Host cell protein clearance by Amsphere™ A3 and other commercially available protein A resins

Qualitative analysis of HCPs by 2D-LC/MS

S. Tsuda[1], T. Tanaka[2], Y. Imanishi[2], K. Itaya[1], T. Ichii[1], Y. Okano[1], T. Matsuda[1], M. Hanamura[1], G. Platteau[3,*], G. Ströhlein[3], T. Arien[3]

[1] JSR Life Sciences Corporation - 25 Miyukigaoka, Tsukuba, Ibaraki, 305-0841, JAPAN
[2] JSR Corporation - 25 Miyukigaoka, Tsukuba, Ibaraki, 305-0841, JAPAN
[3]JSR Life Sciences - Technologielaan 8, 3001 Leuven, BELGIUM

INTRODUCTION

The level of host cell proteins (HCPs) is one of the critical quality attributes of biopharmaceuticals. Clearance of these HCPs is one of the performance parameters of the downstream process (DSP) of antibody production. Generally, the majority of HCPs in harvested cell culture fluid is removed during the affinity capture step using Protein A chromatography. Remaining HCPs are removed in subsequent polishing steps by a combination of ion-exchange, hydrophobic interaction, or mixed-mode techniques. Thus, identification of the remaining HCPs after the Protein A step can provide valuable information for the resin choice and development of the polishing process. In this study, HCPs in elution samples of Protein A chromatography were evaluated with ELISA (quantitative analysis) and 2D-LC/MS (qualitative analysis). Results for Amsphere A3 were compared with two other commercially available resins.

PROPERTIES OF PROTEIN A RESIN AND MODEL FEED

Using two industrial monoclonal antibodies (mAbs) and an Fc-fusion protein, the performance of Amsphere A3 was compared to two of the most commonly used commercial Protein A resins.

Resin	Matrix	Ligand	Modified Protein A domain	Mean particle size (µm)
Amsphere A3	Polymethacrylate	Alkali-stabilized rProtein A	C domain	50
Agarose S	Highly cross-linked agarose	Alkali-stabilized rProtein A	B domain	85
Agarose L	Highly cross-linked agarose	Alkali-stabilized rProtein A	B domain	85

Feed	Vendor	Cell	Class	Titer (g/L)	MW (kDa)	pI	HCP	DNA	Aggregate
							ppm/IgG	ppm/IgG	%
Trastuzumab BS	A	CHO	IgG1	1.10	146	8.5	475000	5600	10
Bevacizumab BS	B	CHO	IgG1	1.66	149	8.3	386000	14400	25
Etanercept BS	A	CHO	Fc-fusion	0.93	51	7.9	600000	10500	29

EXPERIMENTAL CONDITIONS

◆ PURIFICATION

Sample loading (4 min residence time):
(a) Over 10%BT DBC (b) 80% of 10%BT DBC

↓

Wash I: 20 mM Sodium Phosphate, pH 7.5, 2CV

↓

Wash II: 20 mM Sodium Phosphate + 1 M NaCl, pH 7.5, 3CV

↓

Wash III: 20 mM Sodium Phosphate, pH 7.5, 3CV

↓

Elution: 100 mM Sodium Acetate, pH 3.3

- Column (ID: 0.5 cm, BH: 20.0 cm), AKTA prime plus, GE Healthcare.

◆ IMPURITY ANALYSIS

- CHO Host Cell Protein ELISA Kit, 3rd Generation, F550, Cygnus Technologies.
- Quant-iT™ PicoGreen dsDNA Assay Kit, P11496, Thermo Fisher.
- TSKgel Super SW3000, 0.1 M PB, 0.1 M Na_2SO_4, pH 6.7, 0.35 mL/min, Tosoh.

◆2D-LS/MS ANALYSIS

Sample Preparation	1st LC dimension	2nd LC dimension	MS
• Denaturation • Alkylation • Digestion	• Operation at pH 10	• Operation at pH 2.4	• ESI positive

RESULTS

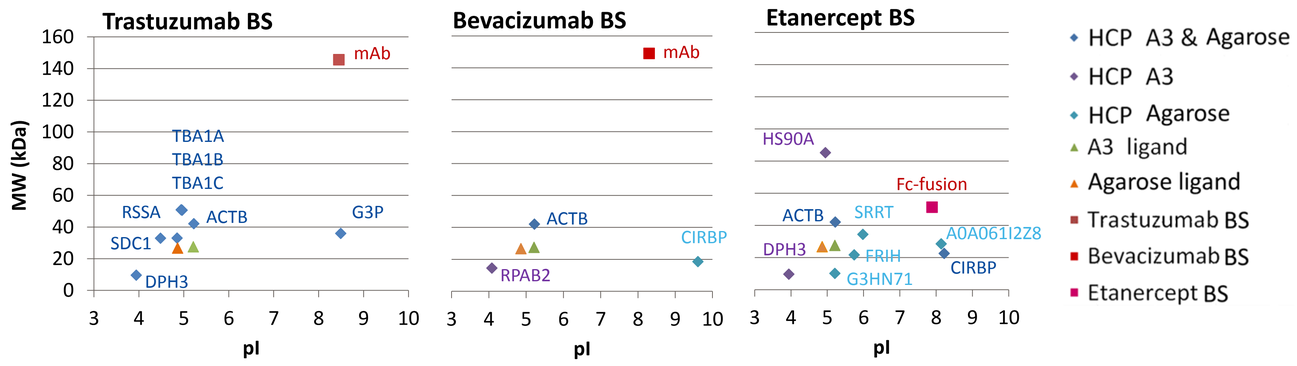
◆ CAPACITY AND IMPURITY CLEARANCE

- Amsphere A3 exhibited higher DBC than both Agarose S/L.
- The impurity levels in the elution pools of Amsphere A3 were as low as Agarose S/L.

Feed	Resin	(a)	(b) 80% load of DBC 10%BT			
		DBC 10%BT	Recovery	HCP	DNA	Aggregate
		g/L	%	Log reduction	Log reduction	%
Trastuzumab BS	Amsphere A3	51.5	103	2.4	2.8	1
	Agarose S	37.5	101	2.2	2.9	1
Bevacizumab BS	Amsphere A3	43.9	111	2.0	2.7	5
	Agarose L	37.2	110	2.0	2.9	7
Etanercept BS	Amsphere A3	13.1	75	1.8	2.2	12
	Agarose L	7.6	79	1.7	2.1	16

◆IDENTIFICATION OF HCPS USING 2D-LC/MS

- Most of the HCPs in the eluates of Amsphere A3 and Agarose S/L were in common.
- HCPs with pI < 6 were detected more than HCPs with pI > 8.
- HCPs with pI values between 6 and 8 were not detected, regardless of the resin used.



DISCUSSION

◆ 2D-SDS/PAGE OF FEED

The feeds for this study contain HCPs with pI from 3 to 10.

loading at pH 7.5

◆CORRELATION BETWEEN AMOUNT OF IgG AND HCP (Trastuzumab BS)

- No significant differences in remaining HCPs are observed, although Amsphere A3 and Agarose S/L have a different base matrix.
- From 2D-LC/MS analysis, pIs for the majority of remaining HCPs are less than 6. Taking into account the pI of the target molecules, non-specific electrostatic interaction between HCPs and the target molecule is considered to be a dominant factor in determining the residual HCPs after Protein A chromatography.
- Quantitative HCP analysis showed a linear correlation between IgG and HCP amounts, which indicates the direct interaction between IgG and HCPs.
- For Amsphere A3 and Agarose S/L, HCPs with pI in the pH range from 6 to 8 were not detected. Therefore, it is assumed that non-specific hydrophobic interaction between HCPs and resin is well suppressed for both resins.

CONCLUSION

- HCPs in elution samples of Protein A affinity chromatography were analyzed quantitatively and qualitatively.
- Amsphere A3 exhibits higher DBC with equivalent yield and impurity clearance as commercially available Agarose S/L.
- The results suggest that the electrostatic charge of HCPs during the load and wash steps is mainly determining whether or not HCPs interact and co-elute with the IgGs.
- Amsphere A3 can replace the existing agarose-based Protein A affinity resins under the same binding/wash conditions without specific adjustment of subsequent polishing processes.

JSR Life Sciences