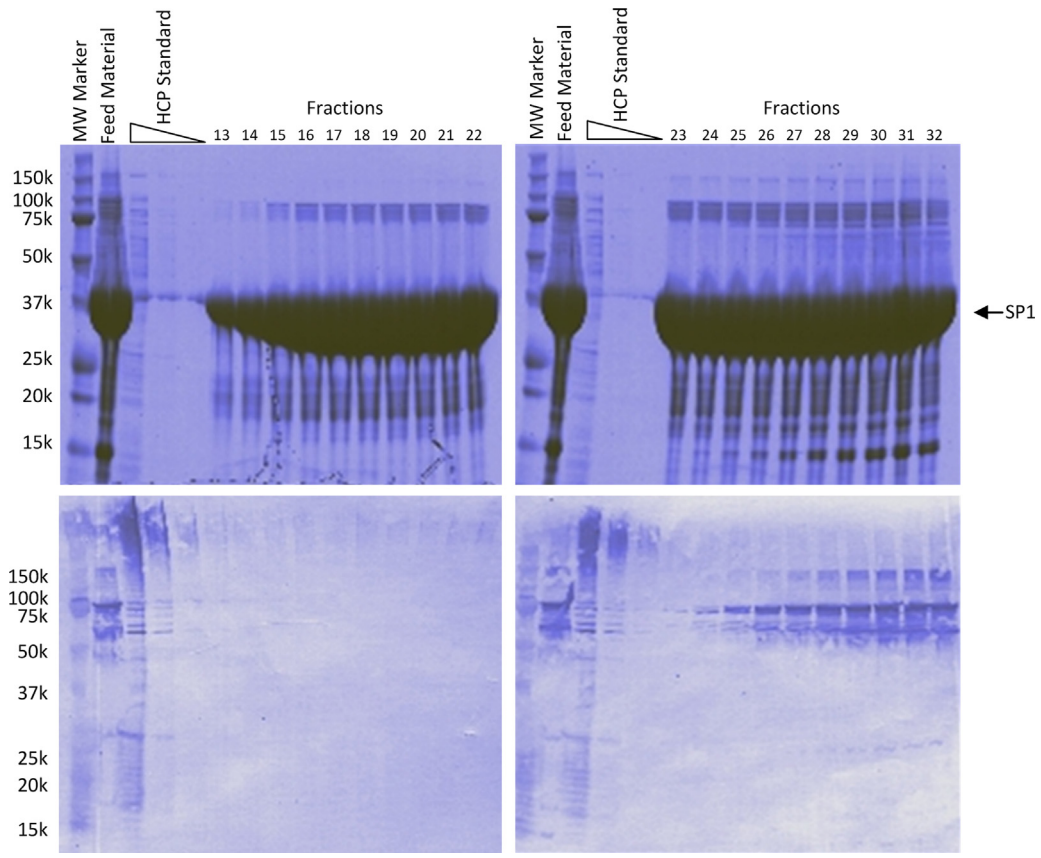

Fig. 1. In-process monitoring of SP1 purification through cation exchange membrane chromatography. The proteins were intentionally over loaded in the SDS-PAGE (Upper panel) to allow for the detection of minor HCP impurities in the corresponding Western blot (lower panel). A rabbit anti-*E. coli* polyclonal antibody was used as the primary antibody (Dako, Cat#B0357), and an alkaline phosphatase-conjugated goat anti-rabbit antibody (Sigma, Cat#A3687) was used as the secondary antibody.

Our investigation began with analyzing the breakthrough curves of SP1 and HCPs. SP1 was overexpressed in *E. coli* in the form of inclusion bodies (IB), and was manufactured using a typical process involving IB solubilization in 8 M urea and purification under denaturant conditions. Sartobind® S SingleSep capsules (Sartorius AG, Goettingen, DE Germany) were used for the purification. The separation of SP1 and HCPs through this process was demonstrated by SDS-PAGE (to visualize SP1) and anti-HCP Western blot (to illustrate HCPs). As shown in Fig. 1, high concentration of SP1 and SP1 isoforms (oligomers and fragments as determined by Mass Spectrometry, data not shown) started to flow through the membrane at fraction 13 (Fig. 1 upper panel), much earlier than HCPs which started to breakthrough at fraction 24 (Fig. 1 lower panel). The fractions between 13 and 24 constituted the operating window of this step. Based on the concentrations of SP1 and HCPs in each fraction, breakthrough curves can be constructed.

To compare the performance of multiple membrane modules in series and in parallel, small scale modules (1 mL MV per module) were assembled as shown in Fig. 2, with sampling ports located at the outlets of each membrane. Breakthrough curves from each membrane module and the main outlet were constructed and overlaid in Fig. 2. The *y*-axis, C/C_0 describes the ratio between solute concentration in the flow-through (C) and in the feed stream (C_0). The breakthrough curves at the final outlet in the parallel system were nearly identical to those from membrane #1 and #3 (Fig. 2A). Hence, the separation power of a parallel system is no better than that of a single module. In contrast, SP1-HCPs separation was progressively improved from membrane #1 to membrane #3 in the series system (Fig. 2B). The overall performance using in-series and in-parallel systems was compared by overlaying the breakthrough curves at the final outlets, as shown in Fig. 2C. Although SP1 flowed through the modules at very similar times, HCP breakthrough patterns were strikingly different. The window of separation was much larger in the series configuration than in the parallel configuration.

To confirm the superior performance of series-connection observed at small scale, a larger scale experiment was carried out. Three 5-inch modules (70 mL MV for each module) were used (Fig. 3 left panel) to process a large amount of feed material, containing ~50 g of protein. This feed material contained abnormally high concentration of HCPs (data not shown). As a result, there was no measurable separation between SP1 and HCPs after the first membrane (Fig. 3 right panel). In contrast, the separation of SP1 and HCPs can be first seen at the end of membrane #2; the breakthrough curves of SP1 and HCPs were further apart after membrane #3. This experiment confirmed that multiple modules connected in series can offer a much improved process capacity, as well as robustness over multi-modules in a parallel system.

Back pressure from different multi-module designs was also monitored. As expected, multiple modules in parallel resulted in less back pressure compared to one single module (Table 1). As more modules were connected tandemly, increases in back pressure were encountered. However the back pressure in the 3-module in series design was well within the pressure limit for these membrane modules (4 bar). This level of back pressure can be handled well with commonly used chromatography equipment.

In this study, a typical flow-through mode application of membrane chromatography was used as an example to compare the performance of multiple membrane modules in series and in parallel. It was observed that the series connection provided superior separation between the target protein SP1 and HCPs. Better separation in this case led to more than 30% increase in process capacity (Fig. 2C). The series connection was also more robust