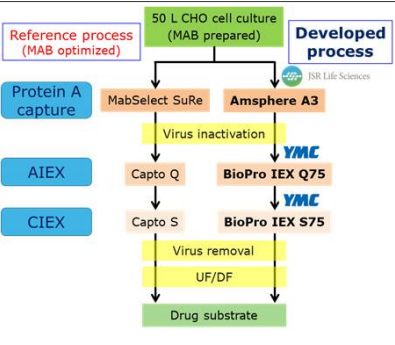


Introduction

The development of advanced downstream technology for biopharmaceutical antibody production was conducted. The verification of monoclonal antibody purification from 50 L CHO cell culture has been conducted at a GMP Facilities by 3-step process using Amsphere A3, Protein A affinity chromatography resin, and BioPro IEX S75/Q75 as ion-exchange resin. As a result, our process could obtain purified antibodies on high purity and high efficiency compared with a process using competitor's resin. In this poster, we will report the detail of investigating process parameter and scaling up. This work is supported by Manufacturing Technology Association of Biologics (MAB) which aims to establish an industrial technology platform for biopharmaceuticals as a technology research association developing next-generation production technologies for modern- and next-generation biopharmaceuticals in Japan.

Our process flow



Packing material using this study

Amsphere™ A3

Outstanding dynamic binding capacity

- 20%-50% higher DBC compared to market standard product
- All 1-2 min residence time, more than 50% higher

Superior base stability

Good Pressure Flow Properties

Excellent impurity clearance

Amsphere A3 showed high capacity especially for shorter residence time.

BioPro IEX Q / BioPro IEX S

Features

- High productivity on purification
- Hydrophilic polymer beads with low nonspecific adsorption
- High binding capacity/high recovery/high resolution/low backpressure
- Suitable for capture step and intermediate purification step

Specifications

	Anion exchanger BioPro IEX Q75	Cation exchanger BioPro IEX S75
Matrix	Hydrophilic porous polymer	
Particle size (mm)	75	
Ion exchanger	-R-N(CH ₃) ₃	-R-SO ₃
Usable pH range	2~12	
Ion exchange capacity (meq/mL-resin)	> 0.10	
Dynamic binding capacity (DBC) (mg/mL-resin)	> 160 (BSA)	> 160 (lysozyme)

DBC for Lysozyme (mg/mL-resin, 10% breakthrough)

Process development (in Lab scale)

① Sample loading amount

Experimental condition

Column: Amsphere A3, MabSelect SuRe

Bed height: 20 cm

Loading sample: Cell culture (Titer: 1.2 e7/L)

RT: 4min

DBC analysis: HPLC (shimadzu, LC-200R)

② Elution condition

Experimental condition

Column: TSKgel S7500

Resin: Amsphere A3

Bed height: 1.4 cm

Loading amount: Cell culture (Titer: 1.2 e7/L)

Loading amount: 85% of DBC @ 10% RT

RT: 4min

Lution buffer: 0.1M NaOAc, pH3.5-4.0

③ Wash condition

Experimental condition

Column: TSKgel S7500

Resin: Amsphere A3

Bed height: 1.4 cm

Loading sample: Cell culture (1.2 e7/L)

Loading amount: 95% of DBC @ 10% RT

RT: 4min

Lution buffer: 0.1M NaOAc, pH3.5-4.0

AIEX process condition

Column	Resin	Bed height	Loading amount	RT	Lution buffer
Amsphere A3	MabSelect SuRe	20 cm	85% of DBC @ 10% RT	4 min	0.1M NaOAc, pH3.5-4.0
BioPro IEX Q75	BioPro IEX Q75	1.4 cm	85% of DBC @ 10% RT	4 min	0.1M NaOAc, pH3.5-4.0
BioPro IEX S75	BioPro IEX S75	1.4 cm	85% of DBC @ 10% RT	4 min	0.1M NaOAc, pH3.5-4.0

Sample loading amount for CIEX process

DBC breakthrough curve of BioPro IEX S75

Yield (%) and HCP (ppm) for different pH values:

pH	Yield (%)	HCP (ppm)
pH3.0	92	8
pH3.5	90	5
pH4.0	91	5

Yield and HCP was not changed between pH3.0-4.0.

Summary of the results from our purification run (50L cell culture)

Process	Yield (each step) (%)	HCP (ng/mg-IgG)		DNA (pg/mg-IgG)		protein A (ng/mg-IgG)		Monomer (%)	
		P-3	Ref.	P-3	Ref.	P-3	Ref.	P-3	Ref.
Clarified cell culture fluid	90	127,000	-	66,900,000	-	-	-	-	-
Protein A capture	94	194	145	26,200	18,000	7.22	6.41	-	98.6
AIEX	106	4.57	0.64	1.12	<0.44	0.48	0.49	92.2	98.5
CIEX	94	3.04	0.46	1.98	<0.12	0.38	0.67	93.9	98.6
Virus removal	97	3.28	0.71	1.29	<0.25	0.27	0.35	-	-
UF/DF	102	1.47	0.64	1.18	<0.26	0.44	0.71	-	-
Drug substrate		4.9	ND*	ND*	ND*	0.50	1.10	92.6	98.7

ND*: not detectable

Conclusions

- We have successfully developed purification process.
- Our result using Amsphere A3 and BioPro IEX S/Q75 have showed it can be obtained high-quality IgG equal or greater than the process using competitor's resin.

Acknowledgement

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