Two step purification of monoclonal antibodies with Cellufine Affinity and Mixed Mode Chromatography Resins

Cellufine resins are spherical porous cellulose particles that have been surface modified with a range of chromatography chemistries. They are widely used in the manufacture of biologics such as antibodies, vaccines, and therapeutic proteins. Monoclonal antibody (mAb) molecules offer excellent specificity with low side effects. They are forming a large market to replace small molecule drugs. However, since mAb's are large multi-subunit proteins, advanced purification technology workflows are required. Protein A affinity chromatography plays a key capture role at the start of the purification workflow for mAb's. Protein A is extremely expensive and dominates the overall manufacturing cost. Therefore, it is important to select a resin that offers high performance at a competitive price. JNC's Cellufine SPA-HC offers an attractive balance between performance and cost. In this technical report a method for achieving cost effective two-step purification of a mAb will be described. In this workflow, a Protein A capture step is followed by a flow through polishing step employing mixed mode anionic Cellufine MAX IB with minimal pH and ionic strength adjustment required between steps. By efficiently combining capture and polishing it is possible to purify a mAb in a cost-effective workflow with minimal time spent moving between the two chromatography modes.

1. Purification Outline

CHO cell culture and recovery of mAb's presents a purification challenge. In addition to the target antibody there are the following contaminants that must be removed from the final therapeutic product; a) host cell proteins (CHO-HCP), b) host cell-derived dsDNA fragments, c) antibody aggregates and d) leached whole Protein A affinity ligand and proteolytic fragments generated during the initial affinity capture step. To achieve efficient purification of a mAb, a two-step purification workflow has been developed and will be described below.

Step 1 - rProtein A affinity capture

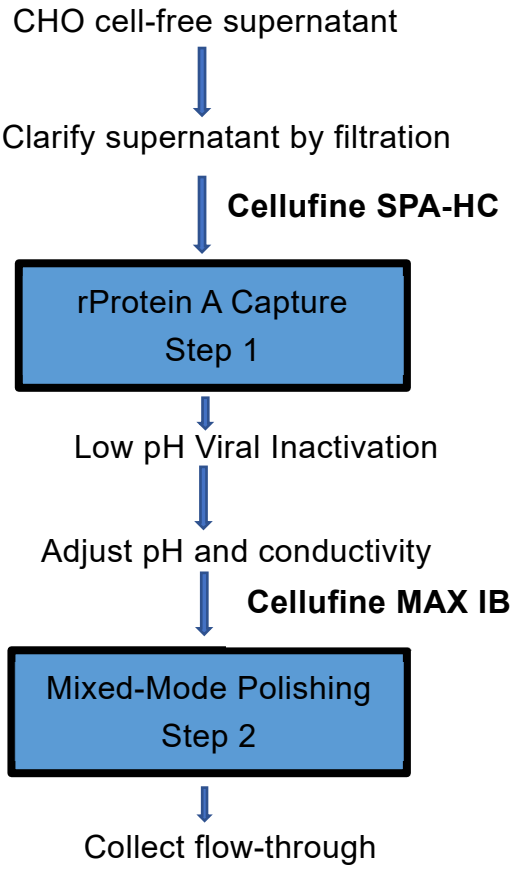
In this first step, a Cellufine SPA-HC rProtein A affinity chromatography column is used to capture mAb from the CHO cell clarified supernatant sample. Due to the high binding affinity of rProtein A for mAb's this step removes 99% of the impurities in the CHO cell supernatant. Cellufine SPA-HC utilizes a novel recombinant rProtein A ligand with high alkali stability. The cellulose base bead is produced with our unique manufacturing technology and is designed to maximize the balance between adsorption capacity and structural stability allowing for the use of high flow rates during processing steps.

Step 2 - Mix mode polishing

After rProtein A affinity capture step, a flow through mixed mode polishing step is used to remove antibody aggregates, leached rProtein A, and CHO-HCP. In this technical report Cellufine MAX IB was used as a mixed mode Anionic-Butyl chromatography resin to remove contaminants after rProtein A capture.

The combined two-step capture and polishing protocol is summarized in Figure 1 below.

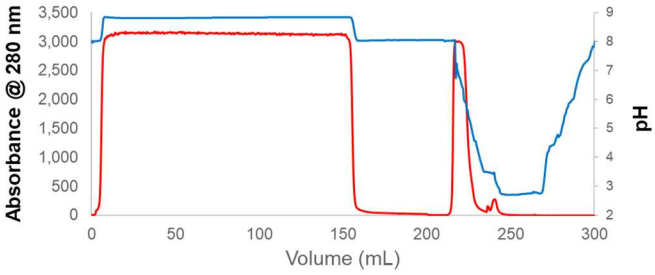
Fig.1 Two-Step mAb purification workflow



2. Protein A Chromatography

After removing the cultured cells, CHO cell culture supernatant was clarified with a 0.22um PES filter to remove high molecular weight impurities. The mAb's in the filtrate were then captured by Protein A chromatography (Fig.2) with a pre-packed Cellufine SPA-HC 5 mL cartridge (ID.14.6mm x H 30mm).

Fig.2 Chromatography with Cellufine SPA-HC



Column: 1.46 cm ID x 3.0 cmL (volume= 5 mL)

Loading: 80% of 10% DBC (50 mg/mL at 4 min residence time) for a 200 mg total load

Antibody titer: 5 mg/mL

Flow Rate: 1.25 mL/min (4 min residence time)

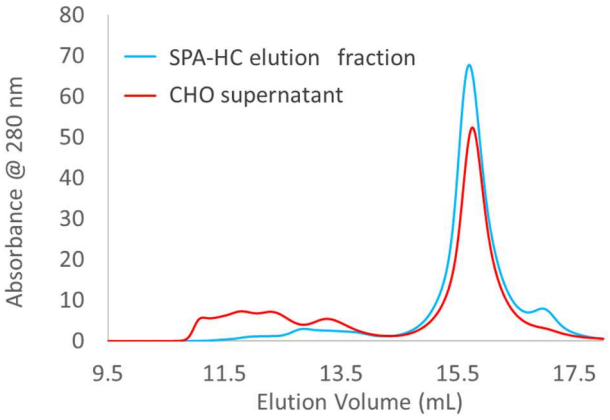
Elution: 10 CV, 60 mM Acetic acid pH 3.5

A high overall mAb antibody recovery rate of 93% was achieved in this initial capture step. Subsequent SEC-HPLC analysis showed that the level of mAb dimers or higher molecular aggregates contaminants was efficiently reduced (Fig. 3 and Table 1).

Table 1 Aggregate removal with Cellufine SPA-HC

	CHO Sup. %	SPA-HC elution frac. %
High M.W. Aggregate	21.3	3.9
IgG dimer	10.1	6.0
IgG monomer	68.6	90.2

Fig 3, SEC Analysis of SPA-HC Elution fractions



Column : TSK gel Super SW mAb HR
Mobile phase : 200 mM Sodium phosphate pH 6.7 + 0.1M Sodium sulfate
UV Detector : PDA 280 nm

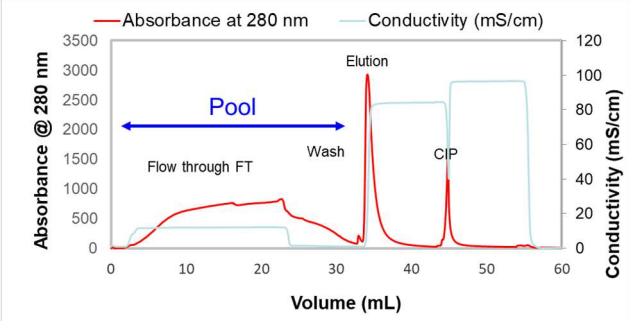
This reduction in the level of these contaminants present in the initial cell culture load, lowered their concentration for subsequent polishing steps. Aggregated mAb's have a lower binding affinity for Cellufine SPA-HC Protein A resin compared to the monomer. This may be due to the nature of the affinity ligand, which consists of a modified C-domain compared to the more widely used Z-domain.

3. Mix mode Chromatography

Flow-through polishing of Protein A elution fractions can be performed using an anionic mixed mode chromatography step. In this mode, contaminants are adsorbed on to the column allowing the mAb's to pass through the column with minimal dilution.

In this polishing process example a Cellufine SPA-HC Protein A low pH eluted fraction was adjusted to pH 7.0 with 1 M Tris base and conductivity to 6 mS/cm with 1 M NaCl. After filtration through a 0.22 µm PES filter, flow through polishing was carried out with Cellufine MAX IB. Results are shown (Fig. 4).

Fig.4 Flow through polishing with Cellufine MAX IB



Column: 1 mL mini column
Load buffer : 10 CV of 60 sodium acetate pH 7
Load sample: 20 mL (120 mg mAb))
mAb titer: 6 mg/mL
Flow: 0.25 mL/min (4 min residence time)
Elution buffer: 1 M NaCl
CIP: 0.5 M NaOH static flow for 30 min

Cellufine MAX IB packed in a 1 mL mini column (6.7 mmID x 3 cmL) and equilibrated with 10 CV (column volume) equilibration buffer (60 mM sodium acetate buffer, pH 7.0). A 20 mL load sample was then prepared at an antibody concentration of 6 mg / mL, and 20 mL (120 mg of antibody load) were pumped on to the column at 0.25 mL/min. The resulting flow-through fraction was collected and then 5 CV of equilibration buffer were used to wash any unretained material in the column. This wash fraction was pooled with the initial antibody flow through fraction. To recover adsorbed fractions, 5 CV of 1 M NaCl was passed through the column and the fractions collected. Finally, 0.5M NaOH was used for clean-in-place (CIP).

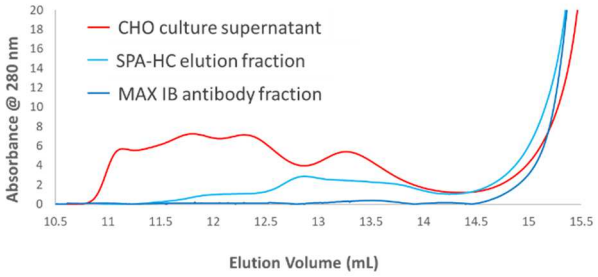
The residual impurities in the recovered mAb fraction and elution fractions were confirmed by SEC analysis (Fig 5 and Table 2).

Table 2, Summary of removal of process impurities by Cellufine MAX IB polishing

	Recovery %	HCP ppm	Leak ProA
Load sample	-	9.5	3.9
Antibody frac.	81	3.9	0.5
Elution frac.	9.1	11.5	3.4

The mAb recovery rate was 81%, with some antibodies retained on the column.

Fig.5, Summary of SEC-HPLC analysis of aggregated mAb fractions



Column：TSK gel Super SW mAb HR

Mobile phase：200 mM Sodium phosphate pH 6.7, and 0.1M Sodium sulfate

UV Detector：PDA at 280 nm

The above data summary clearly showed that mAb aggregates are efficiently removed from the Protein A capture fraction by anionic mixed mode polishing. Aggregates were reduced to 2.5% in the flow through fraction after passing through Cellufine MAX IB.

In this study, to evaluate the performance of Cellufine MAX IB, we used a CHO-cell supernatant sample with a large number of dimers (10.1%) and polymer aggregates (21.3%) to test the performance of our two-step capture-polishing workflow. A summary of the reduction in mAb aggregates across this workflow is shown in Table 3.

Table 3 Reduction of mAb aggregates in two-step workflow

	Culture sup. %	SPA-HC elution %	MAX IB mAb frac. %
High M.W. aggregate	21.3	3.9	0.6
Dimer	10.1	6.0	1.9
Monomer	68.6	90.2	97.6

mAb aggregates are clearly reduced to 0.6% compared to the initial level of 21.3% in the CHO cell culture supernatant. Dimers were also reduced to 1.9% in the final sample from 10.1% in the CHO-cell culture supernatant.

Conclusion

With the progress of cell culture technology, the fermentation titer of monoclonal antibodies is increasing. Along with this progress, side effects such as the formation of antibody aggregates have also become a severe impurity problem. This streamlined two-step capture of mAb by Cellufine SPA-HC and polishing with Anionic mixed mode Cellufine MAX IB described in this report, is a breakthrough in efficiently removing host-derived protein, leached Protein A, and antibody aggregates to a low level. Two-step purification is an excellent method that contributes to cost reduction and lead time reduction in the downstream purification of mAb's. Finally, Table 4 summarizes the overall two-step workflow described in this report.

Table 4, Final Summary of Contaminant Removal in a Two-step mAb purification workflow

Process	CHO-HCP	Protein A Leaching	Aggregation	Recovery
	[ppm]	[ppm]	[%]	[%]
CHO Supernatant	56342	-	31.4	-
Cellufine SPA-HC	17.9	4.3	9.9	92.8
Cellufine MAX IB	4	0.5	2.5	75

Product information

Please see our Web site for detailed product information.

Cellufine SPA-HC

https://www.jnc-corp.co.jp/fine/en/cellufine/grade/grade-9.html

Cellufine MAX IB

https://www.jnc-corp.co.jp/fine/en/cellufine/grade/grade-8.html#maxib

The instruction manual and technical data can be downloaded as pdf from the following website.

https://www.jnc-corp.co.jp/fine/en/cellufine/guide/index.html

Purchase information

Product	Quantity	Catalogue No.
Cellufine SPA-HC	1ml x 1 (Mini-Column)	21900-11
	1ml x 5 (Mini-Column)	21900-51
	5ml x 1 (Mini-Column)	21900-15
	10ml	21900
	50ml	21901
	500ml	21902
	5 lt	21903
	10 lt	21904
Cellufine MAX IB	1ml x 5 (Mini-Column)	21600-51
	5ml x 5 (Mini-Column)	21600-15
	10ml	21600
	50ml	21601
	100ml	21602
	500ml	21603
	5 lt	21604
	10 lt	21605

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