Debottlenecking Antibody Purification Protein A Chromatography with High Titre Feedstocks

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Protein A chromatography has become a widely used platform in monoclonal antibody (mAb) purification. It makes use of the specific interactions that take place between the Fc regions of immunoglobulin G and immobilised Protein A, a cell wall component of Staphylococcus aureus. Its high specificity typically generates more than 95% purity in one step. Remaining host cell proteins (HCP) of the expression cell line flow through the column and are usually reduced by two to three orders of magnitude. Subsequent mAb elution requires a pH shift of the mobile phase. These non-physiological conditions are further applied for acidic virus inactivation and may cause mAb aggregation. Recently, a new generation of high capacity Protein A chromatography resins has been developed. One example is TOYOPEARL AF-rProtein A HC-650F. Herein, we describe the impact of comparably higher mAb loadings on aggregation during Protein A chromatography, as well as the benefits of high capacity Protein A resins for high titre feedstocks.

MAbs are a unique and versatile class of molecules. They combine high target affinity and specificity with various different effector functions, such as complement activation and opsonisation [1]. MAbs are increasingly used in diagnostics and therapy. While the number of approved mAbs for pharmaceutical purposes has grown tremendously [2], mAb production processes have improved, too.

Recent approaches to reduce cost of mAb production focused on increasing the expression rate. MAb titres higher than 5 g/L have been reported [3] and 10 g/L seem close to reach. Today, purification of the target molecules, the so called downstream processing, has become the bottleneck of the production process. Coping with higher titres seems to be a challenge, especially with regards to the expensive Protein A chromatography step.

The new TOYOPEARL AF-rProtein A HC-650F carries a recombinant ligand that is stable in alkali solutions applied in industrial cleaning procedures. It benefits from superior mAb capacity and increases capturing productivity. Besides higher capacities, the mAb uptake behaviour is a major driver in process economics. High capacities at high feed concentrations will lead to concerting effects when it comes to fast and efficient capturing solutions.

mAb Adsorption

The dynamic binding capacity (DBC) of a stationary phase is influenced by the contact time between the sample and the ligand, namely residence time, which decreases when increasing the flow rate. Good mass transfer properties enable a resin to reach a high binding capacity even at high flow rates. The binding capacity also depends on the feed concentration. The capacity of the new resin was tested at various residence times and mAb titres. Dynamic capacities are typically calculated from so called breakthrough curves. A 6.6 mm ID glass column was packed to a bed height of 2 cm.

Z show the breakthrough curves for TOYOPEARL AF-rProtein A HC-650F at two feed concentrations. The resin shows complete mAb adsorption until breakthrough occurs. This remains unaffected for the comparably short residence times of 1 min. The measured capacities refer to a particular example mAb and are higher than 100 mg/ml. Typical mAb capacities range from 50 – 70 mg/ml resin at 2 min residence time and exceed the DBCs of all other known base stable Protein A resins.

A mAb stock solution was diluted to 5 and 10 mg/ml in 100 mM sodium phosphate buffer, pH 6. After bypassing the column until the corresponding maximum absorptions of the mAb solutions were reached, the column was switched into the flow line and loaded. The resin adsorbs the antibody until the capacity of the stationary phase is exhausted and UV signal is increasing because the antibody solution is ‘breaking through’ the column. The DBC of the column is typically calculated from the volume that generated 5% or 10% of the maximum UV absorbance of the sample.

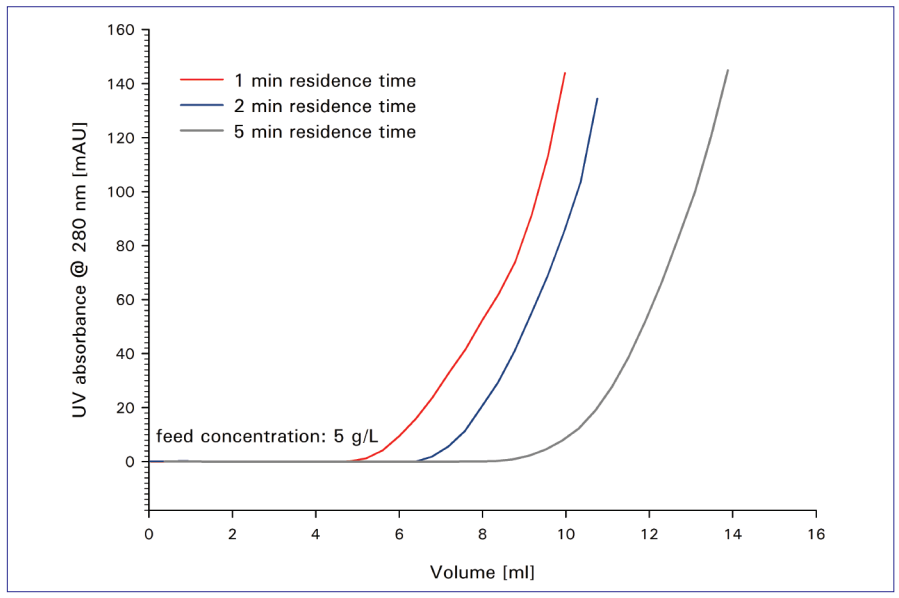

Figure 1a & b. Breakthrough curves of mAb A on TOYOPEARL AF-rProtein A HC-650F packed into a 6.6 mm ID x 2 cm L column. A: 5 g/L mAb. B: 10 g/L mAb.

MAb Elution from High Capacity Resins

A purified humanised monoclonal IgG was diluted in 100 mM sodium phosphate buffer, pH 6.5, to a final concentration of 4.75 g/L. Simulating high HCP density in the Protein A feed, these solutions were spiked with concentrated cell culture fluid. Protein A chromatography with TOYOPEARL AF-rProteinA HC-650F was conducted in 5 mm ID x 1 cm columns using a robotic chromatography station. The total loaded mass was varied from 10 to 50 mg/ml resin. A residence time of 2 minutes (linear velocity of 30 cm/h) was applied. Subsequently, the columns were washed with binding buffer for 20 column volumes. For mAb elution, 100 mM sodium acetate buffer, pH 3.25 was used. More than 95% mAb could be recovered. Due to the acidic pH applied for Protein A elution, mAbs are prone to aggregation. Naturally, high capacity Protein A resins adsorb large amounts of mAb. This might enhance mAb aggregation due to higher target protein concentrations in the elution pool. Thus, special attention was paid to the aggregate content after elution. Size exclusion chromatograms of two mAb elution pools are shown in *Figure 2*.

The elution pools of 10 mg/ml and 50 mg/ml mAb load were injected, respectively. Although the SEC chromatograms seem to show aggregates for the higher loading only, a closer look reveals similar aggregate contents when referring to the corresponding total protein amount detected by SEC. Both pools contain 0.6% aggregates.

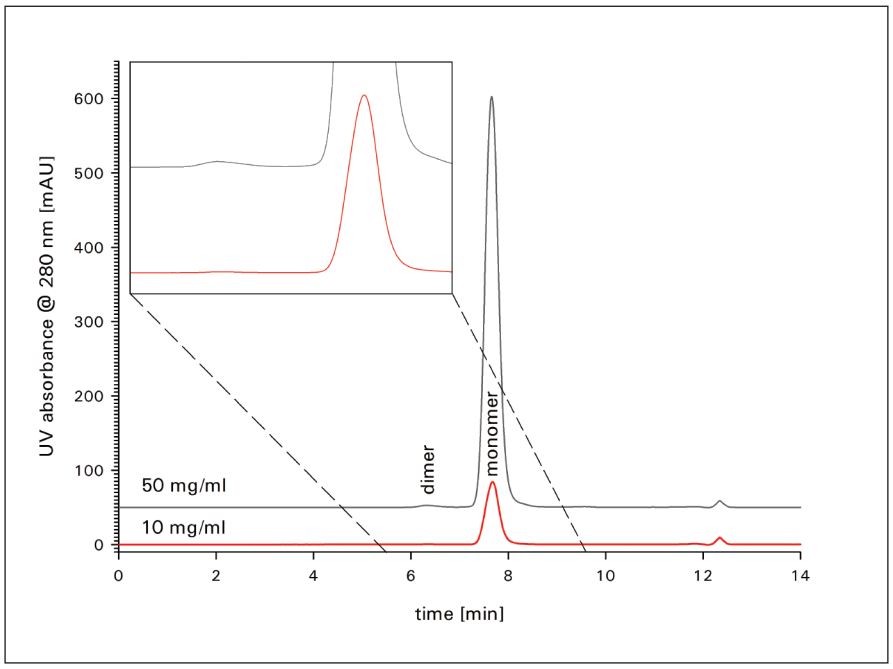

Figure 2. Size exclusion chromatogram of two Protein A elution pools. 50 µl were injected on a TSKgel SuperSW mAb HR U(HPLC) column, 7.8 mm ID x 30 cm L at a flow rate of 1 ml/min, 100 mM sodium phosphate buffer, pH 6.7 + 100 mM sodium sulphate. UV @ 280 nm.

Protein A leaching

In Protein A chromatography used in biopharmaceutical manufacturing, ligand leaching is a major safety concern. Low ligand leaching is crucial and needs to be proofed by ELISA testing. Host cell proteases contained in the cell culture fluid, contribute to ligand leaching [4]. The longer the resin is in contact with the HCP proteases contained in the feed, the more probable seems ligand leaching. From this perspective, high titres are favourable, as they reduce contact time. Further, mAb molecules have been shown to have a ligand detaching effect [4]. Altogether, one would expect increased Protein A leaching for low mAb titre feedstreams and higher absolute loadings. Protein A leaching was analysed for 2.5 g/l, 4.75 g/l and 7 g/L concentrated feed streams. Spiking and residence time were kept constant. *Figure 3* exemplarily depicts the contour plot for the highest load.

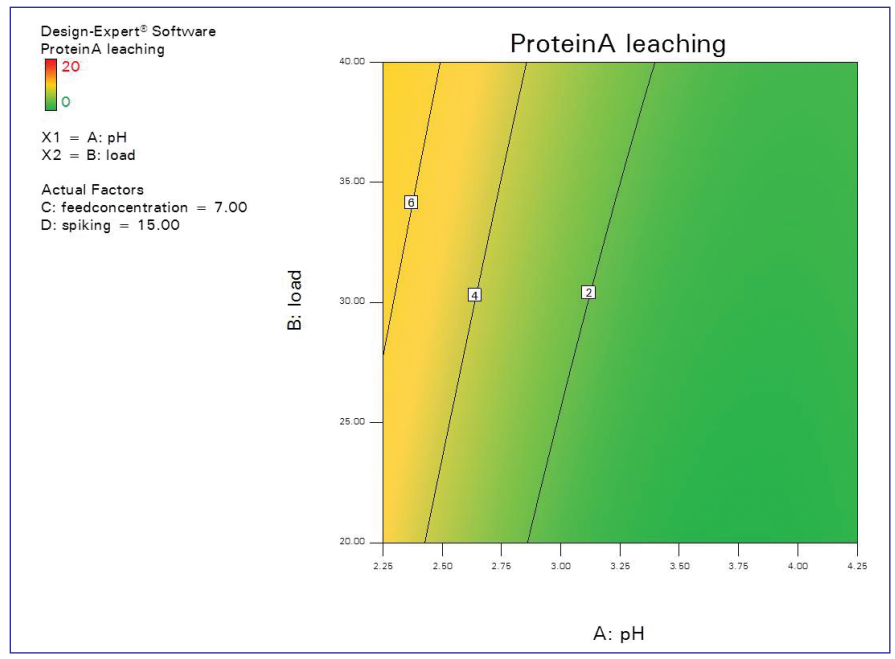

Figure 3. Protein A leaching contour plot for a high load concentration of 7 g/L. Protein A leaching is plotted against pH and absolute load.

According to these results, the absolute load has little influence on numeric Protein A leaching. Overall, Protein A leaching does not exceed 45 ppm for any of the tested pH and load conditions. Higher absolute mAb loadings seem to be advantageous, since the relative Protein A content of the mAb pool decreases.

Considering the obtained results regarding Protein A leaching, aggregate content and protein adsorption, high titres seem favourable for Protein A chromatography. This mAb seemed unaffected with regards to aggregation, and was efficiently adsorbed, which reduces Protein A cycle time. Further, Protein A leaching was even lower when applying higher titres. Thus, ultra-high capacity Protein A resins offer additional benefits besides reducing costs because less resin volume is needed to purify a given amount of monoclonal.

References

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