TOSOH

Application Note

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PROTEIN A CHROMATOGRAPHY – THE PROCESS ECONOMICS DRIVER IN mAb MANUFACTURING

THE OPTIMIZATION OF THE PROTEIN A CAPTURE STEP IN DOWNSTREAM PROCESSING PLATFORMS CAN CONSIDERABLY IMPROVE PROCESS EFFICIENCY AND ECONOMICS OF INDUSTRIAL ANTIBODY MANUFACTURING. PARAMETERS LIKE RESIN REUSE AND ITS CAPACITY CONTRIBUTE CONSIDERABLY TO THE PRODUCTION COSTS. THE USE OF A HIGH CAPACITY PROTEIN A RESIN CAN IMPROVE THE PROCESS EFFICIENCY AND ECONOMICS. THIS PAPER PRESENTS THE KEY FEATURES OF A NEW CAUSTIC STABLE PROTEIN A RESIN PROVIDING EXTREMELY HIGH IgG BINDING CAPACITIES.

Biopharmaceuticals represent an ever growing important part of the pharmaceutical industry. The market for recombinant proteins exceeded $ 100 billion in 2011 with a contribution of 45% sales by monoclonal antibodies (mAbs) (1). The introduction of the first mAb biosimilars in Europe raised the competitive pressure in an increasingly crowded market place. The industry faces challenges, such as patent expirations accompanied by approvals of corresponding biosimilars, failures in clinical trials/rejections or the refusal of health insurers to pay for new drugs.

These challenges force the industry to minimize risk and time-to-market and to proceed more cautiously. Standardization and platform strategies in development and manufacturing, as well as process optimization become a focus for the industry. Biosimilar manufacturers need to adopt the most cost-effective manufacturing technologies including efficient and cost effective purification operations in order to realize highly competitive pricing compared to the original biologic.

Protein A affinity resins are dominating the Cost of Goods (COGs) of mAb manufacturing. Bioreactors at the 10.000 L scale operating at a titer of about 1g/L typically generate costs of $ 4-5 million (2). Therefore the Protein A capturing step is the key driver to improve process economics. Besides the capacity of the resin, life time and cycle numbers significantly contribute to the production costs in mAb manufacturing.

Today, the IgG binding capacities of most Protein A resins are in the range of 30-50 g/L, offering significant advantages for the processing of high-titer feedstreams when compared to resins with capacities of about 20 g/L. The new Protein A resin TOYOPEARL® AF-rProtein A HC-650F exhibits binding capacities of greater than 70 g/L, almost approaching level known from ion exchange chromatography. These high capacities result in even smaller in-process pools, reduced buffer consumption and potentially lower COGs.

PLATFORM STRATEGY

The implementation of a platform process for mAb purification offers numerous advantages compared to a traditional “one step-at-a-time” approach. It accelerates process development while minimizing costs. Typical mAb platforms utilize a common sequence of unit operations for downstream processing, starting with Protein A affinity chromatography accompanied by ion exchange, mixed-mode, or hydrophobic interaction polishing steps.

Protein A chromatography has become a standard technique in the purification of monoclonal antibodies. Due to its selectivity, this affinity chromatography step efficiently removes host cell contaminants, while requiring comparably low method development efforts. On the other hand,

PROTEIN A CAPTURE STEP AND PROCESS ECONOMICS

A systematic process simulation of a standard mAb manufacturing process with 25 unit operations with SuperPro Designer® combined with design of experiments (DoE) identified a significant dependency of unit production costs on the IgG capacity of the Protein A resin (Figure 1).

The simulated downstream purification scheme comprised of Protein A, anion exchange and HIC chromatographic steps. Besides the IgG binding capacity, which had the highest impact on unit production costs, the mAb titer and the number of Protein A cycles per fermentation batch had been identified as key factors for cost optimization (3).

EFFECT OF BINDING CAPACITY ON OPERATING COST

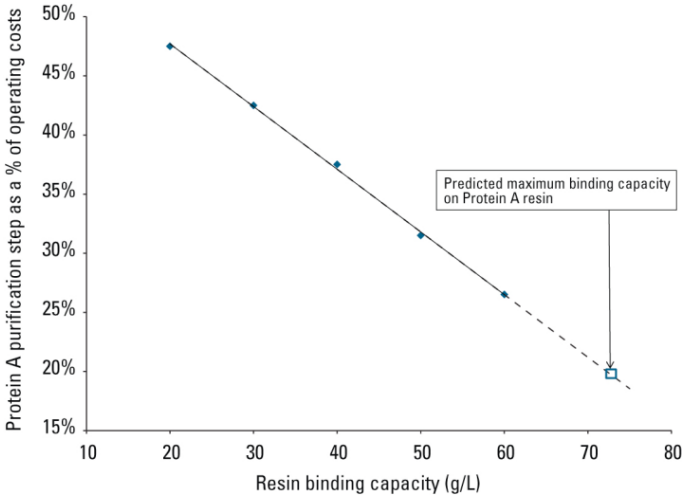

Figure 1

Figure 1 shows that the percentage share of the Protein A capturing costs in total operation costs is decreasing with rising resin capacity. This implicates that replacing a traditional Protein A resin with a dynamic binding capacity (DBC) of 20 g/L by the new high capacity TOYOPEARL AF-rProtein A HC with a DBC of 70 g/L would reduce the relative costs of the Protein A step more than half. Similar findings were reported by Kobayashi et al. for another example comparing protein A resins with 20, 30 and 50 g/L IgG binding capacity (4).

IgG BINDING CAPACITY AT HIGH TITER

Upstream efficiency improvements resulting in high fermentation titer have created significant DSP bottlenecks. Rising titers are a challenge for downstream processing, especially with regards to the expensive Protein A capturing step. TOYOPEARL AF-rProtein A HC-650F benefits from superior mAb capacity and beneficial mAb uptake behavior and can thereby increase capturing productivity.

Since DBC depends on flow and feed concentration, the binding capacity was tested at various residence times and various IgG and mAb titers. Figures 2 and 3 show the DBCs of TOYOPEARL AF-rProtein A HC-650F at three feed concentrations and three residence times. For both, polyclonal IgG and a monoclonal antibody, the dynamic capacities increase with rising titers.

The DBC is also a function of the residence time and is increasing with residence time, as can be expected. In contrast to other high capacity Protein A resins on the market, the binding capacity of this resin is considerably high even at a very short residence time of 1 min. This broad range of applicable flow rates enables the user to tune the capturing throughput in a way that is ideally fitting into the complete workflow. Most interesting is the observation of rising DBC with high titer. Even at short residence times the

DBC OF IgG AT DIFFERENT LOADS AND RESIDENCE TIMES

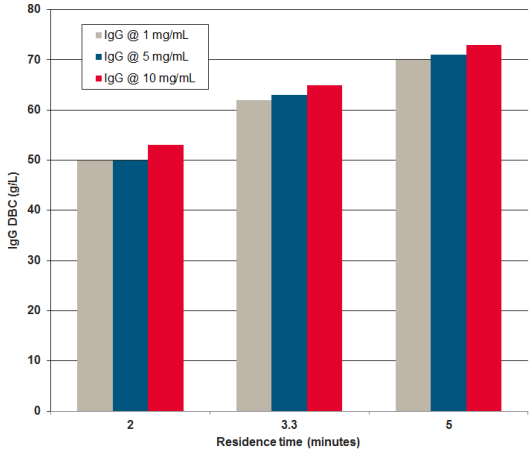

Figure 2

Column: TOYOPEARL AF-rProtein A HC-650F (5 mm ID × 5 cm)
Mobile phase: 20 mmol/L sodium phosphate, 150 mmol/L NaCl pH 7.4;
Residence time: 2, 3.3, 5 min; Detection: UV @ 280 nm
Sample: polyclonal human IgG @ 1, 5, 10 g/L in mobile phase
DBC measured at 10 % breakthrough

DBC OF A MONOCLONAL IgG AT DIFFERENT LOADS AND RESIDENCE TIMES

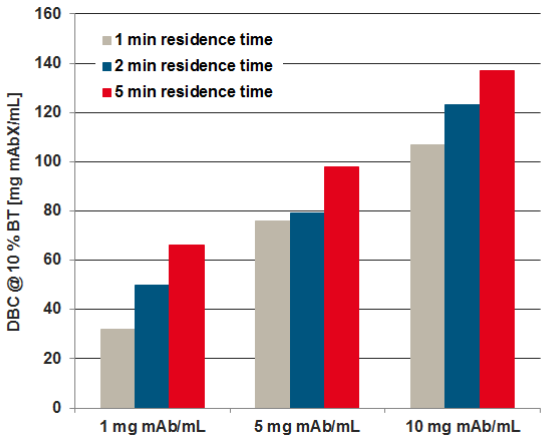

Figure 3

Column: TOYOPEARL AF-rProtein A HC-650F (6.6 mm ID × 2 cm)
Mobile phase: 100 mmol/L sodium phosphate pH 6.5;
Residence time: 1, 2, 5 min; Detection: UV @ 280 nm
Sample: monoclonal antibody mAbX @ 1, 5, 10 g/L in mobile phase
DBC measured at 10 % breakthrough

PROTEIN A LEACHING

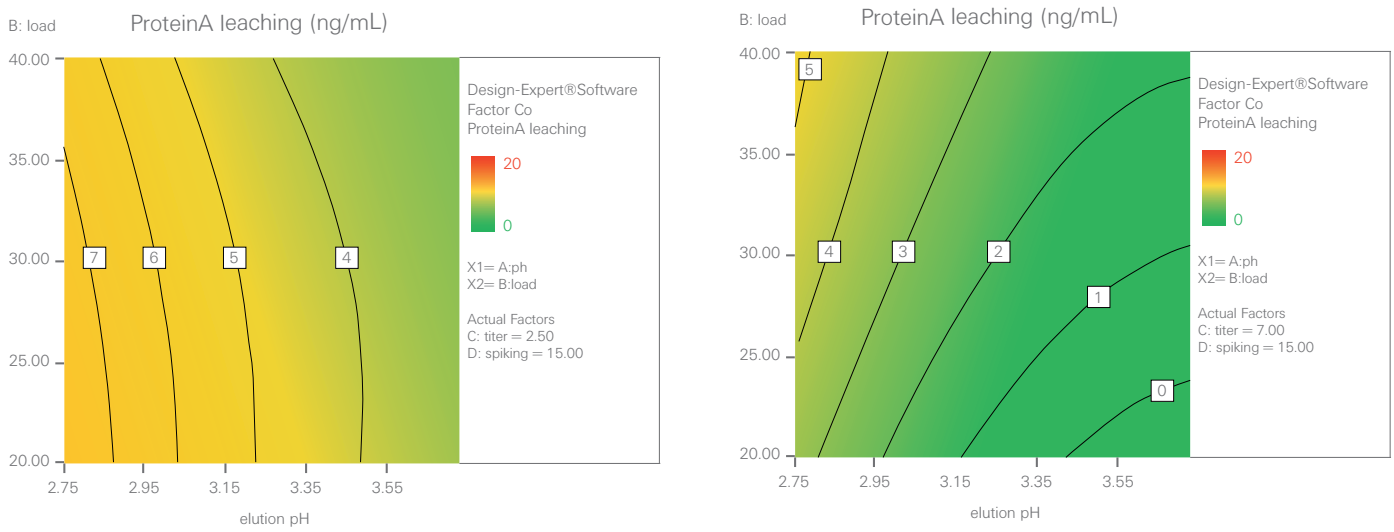

Figure 4

Contour plots for two different load concentrations. Protein A leaching is plotted against pH and absolute load. A: 2.5 g/L. B: 7 g/L.

protein adsorption is high at high feedstock titer. Measured capacities of greater than 100 g/L exceed the DBCs of all other commercially available caustic stable Protein A resins. The high capacities of TOYOPEARL AF-rProtein A HC-650F at high feed concentrations enable fast and efficient capturing solutions.

mAb ELUTION AND LIGAND LEACHING AT HIGH LOAD

As high protein loads could have an impact on aggregation and ligand leaching during Protein A chromatography, the elution properties of the new resin at high protein load were analyzed. A monoclonal antibody was diluted to a final concentration of 4.75 g/L and spiked with host cell proteins. Protein A chromatography was conducted in 200 µL RoboColumns® at a RT of 2 minutes.

The total loaded mass was varied from 10 to 50 mg/mL resin and columns were washed with 20 column volumes of loading buffer prior to elution. Recovery was > 95% at pH 3.25. SEC analyses of two elution pools (10 and 50 mg/mL load) revealed similar aggregate contents (0.6%) when referring to the corresponding total protein amount. Thus, large amounts of mAb absorbed by high capacity resins do not in principle enhance the risk for mAb aggregation during elution.

The dependency between the absolute load and protein A leaching at various mAb titers was analyzed for 2.5 and 7 g/L concentrated feed streams. The contour plots (Figure 4) reveal that the absolute load has little influence on numeric Protein A leaching. Ligand leaching was even slightly lower when applying higher titers.

RESIN ROBUSTNESS AND CIP STABILITY

It goes without saying, that the repetitive use of Protein A reins in DSP of mAbs also has a considerable impact on operating costs. Therefore robust and caustic stable Protein A resins are preferable for mAb manufacturing to take advantage of repeated Cleaning-in-Place (CIP) and reuse of the resin. Figure 5 depicts the excellent caustic stability of TOYOPEARL AF-rProtein A HC against 0.2 M NaOH at 15 minute contact time per CIP cycle.

The superior caustic stability underlines the perfect ability for cleaning and reuse in capturing of mAb's. This stability exceeds the requirement for reuse, facilitates long lifetimes and features the possibility to operate highly cost-efficient. At least 300 CIP cycles without significant reduction in DBC have been approved under the conditions described in Figure 5.

CIP STABILITY

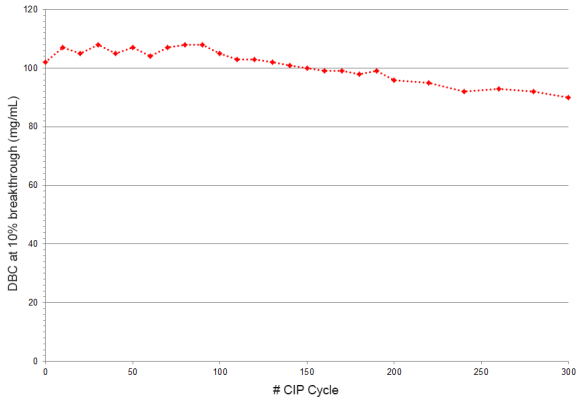

Figure 5

DBC at 10% breakthrough after repeated CIP cycles; 0.2 M NaOH, 15 min contact time mAb DBC at 5 g/L and 2 min. residence time.

HOST CELL PROTEIN CLEARANCE AND LIGAND LEACHING

The CIP stability has also been analyzed with regard to ligand leaching and log reduction of Chinese Hamster Ovary proteins (CHOP). The results shown in Figure 6 and 7 underline the resistance of TOYOPEARL AF-rProtein A HC against 0.2 M NaOH at 15 minutes contact time. Under the described conditions more than 200 CIP cycles have been conducted and both, the ligand leaching with less than 2.5 ppm and the consistent CHOP reduction values of log > 2.5 confirm the robustness of the resin.

The main reason for the caustic stability and low ligand leaching of this resin is the multipoint attachment of an enhanced recombinant protein A ligand to the TOYOPEARL matrix. The capability of TOYOPEARL AF-rProtein A HC to keep its superior performance up to 300 CIP cycles adds to the cost reduction effect of its outstanding binding capacity and can considerably reduce operating costs of mAb manufacturing.

CONCLUSION

The implementation of platform strategies in R&D and manufacturing of mAbs offers great opportunities to safe time, money and to improve the overall economics. Capturing with Protein A has become a standard in these platforms but traditional resins deemed to be very expensive and dominate the CoG in purification of mAbs. Binding capacities, high titer adsorption and alkaline resistance of Protein A resins have a major impact on unit production cost.

New and advanced Protein A resin technologies are addressing the current challenges like resin capacity, robustness and multiple re-use. Hence, the advanced TOYOPEARL AF-rProtein HC-650F offers a multitude of benefits in development and production of antibodies.

The performance offered by TOYOPEARL AF-rProtein A HC-650F enables fast, cost effective, robust and efficient capturing solutions for R&D and manufacturing of antibodies. It is the perfect match for the Biotech Industry to improve process economics, to overcome restrictions in DSP and to face the current challenges in bioprocessing.

1. BioPlan Associates, Inc (2012); 9th Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production
2. Uwe Gottschalk (2009); Process Scale Purification of Antibodies
3. Matthias Franzreb, Egbert Müller, Judith Vajda (2014); under publication
4. Shohei Kobayashi & Yasufumi Ueda (2013); Biopharm International

PROTEIN A LEACHING AS A FUNCTION OF # OF CIP CYCLES

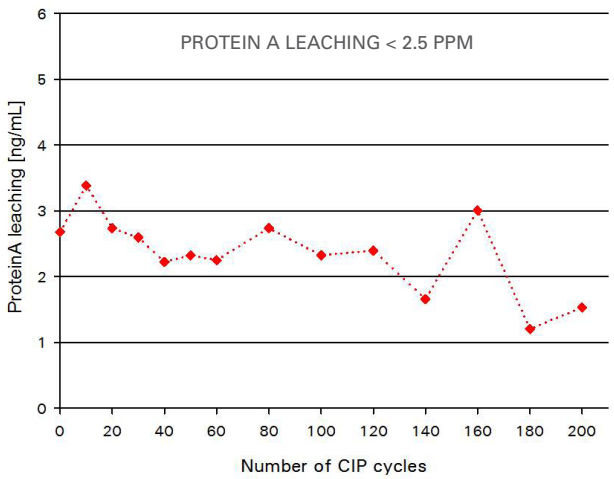

Figure 6

CHOP LOG REDUCTION AS A FUNCTION OF # OF CIP CYCLES

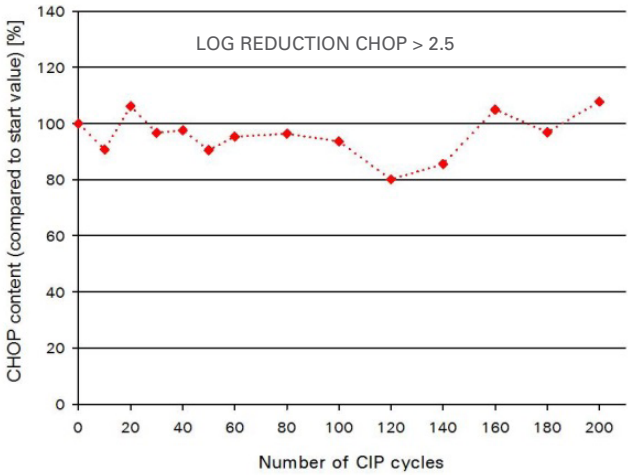

Figure 7