Purification of monoclonal antibody from CHO culture supernatant with Cellufine products

Cellufine is a brand name of chromatography media that surface of the porous cellulose particle are modified by various ligand. Cellufine products are employed in downstream process of bio medicine such as antibody medicine, vaccine, gene therapy and protein medicine. The market of antibody medicine is becoming larger than chemical medicine these days. Because antibody medicines such as monoclonal antibody are made from protein, it is difficult to purify unless sophisticated technique.

Chromatography media purifying antibody are sold by some manufacturer. These media tend to have unique feature by its ligand density, distribution of ligand, particle size and flow property and so on. Protein A affinity chromatography media which are the leading role of downstream process are so very high price that its cost become main factor to increase production cost. So it is important to select protein A chromatography media to save production cost according to desired production scale.

Basically, there are three step chromatography to purify monoclonal antibody. First step is used protein A chromatography, second and third step are used deferent type of ion exchange chromatography. Cellufine brand line up these chromatography resins, Cellufine SPA-HC as a protein A resin, Cellufine MAX Q-h as a anion ion exchanger, Cellufine MAX GS as a cation ion exchanger. These resins are highly crosslinked to tolerate high pressure which enable to employ high flow rate.

This report introduce that conventional three step chromatography to purify monoclonal antibody from CHO supernatant with Cellufine chromatography media efficiently.

1． Purification procedure

Impurity from CHO host cell include host cell protein; CHP, host DNA and protein A leakage after protein A step. To purify these impurities, it is need to use three step chromatography process.

1-1. Protein A chromatography

Protein A chromatography step should be selected as a first step to purify monoclonal antibody. Protein A is the protein from *Staphylococcus aureus* which adsorb antibody highly selectivity. Protein A chromatography employs extensively highly purification; More than 99 percent impurity remove from objective sample. Cellufine SPA-HC (https://www.jnc-corp.co.jp/fine/en/cellufine/grade/grade-9.html) is immobilized alkaline resistant recombinant protein A ligand. According to unique particle control technology, Cellufine SPA-HC enable to increase high adsorption capacity and high flow property. This is because that Cellufine SPA-HC is superior to other competitor resin in terms of cost performance.

1-2. Anion ion exchange chromatography

In this experiment, anion ion exchange chromatography was employed as a second step. In this step, DNA from host cell was adsorbed to the resin because of its negative charge of the phosphate residue. HCP and protein A leakage derived from first chromatography step also be removed from the monoclonal antibody sample. Second step was conducted by flow through mode, i.e. antibody was not adsorb to the resin, though impurities were adsorbed to the resin. In this step, Cellufine MAX Q-h (https://www.jnc-corp.co.jp/fine/en/cellufine/grade/grade-6.html) which features high adsorption capacity and low backpressure was employed as a second step.

1-3. Cation ion exchange chromatography

It is important for the third step to purify high level purity up to bulk drug level. Slightly, there are some impurities such as HCP, protein A leakage after the second step. Most severe impurity may be dimer or aggregation of the monoclonal antibody caused in cell culture or chromatography steps.

In this experiment, third step was employed Cellufine MAX GS (https://www.jnc-corp.co.jp/fine/en/cellufine/grade/grade-6.html) as a cation ion chromatography step. Cellufine MAX GS is the state of the art cation ion exchanger to be able to separate monomer from aggregation of the antibody. According to increasing antibody titer at CHO culture step, antibody aggregation contamination has been becoming severe problem of impurity profile. Cellufine MAX GS is appropriate media to solve this aggregation problem.

1-4. Conclusion of procedure

Whole process procedure in this experiment is showed in figure 1. In this experiment, CHO supernatant was purified with three step chromatography.

2. Protein A chromatography step

Cellufine SPA-HC was packed in the open column (Empty 5 mL Mini Column; ID.14.6mm x H 30mm, catalogue No.: EMC5S, JNC corporation, https://www.jnc-corp.co.jp/fine/se/english/product/mini.html)

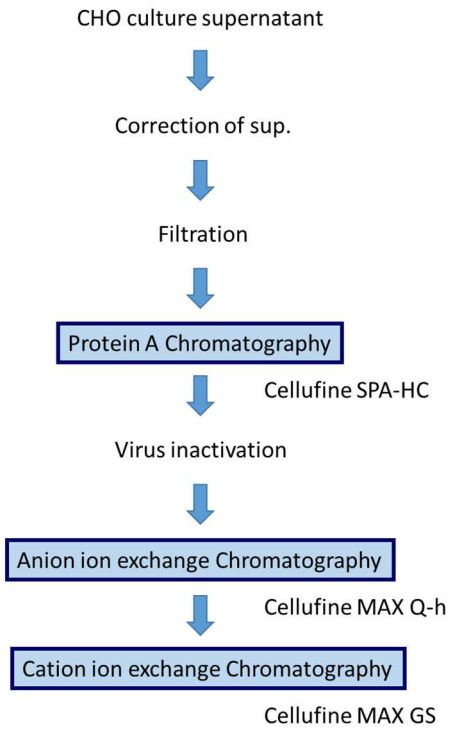

Figure 1 Procedure of the chromatography steps

After removed CHO cell from the culture supernatant, then the sample was passed 0.22 um filter to remove insoluble impurity. The

sample to be load to the Cellufine SPA-HC was prepared above methods. Monoclonal antibody titer of the sample was 1.0 mg/ ml.

The process conditions of protein A chromatography were conducted described in Table 1. Load sample volume was calculated as below. First of all, dynamic binding capacity (DBC) of the Cellufine SPA-HC at residence time 4 min in 10 % breakthrough point was measured in advance. DBC was 67 mg/ ml in this conditions. To avoid unbound antibody to the column, 80 % of the DBC was decided to load to Cellufine SPA-HC.

Load sample calculation

67x0.8 = 53.6mg mab/ mL-column

Step	Load vol. [CV]	Buffer
Equilibrium	5	20 mM Tris-HCl, 0.15 M NaCl, pH7.5
Sample load	267 mL	mab conc. 1.0 mg /ml
Wash	10	20 mM Tris-HCl, 0.15 M NaCl, pH7.5
Elution	5	60 mM Acetate-Na, pH3.5
Acid wash	5	0.1M Acetic acid solution
Wash	3	20 mM Tris-HCl, 0.15 M NaCl, pH7.5
CIP	5	0.1M NaOH
Equilibrium	10	20 mM Tris-HCl, 0.15 M NaCl, pH7.5

Table 1. Process condition of Cellufine SPA-HC

Column: Empty 5 mL Mini Column

(ID.14.6 mm x H 30 mm, Catalogue No.: EMC5SK)

Flow rate: 1.265 ml/ min (Residence time 4 min)

As Cellufine SPA-HC was packed in 5 mL volume column, final sample volume to load to the column was decided up to 268 mg.

Chromatograph of the experiment shows figure 2.

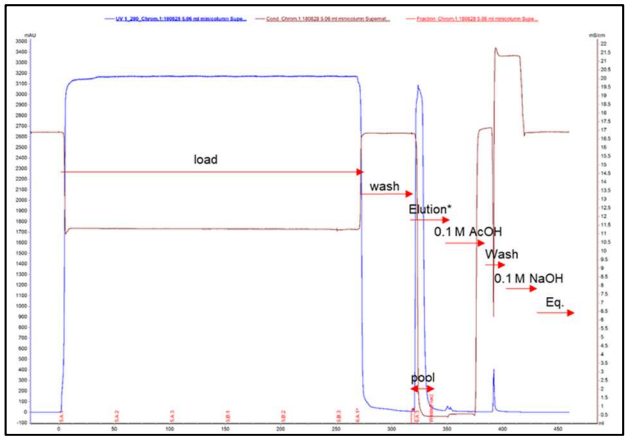

Figure 2 . Chromatogram of Cellufine SPA-HC

Blue line; UV value, brown line; conductivity

After sample loading, the antibody bound to the resin were eluted with 5 column volume (CV) of 60 mM sodium acetate, pH3.5. Collected elution sample was measured impurities described in Table 2. Recovery after elution became more than 95 % with 5 CV of elution buffer. If correction volume is higher, recovery rate will be increased according to correction volume. But this case will cause another inefficiency in sequential process after protein A chromatography.

After purification of Cellufine SPA-HC step, more than 99.7 % of CHO impurity was removed from sample. Thus protein A chromatography step is the most important step to purify monoclonal antibody in downstream process.

Process	CHO-HCP [ppm]	Pro A leakage [ppm]	Antibody Aggregation [%]	Recovery [%]
CHO Sup.	928000	-	-	-
Cellufine SPA-HC	2350	14.2	1.8	95

Table 2 Impurities after protein A chromatography

CHO-HCP and ProA leakage were measured by commercial ELISA. Recovery of the antibody was measured by spectrophotometer. Antibody aggregation was calculated by SEC column by measuring its area.

3. In activation of virus with acid treatment

After protein A chromatography step, elution sample was treated acid condition to inactivate endogenous virus. First, elution sample was added 1 M acetic acid and adjusted pH3.4, then the sample settled in an hour, room temperature.

After virus inactivation, added 1 M Tris-HCl and pure water to adjust pH7.5 and conductivity 6 mS/ cm to prepare as a next step of load sample.

4. Anion ion exchange chromatography step

Next step of the protein A chromatography, anion ion exchange chromatography was employed by flow through mode, i.e. impurities were adsorbed to the column, but monoclonal antibody was not adsorbed to the column. In this step, Cellufine MAX Q-h which is excellent adsorption capacity and high flow rate was employed. The process condition of anion ion exchange chromatography were described in table 3.

Process	Load vol. [CV]	Buffer
Equilibrium	5	20 mM Tris-HCl , pH7.5
Sample load	26.5 mL	7.40 mg_mab/ml, pH7.5, 6 mS/cm
Wash	5	20 mM Tris-HCl , pH7.5
Salt wash	5	1 M NaCl
CIP	5	0.5 M NaOH
Equilibrium	15	20 mM Tris-HCl , pH7.5

Table 3 Process condition of Cellufine MAX Q-h

Column: Empty 1 mL Mini Column

ID.6.7 mm x H 3 0mm, catalogue No.: EMC1SK

Flow rate: 0.265 ml/min (residence time 4 min)

In this step, anion ion exchange chromatography was employed by flow through mode. As chromatogram after this step described in fig.3, UV value was detected instantly after load sample. It was need to correct this flow through UV fraction. After loading sample, washed by 1 M sodium chloride to remove impurities which bond to the column. Then cleaning in place (CIP) was conducted to remove impurity residues.

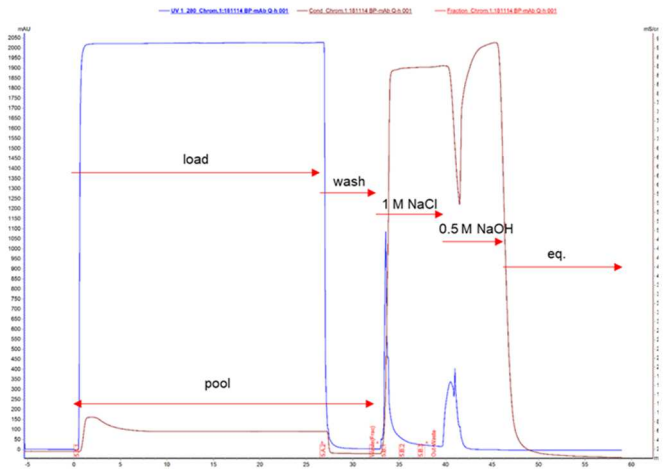

Fig. 3 Chromatogram of Cellufine MAX Q-h

Blue line; UV value, brown line; conductivity

After purification of Cellufine MAX Q-h step, impurities of the elution sample were measured described in Table 4. After second step, HCP became 49 ppm and recovery of the antibody excess 98 %. This highly recovery rate indicate that flow through mode was effective for removal impurities efficiently, though antibody was pass the column perfectly.

Process	CHO-HCP [ppm]	ProA leakage [ppm]	Antibody Aggregation [%]	Recovery [%]
Cellufine SPA-HC	2350	14.2	1.8	95
Cellufine MAX Q-h	49	8	1.2	98

Table 4 Impurities after Cellufine MAX Q-h

Measurement methods were described in table 2.

In this experiment, loading sample was adjusted in pH 7.5 and conductivity 6 mS / cm, but these conditions will be different from antibody its intrinsic isoelectric point. So sample conditions must be decided in advance that antibody wasn't adsorbed to the column.

5. Cation ion exchange chromatography step

The final step conducted with cation ion exchange chromatography step. In this step, it is important to remove aggregation from monoclonal antibody and to purify highly up to bulk drug level. Cellufine MAX GS which is unique property to remove aggregation from monoclonal antibody selectively was employed third step.

After second step, corrected sample was added 1 M acetic acid and pure water to adjust pH 5 and conductivity 6 mS/ cm. This process was conducted the conditions described in table 5.

Process	Load vol.[CV]	Buffer
Equilibrium	5	20 mM Acetate-Na, 50 mM NaCl, pH5.0
Load sample	10.7 mL	7.40 mg_mab/ml, pH5.0, 6 mS/cm
Wash	5	20 mM Acetate-Na, 50 mM NaCl, pH5.0
Wash	10	20 mM Acetate-Na, 0.19 M NaCl, pH5.0
Salt wash	5	1M NaCl
CIP	5	0.5M NaOH
Equilibrium	20	20 mM Acetate-Na, 50 mM NaCl, pH5.0

Table 5 Process condition of Cellufine MAX GS

Column: Empty 1 mL Mini Column

ID.6.7 mm x H 3 0mm, catalogue No.: EMC1SK

Flow rate: 0.265 ml/min (residence time 4 min)

Cellufine MAX GS was employed by bind and elute mode; i.e. monoclonal antibody was adsorbed column and removed impurity from the sample. Chromatogram of the experiment was described in Fig.4.

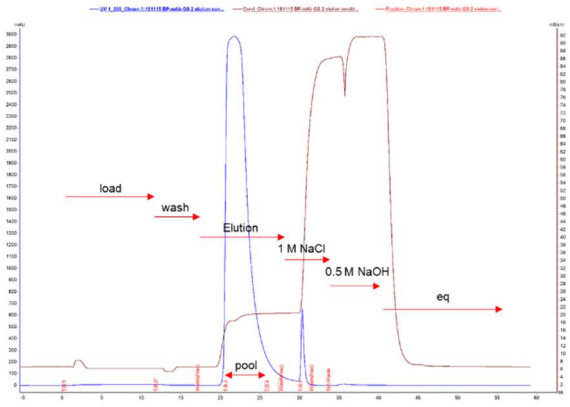

Fig.4 Chromatogram of Cellufine MAX GS

Blue line; UV value, brown line; conductivity

After purification of Cellufine MAX GS step, impurity of the elution sample were measured described in Table 6. After final step, HCP became 5 ppm, protein A leakage was 0.5 ppm and recovery of the antibody approximately 90 %. The recovery was a little lower compare to other chromatography steps. This is because antibody was not to elute from the column but to correct low elution volume. Aggregation of the monoclonal antibody was adsorbed to Cellufine MAX GS more strongly than monomer antibody. So according to reduce correction volume, strong binding aggregate antibody was removed from monomer sample efficiently.

Process	CHO-HCP [ppm]	ProA leakage [ppm]	Antibody aggregation [%]	Recovery [%]
Cellufine MAX Q-h	49	8	1.2	98
Cellufine MAX GS	5	0.2	0.5	90

Table 6 Impurities after Cellufine MAX GS

Measurement methods were described in table 2.

This is unique feature for Cellufine MAX GS, although other cation ion exchange media don't separate monomer from aggregate. If there are much aggregate in the monoclonal antibody sample, Cellufine MAX GS must be the best choice to purify these samples.

Conclusion

In this experiment, monoclonal antibody derived from CHO cell was highly purified by three step chromatography with Cellufine products. According to increasing antibody titer at CHO culture step, antibody polymer contamination has been becoming severe problem of impurity profile. After three step chromatography, monoclonal antibody sample were highly purified without aggregation antibody described in table 7. This method will be effective to purify monoclonal antibody from CHO cell supernatant efficiently.

Process	CHO-HCP [ppm]	ProA leakage [ppm]	Antibody aggregation [%]	Recovery [%]
CHO Supernatant	928000	-	-	-
Cellufine SPA-HC	2350	14.2	1.8	95
Cellufine MAX Q-h	49	8	1.2	98
Cellufine MAX GS	5	0.2	0.5	90

Table 7 Impurity profile of three step chromatography

Information of products

Please check Web site of Cellufine information.

Cellufine SPA-HC

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

Cellufine MAX Q-h, Cellufine MAX GS

https://www.jnc-corp.co.jp/fine/jp/cellufine/grade/grade-6.html

Operation instruction and technical information are available from below WEB site.

https://www.jnc-corp.co.jp/fine/jp/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 Q-h	Robo Column® 5-10	20600-802
	1ml x 5 (Mini-Column)	20600-51
	5ml x 5 (Mini-Column)	20600-55
	100ml	20600
	500ml	20601
	5 lt	20602
	10 lt	20603
Cellufine MAX GS	1ml x 5 (Mini-Column)	21300-51
	5ml x 5 (Mini-Column)	21300-55
	100ml	21300
	500ml	21301
	5 lt	21302
	10 lt	21303

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