New High Capacity TOYOPEARL Resins for Separation of Monoclonal Antibody

Tosoh corporation

March/April 2014

TOPICS

- High capacity, alkaline-resistant Protein A resin
  - TOYOPEARL AF-rProtein A HC-650F
- Salt-tolerant (mixed-mode) anion-exchanger
  - TOYOPEARL NH2-750F
- Salt-tolerant (mixed-mode) cation-exchanger
  - TOYOPEARL MX-Trp-650M
- No-salt/low-salt HIC application
  - TOYOPEARL Hexyl-650C, Phenyl-650M

Issues in Mab Production

TOSOH

■**Mab biopharmaceuticals are too expensive!!!**

–Not recommend to apply in some case.

–Health insurance rejected in UK (Avastin, Erbitax, -2009)

(ex.; Abastin 400 mg/ $2,000 per injection, total cost; $30,000- 50,000)

– Cost reduction required for Mab product!!!

■**Improvement for reduction of production cost**

•Production process

–Higher titer and large scale fermentation

–Improvement in source; prokaryote, animal cell, transgenic animal/plant

–Chromatography step； high capacity, high selectivity, high throughput, single-use

–Non-chromatography step; selective precipitation

•Mab molecule

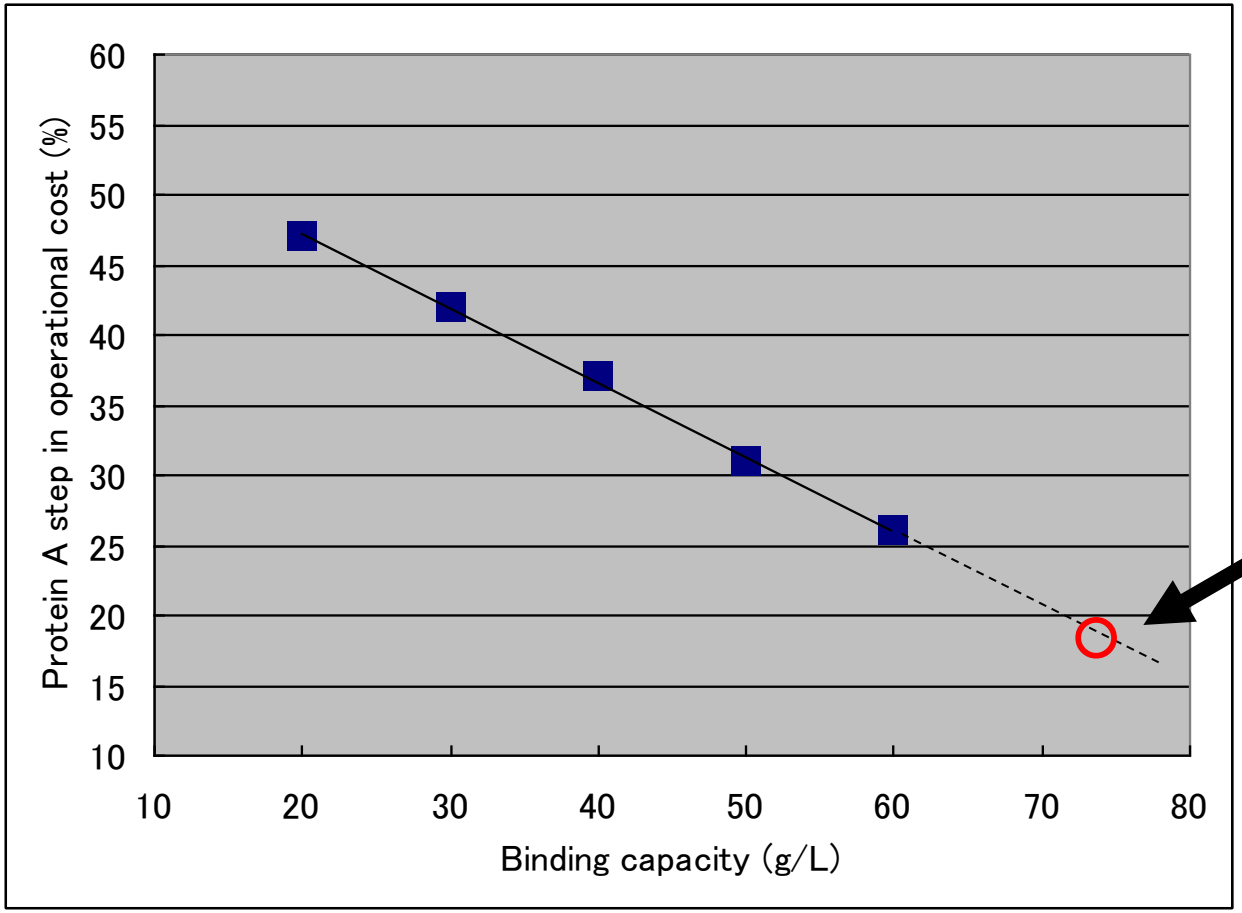
–Recycling Mab, sweeping Mab, bi-specific Mab, potelligent Mab

–Single body, fragment Mab, antibody-drug conjugate (ADC)

–DDS technology, PEGylation

New High Capacity, Alkaline-Resistant rProtein A Affinity Chromatography

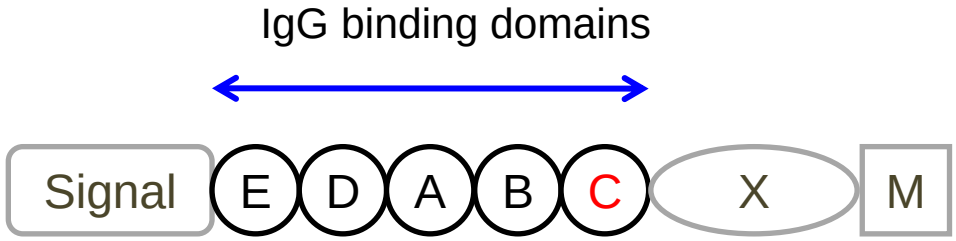
Impact of Binding Capacity on Protein A Resin for Protein A Process Operational Cost

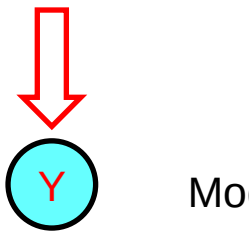


Predicted maximum/limit binding capacity on Protein A resin

Ref.; Prof. Dr.-Ing.Matthias Franzreb, Report “Effect of variations within Protein A chromatography step onto process economy of industrial Mab production” (2013), unpublished, partially modified

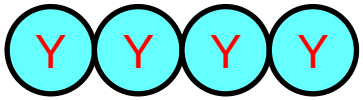
Structure of rec Protein A Variants





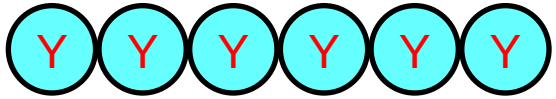
Modified, code-optimized C domain

High throughput type;



New alkali-stable ligand
containing multiple coupling sites

High capacity type;



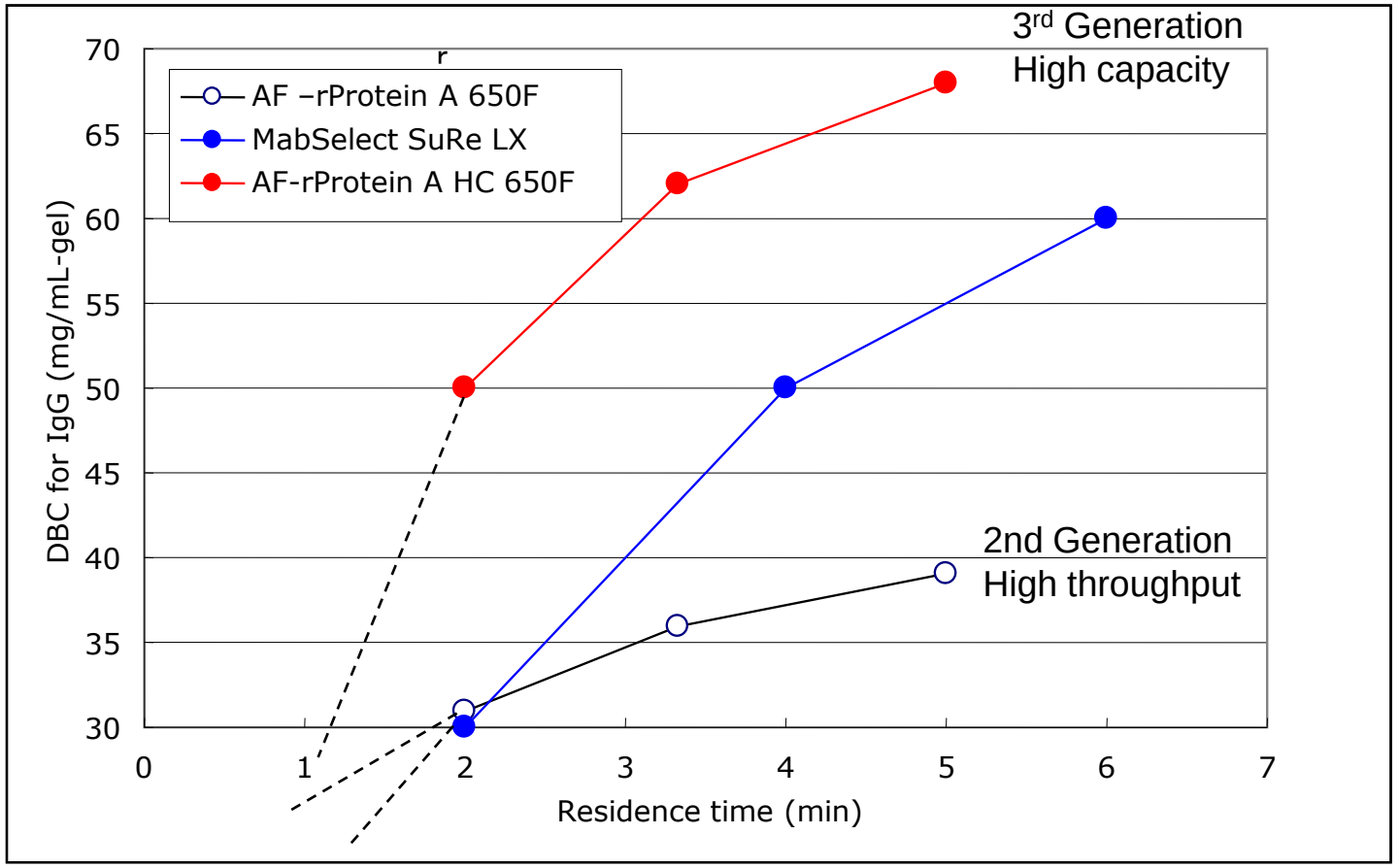
- rProtein A binds to Fc region of IgG.
- Weak binding of Fab region obserbed as well as domain B-Protein A
- Toxity; single dose intravenous administration of rat; LD50, > 150 mg/Kg

TOYOPEARL AF-rProtein A HC-650F for High Capacity Separation

■Particle size	30 - 60 micron (> 80 %)
■Ligand	rec Protein A (*E. coli*)
■Ligand coupling	multi-point attachment
■IgG capacity(static)	> 68 g/L
	(DBC; > 65g/L at 5 min residence time)
■Bioburden	
•Bacterial count	< 100 colonies/mL
•Endotoxin	< 10 EU/mL
■Foreign substances	< 6 paritcles/100 mL
■No animal source used	
■Shipping solvent**	20 % ethanol
■Protein A ELISA kit available	

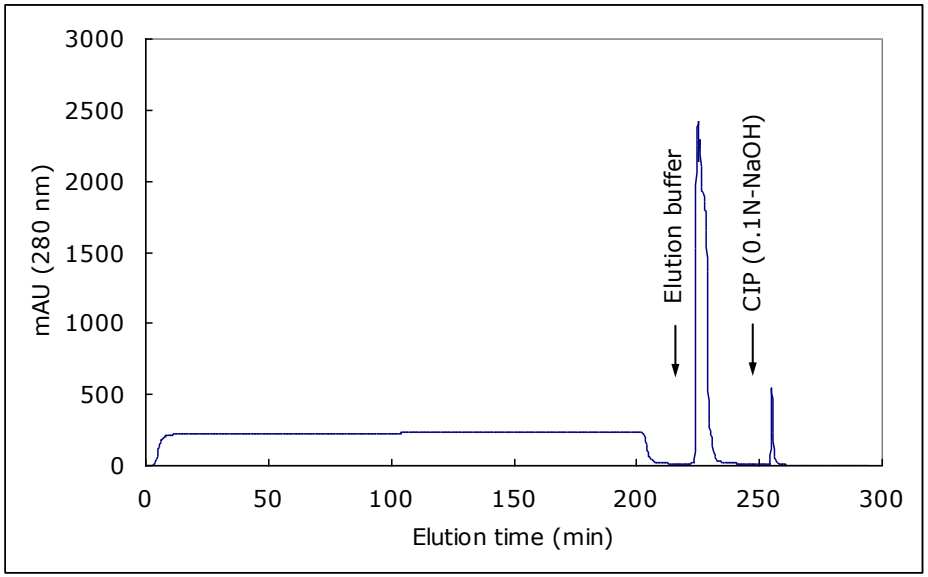
•2 % benzylalcohol is effective for storage.

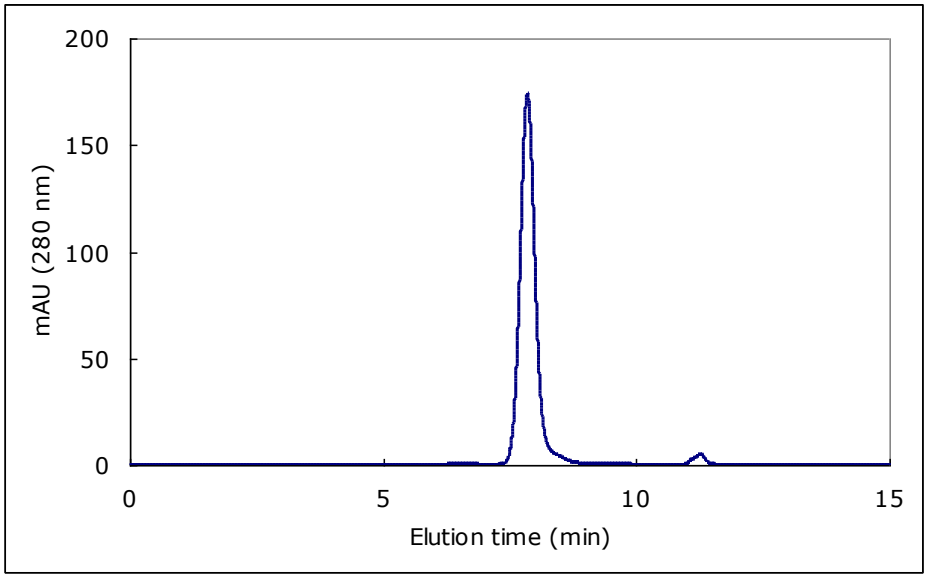
Comparison of DBC at Various Residence Time with TOYOPEARL AF-rProtein A HC-650F



* MabSelect SuRe LX: DBC from Brochure data

Purification of Monoclonal Antibody from CHO Cell Culture on TOYOPEARL AF-rProtein A HC-650F





Column; TOYOPEARL AF-rProtein A HC-650F (5 mm I.D. x 5 cm)
Binding; 0.02 mol/L sodium phosphate buffer (pH 7.4) containing 0.15 mol/L NaCl
Elution; 0.1 mol/L citrate (pH 3.0)
Flow rate; 0.2 mL/min Detetion; UV (280 nm)
Sample; 40 mL feedstock from cell culture (humanized IgG1: 1 g/L)
Purity check by TSKgel G3000SWXL (right figure)

TOYOPEARL AF-rProtein A HC-650F/-650F
Typical Chromatographic Conditions (1)

For regular binding antibody

- Humanized Mab

- Binding and washing
  - 0.02 mol/L sodium phosphate buffer (pH 7.2), 0.15 mol/L NaCl
  - (When other impurities like green pigment is adsorbed, additional washing with stepwise buffer at pH above 4 may be effective.)
  - (Additional pre-washing with 50 mM Tris-HCl (pH 8.0), 1 mol/L NaCl [*1])
- Elution
  - 0.1 mol/L acetate or citrate (pH 3.7-3.0) or 0.1 mol/L glycine-HCl (pH 3.7-3.0)
  - **Elution pH is 0.2 point lower** than other commercial Protein A resin
- Cleaning
  - Acidic solution of pH 2.7-2.5
  - 0.1 mol/L NaOH, To remove accumulated DNA/chromatin , 0.5 mol/L NaOH is effective[*1], while the cleaning conditions degradate Protein A resin.
  - For hydrophobic impurities, 20-30% ethanol may be effective.
  - *1; Reference; P. Gagnon et al., J. Chromatogr., A, 1340 (2014)68-78

TOYOPEARL AF-rProtein A HC-650F/-650F
Typical Chromatographic Conditions (2)

Weak binding antibody

- Fab, IgM, Mouse IgG1

- Binding and washing
  - Higher pH buffer without salt is recommended.
  - 0.02 mol/L sodium phosphate buffer (pH 7.2), no salt
  - 0.02 mol/L Tris-HCl (pH 8.4), no salt
- Elution
  - Salt gradient from 0 to 0.5 mol/L NaCl in binding buffer
  - 0.1 mol/L glycine-HCl (pH 3.7-3.0) or 0.1 mol/L acetate buffer (pH 3.7-3.0)
- Cleaning
  - Acidic solution of pH 2.7-2.5
  - 0.1 mol/L NaOH, To remove accumulated DNA/chromatin , 0.5 mol/L NaOH is effective[*1], while the cleaning conditions degradate Protein A resin.
  - For hydrophobic impurities, 20-30% ethanol may be effective.
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Comparison of 3 different ProteinA resins and in a DoE approach using parallel chomatography

- Experimental set-up
  - Equipment; Robot, Freedom Evo (Tecan)/DoE; Design Experts Ver. 9.0
  - Column; MediaScout RoboColumn 5 mm I.D. x 1 cm (0.2 mL)
  - 3 rProtein A resins
    - MabSelect SuRe LX (GE Lifescience)
    - TOYOPEARL AF-rProtein A HC-650F, AF-rProtein A-650F (Tosoh)
    - Binding: 100 mM phosphtate (pH 6.5), Elution; 100 mM citrate or acetate at various pH
    - Flow rate; 30 cm/hr - 150 cm/hr (residence time; 0.4 – 2 min)

CIP Study with 0.2 N NaOH on TOYOPEARL AF-rProteinA HC-650F

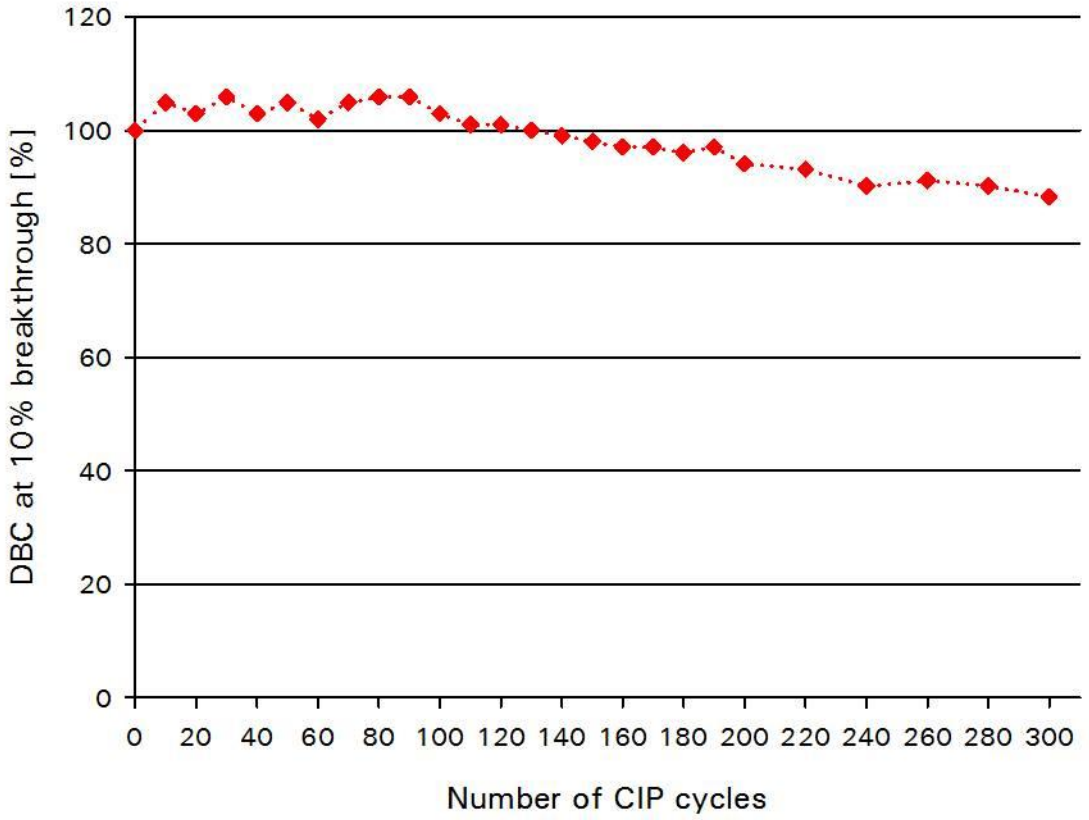


Figure: DBC at 10% breakthrough is stable over 300 CIP cycles using 0.2 M NaOH with 15 min contact time per cycle. The binding capacity begins to decline after 200 cycles. DBC is measured with monoclonal IgG (BImAb04) with a titer of 5 g/L and a residence time of 2 minutes and reaches values of 102 mg/mL (=100%)

CIP Study of CHOP Removal on TOYOPEARL AF-rProteinA HC-650F

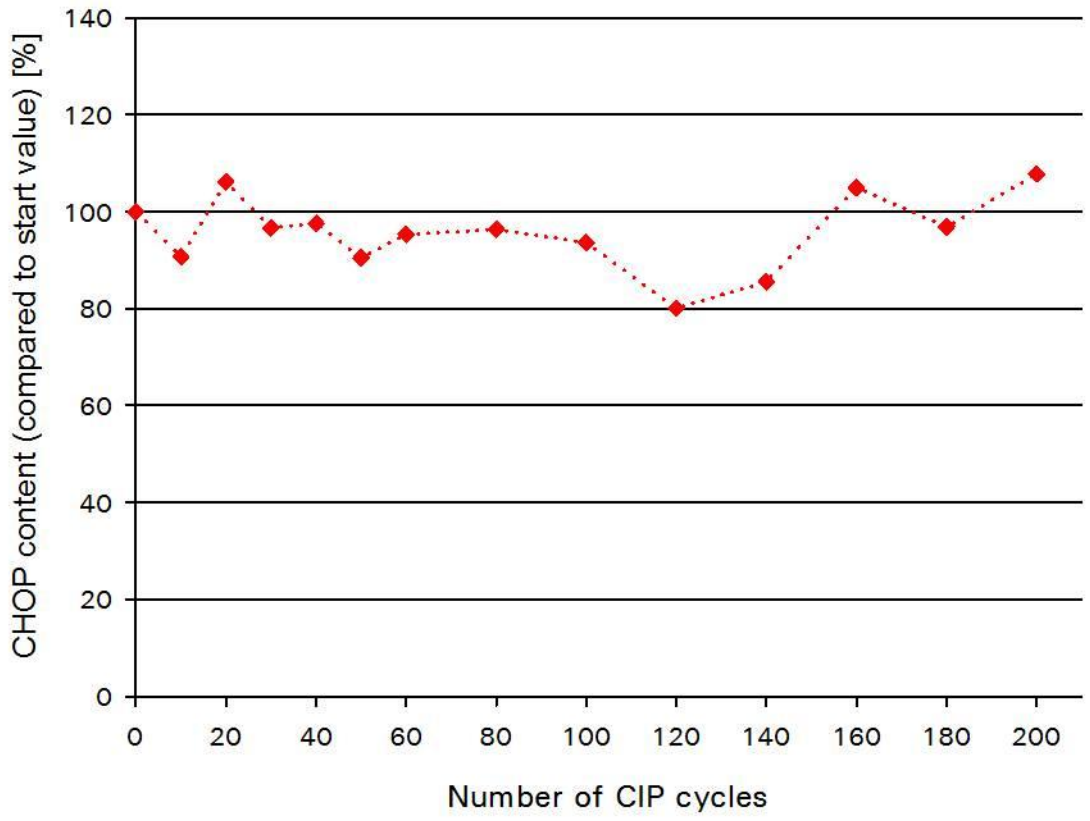
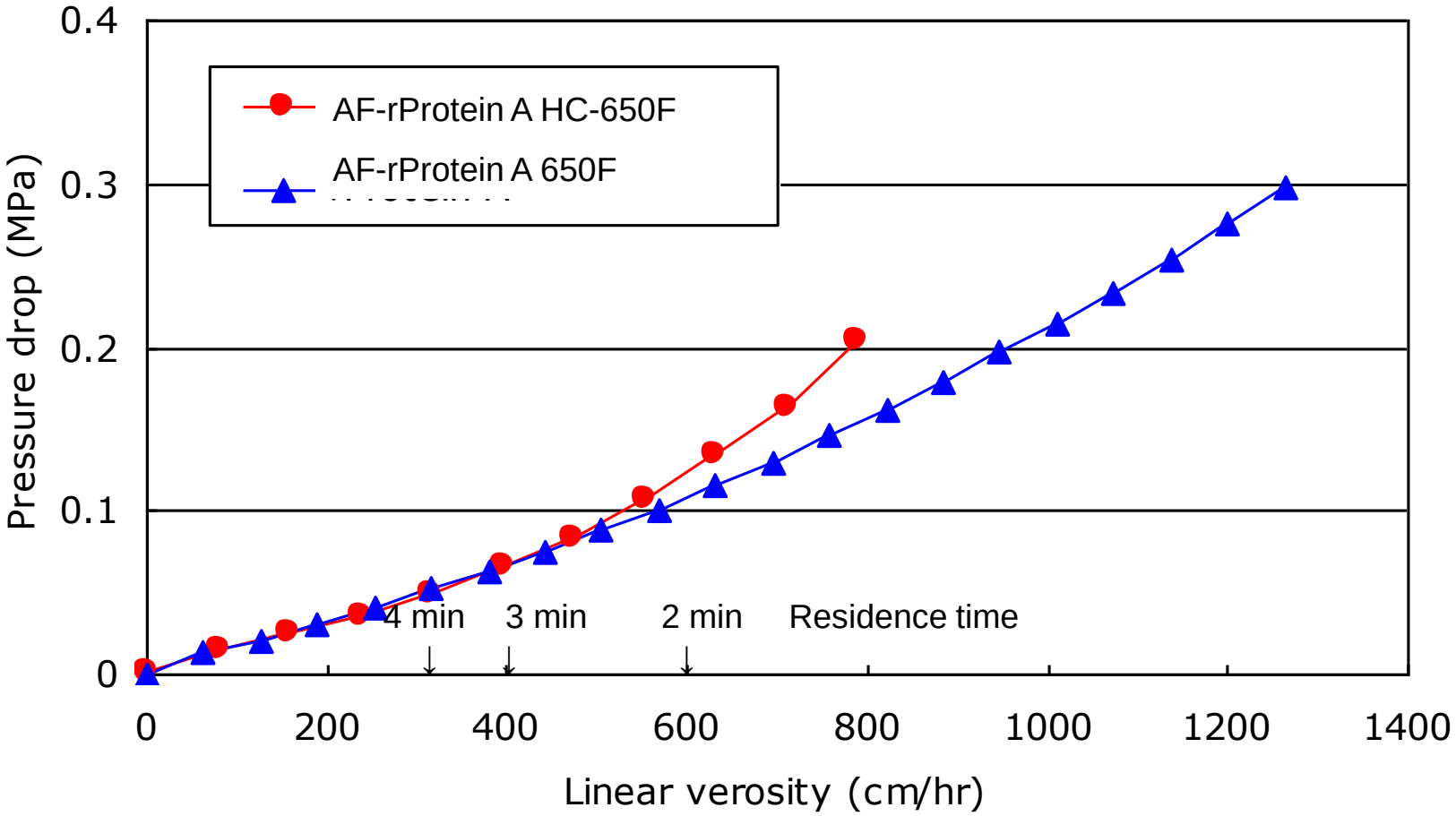


Figure: Purity is stable over 200 CIP cycles using 0.2 M NaOH with 15 min contact time per cycle.

Summary of DoE Evaluation

	TOYOPEARL AF-rProtein A HC-650F	MabSelect SuRe LX
Binding capacity (DBC)	> 60 g/L, flow rate dependence. Higher DBC at residential time over 4 min.	> 50 g/L. flow rate dependence. Higher DBC at residential time over 4 min
Recovery	Almost quantitative. Stronger binding of IgG requires 0.2 point lower pH for elution.	Almost quantitative
HCP removal	Impurities reduction as 2.5 -3.5 log. High capacity Protein A may not be the best for removal of impurities.	Impurities reduction as 2.5 -3.5 log. High capacity Protein A may not be the best for removal of impurities.
Aggregate removal	Similar to regular protein A resin. Although elution pH is 0.2 point lower but no increase of aggregate during elution.	Similar to regular Protein A resin
Alkaline resistance	CIP 200-300 cycles with 0.1-0.2 N NaOH. After CIP 200 cylces, still the same purification efficiency due to stable protein A ligand.	200-300 cycles with 0.1-0.2 N NaOH
Ligand leakage	Relatively low, due to highly stable chemical immobilization and multi-point attachment of ligand to resin	Slighly higher lekage. Protein A ligand attached by one-site at C-terminal of Protein A ligand

Resin Rigidity; Pressure/Flow Curve



Column size: 22 mm I.D. x 20 cm

Comparison of Protein A Resins

Capacity/Price/Rigidity/Residence Time

- GE; MabSelect SuRe LX
- Novasep; AbSolute High Cap
- Merk; Eshmuno A, ProSep UltraPlus
- Thermo/Life Technologies; POROS Protein A
- Kaneka; KanCap Pro A, JSR; Amsphere Protein A

Product name	Supplier	Base matrix	Particle size (micron)	Binding capacity (g/L)*			Chemical stability	List price ($/L)**	Comment
				SBC	DBC (2 min)	DBC (5 min)			
TOYOPEARL AF-rProtein A HC-650M	Tosoh	Polymer	45	>68	50	70	NaOH durable	13,500 (tentative)	rProtein A; C-domains, Multipoint attachement
TOYOPEARL AF-rProtein A 650M	Tosoh	Polymer	45	>45	30	40	NaOH durable	12,000	rProtein A; 4 C-domains, Multipoint attachement, high flow type
MabSelect SuRe LX	GE	Agarose	85	ND	30	58	NaOH durable	21,300	rProtein A; 4 B-domains. Single point attachement
MabSelect SuRe	GE	Agarose	85	ND	30	40	NaOH durable	17,500	rProtein A; 4 B-domains, Single point attachement
AbSolute	NovaSep	Silica glass	44	70	40	45	pH 1.5 - 9.0	18,000	High flow type, Bed height max 40 cm, 1 % benzyl alcohol/acetate buffer, < 10 CFU/mL
AbSolute High Cap	NovaSep	Silica glass	35	90	65	70	pH 1.5 - 9.0	> 18,000?	High capacity type;
Eshumuno A	Merck	Polymer	50	ND	37	45	NaOH durable	?	High purity even after CIP
ProSep UltraPlus	Merck	Porous Glass	60	> 67	45	50	pH 1.5 - 8.5	15,000	rProtein A; natural type. Multipoint attachement. High flow type. Bed height max
KanCapPro A	Kaneka	Cellulose	75	ND	30	50	NaOH durable	?	rProtein A; 5 C-domains, announced to expand production scale in 2013
Amsphere Protein A JWT203	JSR	Polymer	50	ND	33	43	NaOH durable	?	Hydrophobic properites?

* Catalogue data ** Estimated price

Product Lines;

Bulk Resin and Screening Columns

P/N	Product name	Volume size
0023425	TOYOPEARL AF-rProtein A HC-650F	10 mL
0023426	TOYOPEARL AF-rProtein A HC-650F	25 mL
0023427	TOYOPEARL AF-rProtein A HC-650F	100 mL
0023428	TOYOPEARL AF-rProtein A HC-650F	1 L
0023429	TOYOPEARL AF-rProtein A HC-650F	5 L
0023434	TOYOPEARL AF-rProtein A HC-650F	50 L
0023430	ToyoScreen AF-rProtein A HC-650F	1 mL x 5
0023431	ToyoScreen AF-rProtein A HC-650F	5 mL x 1
0023432	ToyoScreen AF-rProtein A HC-650F	5 mL x 5
0021400	ToyoScreen Holder	
0020028	T-F Union (M6 10-32)	Connecter for FPLC system
0023433	ELISA for Protein A-R40	ELISA kit for TOYOPEARL AF-rProtein A HC-650F

Conclusion;

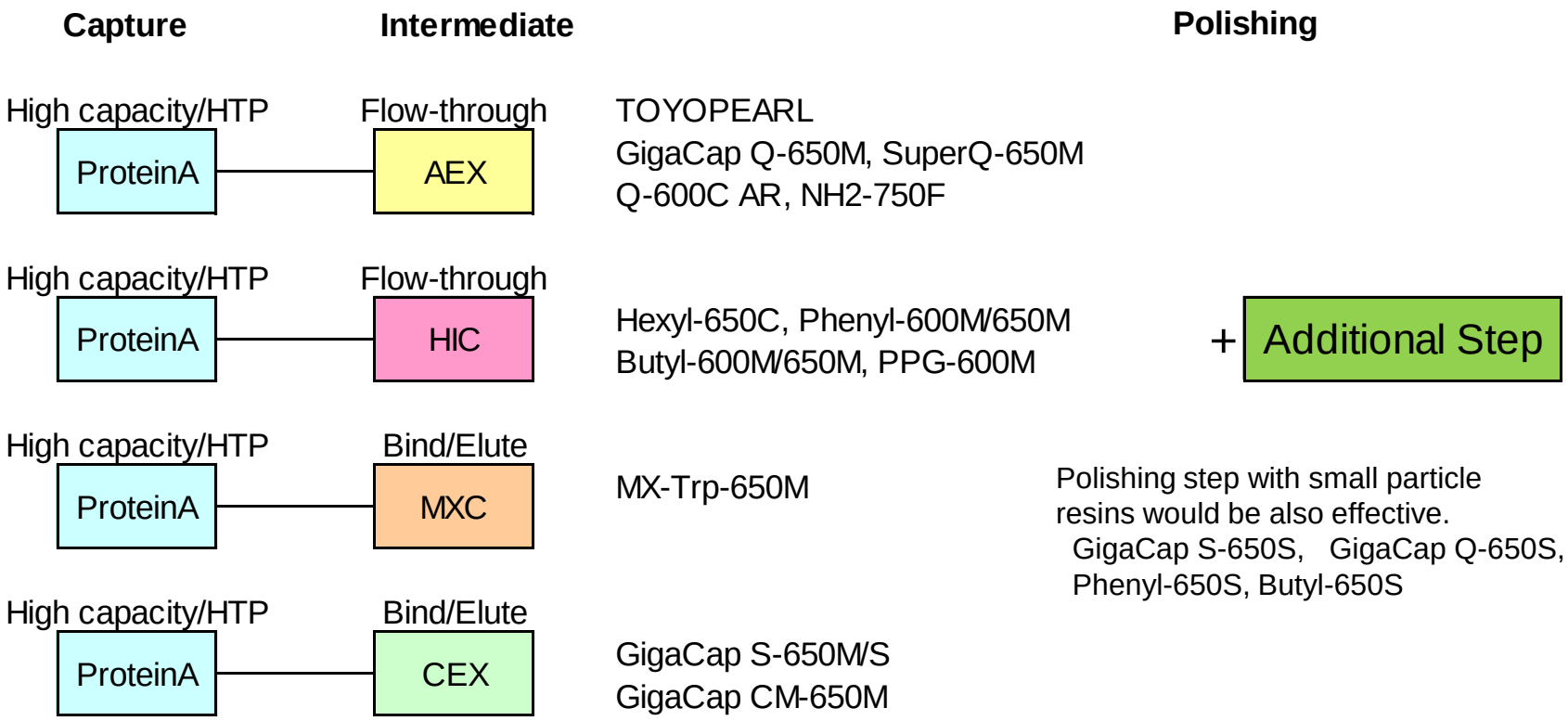
New High Capacity, Alkaline-Resistant, 3rd Generation, TOYOPEARL AF-rProtein A HC-650M

- Higher capacity among Protein A resins
  - 70 g/L IgG capasity at 5 min residence time
  - 50 g/L IgG capacity at 2 min residence time
- Advantage of new Protein A resin
  - Higher capacity; big impact to reduce production cost
  - Alkaline resistance; stable for 200 CIP cycles
  - Resin rigidity; applicable to large scale column
  - Less leakage of Protein A ligand
- Selection of Protein A resins
  - TOYOPEARL AF-rProtein A HC-650F for ordinary process with higher capacity (residence time at 4-6 min)
  - TOYOPEARL AF-rProtein A 650F for high-throughput, high flow rate process (residence time less than 3 min)

Post-Protein A Process Separation

- Flow through anion-exchange chromatography
  - TOYOPEARL GigaCap Q-650S/M, SuperQ-650M, Q-600C AR
- Salt-tolerant anion-exchange chromatography
  - TOYOPEARL Q-600C AR
  - TOYOPEARL NH2-750F (New, June 2014)
- High capacity cation-exchange chromatography
  - TOYOPEARL GigaCap S-650S/M, GigaCap CM-650M
- Salt-tolerant cation-exchange chromatography
  - TOYOPEARL GigaCap CM-650M
  - TOYOPEARL Sulfate-650M (2H, 2014)
- Mixed-mode, salt-tolerant chromatography
  - TOYOPEARL MX-Trp-650M
- “No-salt” hydrophobic interaction chromatography
  - TOYOPEARL Hexyl-650C, Phenyl-650M
  - (TOYOPEARL Phenyl-600M, Butyl-600M, PPG-600M, Butyl-650M)

Purification Process for Mab with Protein A (Protein A and the following process)



* Non-Protein A process would be also effective with CEX, AEC, MXC and HIC due to reduction of production cost.

TOYOPEARL® High-Capacity Resins (Ion-Exchange and Mixed-Mode Chromatography)

Ion-exchanger/Mixed-mode	Type	Pore size	Particle size (micron)	Specific application
TOYOPEARL GigaCap Q-650M, Q-650S	AEX	medium	75, 35	IgG, protein, saccharide
TOYOPEARL GigaCap DEAE-650M	AEX	medium	75	Insulin, Factor VIII
TOYOPEARL SuperQ-650S, M, C	AEX	medium	30, 60, 100	protein, peptide
TOYOPEARL Q-600C AR	AEX	medium	75	Aggregate, Alkaine stable
TOYOPEARL NH2-750F	AEX	medium	45	Aggregate, salt tolerant
TOYOPEARL Q-750M (R&D)	AEX	large	60	IgM
TOYOPEARL DEAE-650S, M, C	AEX	large	30, 60, 100	Factor VIII
TSKgel SuperQ-5PW(20), (30)	AEX	medium	20, 30	OligoDNA
TSKgel DEAE-5PW(20), (30)	AEX	large	20, 30	protein, peptide
TOYOPEARL GigaCap S-650M, S-650S	CEX	medium	75, 35	IgG
TOYOPEARL GigaCap CM-650M	CEX	medium	75	IgG, insulin, salt-tolerant
TOYOPEARL Sulfate-650M (R&D)	CEX	medium	60	Aggregate, salt tolerant
TOYOPEARL SP-650S, M, C	CEX	large	30, 60, 100	protein, peptide
TOYOPEARL CM-650S, M, C	CEX	large	30, 60, 100	Antibiotics, peptide
TOYOPEARL SP-550C	CEX	small	100	Small protein, peptide
TOYOPEARL MegaCap II SP-550EC	CEX	small	200	Insulin, small protein
TSKgel SP-3PW(30)	CEX	small	30	Insulin, small protein
TSKgel SP-5PW(20), (30)	CEX	large	20, 30	protein, peptide
TOYOPEARL MX-Trp-650M	Mixed	medium	60	IgG, salt-tolerant

TOYOPEARL® High-Capacity Resins (HIC and Affinity Chromatography)

Hydrophobic interaction chromatography	Type	Pore size	Particle size (micron)	Specific application
TOYOPEARL PPG-600M	HIC	medium	60	IgG, hydrophobic protein
TOYOPEARL Butyl-600M	HIC	medium	60	IgG
TOYOPEARL Phenyl-600M	HIC	medium	60	IgG
TOYOPEARL Phenyl-650S, M, C	HIC	large	30, 60, 100	
TOYOPEARL Phenyl-750M (R&D)	HIC	large		IgM
TOYOPEARL Butyl-650S, M, C	HIC	large	30, 60, 100	
TOYOPEARL SuperButyl-550C	HIC	small	100	Small protein
TOYOPEARL Ether-650S, M	HIC	large	30, 60	IgM, hydrophobic protein
TOYOPEARL Hexyl-650C	HIC	large	100	Hydrophilic protein, plasmid
TSKgel Phenyl-5PW(20), (30)	HIC	large	20, 30	
TSKgel Ether-5PW(20), (30)	HIC	large	20, 30	Hydrophobic protein

Affinity chromatography	Type	Pore size	Particle size (micron)	Typical application
TOYOPEARL AF-rProtein A-650F	AFC	large	45	IgG, IgM
TOYOPEARL AF-Heparin HC-650M	AFC	large	60	Coagulation factor
TOYOPEARL AF-Blue HC-650M	AFC	large	60	Nucleotide dependent protein
TOYOPEARL AF-Red-650M	AFC	large	60	Nucleotide dependent protein
TOYOPEARL AF-Chelate-650M	AFC	large	60	His-tag protein
TOYOPEARL AF-Epoxy-650M	AFC	large	60	Activated resin
TOYOPEARL AF-Tresyl-650M	AFC	large	60	Activated resin
TOYOPEARL AF-Formyl-650M	AFC	large	60	Activated resin
TOYOPEARL AF-Amino-650M	AFC	large	60	Activated resin
TOYOPEARL AF-Carboxy-650M	AFC	large	60	Activated resin

For AFC, high capacity was achieved by optimization of pore size, modification, spacer arms, ligand density and particle size.

Screening of Anion-exchangers after Protein A Affinity Chromatography (Flow-through mode)

Resin	Yield (%)	HMW % removal	LMW % removal	CHO % removal	ProA % removal
Capto Q	97.4	26%	NSR *	86%	34%
TOYOPEARL® GigaCap Q-650M	98.2	33%	NSR *	86%	40%
Poros Q	97.1	31%	NSR *	79%	22%
Fractogel TMAE	95.2	31%	NSR *	78%	3%
Fractogel TMAE Hicap	100.0	35%	NSR *	85%	32%
UNO-Sphere Q	97.0	37%	NSR *	88%	30%

* NSR; No significant reduction detected

Conditions; Column: 1 cm I.D. x 10 cm (7.85 mL)
Sample was charged at 125 g/L resin with buffer at pH 8.5,
and conductivity of 3 mS/cm.

Reference; The poster by J. Tetrault et al.,Waterside Conference, US (2008)

Better results on Toyopearl Q-600C AR as flow through mode.

Comparison of HIC and HCIC (Mixed-Mode) for Whole Mab Purification Steps

	ProA-HIC-AEX	ProA-HCIC-AEX
Process parameters	Mab protein specific	More general for Mab
Process development efforts	Complex	Simple
Sample manipulation	Significant	Minor
Overall process yields (%)	50-60	50-60
HMW % by SEC HPLC	1.00	1.73
LMW % by SEC HPLC	Not detected by HPLC	0.49
CHO HCP level (ppm)	0.74	2.65
Leached Protein A level (ppm)	1.70	2.23

Ref.; J. Chen et al., J. Chromatogr. A, 1177(2008) 272-281

Specification of TOYOPEARL NH_2-750F

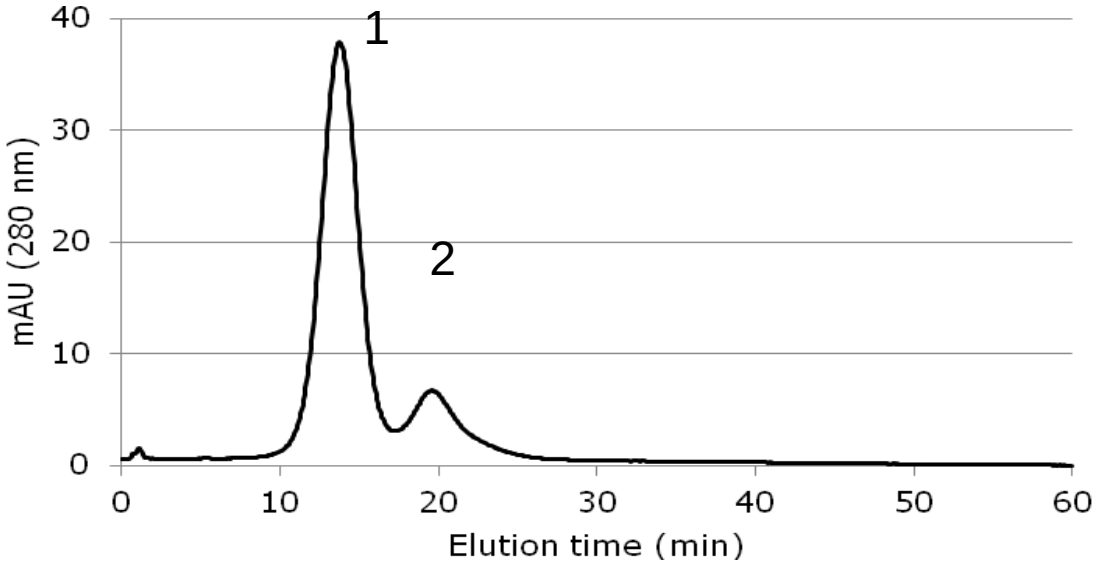
Items	Result
Product name	TOYOPEARL NH_2-750F
Ligand	Primary amine (polyamine)
Ion-exchange group (meq./L)	0.07 – 0.13
Particle size distribution (um)	30 – 60
Static binding capacity (IgG, g/L)	≧ 70
Bacterial counts (CFU/mL)	≦ 100
Endotoxin (EU/mL)	≦10
Foreign subustances	≦ 6
Eluable matter	≦ 0.2 %

* Estimate pore size of the resin may be 100 – 150 nm.

Expected Application of New Salt-tolerant TOYOPEARL NH2-750F

- Salt-tolerant separation（Mab, others)
  - Binding of proteins even at above 0.15 mol/L NaCl in buffer
  - Capture of feedstock sample without dilution of salt
  - Aggregate removal by flowthrough or gradient mode
  - Large pore resin for IgG and high molecular weight samples
  - Protein may be bound even at pH below pI in eluent
- Chromatographic conditions
  - Binding buffer pH lower than pI of target protein
  - Presence of salt in buffer may enhance binding capacity.
  - Higher pH in binding buffer, the higher conc. of salt for binding up to max. 0.3 mol/L NaCl in buffer
  - Different binding and selectivity from ordinary anion-exchanger

Separation of Aggregates from Monoclonal Antibody (IgG1) on TOYOPEARL NH_2-750F



Chromatographic conditions

Column; TOYOPEARL Aminated-750F (5 mm I.D. X 5 cm)

Elution; A 60-min linear gradient from 0 to 1 mol/L NaCl in 20 mmol/L Tris-HCl (pH 8.0)

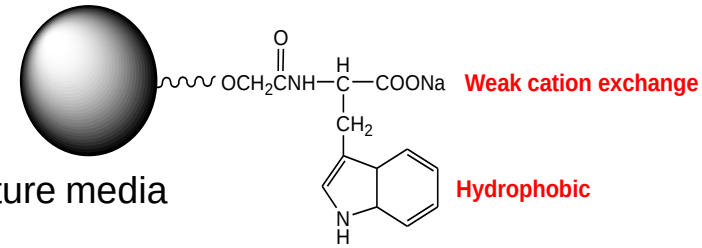
Flow-rate; 1.0 mL/min, sample; Detection; UV (280 nm)

Sample; Mab (IgG1, 0.5 mg)

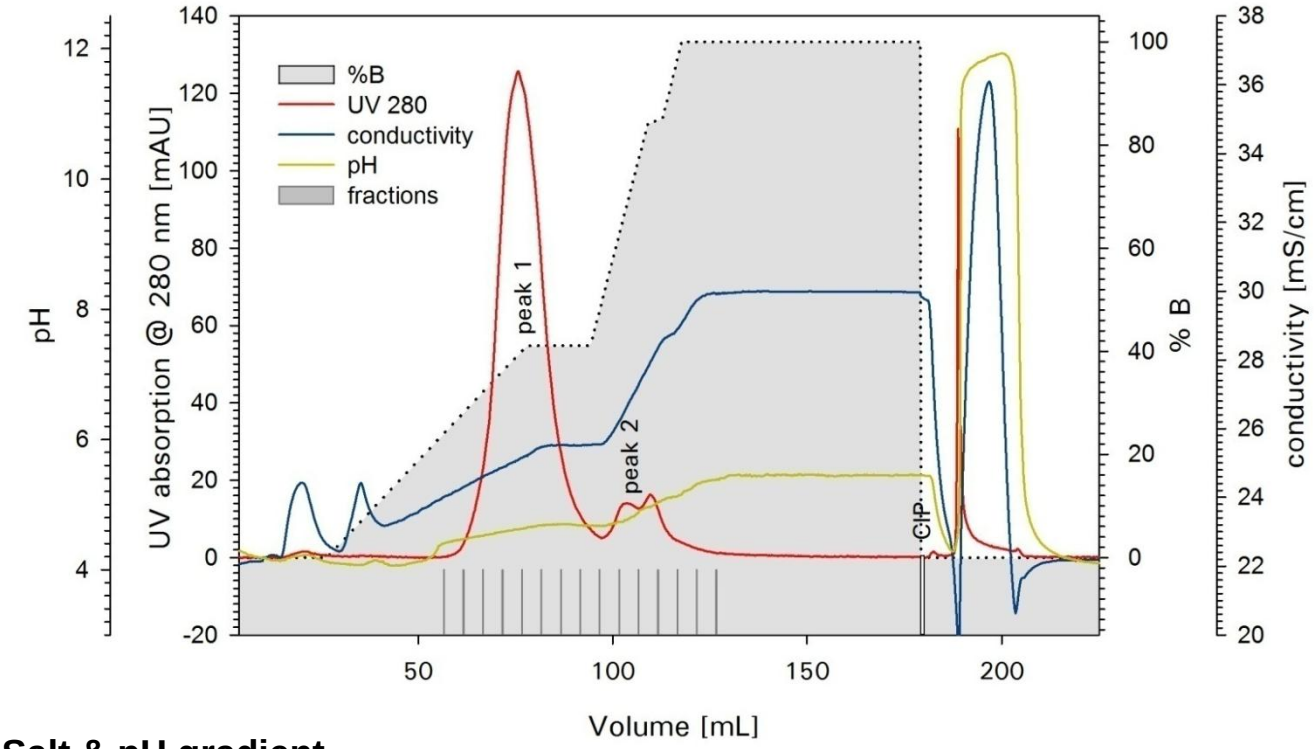
* Effective flow-through mode application was also confirmed by a customer.

Expected Application of Mixed-Mode, TOYOPEARL MX-Trp-650M

- High capacity (> 75 g/L IgG) and high selectivity
- Both ionic and hydrophobic resin features
  - Ligand; tryptophan (cationic/HIC type); carboxyl/imidazole
    - Ref.; anionic/HIC type; GE CaptAdhere, Pall MEP HyperCell
- Salt tolerant
  - Adsorbs proteins even at high salt up to 0.2 mol/L NaCl
  - Adsorbs protein at lower salt concentration than HIC
  - Most proteins are adsorbed and separated.
- Different selectivity
- Similar effect to Protein L chromatography
- Target molecules
  - Aggregate removal, Fab fragments, scFV
  - Antibody and other recombinant proteins from cell culture media
  - Neutral and basic pI proteins
  - Required optimization of adsorption and elution conditions

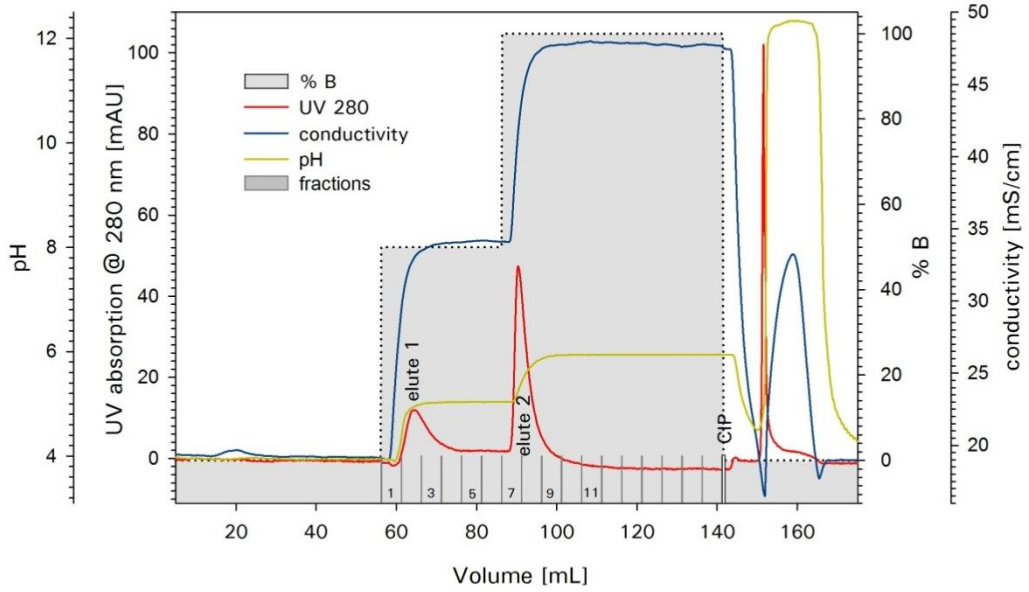


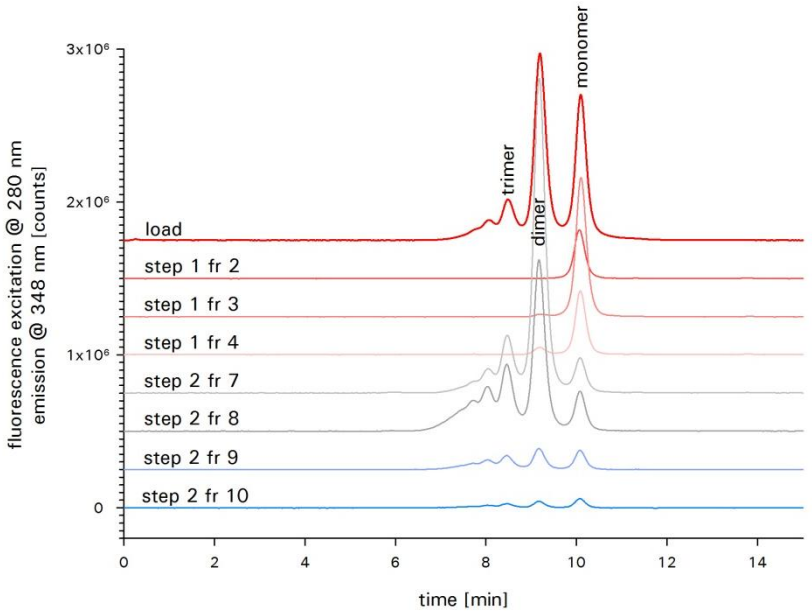
Aggregated MAb – Bind-and-Elute Mode on Toyopearl MX-Trp-650M (optimized)



Salt & pH gradient
Loading: 0.1 M acetate + 0.2 M NaCl, pH 4.3
Elution: 0.1 M acetate + 0.4 M NaCl, pH 5.6
Flow rate; 150 cm/h
Sample; 10 mg antibody per ml resin were loaded, 5ml-samples were fractionated
A step gradient can be applied. (aggregate content ~ 17 %)

scFv Polishing with MX-Trp-650M; Gradient Optimization





Sample: 10 µg scFv
A: 0.1 M acetate + 0.2 M NaCl, pH 3.5
B: 0.1 M acetate + 0.5 M NaCl, pH 5.6
Gradient: 45 CV 45 % B, 75 CV 100 % B

Sample: fractions from Trp run
Column: TSKgel G3000SWXL
Liquid Phase: 0.1 M sodium phosphate + 0.1 M sodium sulfate, pH 6.7

Purification of scFv on Mixed-Mode Resin and Protein L Affinity Resins

	Protein L	MX-Trp-650M linear gradient	MX-Trp-650M step gradient
Aggregate content [%]	15	3	3
Aggregate removal factor	4	22	22
Monomer yield [%]	62	79	93
Linear velocity	150 cm/h	150 cm/h	150 cm/h
DBC	20 mg/ml at 50 cm/h	50 mg/ml	50 mg/ml

Flow-Through HIC Separation

REPORT

mAbs 5:5, 795–800; September/October 2013; © 2013 Landes Bioscience

Purification of monoclonal antibodies by hydrophobic interaction chromatography under no-salt conditions

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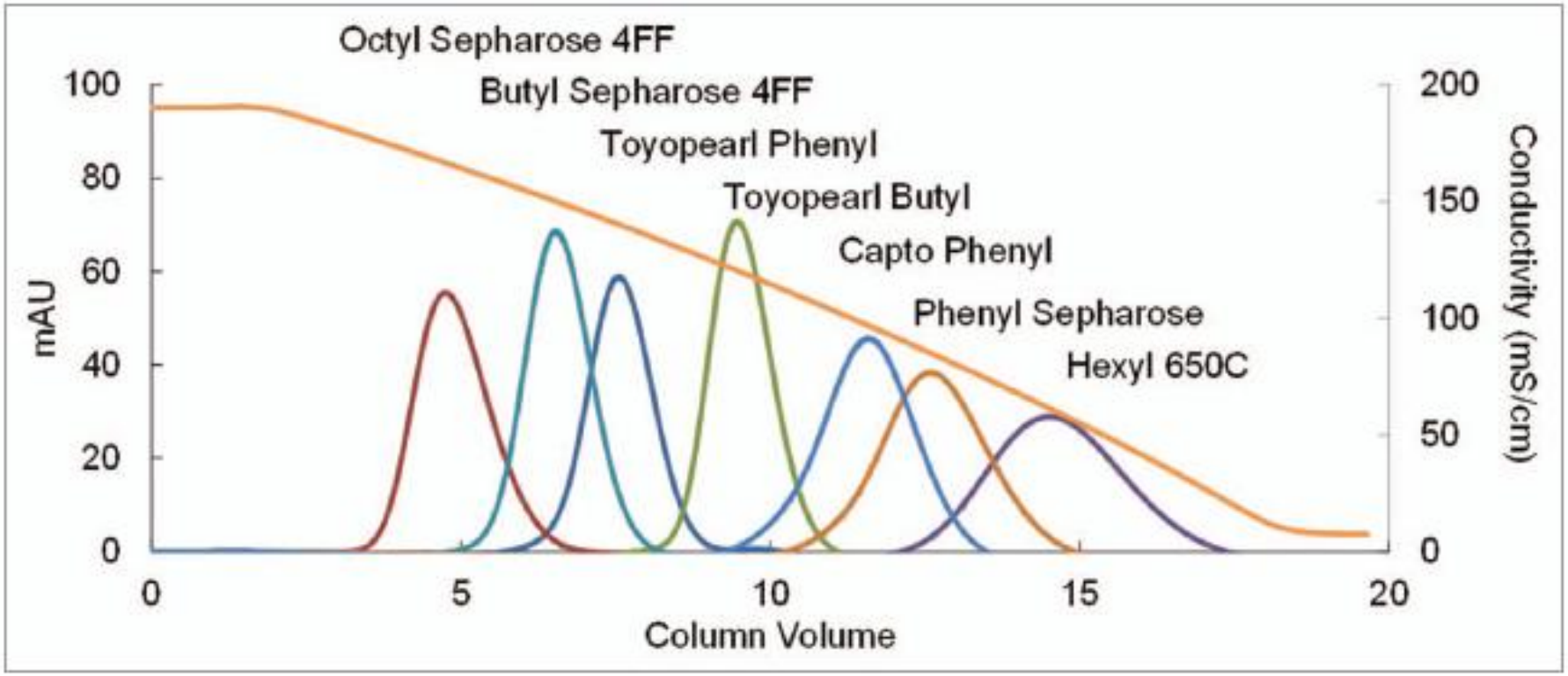
Keywords: HIC, flowthrough, monoclonal antibodies, no salt, aggregates

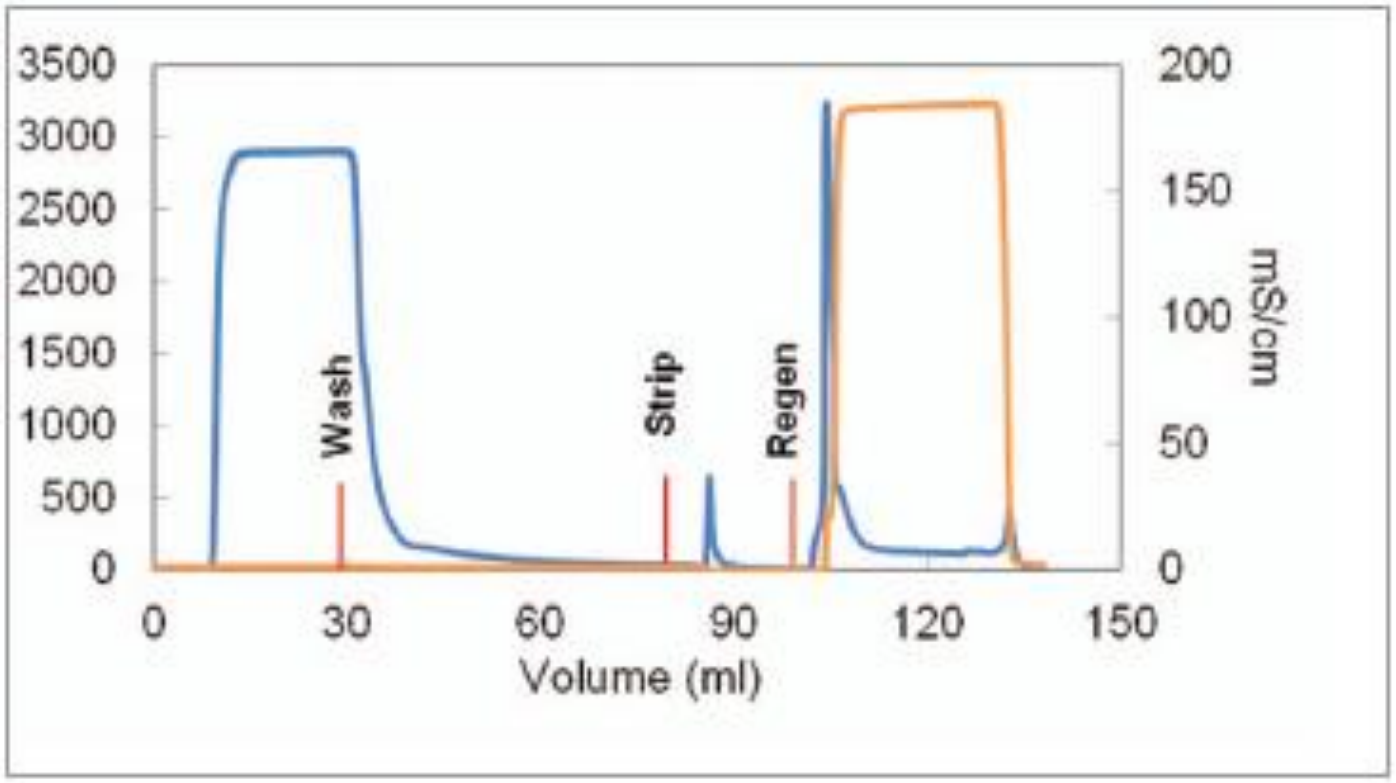
To overcome cumbersome, high salt concentration buffer on HIC,
no salt or low salt HIC separation was evaluated with TOYOPEARL Hexyl-650C and Phenyl-650M.

Linear Gradient Separation of Lysozyme on 7 Commercially Available HIC Resins



Ref. S. Ghose et al., mAbs, 5:5, 795-800, September/October, 2013

No-Salt HIC Flow-Through Step



Ref. S. Ghose et al., mAbs, 5:5, 795-800, September/October, 2013

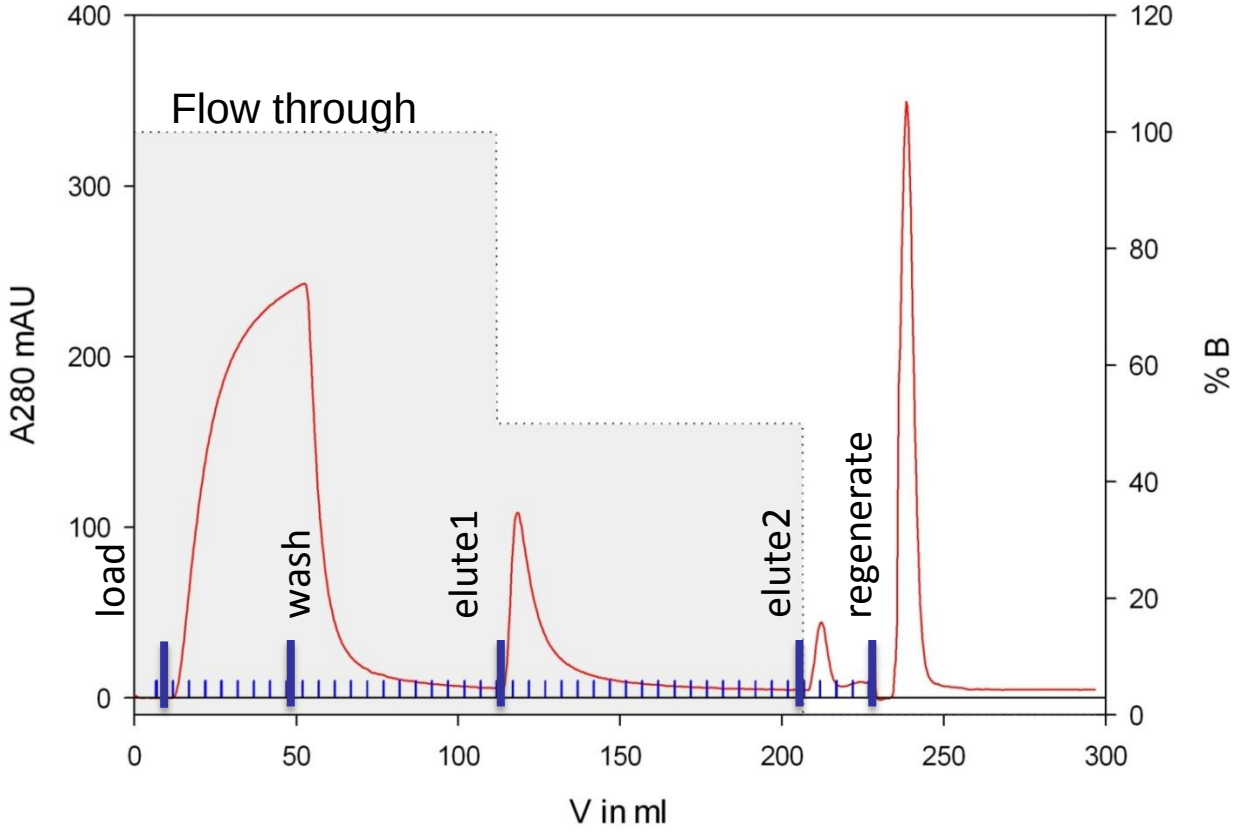
Process Performance on Flow-Through HIC Step on TOYOPEARL Hexyl-650C

Mab	Protein A fraction loading (g/L)	Mobile phase composition for flow-through	Mobile phase conductivity (ms/cm)	Recovery (%)	Impurity HMW (%)	HCP (ppm)
A	35	Original sample	-	100	0.80	10.0
		10 mM sodium citrate (pH 5.5)	2.6	86	0.21	3.8
B	65	Original sample	-	100	0.70	25.0
		5 mM sodium citrate (pH 6.0)	1.3	88	0.13	4.7
C	70	Original sample	-	100	2.50	100.0
		10 mM sodium citrate (pH 5.5)	2.6	88	0.34	10.0
D	55	Original sample	-	100	2.20	10.0
		10 mM sodium citrate (pH 6.0)	2.6	90	0.37	< 1.4

Ref. S. Ghose et al., mAbs, 5:5, 795-800, September/October, 2013, modified

Recovery and purity for Mabs depend on properties of Mab molecules.

Flow Through HIC Chromatography with Dual Salts Eluent



Column; TOYOPEARL Phenyl-650M
Flow rate; 450 cm/h loading velocity
Buffer A; 1 M NaCl + 200mM citrate in 20 mM phosphate buffer (pH 7)(Buffer B)
Sample; IgG1, 10 mg/mL gel

Conclusion

- High capacity alkaline-durable Protein A resin
  - TOYOPEARL AF-rProtein A HC-650F for high capacity
  - TOYOPEARL AF-rProtein A-650F for high throughput
- Post-Protein A chromatography on AIEC, CIEC, HIC and MMC.
  - TOYOPEARL NH2-750F for salt tolerant, aggregate removal
  - TOYOPEARL Hexyl-650C for no (low) salt flow-through
  - TOYOPEARL MX-Trp-650M for salt tolerant, aggregate removal
  - TOYOPEARL GigaCap S-650S for higher resolution with 35 um
- Non-Protein A process
  - Capture step on TOYOPEARL GigaCap S-650M
  - Mab and single body purification by TOYOPEARL MX-Trp-650M, which may replace Protein L affinity chromatography

TOSOH

Thank you for your attention.