Life Sciences

Application Note

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Optimization and Scale-up of Antibody Purification Conditions by Hydrophobic Charge Induction Chromatography (HCIC) on MEP **HyperCel**™ Sorbent: Application to Monoclonal Mouse IgG_1

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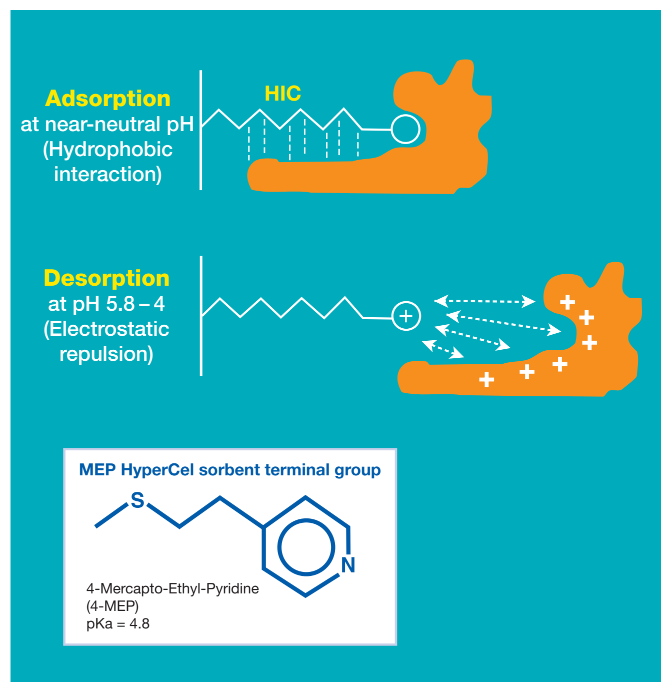
Optimization and Scale-up of Antibody Purification Conditions by HCIC on MEP **HyperCel** Sorbent: Application to Monoclonal Mouse IgG_1

What this Study Demonstrates

This study of HCIC on MEP **HyperCel** sorbent demonstrates that high-purity antibody can be directly recovered at high yields in a single capture step, even from feedstocks containing substantial concentrations of contaminating proteins such as albumin. The HCIC dual-mode mechanism allows a fine tuning of the elution conditions, based on relative hydrophobicity of the proteins in the sample and pH step-elution. This mechanism allows an efficient selective desorption of the target antibody from contaminants. After optimization using conventional low-cost buffers, a 97 % purity and a 97 % yield are obtained and are similar to those obtained using affinity on protein A resins by a direct capture from a non-concentrated feedstock.

Finally, MEP **HyperCel** is an industry-scalable sorbent, that can be treated with 1M sodium hydroxide for cleaning and represents a cost-effective option for large-scale IgG capture.



*Adsorption / elution mechanism of MEP **HyperCel** sorbent.*

1. Introduction

Hydrophobic Charge Induction Chromatography (HCIC) on BioSepra MEP **HyperCel** sorbent is a chromatography method specifically developed to purify and recover antibodies from various feedstocks. The method allows a direct capture of antibodies at physiological pH and the elution is performed at mild acidic pH. High yield and purity levels are usually reached after short optimization studies, therefore positioning MEP **HyperCel** sorbent as a cost-effective and powerful alternative to affinity resins.

This study shows that MEP **HyperCel** sorbent can be advantageously used for efficient capture and purification of mouse IgG_1 in a single step operation.

2. Objective

Optimize Hydrophobic Charge Induction Chromatography (HCIC) as a potential replacement of affinity (protein A) for the direct capture of monoclonal antibodies intended for therapeutic or diagnostic use. The final goal was to design a robust and scalable method for capturing mouse IgG_1 from albumin containing feedstocks, using MEP **HyperCel** sorbent, in a single step, with at least 90% purity and yield.

3. Materials and Methods

- **Samples:** Optimization was performed with two different cell culture supernatants (CCS), containing albumin and transferrin from the cell culture medium: a 30-fold concentrated CCS [mouse IgG_1: 1.5 mg/mL] and a non-concentrated CCS [IgG_1: 0.043 mg/mL].

- **Chromatography:** HCIC chromatography on a MEP **HyperCel** sorbent (Pall), equilibration in PBS, pH 7.4. The CCS were loaded directly on the column; after the runs, the MEP **HyperCel** sorbent was regenerated using approximately 5 column volumes of 1 M NaOH. Small-scale runs were performed on an ÄKTA* Explorer 100 (GE Healthcare) and scale-up runs on a PKL6 chromatography system (Pall).

- **Analytics:** The fractions were analyzed using both SDS-PAGE (12% polyacrylamide gels) and SELDI on the ProteinChip* System (Ciphergen Biosystems, Inc.). IgG purity was assessed by SEC HPLC on a TSKgel* G4000SWXL column (Tosoh Bioscience). Protein concentration was determined by BCA standard assay (Interchim).

Optimization and Scale-up of Antibody Purification Conditions by HCIC on MEP **HyperCel** Sorbent: Application to Monoclonal Mouse IgG_1

4. Chromatography Optimization Methodology by HCIC on MEP **HyperCel** Sorbent.

4.1. pH step-elution and specific washing procedures

MEP **HyperCel** sorbent captures antibodies directly from crude feedstreams by hydrophobic charge induction mechanism. IgG capture is achieved at physiological pH, and elution is prompted by decreasing and adjusting the pH by step elution (typically in the pH range from 6 to 3). Contaminants from cell culture such as albumin, transferrin or other hydrophobic species may also bind to the MEP ligand. A fraction of these contaminants can be removed by specific washing steps, typically water and sodium caprylate washes (see Figure 1), but tuning of the mobile phase pH and composition is necessary to achieve optimal purity (see Section 5.2).

4.2. Flow Rate and Residence Time Optimization

Residence time also influences the separation quality (yield) and duration. The initial experiments allowed for a 5 minutes residence time. Studies on yield and purity versus residence time are performed to reduce the cycle time during the scale-up phase (see Section 5.3).

4.3. Scale-up

Once conditions (pH elution and residence time) are determined, scale-up is achieved by increasing the column diameter at constant bed height. MEP **HyperCel** sorbent is process-scalable, and can be used from mL to 100 L column sizes.

5. Direct Capture of IgG_1 from Concentrated Cell Culture Supernatant: Optimization

5.1. Determination of the Initial IgG_1 Capture and Elution Parameters

As shown in Figure 1, the concentrated CCS [IgG_1: 1.5 mg/mL] was loaded on the MEP **HyperCel** column equilibrated in PBS, pH 7.4. After injection, a three-step washing sequence was applied (PBS, H_20, sodium caprylate, W1, W2,W3) and step elution was performed at pH 4.5 (E1), 4.0 (E2), and 3.0 (E3).

SDS-PAGE analysis (Figure 2) showed that, as expected from the cell culture conditions, the loading fraction was heavily contaminated by albumin. The washing steps with deionized water and sodium caprylate desorbed only a small fraction of the albumin (W2 and W3). The target IgG_1 was mainly recovered after the pH 4.5 elution with an estimated purity of 70-80% (SDS-PAGE). Some of the IgG was also desorbed during washing, due to column overloading.

Conclusion: **In the non-optimized initial sequence, the final IgG fraction was, however, still contaminated by albumin and transferrin and therefore required further optimization (see Section 5.2).**

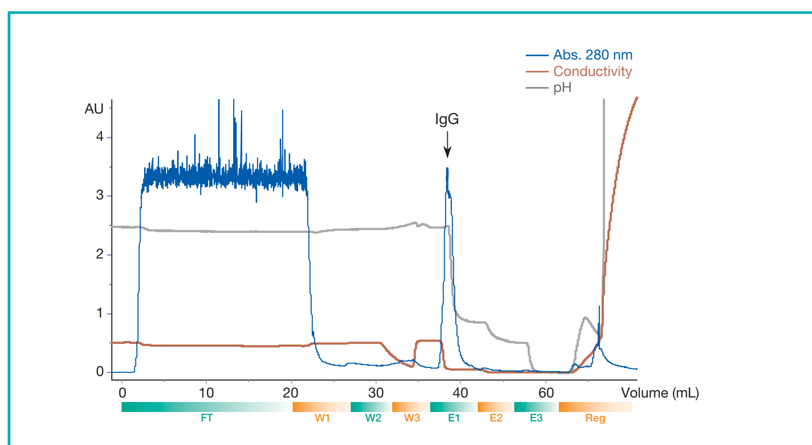


Figure 1. Purification of a mouse IgG_1 from a concentrated feedstock with a direct elution at pH 4.5.

Column: 0.46 cm I.D. x 5 cm height, volume: 0.83 mL; Sample: concentrated IgG_1 feedstock; Load: 20 mL; Equilibration buffer: PBS, pH 7.4; Wash buffers: PBS, pH 7.4 (W1), deionized water (W2), 25 mM sodium caprylate in PBS (W3); Elution buffers: 50 mM sodium acetate, pH 4.5 (E1), pH 4.0 (E2), pH 3.0 (E3); Regeneration: 1 N NaOH; Flow rate: 60 cm/h (residence time: 5 min.)

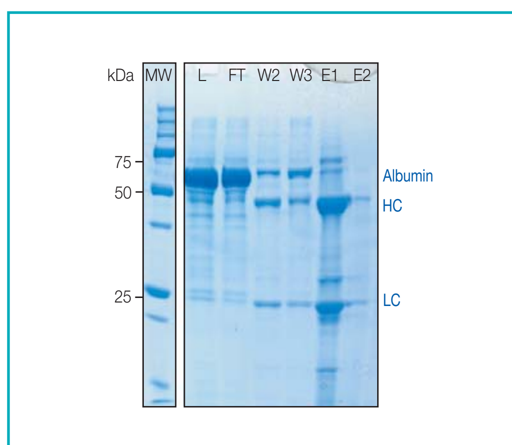


Figure 2. SDS-PAGE analysis in reduced conditions.
L: Load
FT: Flowthrough
W2: Wash 2 (deionized water)
W3: Wash 3 (sodium caprylate)
E1: Elution pool 1 (pH 4.5)
E2: Elution pool 2 (pH 4.0)

Optimization and Scale-up of Antibody Purification Conditions by HCIC on MEP **HyperCel** Sorbent: Application to Monoclonal Mouse IgG_1

5.2. Principle of Purity Optimization Based on HCIC Mechanism: Buffer Salt Concentration and Elution pH

HCIC is based on the pH-dependent behaviour of a dual mode synthetic ligand (4-mercapto-ethyl-pyridine). On MEP **HyperCel** sorbent, after physiological capture, the IgG desorption is triggered by electrostatic charge repulsion: this is achieved by reducing the pH of the buffer, which establishes opposing (positive) charges on both the ligand and the antibody. When the pH of the buffer is reduced, the magnitude of the opposing charges depends on the isoelectric point of the antibody, the feedstock impurities, and the pKa of the ligand.

Therefore, the HCIC mechanism allows a fine tuning of the separation based on both pH elution and relative hydrophobicity of the different species co-existing in a complex feedstream. In practice, this is done by adjusting two operational parameters: the salt concentration and the pH of the buffer (during step-elution).

The different conditions tested are summarized in Figure 3. In this case, after standard loading and equilibration in PBS, optimization resulted in the addition of 0.5 M NaCl in the elution buffer at pH 5.5 (thus enhancing the selectivity of the IgG elution). This was followed by one pH elution step at pH 5 without salt (E2) (two other steps tested at pH 4 and 3 did not result in desorption of additional IgG and were dropped in the final sequence).

The final elution sequence adopted was therefore:

1. Albumin desorption: First elution step (E1) at pH 5.5 in the presence of 0.5 M NaCl.

2. IgG elution: Second elution step (E2) at pH 5: The IgG was totally eluted during this step – yield 93%, estimated purity by SEC HPLC 97% – see Figure 4).

Conclusion: **As shown in Figures 5 and 6, and in Table I, the optimization of the elution parameters resulted in a significant enhancement of both purity and yield of the IgG_1 compared to the initial conditions described in Section 5.1.**

Conditions	Results	Conclusions
Standard Conditions		
• Binding: PBS, pH 7.4 • Wash: PBS, water, sodium caprylate • Elution: pH 4.5, 4.0, 3.0	70 – 80 % purity (albumin contamination)	• Fine tuning elution pH • Increase selectivity through NaCl additions in elution buffers
Elution Optimization		
• Binding: PBS, pH 7.4 • Wash: PBS, pH 7.4 • Elution: pH-step elution (from pH 5.5 to 3.0) – with or without addition of 0.5 M NaCl in elution buffers	Selectivity improved by differential elution of IgG versus albumin	• Albumin is eluted at higher pH than IgG when the buffers contain NaCl • Complete elution of IgG when NaCl concentration decreases
Optimized Conditions		
• Binding: PBS, pH 7.4 • Wash: PBS, pH 7.4 • Elution: First elution step: pH 5.5 + 0.5 M NaCl Second elution step: pH 5.0 - no salt	Purity > 90 % Yield > 90 %	• First elution step: albumin desorption • Second elution step: IgG elution

Figure 3. Optimization principles

Optimization and Scale-up of Antibody Purification Conditions by HCIC on MEP **HyperCel** Sorbent: Application to Monoclonal Mouse IgG_1

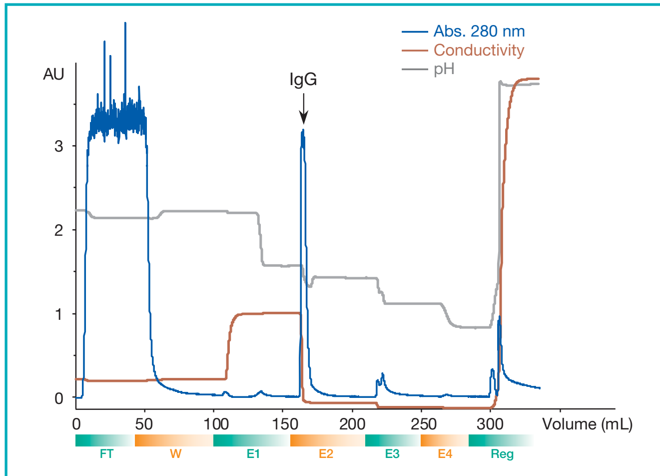


Figure 4. Purification of a Mouse IgG_1 from a concentrated feedstock on MEP **HyperCel** sorbent (optimized protocol).

Column: 1 cm I.D. x 9.7 cm height, volume: 7.6mL; Sample: concentrated IgG_1 feedstock; Load: 45 mL; Equilibration: PBS, pH 7.4; Wash: PBS, pH 7.4; Elution buffers: 100 mM sodium acetate, pH 5.5 + 0.5 M NaCl (E1), 50 mM sodium acetate, pH 5.0 (E2), 50 mM sodium acetate, pH 4.0 / 3.0 (E3 / E4); Flow rate: 80 cm/h (residence time: 7.5 min.).

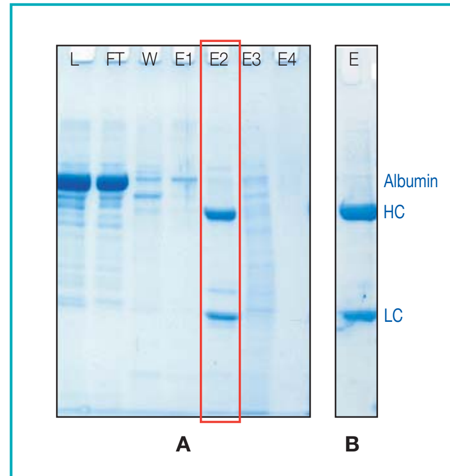


Figure 5. SDS-PAGE analysis in reduced conditions. Fractions from the purification of a mouse IgG_1 from a concentrated feedstock.

A: *Optimized purification on MEP* ***HyperCel*** *sorbent.*

B: *Purification on Protein A Ceramic HyperD® F sorbent.*

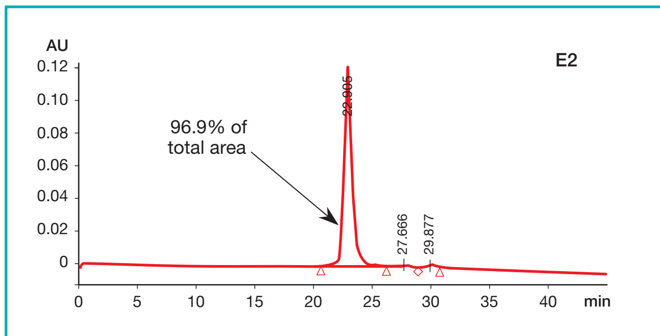


Figure 6. Analysis on a TSKgel* G4000SWXL gel filtration column of elution pool E2 from purification of concentrated Mouse IgG_1 feedstock on MEP **HyperCel** sorbent (optimized protocol).

Table I. Yields and purification factor for the purification of concentrated CCS on MEP HyperCel sorbent following an optimized protocol.

Step	[IgG_1] (mg/mL)	Purity (%)	Purification factor	Yield (%)
Conc. CCS	1.5	4	1	100
Elution pool E2	2.6	97	24	93

Optimization and Scale-up of Antibody Purification Conditions by HCIC on MEP **HyperCel** Sorbent: Application to Monoclonal Mouse IgG_1

5.3. Optimization of Operational Parameters for Scale-up (cycle duration)

For scale-up considerations, the best trade-off between cycle duration and yield was optimized by studying the influence of various residence times. Typically, on MEP **HyperCel** sorbent, the users should allow a minimum residence time of 5 – 8 minutes for optimal results (capacity, yield). In this case, the results summarized in Figure 7 showed that the minimum residence time to achieve 90% IgG yield was estimated at approximately 7 minutes. In the final process, the total cycle duration was further optimized by reduction of elution time. Residence time is longer than normal due to the high protein concentration of the sample.

Conclusion: **The final total cycle duration was significantly reduced and resulted in the purification of 67 mg of 97% pure IgG_1 from 45 mL of feedstock on a 7.6 mL MEP HyperCel column.**

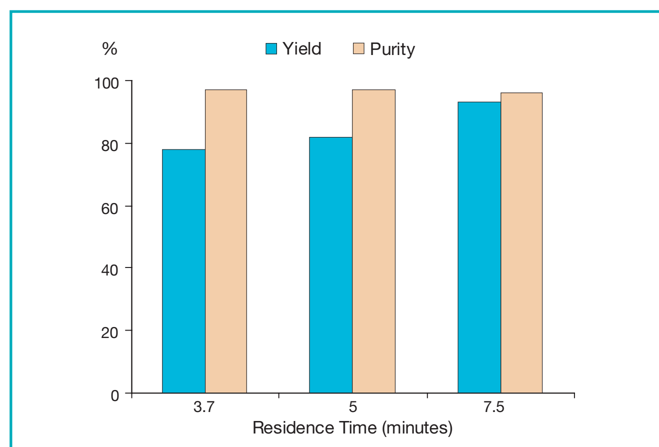


Figure 7. Evolution of yield and purity versus residence time during the purification of a 30-fold concentrated feedstock on MEP **HyperCel** sorbent through an optimized protocol.

Optimization and Scale-up of Antibody Purification Conditions by HCIC on MEP HyperCel Sorbent: Application to Monoclonal Mouse IgG_1

6. Direct Capture of IgG_1 from Non-Concentrated Cell Culture Supernatant: Optimization and Scale-up

The previously optimized protocol was successfully applied to the capture of IgG_1 from the non-concentrated feedstock [IgG_1: 0.043 mg/mL]. The conditions are summarized in Figure 8, and results in Figure 9 and Table II.

The lower protein concentration allowed to increase the efficiency of the capture and therefore to significantly decrease the residence time to 3 – 5 minutes without any loss of IgG yield.

Conclusion: A 60-fold scale-up was achieved in increasing the column diameter. MEP HyperCel sorbent achieved efficient IgG_1 capture (yield 97%, purity 97%) from the non-concentrated CCS, with shorter residence times, suggesting its use as a capture device and eliminating the need for the initial concentration step.

Table II. Yield and purification factor for the scale-up of the purification of a non-concentrated CCS on MEP HyperCel sorbent.

Step	[IgG_1] (mg/mL)	Purity (%)	Purification factor	Yield (%)
CCS	0.043	2	1	100
Elution pool E2 Scale up	0.77	97	45	97

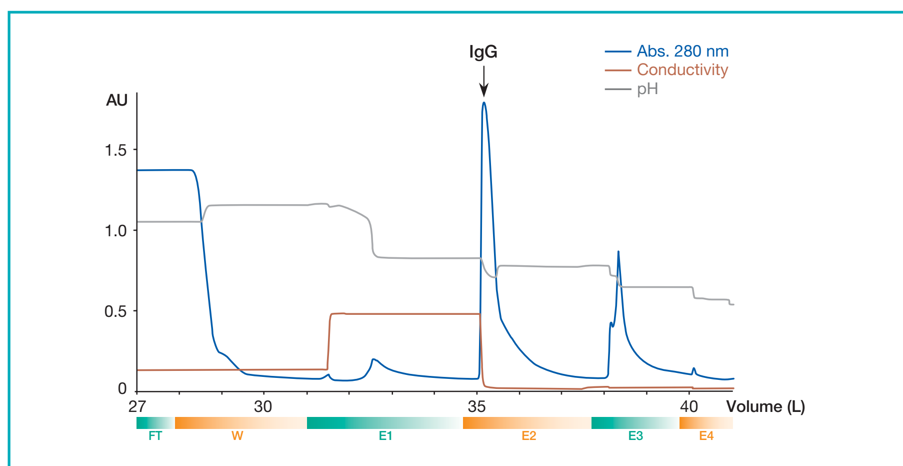


Figure 8. Purification of a mouse IgG_1 from 28 L of non-concentrated cell culture supernatant on MEP **HyperCel** sorbent.

Column: 5 cm I.D. x 17.5 cm height, volume: 343 mL; Sample: non-concentrated cell culture supernatant; Load: 28 L; Equilibration + Wash: PBS, pH 7.4; Elution: 100 mM sodium acetate, pH 5.5 + 0.5 M NaCl (E1); 50 mM sodium acetate, pH 5.0 (E2); 50 mM sodium acetate, pH 4.0 / pH 3.0 (E3 / E4); Flow rate: Loading: 230 cm/h (residence time: 4.6 min), Wash / elution: 300 cm/h (residence time: 3.5 min)

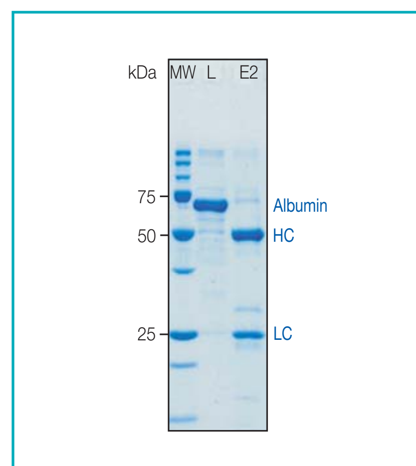


Figure 9. SDS-PAGE analysis in reduced conditions.

Optimization and Scale-up of Antibody Purification Conditions by HCIC on MEP **HyperCel** Sorbent: Application to Monoclonal Mouse IgG_1

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Ordering Information

Sorbent	Pack size	Part Number
MEP **HyperCel**	5 mL	12035-069
	25 mL	12035-010
	100 mL	12035-028
	1 L	12035-036
	5 L	12035-040
	10 L	12035-044

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