Screening AEX Resins in the Development of an Optimal AEX Purification Process for Specific Monoclonal Antibody Product

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Abstract

Anion exchange (AEX) is an important chromatography tool for the removal of contaminants and impurities post-ProteinA capture during the purification of monoclonal antibodies. Process impurities can include host-related proteins, leached ProteinA, and other high molecular weight components. The negative charge of most of these impurities binds them to the positively charged beads and allows for the relatively higher pI antibody product to flow through. The optimal AEX step can effect partial to complete removal of contaminants and impurities without binding the antibody. There are many AEX resins commercially available including many new generation high capacity resins and mixed-mode AEX resins. We screened seven different anion exchange resins including Capto-Q, Capto adhere, Unosphere-Q, Toyopearl Gigacap Q, Poros Q, Fractogel Q and Fractogel-hicap Q, under various operational condtions using JMP statistical modeling software. Process yield, product purity, and contaminants profile are used as responses for detecting resin performance trends and the study results allowed us to select top candidates for further process development.

Introduction

AEX chromatography is one of the most effective separation tools used in protein purification. In the mAb downstream purification process described in this poster, the AEX process step uses Fractogel TMAE(M) resin from EMD and immediately follows the ProA capture and UF/DF conditioning steps. Because the mAb pI is higher than those of most CHO host expression system derived impurities, the AEX step is operated in flow-through mode. Often, an AEX flow-through step is used as the final polishing stage of a purification train. However, manufacturing facility constraints dictated that the AEX step occur early in the process train. As a result, the levels of impurities and contaminants in the AEX load are relatively high and the total column load must be reduced to maintain good step performance. For example, the top panel in Figure 1 demonstrates that an unacceptable level of impurity breakthrough (back peak) occurs during the column wash when the AEX column is loaded at levels typical for a final polishing step. Chromatographic performance is greatly improved when the load is substantially reduced (lower panel). As part of process optimization, we desired to restore high load capacities without affecting step performance.

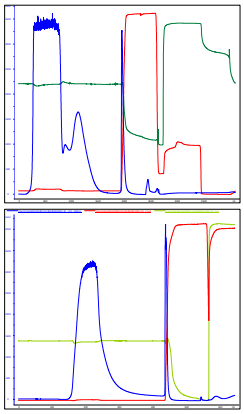


Fig. 1: UV280nm, conductivity, pH. Example chromatogram from AEX process at initially developed loading capacity when switched TMAE Fractogel AEX process from last polishing step to intermediate purification step (Figure 1a), and the chromatogram from AEX process at reduced loading.

Recent years have seen the development of new generation of AEX resins with significantly improved binding capacities and other process benefits. New column chemistries include mixed-mode binding (*e.g.*, the newly-introduced AEX resin, Capto adhere (GE Health Inc.). We designed a systematic four factor by two level DOE study, using JMP software, to screen a set of commercially available AEX resins. The investigation used a D-optimal screen design to focus on seven strong AEX resins (Fractogel-TMAE, Fractogel TMAE-hicap, Unosphere-Q, Toyopearl Gigacap-Q, Poros 50 HQ, Capto Q and Capto adhere) under 4 different loading parameters (loading pH, loading conductivity, loading capacity for IgG, and loading operating column residence time). The true measure of AEX process performance is its ability to bind the various impurities and contaminants, but for practical reasons we use mAb product loading mg/mL resin to define AEX load capacity while monitoring the breakthrough of various impurities and contaminants. Table 1 lists the physical and chemical properties of the seven AEX resins tested.

Results

Table 1: Resins selected for screening

	Capto Adhere	CaptoQ	GigaCapQ	Poros HQ 50	TMAE (M)	TMAE hicap (M)	UNO-Sphere Q
MFR	GE Healthcare	GE Healthcare	TOSOH	AppliedBio systems	EMD	EMD	BioRad
Matrix	Highly crossed-linked agarose	Highly crossed-linked agarose with dextran surface extender	Modified Methacrylic polymer	Poly-styrene-divinyl-benzene	Polymer	Polymer	Hydrophilic acrylamide
Dynamic Binding Capacity	200mg BSA/ml	100mg BSA/ml	175mg BSA/ml	55mg BSA/ml	80-120mg BSA/ml	160-200mg BSA/ml	125-180mg BSA/ml
Particle Size	75µm	90µm	50-100µm	50µm	40-90µm	40-90µm	120µm
Flow Rate / Pressure	3 bar@ 600cm/hr	3 bar @ 700cm/hr	3 bar	1000-5000cm/hr	760cm/hr	760cm/hr	2 bar@ 1200cm/hr
Ion Exchange Type	Multimodal Strong Anion	Strong Anion	Strong Anion	Strong Anion	Strong Anion	Strong Anion	Strong Anion

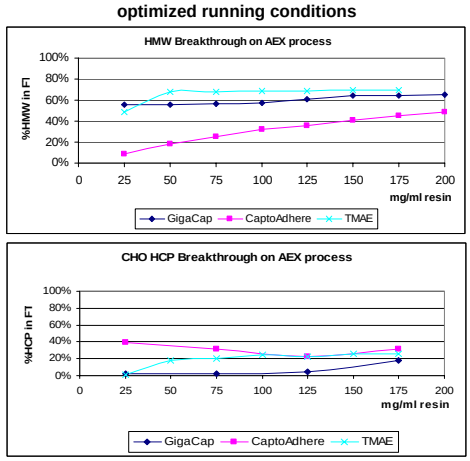
All screening AEX runs were carried out using AP1 columns (1cm x 10cm) with packed bed heights of approximately 10cm on an AKTA chromatography system. The set of analytical assays used to assess process yield and product purity include A280, analytical GP-HPLC, CHO HCP ELISA, and rProteinA detection ELISA (commercial kits from Cygnus Technologies and Repligen, respectively). Table 2 presents the relative effectiveness of impurity clearance of each AEX resin when run under the best operating conditions tested. Fig. 2 represents an example of breakthrough study, performed after the JMP DOE screen, which demonstrates the impurities-removal performance differences among the various resins.

Table 2: Example of AEX resin performance under best tested running conditions and high load

Resin	pH	Cond (mS/cm)	Load (mg/ml resin)	Yield%	HMW % removal	LMW % removal	CHO % removal	ProA % removal
Capto Adhere	7	12	125	93.9	**55%**	NSR*	79%	**97%**
CaptoQ	8.5	3	125	97.4	26%	NSR	86%	34%
GigaCapQ	8.5	3	125	98.2	33%	NSR	86%	40%
PorosQ	8.5	3	125	97.1	31%	NSR	79%	22%
TMAE	8.5	3	125	95.2	31%	NSR	78%	3%
TMAE Hicap	8.5	3	125	100	35%	NSR	85%	32%
UNOQ	8.5	3	125	97	37%	NSR	**88%**	30%

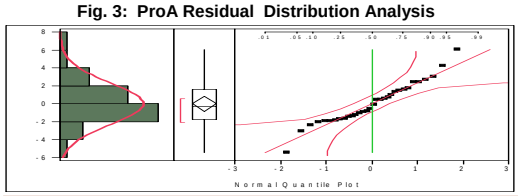
*NSR = No significant reduction detected.

Fig. 2: Impurities breakthrough under optimized running conditions



The DOE study analysis is conducted with JMP software. Fig. 3 shows an example of a residual distribution plot (for Protein A removal) which demonstrated our design model is appropriate and the assumptions are reasonable for subsequent ANOVA analysis. Fig. 4 is the ANOVA analysis for the relative effectiveness of impurity clearance among different AEX resins, and Fig. 5 is an example of a trend analysis of the individual effects that the four loading parameters exert on high molecular weight aggregates removal.

Fig. 3: ProA Residual Distribution Analysis



Goodness-of-Fit Test for ProA residual distribution
Shapiro-Wilk W Test

W	Prob<W
0.973230	0.5929

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Results

Fig 4: ANOVA Analysis of AEX clearance effectiveness for various impurities

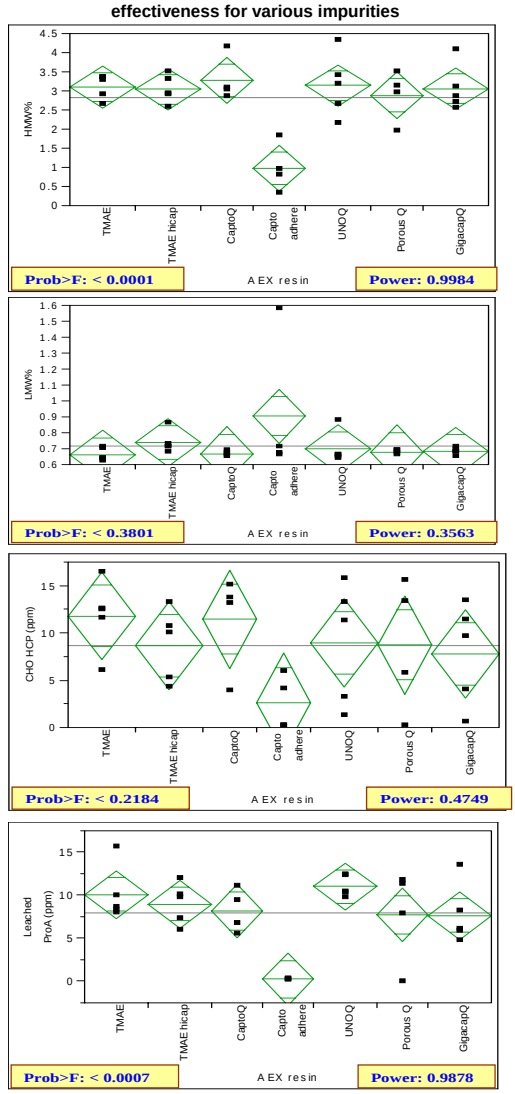
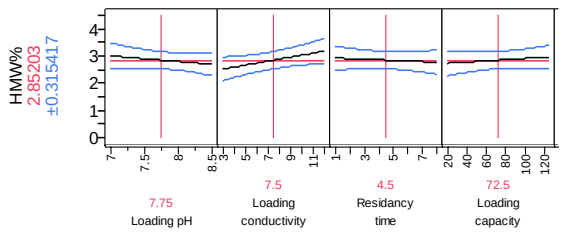


Fig. 5: Trend analysis of load parameter effects on HMW removal



Discussion

Statistical analysis shows that there is a significant benefit in using the mixed-mode Capto adhere resin compared to conventional AEX resins with respect to high molecular weight aggregates and leached ProA removal. To some extent this is expected, since studies have shown that aggregates are usually associated with leached ProA. However, our study results also revealed that Capto adhere is the least robust resin, with overall performance (especially yield) being strongly influenced by process conditions. The conventional AEX resins uniformly showed excellent recovery of antibody product. Because Capto adhere is optimally operated under slightly higher conductivity and lower pH loading conditions, a subset of CHO HCP does not bind to the resin and is present in the flow-through antibody product pool. In contrast, conventional AEX resins bind the majority of CHO HCP with classical breakthrough curves. Finally, it appears that Capto adhere resin can also resolve low molecular weight (LMW) impurities to some degree, even when operated under flow-through mode. In some cases, LMW impurities are enriched in the leading edge of the flow-through product pool, which could be cut out to increase product quality but would affect process yield.

In summary, we have developed a new DOE design screening method which allows us to screen for the best resin for our specific AEX process. Based on the screen results, we chose the conventional AEX resin, Gigacap-Q, and mixed-mode AEX resin, Capto adhere, for further in-depth development studies.