Efficient mAb purification process using Cellufine™ cellulose base chromatography media

INTRODUCTION

In most downstream monoclonal antibody (mAb) purification processes, initial Protein A capture is usually followed by two polishing steps such as CEX and AEX.

Here, we propose an improved polishing workflow by directly combining two flow-through chromatography steps (FT-FT mode), mixed mode and cation exchange chromatography's (Figure1). The proposed combined polishing steps efficiently removes impurities in the mAb polishing workflow avoiding major changes in pH or conductivity.

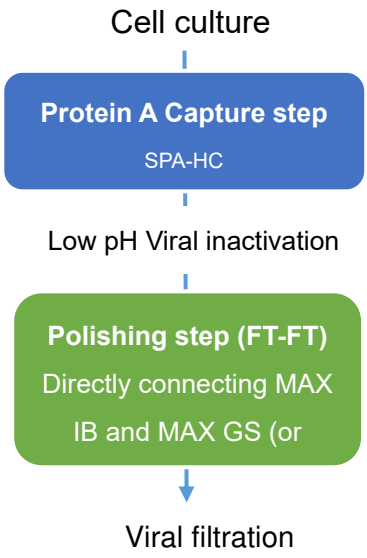

Figure 1. Proposed mAb purification process

EXPERMENTAL PROCEDURE

Materials

Protein A capture resin: Cellufine SPA-HC (JNC)
Mix mode polishing resin: Cellufine MAX IB (JNC)
Cation exchange polishing resin: Cellufine MAX GS and Cellufine MAX DexS HbP (JNC)
Glass chromatography column: 5 mm I.D. x 25 mm H (max. 0.5 mL)
CHO supernatant containing mAb from Manufacturing Technology Association of Biologics (MAB), .
HPLC column: TSKgel SuperSW mAb HR(Tosoh)
HCP ELISA Kit: CHO Host Cell Protein ELISA Kit, 3rd Generation F550 (Cygnus)
Protein A ELISA Kit: Tosoh R40 and R28 Protein A Mix-N-Go ELISA F910 (Cygnus)
HCD qPCR Kit: CHO DNA Amplification Kit in Tubes D555T (Cygnus)

Sample preparation for FT-FT mode

After Protein A resin capture and elution with acetate buffer, the pooled mAb peak sample was adjusted to pH 3.5 for virus inactivation using 0.1 M hydrochloric acid. After this step pH and conductivity were then adjusted to pH 7.0 (±0.1) and 6 (±0.1) mS/cm using 1 M Tris base solution and 5 M NaCl solution. Followed by dilution to 10 g/L of mAb concentration in buffer 20mM Tris Acetate pH 7.0 + cond.6 mS/cm NaCl.

FT-FT mode Polishing chromatography

Empty glass columns were packed with the mix mode resin (Cellufine MAX IB) and cation exchange resin (Cellufine MAX GS or DexS HbP). Two columns were connected directly as shown in Figure 2. A total of 305 mg (1020 mg/ml-resin) of mAb was applied to the connected columns and the flow-through fractions were collected. The detailed chromatographic conditions are listed below.

Column volume: 0.3 mL

Column size: 5 mm I.D. × 15 mm bed

Flow rate: 0.075 mL/min

Residence time: 4 min

Load sample: 10 mg/mL mAb in 20 mM Na Acetate pH7.0 6 mS/cm

Load volume: 29.5 mL

Program: see **Table 1** below

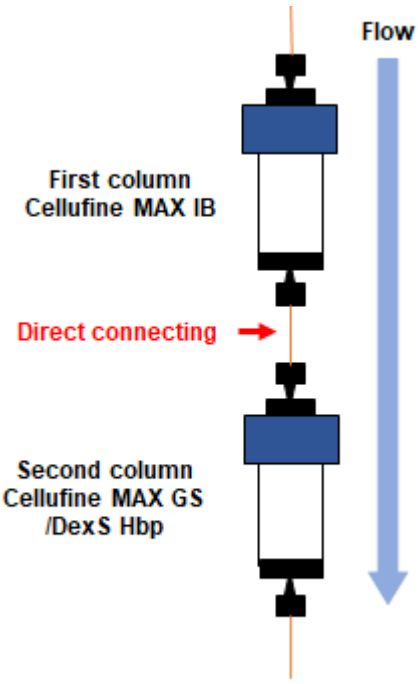

Figure 2. Directly connected columns

Step	Buffer or sample	Load volume (CV)	Flow rate (mL/min)	Fraction
Equilibrium	20mM Tris Acetate pH 7.0, 6 mS/cm NaCl	2	0.075	Waste
Load	10.4 mg/mL mAb pH 7.0 at 6 mS/cm NaCl (agg. 2.3%)	98	0.075	Collect
Wash	20 mM Acetate-NaOH pH7.0, 6 mS/cm NaCl	15	0.075	Collect

Table 1. Polishing step program

Analysis

Quantification of monoclonal antibody concentration was calculated as 1.4AU (A_{280}) = 1g/L immunoglobulin. The monoclonal antibody aggregate content was determined by HPLC-SEC analysis. Residual CHO host cell protein and leached Protein A were analyzed by ELISA. Residual dsDNA was analyzed by a qPCR method.

RESULTS

Combined FT-FT polishing of a Protein A captured mAb are summarized in Table 2. Two cases were tested; a) Cellufine MAX IB followed by Cellufine MAX GS, and b) Cellufine MAX IB followed by Cellufine MAX DexS HbP. In both cases, the recovery of mAb was >93% within acceptable target

requirements set for this FT-FT polishing process. Residual DNA values was lower than detectable limits before and after polishing. Other impurities, such as leached Protein A, CHO-HCP and aggregate content were significantly decreased.

Polishing Workflow	Load (mg/mL-resin)	Monomer recovery (%)	Aggregate (%)	HCP (ppm)	Leached ProA (ppm)	DNA (ppb)
Requirement	**-**	**> 80**	**< 2.0**	**< 100**	**<10**	**<10**
Loading sample	-	-	2.3	648	5.1	<1
MAX IB / MAX GS	1020	93	1.0	13.8	<2	<1
MAX IB / MAX DexS HbP	1020	93	1.0	9.2	<2	<1

Table 2. Results of FT-FT polishing

DISCUSSION

In mAb purification processes, polishing steps are necessary to decrease the level of contaminants, such as leached Protein A, CHO-HCP as low as possible to meet regulatory requirements. Although conventional IEX resins and HIC resins have been often adopted in the polishing steps, these conventional resins each require different loading buffer condition for optimal polishing performance. In these cases, additional equipment (tank, UF etc.) or operations (conditioning, buffer-exchange etc.) are required between the two columns. These additional steps have made polishing cumbersome, time consuming and costly.

In this study, the proposed FT-FT mode workflow efficiently reduced impurities to the required level without any intermediate step between the two polishing columns. Strong cation exchange resins (such as Cellufine MAX GS and DexS HbP) are well-known for removal of mAb aggregates. Cellufine anionic mix mode resin Cellufine MAX IB has unique aggregate binding properties and can be used to remove other impurities in the CEX-working range conditions. Direct connection of these two Cellufine resins could be useful to make antibody drug purification easier.

ACKNOWLEDGEMENT

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