Purification of biopharmaceuticals using small particle polymer media

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Shinya Nozaki*, Kazuhiko Tokunaga, Yoshito Fukuda, Noriyuki Yasuda, Shouhei Ohara, Masato Towata and Tadashi Adachi
Separation Materials Laboratories, R&D Center, Kurosaki Plant, Mitsubishi Chemical Corporation, Kitakyushu 806-0004, Japan
*E-mail: nozaki.shinya@md.m-kagaku.co.jp

1. Introduction

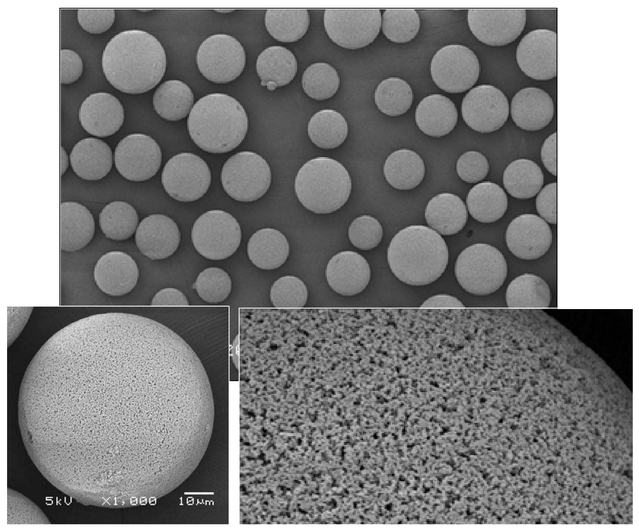
・ Cation exchange chromatography is a well-established unit operation in the downstream processing of monoclonal antibodies[1,2]. In this work, our cation exchange media, ChromSpeed™ S series, are introduced and their adsorption and separation characteristics are discussed. The base matrix is highly porous rigid polymethacrylate, and the average particle diameter is 30 micrometer for S101, and 60 micrometer for S103, respectively[3].

・High dynamic binding capacities and good separation were observed when they were applied for human antibodies, presumably owing to rapid intra-particle diffusion rate. As purification media, ChromSpeed™ will meet the demands of biopharmaceutical manufactures by improving productivity and reducing process time.

References
[1] A. Shukla et al., J. Chromatogr. B, 848 (2007) 28
[2] A. Stein et al., J. Chromatogr. B, 848 (2007)151
[3] K. Tokunaga et al., poster presentation at PREP2014, Boston (2014)

2. Chromatography media for biopharmaceutical purification

- **ChromSpeed™**



ChromSpeed™ S,CM series		
	S103, 101	CM103,101
Type	Strong cation exchange	Weak cation exchange
Functionality	$-SO_3^-$	$-COO^-$
Base polymer	Polymethacrylate	
SBC (human IgG, g/L-resin)	>100	
Particle diameter (μm)	60, 30	60, 30
pH	3 – 12	

- ChromSpeed™ Q, DA series: Strong, weak anion exchangers
- MabSpeed™ series: Protein A affinity media

ChromSpeed™ and MabSpeed™ chromatographic resins are specially designed material for the purification of biopharmaceuticals (antibody-drugs, protein-drugs, etc.). They are based on hydrophilic polymethacrylate matrix with spherical, totally porous structure. The rigid, uniform-particle sized matrix enables high speed chromatographic operation with high production efficiency. Various product lines can meet variety of demands for separation and purification.

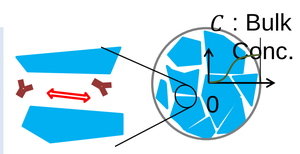
3. Dynamic Binding Capacity (DBC)

- DBC comparison in IgG conc. and residence time

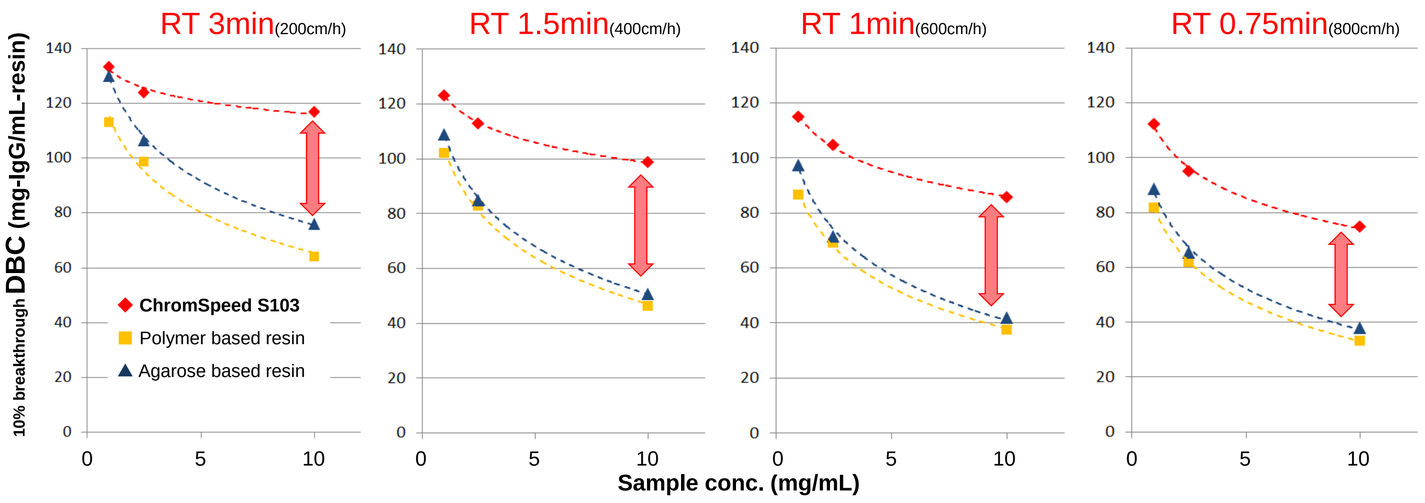
RESINS

	SBC (mg-IgG/mL-resin)	Particle size (μm)
ChromSpeed™ S103	**143**	60
Polymer based resin	**143**	75
Agarose based resin	**144**	90

CONDITIONS
Column 5 x 100 mmH (BV:2.0mL)
Buffer 20mM Sodium Acetate (pH5.5)
Sample gamma-globulin (IgG)
Residence time 0.75-3.0 min (flow rate 200-800 cm/h)



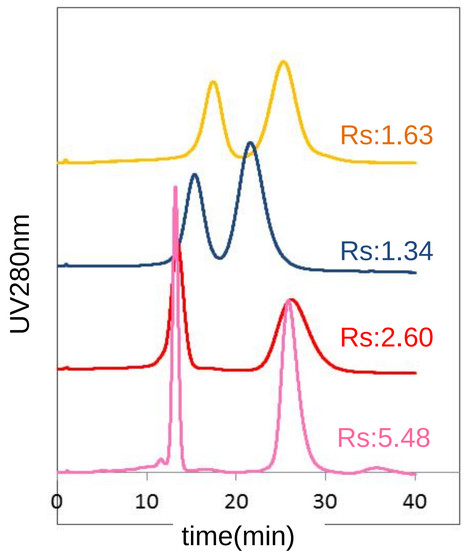
IgG diffusion in the particle



・The highest dynamic binding capacity was observed for ChromSpeed™ S103.
・Moreover, DBC showed less dependence on the IgG concentration for ChromSpeed™ S103.

4. Selectivity

- Resolution of two different protein in saline gradient elution



Column	5 x 100 mmH (BV:2.0mL)
Eluent A	20mM Na-phosphate (pH6.5)
Eluent B	A + 1.0M NaCl
Flow rate	1.0mL/min (R.T. 2min)
Gradient	0-100% B over 60min
Sample	cytochrome C + lysozyme = 125μg / 125μg in 50μL aq.

Polymer based resin
Agarose based resin
ChromSpeed S103
ChromSpeed S101

・Better selectivity was observed for ChromSpeed™ resins, especially in smaller particle S101 (30μm).
・S103 and S101 can be applied to high-flow-rate conditions with supreme selectivity due to rapid intra-particle diffusion rate.

5. Applications

- 3 step mab purification from cell culture

Cell culture
↓ Clarified CHO cell culture, 0.5mg/mL mab (20.8mg/mL-resin)
Protein A affinity : MabSpeed™ rP111
mab capture & removal of aggregates and HCP
↓ Column MabSpeed™ rP111, 10 x 150mmH(BV: 12mL); Start buffer Phosphate buffered saline; Elution buffer 20mM Sodium Citrate, pH3.4, Flow rate 300 cm/h, R.T. 3min
Cation exchange : ChromSpeed™ S101
removal of aggregates, HCP, PrA
↓ Column ChromSpeed™ S101, 5 x 200mmH(BV: 4mL); Sample mab (57.5 mg/mL-resin), pH adjusted to 5.5; Eluent A 20mM Sodium acetate + 50mM NaCl, pH5.5; Eluent B 20mM Sodium acetate, 1M NaCl, pH5.5; Flow rate , 300 cm/h, R.T. 4min; Elution stepwise gradient, B=5% (10CV), 15% (20CV), 100% (10CV)
Anion exchange : ChromSpeed™ Q101
removal of DNA, endotoxin, …
Column ChromSpeed™ Q101, 5 x 50mmH(BV: 1mL); Sample mab (173 mg/mL-resin), eluate after ChromSpeed™ S101, pH adjusted to 6.0; Start buffer 20mM Sodium acetate, pH6.0; Flow rate 300 cm/h, R.T. 1min

3 steps: Affinity → Strong Caion Exchange → Strong Anion Exchange

	accum. Yield (%)	Dimers and Aggregates (%)	Protein A (ppm)	HCP (ppm)
start material	100	–	–	35717
MabSpeed™ rP111	98	<0.5%	4.4	3.1
ChromSpeed™ S101	90	<0.5%	2.6	<0.5
ChromSpeed™ Q101	88	<0.5%	2.4	<0.5

・Each step gives good accumulated yield.
・Host Cell Proteins (HCP) is well removed at affinity step, and strong cation exchange (ChromSpeed S101) can remove the residue very well.
・Dimers and aggregates removal is not well studied due to less impurity in the cell culture.
・Protein A removal might get better by optimization of the elution condition at ion exchange steps.

6. Conclusion

Higher Capacity
Polymer media for cation exchange chromatography have been developed for purification of biopharmaceuticals. The base matrix is highly porous rigid polymethacrylate with the average particle size 30, 60 microrrmeter. Smaller diameter of the particle and larger pore size can provide higher capacity and better mass transfer properties than typical commercial media.
Especially, higher concentration of target proteins can be applied without depressing high DBC.

For Process Scale
Higher DBC, higher selectivity and high flow rate applicability make it possible to purify large amount of molecule antibodies in process scale.
ChromSpeed and MabSpeed are stably manufactured in industrial scale at Mitsubishi Chemical Corp.