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Efficient Contaminant (HCP, DNA) Removal Using MEP HyperCel Chromatography Resin for Monoclonal Antibody Purification

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1. Abstract

This study shows the efficiency of HCIC (Hydrophobic Charge Induction Chromatography) on MEP HyperCel resin in removing contaminants (DNA and HCP) during the capture step of three different monoclonal antibodies. The importance of the optimization of elution pH, washing steps, and buffer composition during methods development is highlighted.

Data demonstrate that MEP HyperCel contributes very efficiently (4 to 5 Log reduction) to DNA removal during the capture step.

2. Introduction

Monoclonal antibody (MAb) purification processes usually comprise three chromatographic steps. This study focuses on the capture step, which is critical for primary isolation of the antibody from the feed and for contaminant removal. Protein A affinity chromatography is usually employed for antibodies because of the high purity levels achieved. Drawbacks associated with its use have, however, prompted the evaluation of alternative resins for this capture.^[1] Among them, hydrophobic charge induction chromatography (HCIC) on MEP HyperCel resin is a chromatography method specifically developed to purify antibodies from various feedstocks.^{[2],[3]} Efficient binding is achieved in physiological conditions, allowing for direct loading after primary recovery of the feed.^[4] Elution is performed in mild acidic conditions, allowing for an easy connection with chromatography techniques usually employed as intermediate purification steps such as ion exchange. In this study, the performance of MEP HyperCel resin in capturing different MAbs from various cell culture supernatants was investigated.

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3. Materials and Methods

3.1. Monoclonal Antibodies

This study was performed using three different cell culture supernatants (CCS). Feedstock 1 contained MAb expressed in Chinese hamster ovary cells (CHO cells) at a concentration of 0.9 mg/mL. Feedstock 2 was a SP2/0 protein-free CCS expressing MAb at 0.5 mg/mL. Feedstock 3 contained MAb expressed at 0.7 mg/mL in SP2/0 cells also, but in this case the culture medium was supplemented with albumin and transferrin.

3.2. Chromatography

All runs were performed on an ÄKTAexplorer* 100 chromatography system (Cytiva), using columns ranging from 5 to 25 mm internal diameter (ID).

The clarified and 0.2 µm-filtered feedstocks were loaded directly on MEP HyperCel columns equilibrated in PBS pH 7.1 or 7.4 or 50 mM TRIS buffer pH 8.0. The column was washed with equilibration buffer, and the elution was performed using sodium acetate or sodium citrate buffers at pH ranging from 5.6 to 3.0. The elution was either performed through a sequence of buffers of decreasing pH, or using a single elution buffer after optimization. Additionally, a second washing step could be performed before the elution started, using either an elution buffer containing 0.5 M NaCl at pH 6.5 to 5.5, depending on the MAb elution pH, or using a sodium phosphate buffer at low concentration (10 mM) at equilibration buffer pH. Each chromatography run was completed by a strip at pH 3.0 and a cleaning procedure using 1 M NaOH (4 to 5 column volumes (CV), 30 to 60 minutes contact time).

3.3. Analytics

Analytical Size Exclusion Chromatography (SEC) was performed using a TSKgel* G3000SWXL column (Tosoh Bioscience). Host Cell Protein (HCP) concentrations were determined using a HCP Elisa kit (Cygnus Technologies) according to the manufacturer protocols. DNA concentrations were analyzed using a Quant-iT* PicoGreen* kit (Invitrogen). SDS-PAGE analyzes were performed using NuPAGE* Novex* Bis-Tris 4-12% gels (Invitrogen).

4. Results and Discussion

4.1. Case 1: Purification of a MAb from Protein-Free CHO Cell Culture Supernatant

4.1.1. Capture Step Development and Optimization Process

The development of an efficient capture step on MEP HyperCel includes typically:

1. MAb elution pH optimization using buffers with incrementally decreasing pH
2. Determination of adapted washing steps to achieve optimal MAb purity
3. Flow rate and residence time optimization

4.1.2. Determination of Elution Conditions by Decreasing pH Steps⁽¹⁾

The objective is to determine the highest possible pH allowing good MAb recovery with the lowest contaminant level. After direct loading of the CCS (Feedstock 1, CHO cell culture) on the MEP HyperCel column, a pH elution study is performed from pH 5.5 to 3.0 (e.g. pH 5.5 | 5.0 | 4.5 | 4.0 | 3.0).

In this first experiment, the column was equilibrated in 50 mM TRIS pH 8.0. About 10 mg IgG/mL of resin was loaded; the column was washed with equilibration buffer (wash 1), and the elution performed through a sequence of steps of decreasing pH from 5.6 to 3.0. Finally, the column was cleaned with 100 mM EDTA pH 7.2 to prevent fouling due to components from the protein free cell culture media and regenerated using 1 M NaOH.

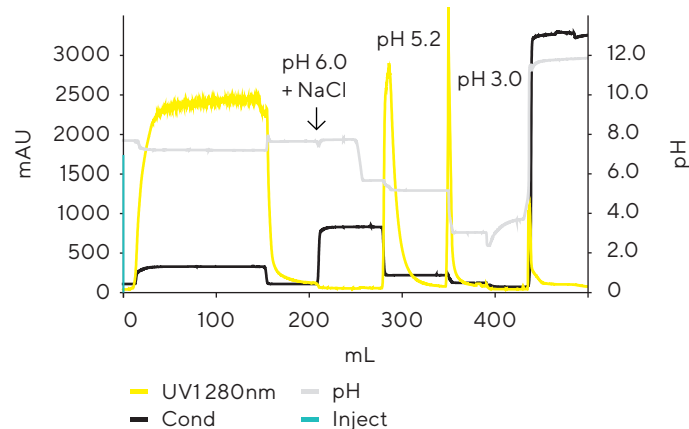
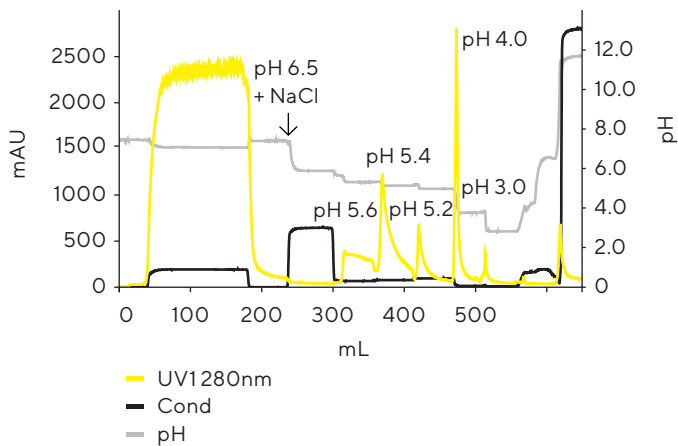


Figure 1: Development of a MAb Capture Step on MEP HyperCel from CHO Protein-Free CCS (Feedstock 1) – Direct Loading and pH Step Elution (pH 5.6 – 3.0)

Figure 2: Optimized MAb Capture Step on MEP HyperCel from CHO Protein-Free CCS (Feedstock 1)

Column:	10 mm ID x 18 cm bed height packed with 14 mL MEP HyperCel resin
Equilibration Wash 1:	50 mM TRIS pH 8.0 (4 CV each)
Sample:	Filtered CHO cell culture supernatant
Wash 2:	50 mM sodium phosphate, 0.5 M NaCl, pH 6.5
Elution:	100 mM sodium acetate, pH 5.6; pH 5.4; pH 5.2 (4 CV each); 50 mM sodium acetate, pH 4.0 (4 CV), pH 3.0 (2.3 CV)
Cleaning:	100 mM EDTA, pH 7.2 (3 CV); 1 M NaOH (5 CV)
Flow rate:	154 cm/h (residence time: 7 min.)

Column:	25 mm ID x 10.8 cm bed height packed with 53 mL MEP HyperCel resin
Equilibration wash 1:	50 mM TRIS pH 8.0 (4 CV each)
Sample:	Filtered CHO cell culture supernatant
Wash 2:	50 mM sodium phosphate, 0.5 M NaCl, pH 6.0 (4 CV)
Elution:	100 mM sodium acetate, pH 5.2 (4 CV each); 50 mM sodium acetate, pH 3.0 (3 CV)
Cleaning:	1 M NaOH (4 CV)
Flow rate:	93 cm/h (residence time: 7 min.)

4.1.3. Determination of the Washing Conditions

Evaluation of an Additional Wash Step

This wash (Wash 2, Figure 1) in the presence of 0.5 M NaCl, performed at an intermediate pH between the equilibration pH and the highest elution pH, was initially intended to desorb unbound impurities while retaining the antibody. In fact, this washing step 2 did not desorb significant amount of proteins; however, it accelerated the return of the UV signal to the baseline (Figure 1). Then, three successive peaks were eluted at pH 5.6, 5.4, and 5.2, with similar intensities. The analysis of the fractions showed that most of the MAb was recovered between pH 5.6 and 5.2. At pH 4.0, only 5% IgG remained on the column.

The HCP analysis showed that the HCP level was significantly lower in the elution pool pH 5.6 – 5.2 (1.9 µg/mL) than in the elution fraction at pH 4.0 (51.2 µg/mL), evidencing clearly the importance of this elution pH optimization. DNA was also analyzed and evidenced that in the elution fractions (pH 5.6 – 5.2), the concentration was below the limit of detection of the test.

From these results, the selected elution pH was pH 5.2 and the additional washing step was kept. Since no protein peak and especially no IgG was desorbed at pH 6.5, a lower pH was investigated to increase the rapidity of the return of the UV signal to the baseline: pH 6.0, with 0.5 M NaCl. A thorough washing step and the complete return of the UV signal to the baseline are important for efficient contaminants removal before the elution of the MAb. (Figure 2)

Purification Performance after Optimization

Fraction	Start Feedstock	MEP HyperCel
IgG yield total recovery [%]	100	93
HCP [ng/mL]	92000	8600
HCP [ng/mg IgG]	102000	1200
HCP (Log ¹⁰ reduction)	-	1.9
DNA [ng/mL]	705	< 0.1
DNA ng/mg IgG	781	< 0.014
DNA (Log ¹⁰ reduction)	-	> 4.7

Table 1: Results Achieved after the Purification of a MAb (Feedstock 1) on MEP HyperCel Resin Contaminant Removal Efficiency (MAb Expressed in Protein-Free CHO cell culture)

The data in Table 1 demonstrate a 100-fold reduction in HCP and particularly a significant DNA removal during the MEP HyperCel capture step with a reduction factor estimated to minimum 4.7 Log (DNA concentration was below 0.1 ng/mL, the detection limit of the test). With the final DNA level already below the acceptable limit for the final purified product, complementary steps in the purification process could focus on remaining contaminants such as HCP.

4.2. Case 2: Purification of a MAb from Protein-free SP2/O Cell Culture Supernatant

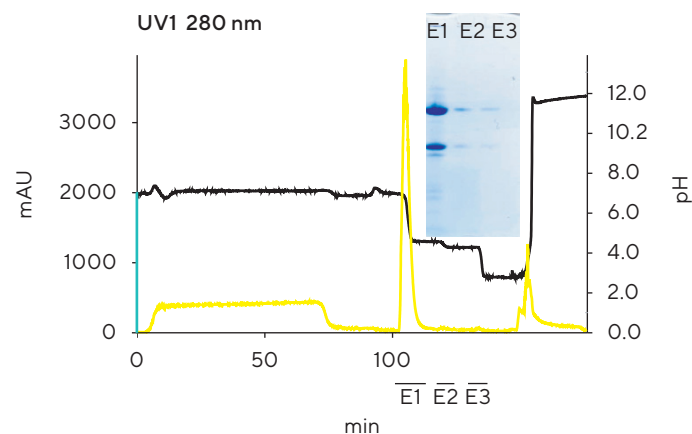
Sample Courtesy of Dr Serenella Serani, TecnoGen, Piana di Monte Verna, Italy

A SP2/O CCS containing MAb at a concentration of approximately 0.5 mg/mL (Feedstock 2) was purified with MEP HyperCel resin after optimization of the elution pH and flow rate parameters. A study was performed to specifically test the impact of the buffer nature selection on antibody purity and recovery.

4.2.1. Influence of the Elution Buffer Type on the Purification Efficiency

The elution buffer nature has an impact on the MAb final purity and the contaminant profile. After elution pH optimization using sodium acetate buffers, antibody elution ended at pH 4.8 (Figure 3A, fraction E1). Only slight IgG traces were detected in subsequent elution fractions (E2, E3) at pH 4.5 and 3.0. A similar experiment (B) employing sodium citrate evidenced a different elution profile with antibody still significantly eluted at pH 4.5 (Figure 3B, fraction E2). This example suggests that buffer types must be carefully selected, and that in some cases sodium acetate could allow for a complete elution at higher pH than sodium citrate, which can be of interest in applications where target proteins have a limited stability at acidic pH.

A.



B.

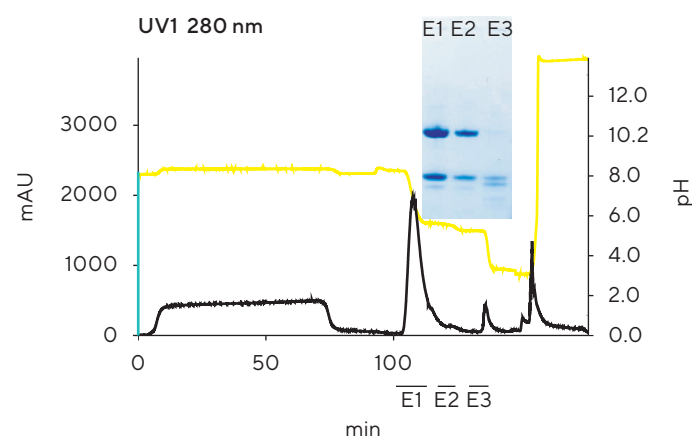


Figure 3: MAb Capture Step on MEP HyperCel (Feedstock 2) - Sodium Acetate Elution (A) versus Sodium Citrate Elution (B)

Column:	PRC column, 5 mm ID x 5 cm bed height, pre-packed with 1 ml MEP HyperCel resin
Equilibration wash 1:	PBS pH 7.1
Sample:	Filtered SP2/O cell culture supernatant
Wash 2:	10 mM sodium phosphate, pH 7.1
Elution:	100 mM sodium acetate (Figure 3A) or 50 mM sodium citrate (Figure 3B) pH 4.8 (E1); 4.5 (E2); 3.0 (E3)
Cleaning:	1 M NaOH
Flow rate:	100 cm/h (residence time: 3 min.)

Purification Performance

Fraction	Start feedstock	MEP HyperCel
IgG yield total recovery [%]	100	87
IgG [mg/mL]	0.67	7.69
HCP [ng/mL]	3300	2700
HCP [ng/mg IgG]	6600	351
HCP (Log ¹⁰ reduction)	-	1.3
DNA [ng/mL]	6000	1.2
DNA [ng/mg IgG]	12000	0.15
DNA (Log ¹⁰ reduction)	-	4.9

Table 2: Contaminant Removal Efficiency
(MAb Expressed in Protein-free SP2/O Cell Culture)

The MAb was significantly concentrated through this capture step. HCP level was reduced about 20-fold from 6600 to 351 ppm. Complementary steps would be necessary to achieve acceptable HCP level in the final purified product. Finally, these results confirmed the high performance of MEP HyperCel resin in DNA elimination, since DNA was decreased to 1.2 ng/mL (0.15 ppm), which corresponds to a 4.9 Log reduction.

4.3. Case 3: Purification of a MAb from a Protein-Supplemented SP2/O Cell Culture Supernatant in the Presence of Cell Culture Supplements (Albumin)

A mouse monoclonal antibody expressed in SP2/O cells (Feedstock 3) was captured on MEP HyperCel resin. Cell culture conditions required the addition of different supplements, such as albumin, which was present at high concentration in the feedstock. Purity of the antibody in the crude was estimated to be less than 5% using Size Exclusion Chromatography (SEC). After optimization of the capture step (Figure 4), purification performance in terms of contaminants and DNA elimination was evaluated (Table 3).

Fraction	Start Feedstock	MEP HyperCel eluate E1
IgG yield total recovery [%]	100	97
Purity ⁽¹⁾ [%]	< 5	89
DNA [ng/mL]	60000	68
DNA [ng/mg IgG]	86000	34

⁽¹⁾ SEC determination on a TSKgel G3000SWXL column.

Table 3: Results Achieved after the Purification of a MAb (Feedstock 3. SP2/O CCS) on MEP HyperCel

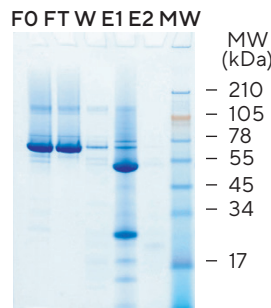
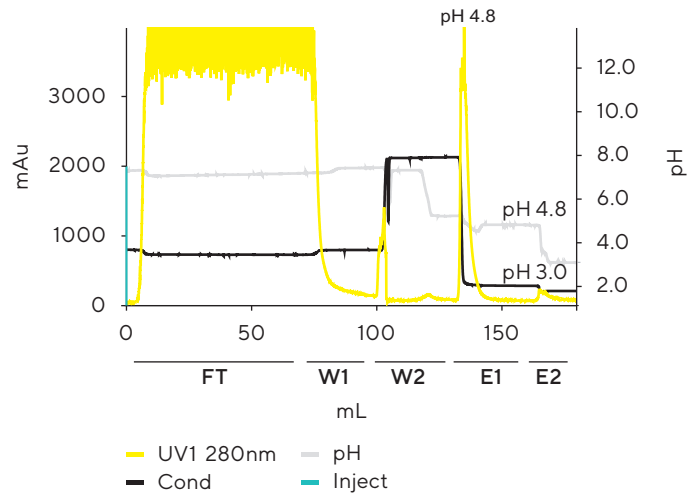


Figure 4: MAb capture step on MEP HyperCel – Application to Feedstock 3. Protein-Supplemented SP2/O Cell Culture Supernatant

Column:	6.6 mm ID packed with 5.3 mL MEP HyperCel resin
Equilibration wash 1:	PBS pH 7.4
Sample:	Filtered SP2/O CCS
Wash 2:	0.1 M sodium acetate, 0.5 M NaCl
Elution:	0.05 M sodium acetate pH 4.8 (E1); 0.1 M sodium acetate pH 3.0 (E2)
Cleaning:	1 M NaOH
Flow rate:	125 cm/h (residence time: 7.5 min.)

The DNA content in the eluate was reduced from 86000 ppm to 34 ppm, which corresponds to a 3.4 Log reduction. Purity of the sample was significantly improved. Albumin was the major contaminant remaining after this capture step and was removed by further chromatographic steps on CM Ceramic HyperD[®] ion exchange resin and Q membrane adsorber (not shown).

5. Conclusion

This study assessed the performance of a MEP HyperCel capture step for monoclonal antibodies. Very efficient DNA removal is accomplished by a single capture step with MEP HyperCel resin. HCP clearance requires an appropriate optimization of elution conditions, and 20 to 100-fold reduction factors are reported in this study. As DNA was efficiently eliminated, complementary chromatography steps in the process can then focus on HCP polishing, which requires an additional reduction to achieve acceptable levels for the delivery of the final product. Finally, the conditions applied for the use of MEP HyperCel resin allow an easy integration of this step in a MAb purification process and improve process flow.

6. References

1. S. Ghose, B. Hubbard, S.M. Cramer, **J. Chromatogr. A** 1122 (2006) 144
2. S.C. Burton, D.R.K. Harding, **J. Chromatogr. A** 814 (1998) 71
3. L. Guerrier, P. Girot, W. Schwartz, E. Boschetti, **Bioseparation** 9 (2000) 211
4. L. Guerrier, I. Flayeux, E. Boschetti, **J. Chromatogr. B** 755 (2001) 37


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