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Use of RoboColumn®* Chromatography Columns for High Throughput Study of Loading Conditions on HyperCel STAR AX and MEP HyperCel Resins for MAb Purification in Flow-Through Mode

Catherine Allioux
Sartorius Stedim Chromatography Resins S.A.S, 48 Avenue des Genottes, 95800 Cergy, France

Correspondence
Dr. Carsten Voss, E-Mail: Carsten.Voss@Sartorius.com

1. Summary

The use of HyperCel STAR AX and MEP HyperCel resins for MAb polishing provides efficient contaminant removal. A study to screen loading conditions for MAb purification in flow-through mode on MEP HyperCel and HyperCel STAR AX resins was conducted using 200 µL RoboColumn® columns. The study demonstrates the suitability of RoboColumn® columns for scale down evaluation of chromatographic parameters, and the good consistency with data obtained on 1 mL PRC prepacked columns.

RoboColumn® columns allow loading of high sample volume per mL of resin and mimic dynamic adsorption phenomena. Those two criteria are of particular interest in flow-through mode purification while the use of 96-well plates is limited for such applications. The miniaturized format offers substantial advantages to conduct high throughput optimization for the use of resins like HyperCel STAR AX and MEP HyperCel for MAb polishing, including limited feedstock consumption and reduced run time.

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2. Introduction

Monoclonal antibodies (MAb) are traditionally purified following a three-step process. After an initial capture with Protein A affinity resin, anion exchangers and mixed-mode resins are frequently involved in subsequent polishing steps. These resins are implemented either in standard bind|elute mode or in non binding (flow-through) mode [1]. Flow-through mode purification offers several advantages over bind|elute mode: ease of operation, reduced process time, and limited resin consumption.

When optimizing conditions for flow-through mode operations, critical parameters like pH, conductivity, residence time, throughput (sample volume per mL of resin) need to be screened. High throughput methods in 96-well filter plates are extensively used for such screening of operating parameters [2]. However, this format is not ideal to mimic protein dynamic adsorption and displacement effects occurring on standard packed bed columns. Additionally, the larger sample volume required in flow-through mode compared to bind | elute mode involves multiple sample loads per well. This significantly limits the use of microplate format and requires the use of regular 1 mL prepacked columns, which subsequently increases the total sample volume and time required to complete the optimization study.

Resins RoboColumn® are minaturized chromatography columns of 200 µL and 600 µL which operate in high throughput mode on robotic liquid handling workstation [3]. They have a resin packed bed maintained between two filter frits. This design allows operating these columns similarly to standard columns in dynamic mode.

This application describes the screening of loading conditions for MAb polishing using two resins: salt-tolerant anion exchange HyperCel STAR AX resin [4], and MEP HyperCel mixed-mode resin [5]. The study was performed in parallel on 200 µL RoboColumn® columns and 1 mL PRC prepacked columns for comparison purposes.
A design of experiment (DoE) approach was developed to evaluate the impact of loading pH and loaded quantity of post-Protein A MAb on the purification performances, including yield, host cell protein (HCP) and aggregates removal.

3. Materials and Methods

3.1. Design of Experiment (DoE) For Screening of Loading Conditions

A DoE approach was applied to evaluate the impact of loading pH and loaded quantity on MAb yield and contaminant removal. The design involved two factors with two levels each (Table 1).

Table 1
Design of Experimental Parameters for the Screening of Loading Conditions

		MEP HyperCel	HyperCel STAR AX
Factor	Loading pH	pH 4.0 and pH 5.0	pH 7.0 and pH 8.0
	Loaded quantity	45 and 90 mg MAb per mL of resin	
Responses		MAb yield of recovery, HCP and aggregates reduction	

3.2. Flow-Through Purification Runs

Load sample was a fraction of post-Protein A affinity MAb, purified from CHO cell culture supernatant. The pH and conductivity of the MAb sample were adjusted according to the loading conditions tested for each resin. Final concentration of MAb in the loaded sample was 3.7 mg/mL. The purification scheme applied on 1 mL PRC prepacked columns and 200 µL RoboColumn® columns is described in Table 2. RoboColumn® columns were operated on a Freedom EVO* workstation (Tecan), PRC prepacked columns were run on an ÄKTAexplorer* 100 system.

Table 2
MAb Purification Sequence in Flow-Through Mode for MEP HyperCel and HyperCel STAR AX resins

	MEP HyperCel resin		HyperCel STAR AX resin		
	Buffer | Solution	**RT [min]**	**Buffer | Solution**	**RT [min]**	**Number of CVs**
Equilibration	50 mM Na acetate, 8 mS/cm	1	50 mM Tris-HCl, 8 mS/cm	1	10
Load	MAb sample	4	MAb sample	2	-
Wash	Equilibration buffer	4	Equilibration buffer	2	10
Strip	50 mM Na acetate, pH 3.0	2	50 mM Tris-HCl, pH 7.0 + 2 M NaCl	2	10
CIP	1 M NaOH, 30 min	5	1 M NaOH, 30 min	5	6
Reequilibration	Equilibration buffer	1	Equilibration buffer	1	10

RT: residence time, CV: column volume. Equilibration buffer pH and MAb loaded quantities: see Table 1

3.3. Analytical Methods

- Total MAb yield of recovery was determined by 280 nm absorbance measurements in UV transparent microplates with a UV | VIS microplate reader:

$$\text{Yield} = \frac{\text{Total MAb recovered in flow-through [mg]}}{\text{Total MAb loaded [mg]}}$$

- CHO host cell proteins (HCP) were quantified using an ELISA assay kit (#F550, Cygnus Technologies) following the manufacturer's protocol.
- Quantification of MAb aggregates was performed using a TSKgel* G3000SWXL column (Tosoh) connected to a Prominence* HPLC system (Shimadzu) following standard operating conditions.

4. Results

4.1. Impact of Loading Parameters on Purification Performances

Figures 1 and 2 present the main effect plots obtained for each resin. These plots correlate the mean of each response with the corresponding factor level applied. The lines plotted for each factor allow to evaluate the effect of this factor on the response tested:

- Horizontal → No effect of the factor
- Slope → Effect

First, the results obtained on 200 µL RoboColumn® columns and on 1 mL PRC prepacked columns are very similar for both resins. For each factor, the effect on the response shows the same trend. In addition, the two column formats have generally comparable magnitude of each effect.

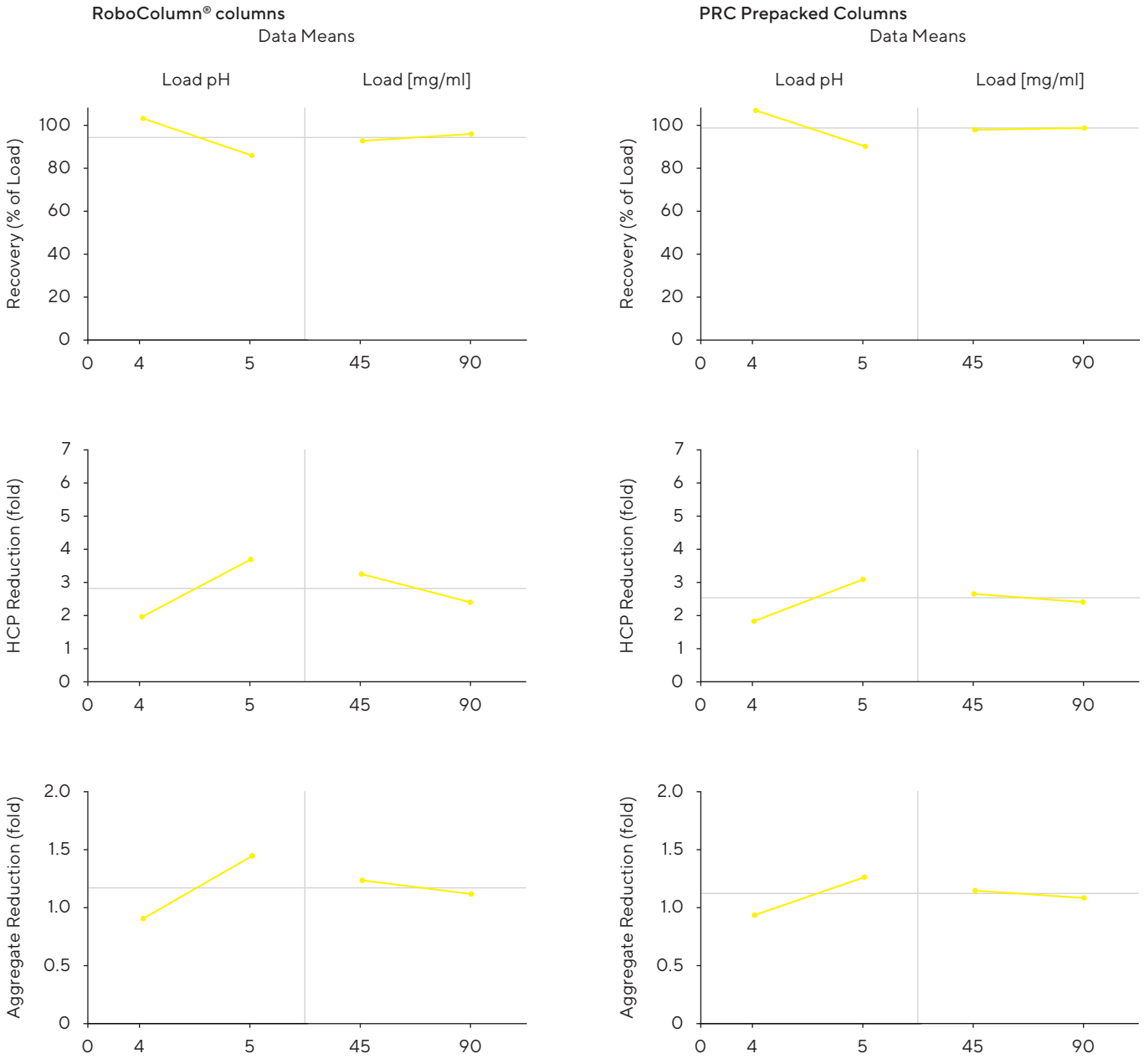
▪ MEP HyperCel Resin

In terms of resin performance, results on MEP HyperCel resin show that purification efficiency is impacted by the loading pH but not by the loaded quantity:

- At low pH (4.0), yield is maximized but lower reduction of HCP and aggregates is obtained
- At high pH (5.0), lower yield but higher contaminant removal

This behavior is in line with the interaction mode of MEP HyperCel resin which binds proteins at physiological pH and triggers elution when lowering pH [6,7]. The data suggests that optimal performances on MEP HyperCel resin may be obtained at an intermediate pH level, between 4.0 and 5.0, for the purification of the targeted MAb in flow-through mode.

Figure 1
Main Effect Plots of Load Factors on MAb Purification Performance Responses Determined by Optimized Regression Models for MEP HyperCel resin

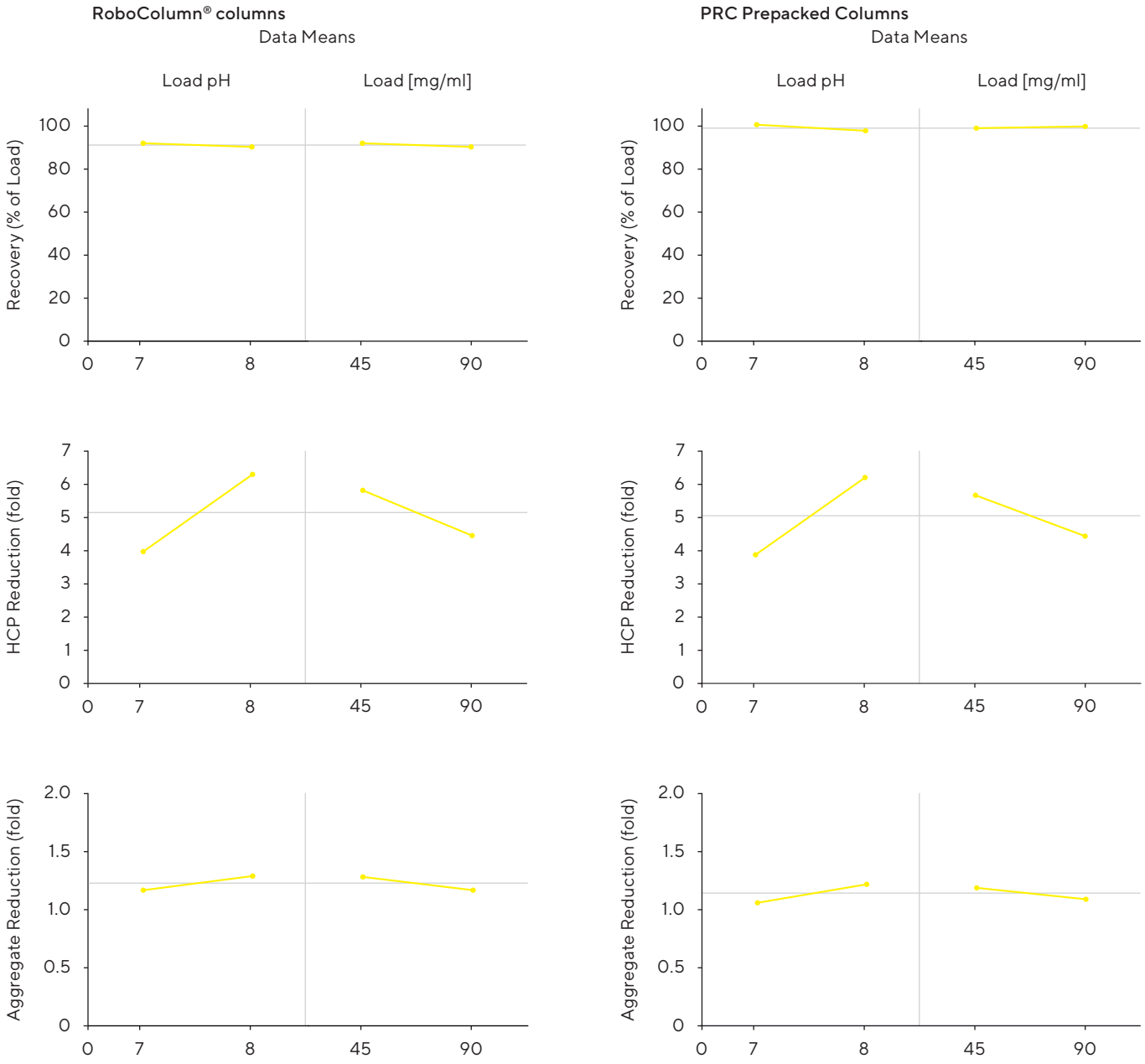


▪ HyperCel STAR AX Resin

For HyperCel STAR AX resin, the results evidence that yield of recovery is not impacted by the load conditions. Aggregate removal was not significant in the conditions tested. In contrary, high HCP reduction can be achieved at high pH level (6- to 7-fold HCP reduction at pH 8.0) but the performance is affected by lowering the pH and | or increasing the loaded quantity.

This behavior is consistent with the chemistry of HyperCel STAR AX resin. At pH 7.0 – 8.0, the anion exchange ligand is positively charged and therefore strongly interacts with negatively charged HCPs. However, acidic HCP proteins hold more negative charges at pH 8.0, thus increasing their adsorption and elimination on the anion exchange resin.

Figure 2
Main Effect Plots of Load Factors on MAb Purification Performance Responses Determined by Optimized Regression Models for HyperCel STAR AX resin



4.2. Sample Volume and Run Time With RoboColumn® columns vs. PRC Prepacked Columns

While the data obtained with RoboColumn® columns and PRC prepacked columns is comparable, the study performed on RoboColumn® provides significant benefits in terms of time and cost savings compared to standard laboratory columns. RoboColumn® requires a 5-fold less amount of loading protein due to the reduced column volume.

As a consequence, this allows significant saving on feedstock usage compared to 1 mL columns. In addition, up to 8 RoboColumn® columns can be operated in parallel with a Freedom EVO workstation, which significantly reduces run time. It also allows to replicate runs and | or screen more conditions.

5. Conclusion

The optimization of loading conditions for MAb purification in flow-through mode on two chromatographic resins was conducted at small scale. The study demonstrates that RoboColumn® columns are able to provide data comparable to standard laboratory scale columns. Similar conclusions on the resin performances could be raised from the analysis of results on both column formats.

This highlights the good suitability of RoboColumn® columns for screening of operating conditions. This is particularly true in flow-through mode where dynamic loading and loaded sample volume generating displacement phenomena can significantly affect the purification performances.

Screening tools such as RoboColumn® columns and 96-well filter plates used in HTPD mode save sample volume consumption and time compared to prepacked columns. This substantially improves screening productivity.

6. References

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Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For further contacts, visit
www.sartorius.com

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