TOSOH

Application Note

MAb Aggregate Removal through IEC in Bind/Elute and Flow-Through Mode

Purification schemes for monoclonal antibodies typically consist of three chromatographic steps accompanied by filtration steps. The common Protein A capturing is typically followed by ion exchange (IEC), hydrophobic interaction (HIC) or mixed-mode polishing steps. Residual DNA, viruses, and host cell proteins are usually removed by flow-through anion exchange chromatography while aggregates can be reduced through a cation exchange, mixed-mode, or HIC step.

The salt tolerant anion exchange resin TOYOPEARL NH2-750F provides a unique selectivity compared to other anion exchange resins and was found to be suited for aggregate removal, too. Herein we describe the development of an anion exchange polishing step for the purification of a monoclonal antibody by using TOYOPEARL NH2-750F. In general, anion exchange resins can be used in bind and elute (B/E) mode as well as in flow-through (FT) mode. Both options were evaluated. To increase the amount of aggregates of the test sample, a monoclonal antibody was aggregated by acidic incubation and subsequently diluted to 1 g/L in loading buffer.

EXPERIMENTAL

Ion Exchange Bind/Elute Mode:

Column:	TOYOPEARL NH2-750F (P/N 0023438), 2.0 mL Omnifit column (6.6 mm ID)
Mobile Phase:	A: 10 mM Tris/HCl pH 8.0 B: 0.35 M NaCl in A
Gradient:	60 column volumes (CV) to 100%B
Linear Flow:	300 cm/h
Detection:	UV @ 280
Load:	5 mg aggregated mAb

Ion Exchange Flow-Through Mode:

Column:	TOYOPEARL NH2-750F, 2.0 mL Omnifit column (6.6 mm ID)
Loading Buffer:	10 mM Tris/HCl pH 7.0, 250 mM NaCL
Load:	100 mg aggregated mAb

SEC Analysis of Collected Fractions:

Column:	TSKgel G3000SWXL, 7.8 mm ID x 30 cm L
Eluent:	100 mM sodium phosphate pH 6.7, 100 mM sodium sulfate, 0.05 % sodium azide
Volumetric flow:	1 ml/min
Detection:	UV @ 280 nm

ELUTION PROFILE OF AGGREGATED ANTIBODY ON TOYOPEARL NH2-750F IN BIND AND ELUTE MODE

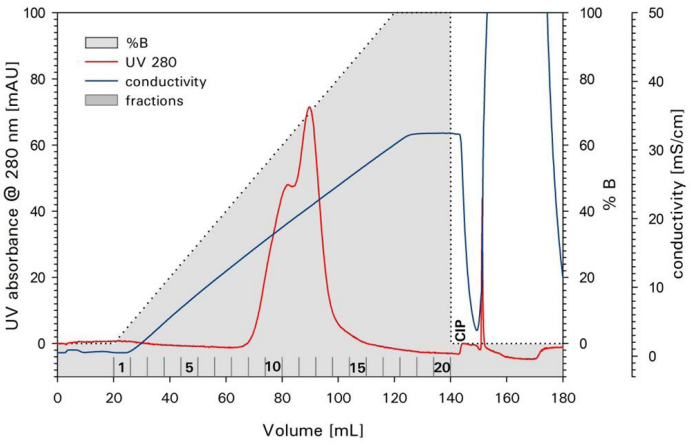

Figure 1

SEC ANALYSIS OF THE BIND/ELUTE OF FRACTIONS OF THE AGGREGATED mAb SAMPLE

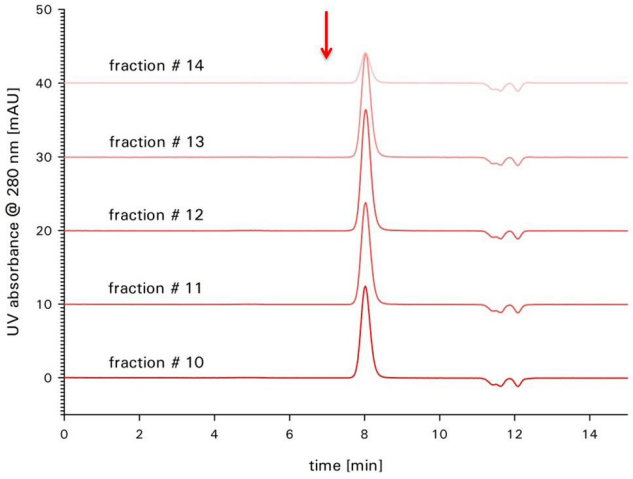

Figure 2

ELUTION PROFILE OF AGGREGATED ANTIBODY ON TOYOPEARL NH2-750F IN FT MODE

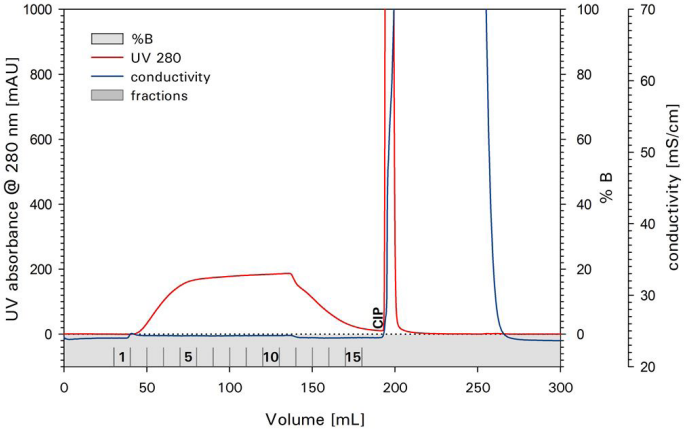

Figure 3

SEC ANALYSIS OF THE AGGREGATED mAb SAMPLE (RED LINE AT 0 MAU) AND FLOW-THROUGH FRACTIONS IN INCREASING FRACTION ORDER FROM BOTTOM TO TOP

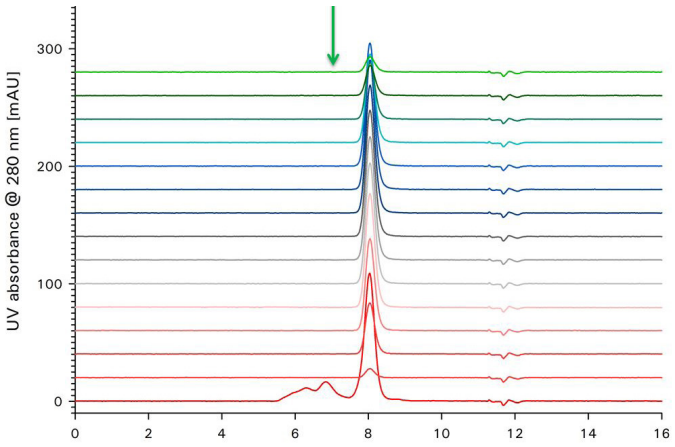

Figure 4

RESULTS

The dynamic binding capacity of TOYOPEARL NH2-750F for the mAb used in this study was evaluated and a value of 95 mg/ml could be reached with 10 mM Tris/HCl, pH 8.0. Figure 1 shows the elution profile of the aggregated antibody on TOYOPEARL NH2-750F in B/E mode. Fractions were collected and analyzed by SEC (Figure 2). The results prove that fractions 10 to 14 have an aggregate content below the limit of detection of SEC. The aggregates did not elute in the salt gradient and remained bound until the column was CIPed with sodium hydroxide.

In order to establish a FT polishing step, buffer conditions were evaluated to optimize non-binding conditions for the monomer by varying pH (pH 7 to 8) and salt content (250 -500 mM NaCl). Best results were obtained with 10 mM Tris/HCl, pH 7.0 at a sodium chloride concentration of 250 mM (Figure 3). To analyze the aggregate removal, 100 mg aggregated antibody were loaded on a 2 mL column and fractions of the flow through were analyzed by SEC. All FT fractions are essentially aggregate free (Figure 4).

CONCLUSION

TOYOPEARL NH2-750F is a salt tolerant anion exchang resin for downstream processing with high binding capacity for immunoglobulin. The resin is ideally suited to develop a polishing step for monoclonal antibodies by either using the resin in BE mode or in FT mode. For both modes ideal conditions for aggregate removal could be established. An additional benefit when using this resin in FT mode is the delivered excellent viral clearance. Typical virus log reduction exceeds five for enveloped and non-enveloped DNA and RNA viruses.