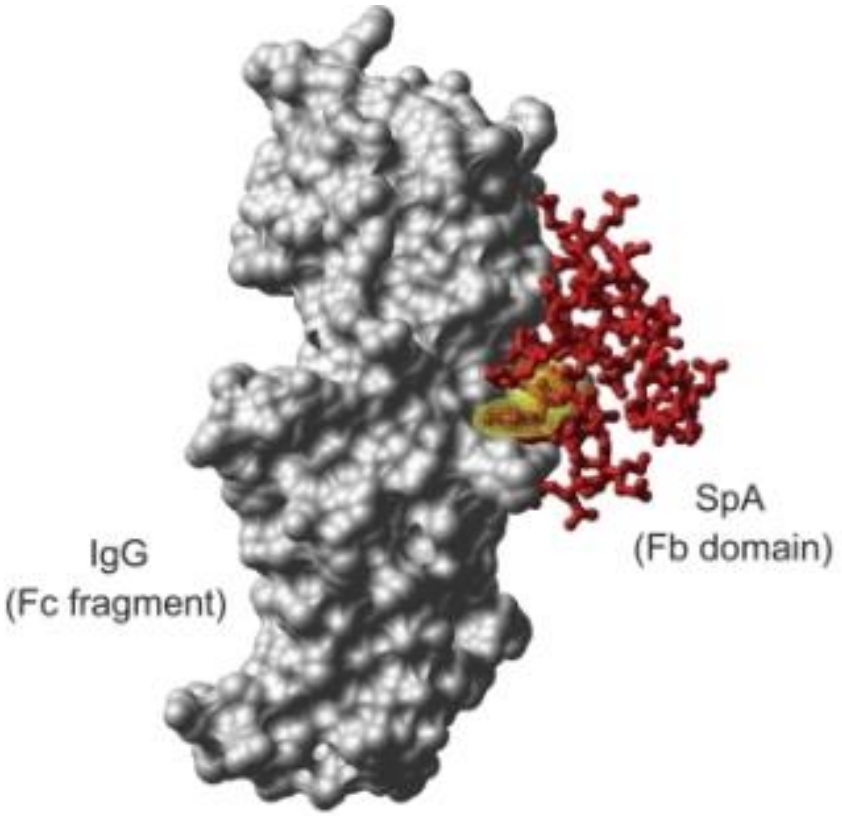
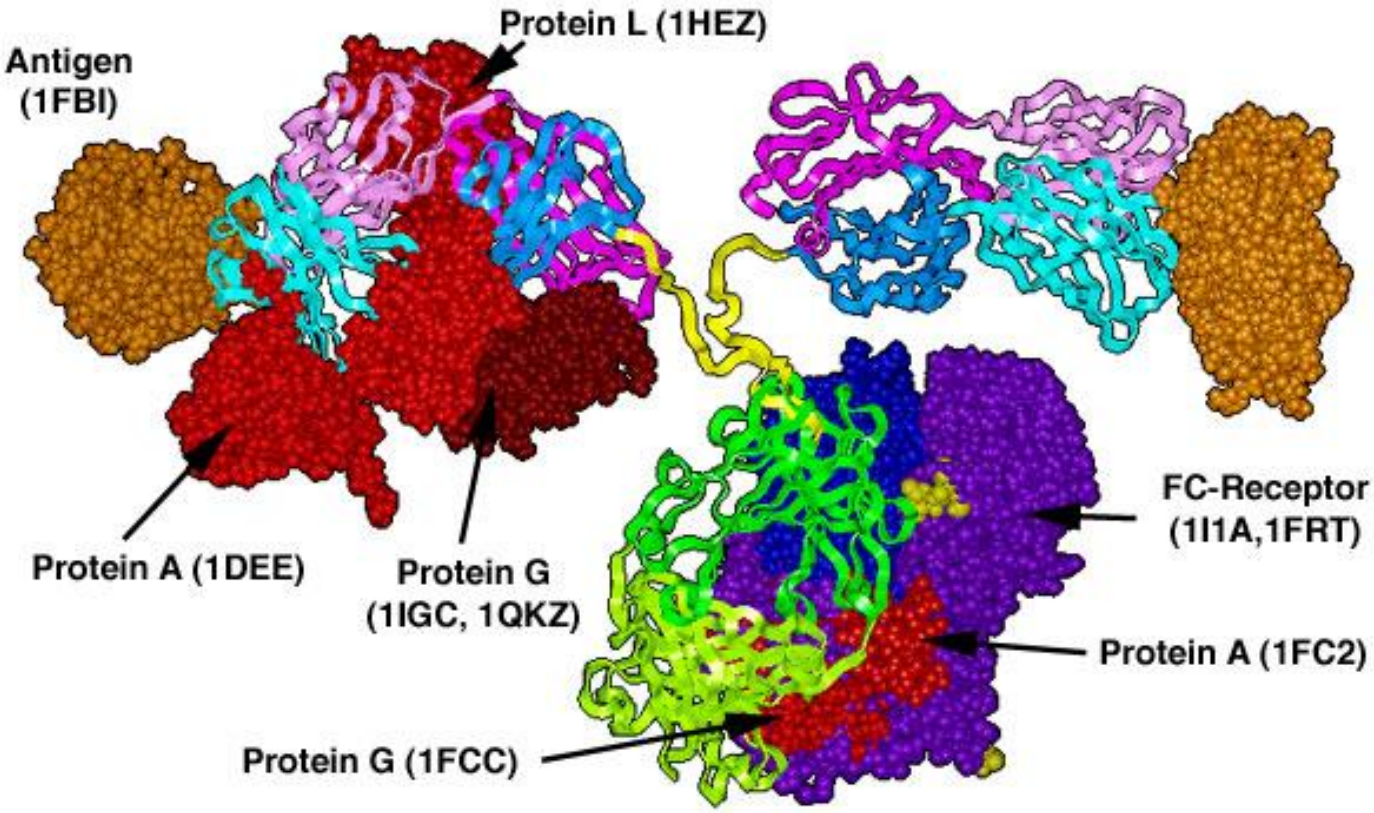
TOSOH



Comparison of 3 different ProteinA resins in a DoE approach using parallel chomatography

Masterthesis
Angelika Wacker
2014
at
TOSOH Bioscience GmbH
(Stuttgart)
tutors: Egbert Müller, Judith Vajda

TOSOH



MAb used in this study was a humanized IgG1

DoE approach to evaluate the optimal process parameters

Variables:
- Antibody titer
- Column load
- CHOP spiking

in

Used experimental design:
central composite design with 4 factors:

Factor	Name	Units	Minimum	Maximum	-1 Actual	+1 Actual	Mean
A	elution pH		2.25	4.25	2.75	3.75	3.25
B	load	mg/mL resin	10.00	50.00	20.00	40.00	30.00
C	titer	g/L	0.25	9.25	2.50	7.00	4.75
D	CHOP spiking	%	5.00	25.00	10.00	20.00	15.00
	CHOP concentration	µg/mL	100	500	200	400	300

out

Variables: - Elution buffer
(Citrate or Acetate)

DOE software : Design-Experts Version 9.0

Center point values

→ All graphs refer to these values if they are not otherwise indicated

Facts

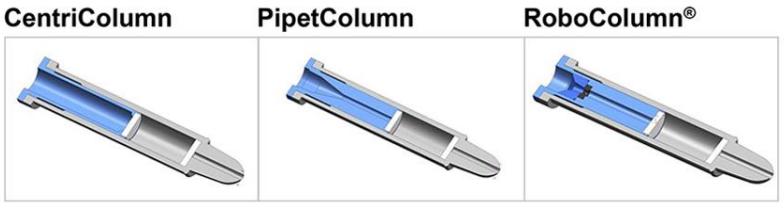
hardware

Experimental set-up:

Robot: **Freedom EVO**

Columns: **MediaScout® RoboColumn®**
bed height : 10.0 mm
d_i : 5 mm
column volume : 200 µL

ATOLL GmbH
convenience in bioscience



Facts
method

Method Protein A :

Used resins: **1. MabSelect SuRe LX**
2. Toyopearl AF - rProteinA - 650F
3. Toyopearl AF - rProteinA HC - 650F

Step	buffer	Volume [CV]	flow [mL/min]	flow [cm/h]
EQ	100 mM Phosphate pH 6.5	20	0.5	150
Load (2min res. time)		according to schedule	0.1	30
Wash	100 mM Phosphate pH 6.5	20	0.5	150
Elution	100 mM Citrate or 100 mM Acetate pH according to schedule	20	0.2	60

Facts – experimental schedule
central composite design with 4 factors

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number	pH Elution	load [mg/mL resin]	titer [g/L]	CHOP spike [%]
1	2,75	20	2,50	10
2	3,25	30	0,25	15
3	2,75	20	7,00	10
4	3,25	30	4,75	25
5	3,75	40	2,50	10
6	4,25	30	4,75	15
7	3,75	40	7,00	20
8	3,75	40	7,00	10
9	2,75	40	2,50	10
10	3,75	20	7,00	20
11	3,25	30	4,75	5
12	3,75	20	2,50	10
13	3,75	40	2,50	20
14	3,25	30	4,75	15
15	3,75	20	7,00	10
16	3,75	20	2,50	20
17	2,75	40	7,00	10
18	3,25	10	4,75	15
19	2,25	30	4,75	15
20	2,75	40	2,50	20
21	3,25	30	4,75	15
22	3,25	30	9,25	15
23	3,25	30	4,75	15
24	3,25	30	4,75	15
25	3,25	50	4,75	15
26	2,75	20	7,00	20
27	2,75	20	2,50	20
28	2,75	40	7,00	20
29	3,25	30	4,75	15
30	3,25	30	4,75	15

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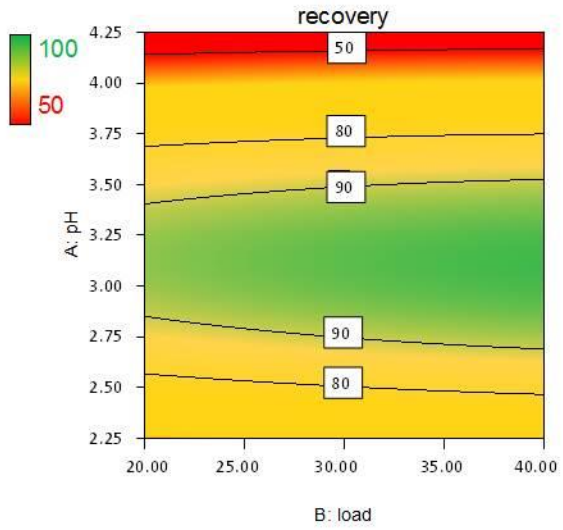
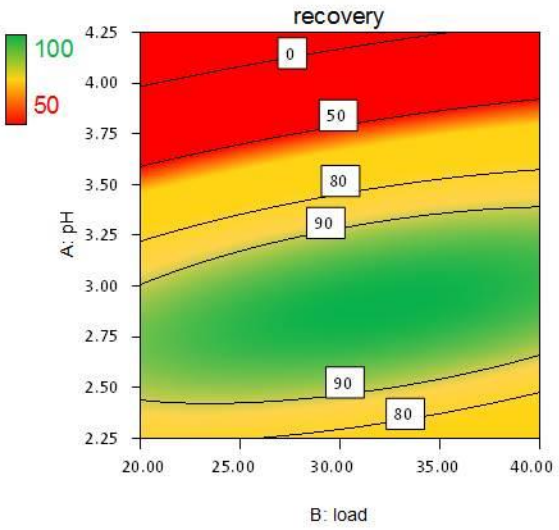
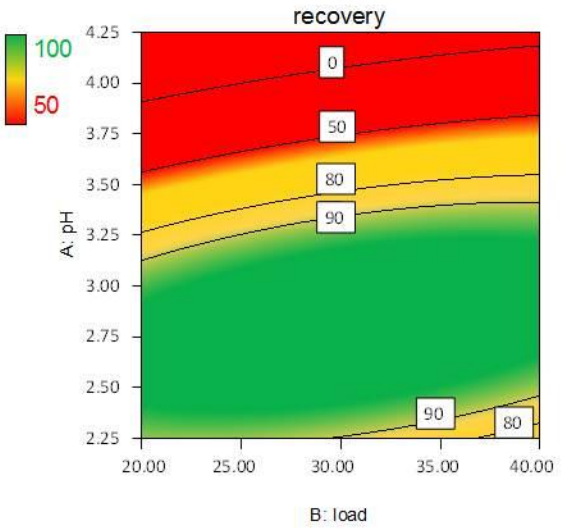
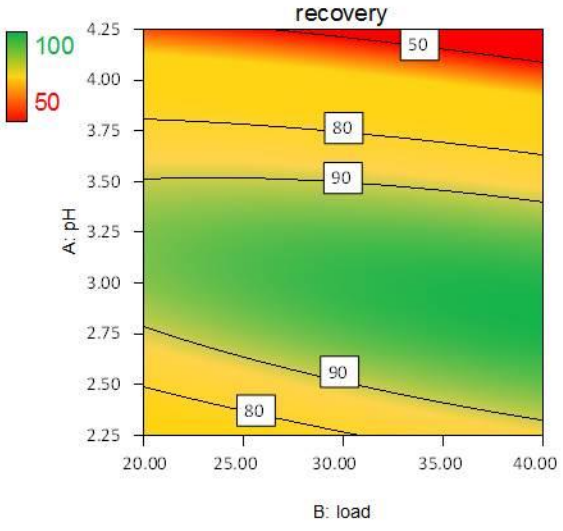
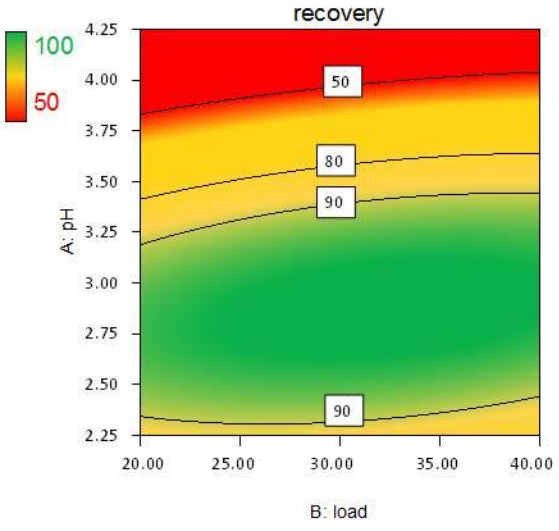
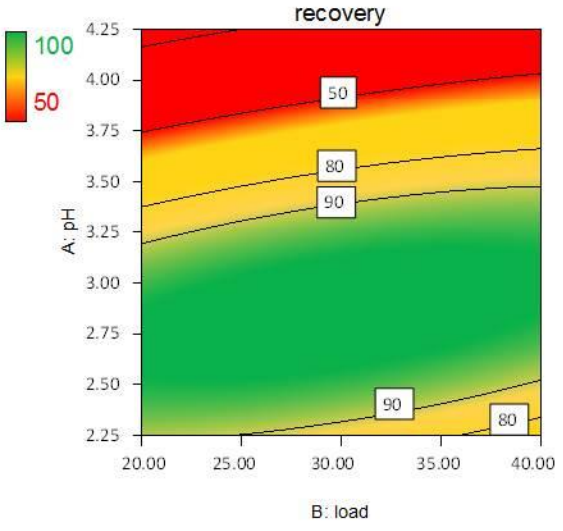
results

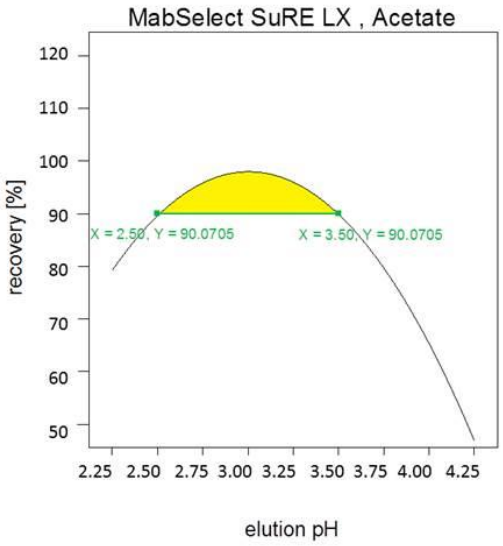
- Recovery
- Aggregate content
- Reduction of CHO proteins
- Ligand leaching
- Dependency of antibody feed titer
- Calculation of optimal parameters
- Longterm stability data

Recoveries [%] – dependency of load

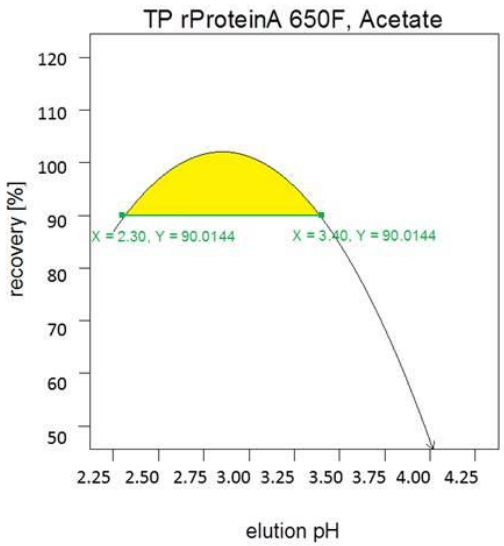
	MabSelect SuRe LX	TP AF rProteinA 650F	TP AF rProteinA HC 650F
Citrate			
Acetate			

Recovery of all Protein A materials - Acetate

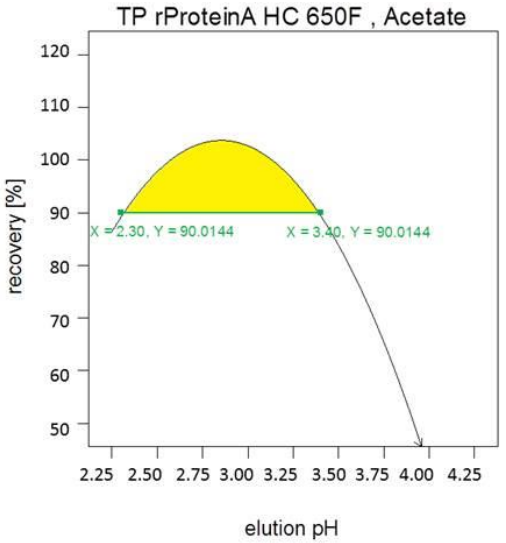
Mab Select SuRe LX



Toyopearl AF-rProteinA -650F



Toyopearl AF-rProteinA HC-650F



Recovery >90% (region marked in yellow) in pH range:

pH 2.5 - 3.5

pH 2.3 - 3.4

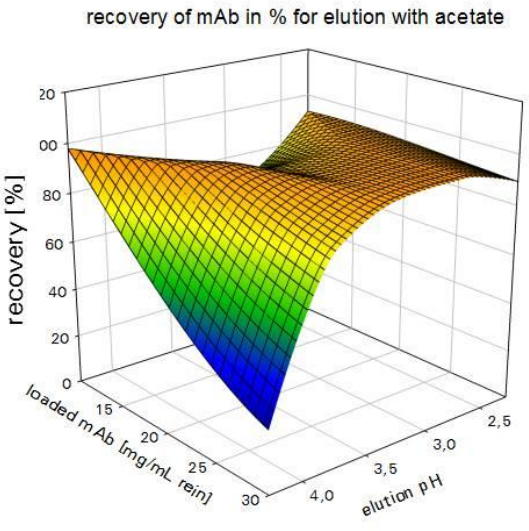
pH 2.3 - 3.4

Conclusions:

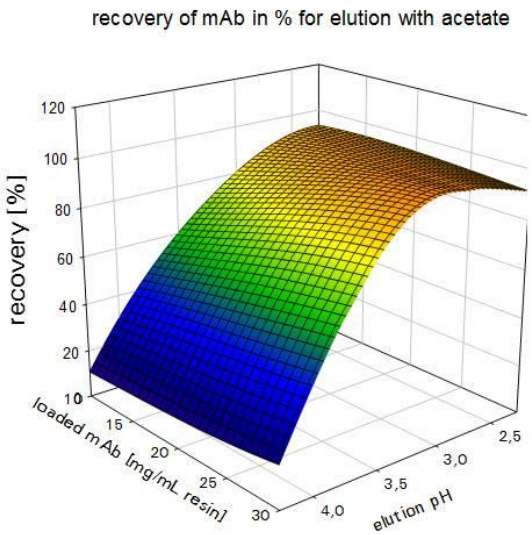
- best recoveries for all resins in the range of pH 2.5 to 3.4
- Recovery window of the competitor resin is slightly shifted towards higher pHs (ΔpH 0.1), but smaller

Recovery of all Protein A materials - Acetate

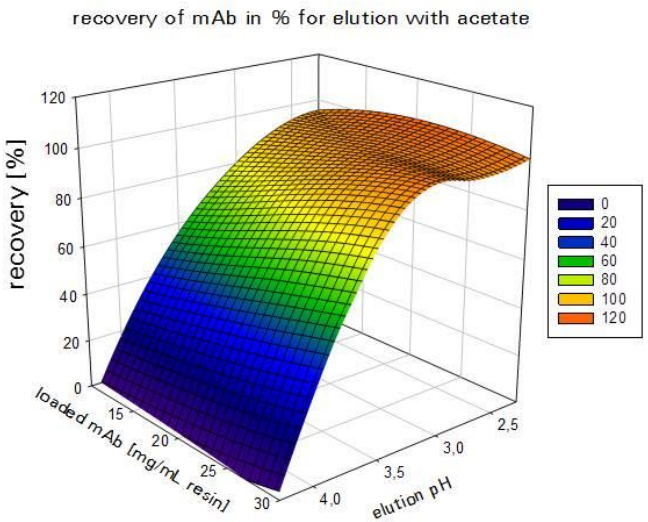
MabSelect SuRe LX



Toyopearl AF-rProteinA -650F



Toyopearl AF-rProteinA HC-650F



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results

- Recovery
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- Dependency of antibody feed titer
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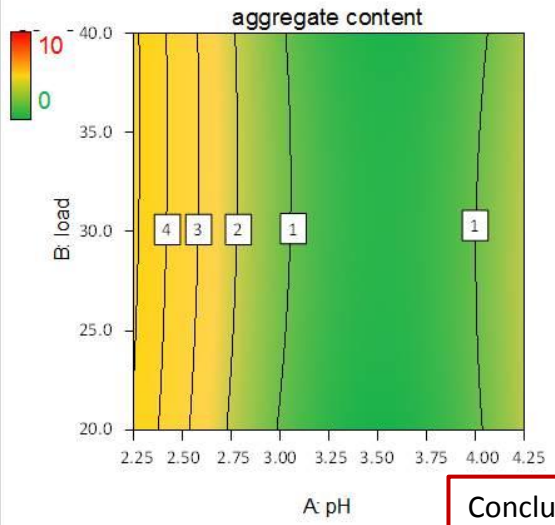
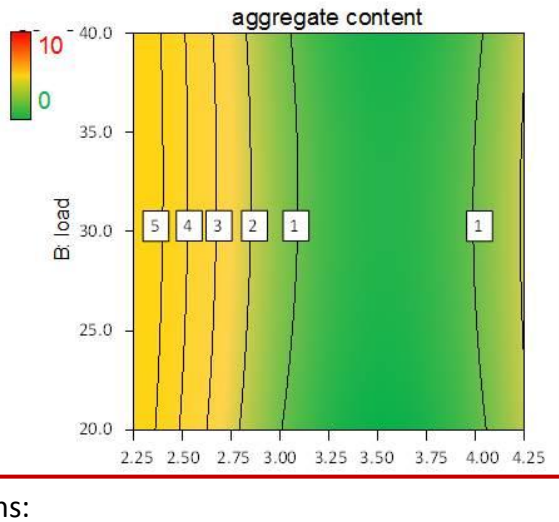
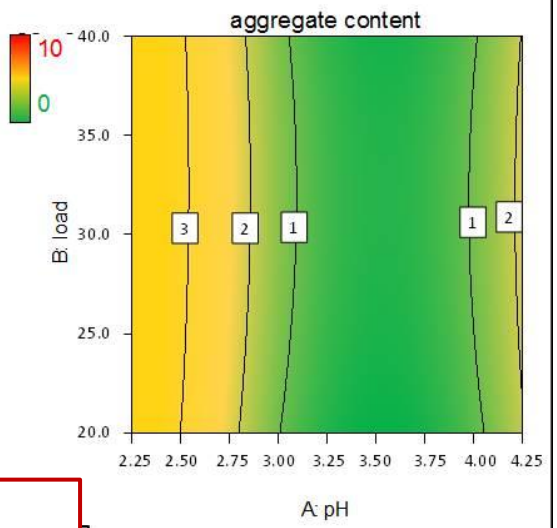
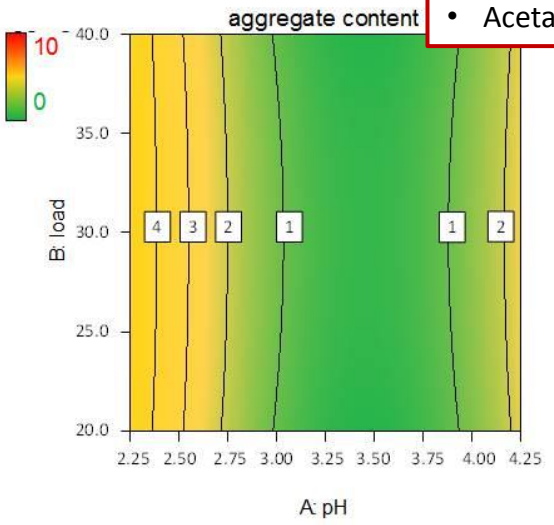
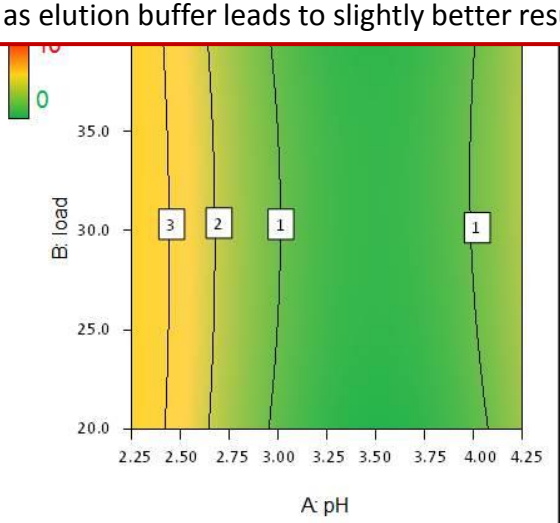
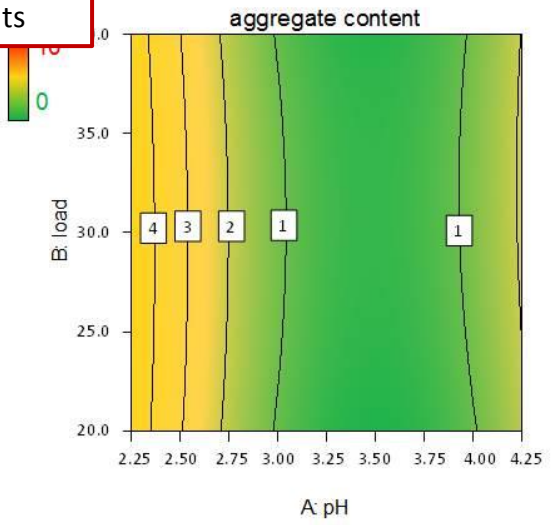
Aggregate content [%]

	MabSelect SuRe LX	TP AF rProteinA 650F	TP AF rProteinA HC 650F
Citrate			
Acetate			

Conclusions:

- All resins show acceptable aggregate contents under 4%
- The lower the pH value the more aggregation occurs
- Acetate as elution buffer leads to slightly better results

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results

- Recovery
- Aggregate content
- Reduction of CHO proteins
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- Dependency of antibody feed titer
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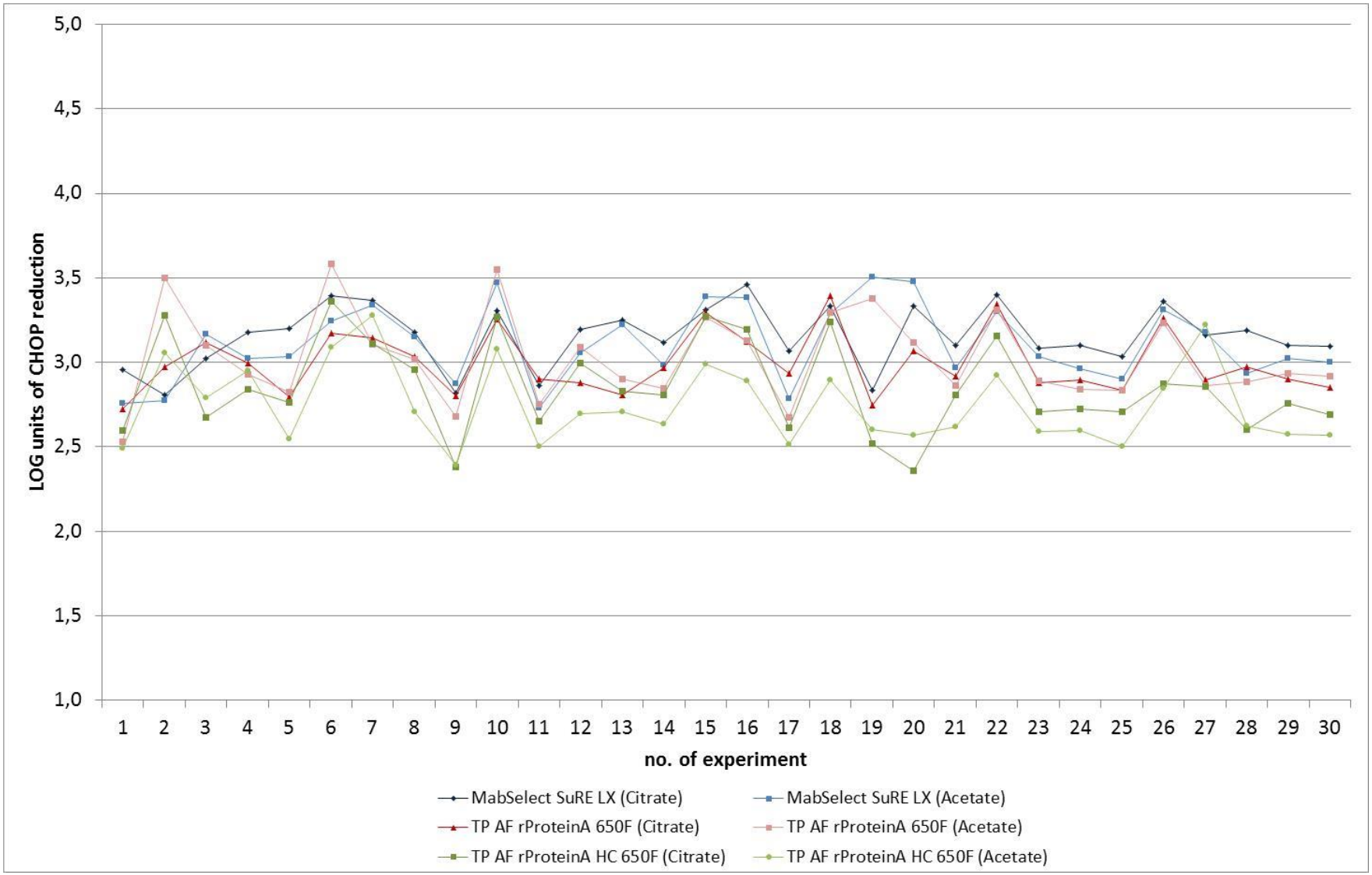
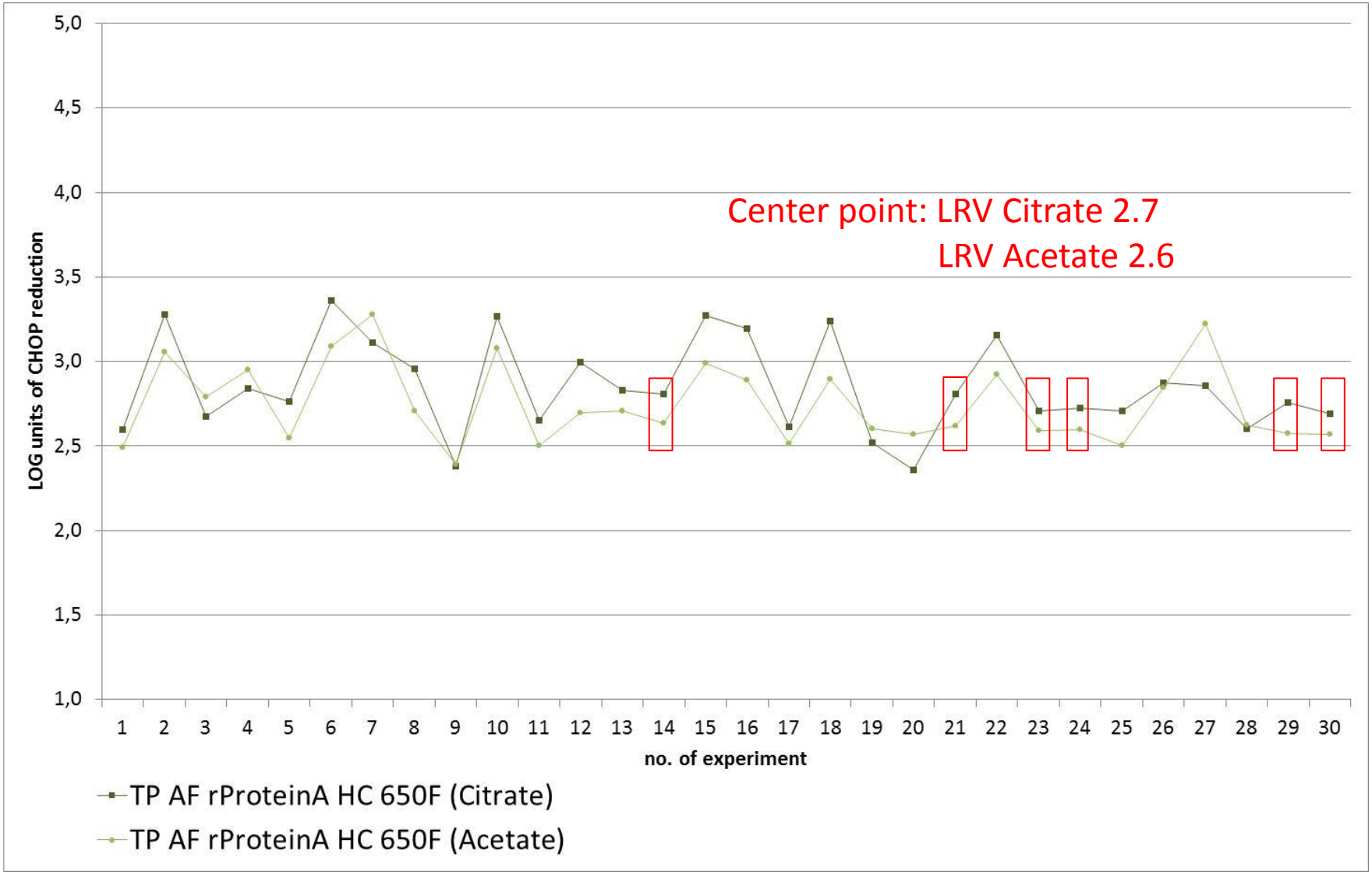
- Longterm stability data

Reduction of host cell proteins



Reduction of host cell proteins

Toyopearl AF- rProteinA HC 650F



TOSOH

results

- Recovery
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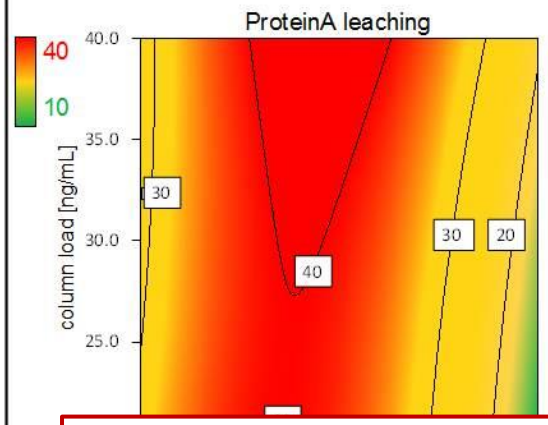
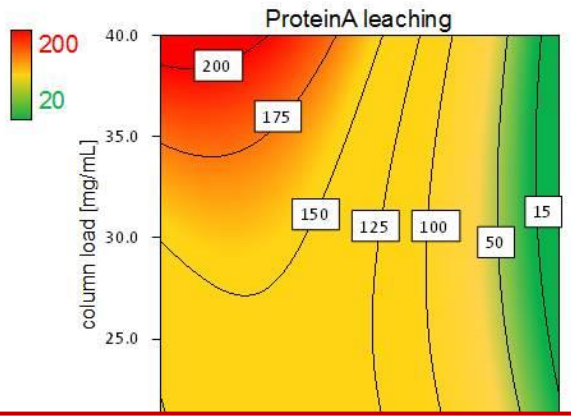
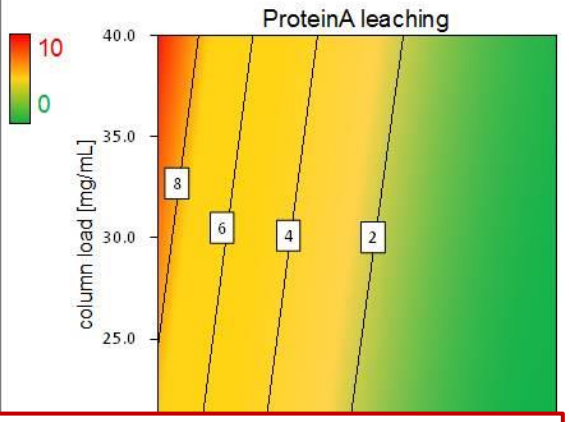
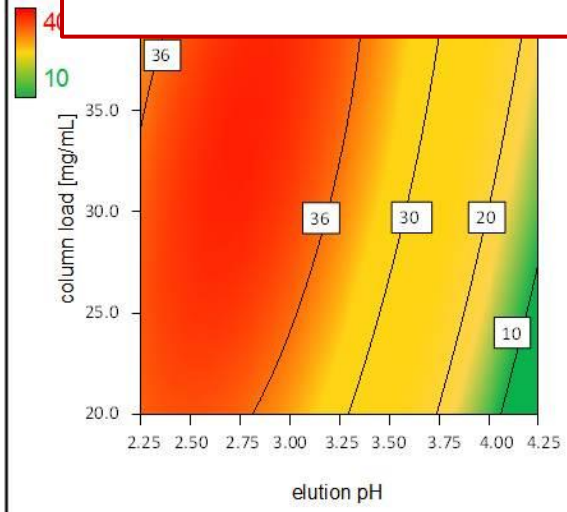
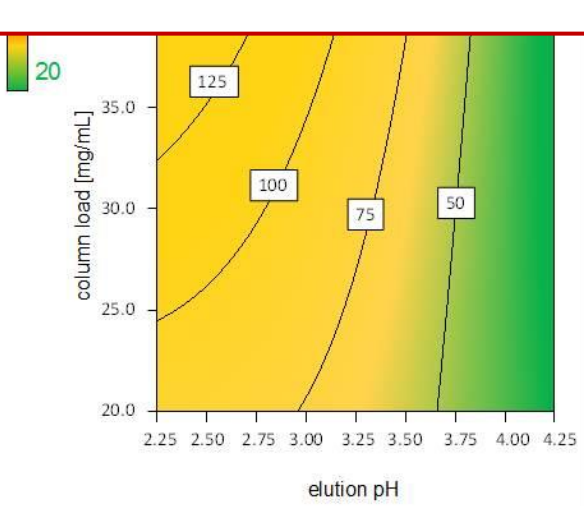
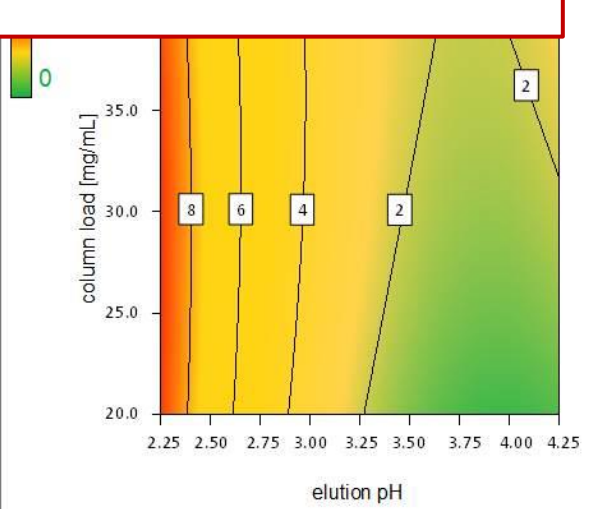
Impurity: ProteinA leaching [ng/mL]

	MabSelect SuRe LX	TP AF rProteinA 650F	TP AF rProteinA HC 650F
Citrate			
Acetate			

Conclusions:

- TP AF rProteinA HC 650F shows very low ProteinA leakage below 15 ng/mL in process relevant pH range for both elution variants
- TP AF rProteinA HC 650F is superior in comparison with MabSelect SuRe LX, which shows moderate Protein A leakage
- Highest leaching (but still acceptable) was detected for TP AF-rProtein A-650F

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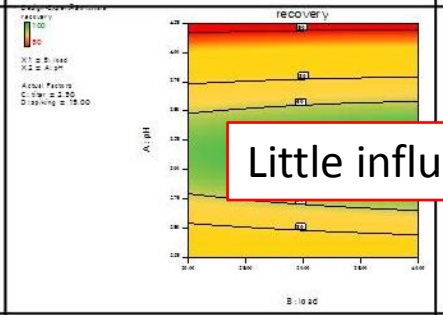
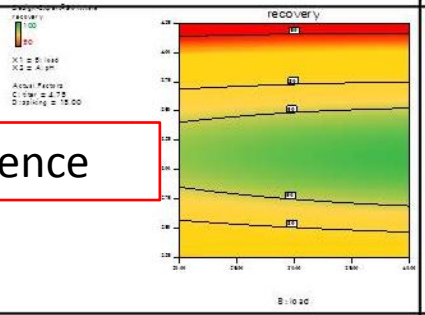
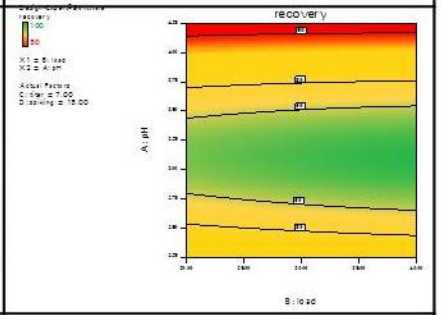
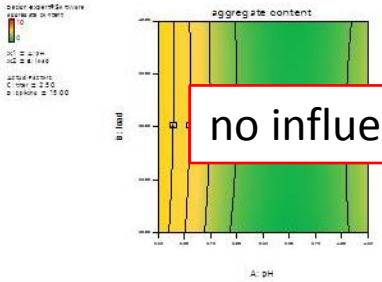
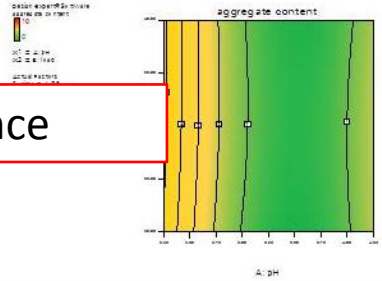
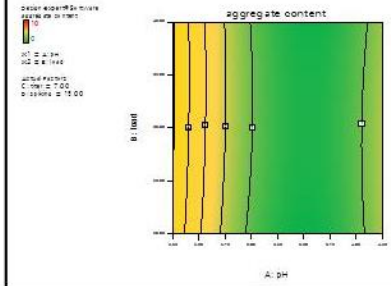
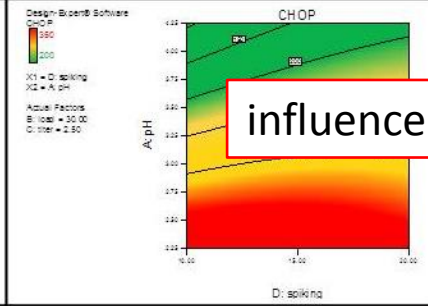
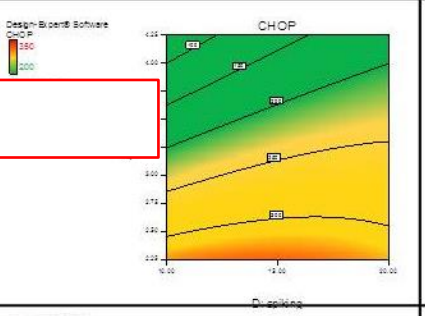
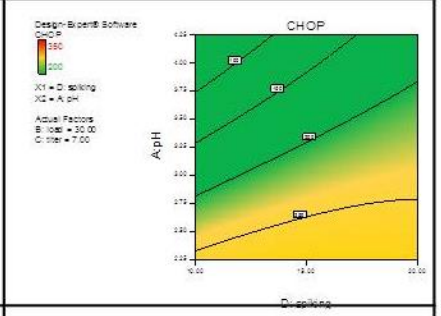
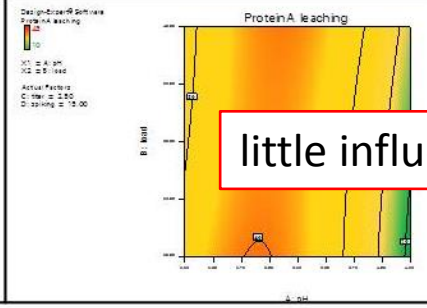
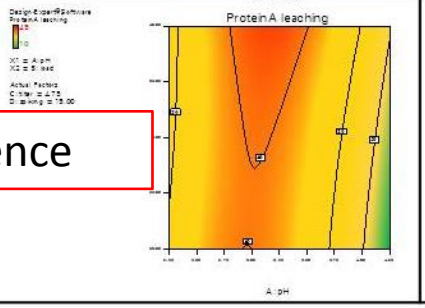
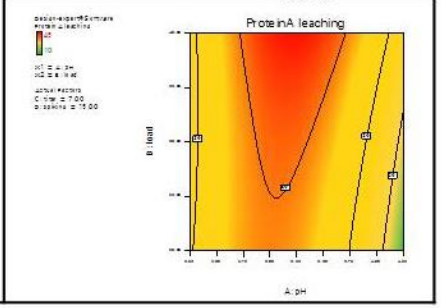
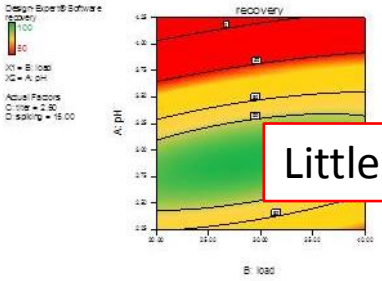
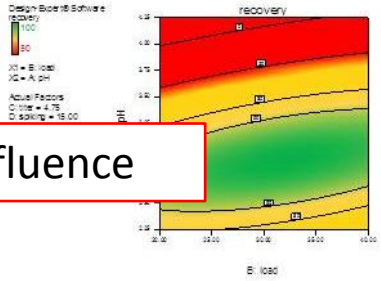
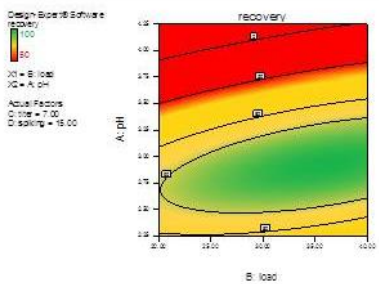
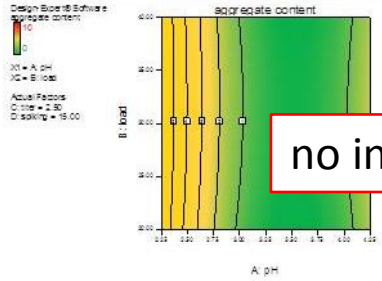
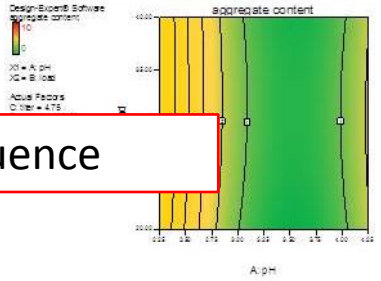
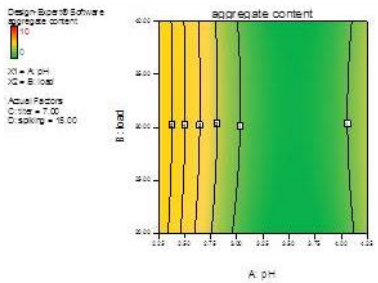
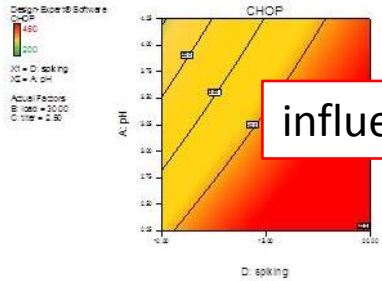
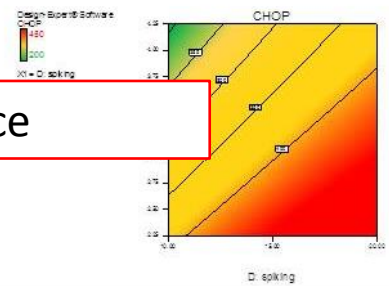
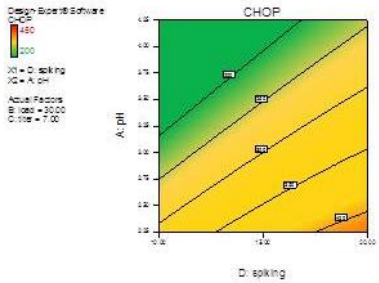
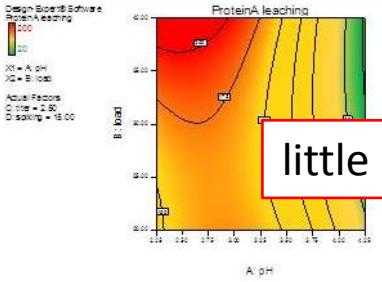
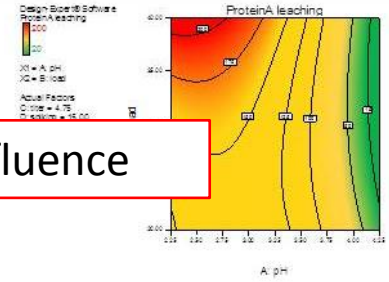
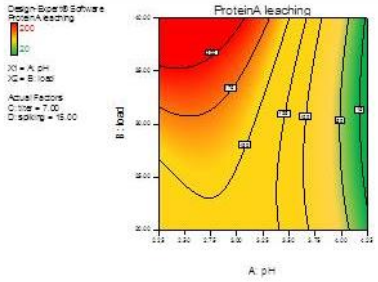
results

- Recovery
- Aggregate content
- Reduction of CHO proteins
- Ligand leaching
- Influence of antibody feed titer
- Calculation of optimal parameters
- Longterm stability data

Dependency of feed concentration example: Mab Select SuRE LX, Citrate Elution

antibody titer	2.5 g/L	4.75 g/L	7 g/L
Recovery [%]	 Little influence		
Aggregate content [%]	 no influence		
CHOP [ng/mL]	 influence		
Protein A [ng/mL]	 little influence		

Dependency of feed concentration
example: TP AF-rProteinA-650F, Citrate Elution

antibody titer	2.5 g/L	4.75 g/L	7 g/L
Recovery [%]			
Aggregate content [%]			
CHOP [ng/mL]			
Protein A [ng/mL]			

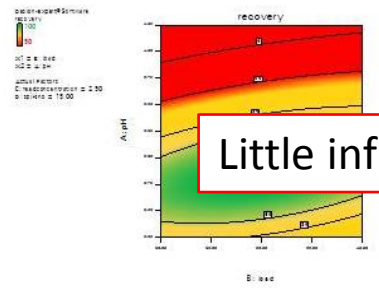
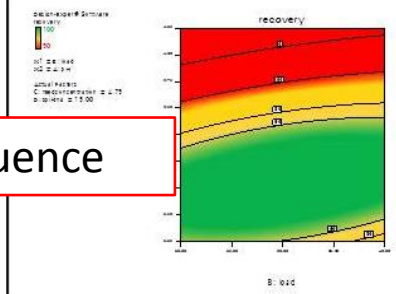
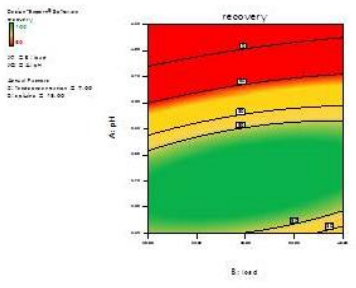
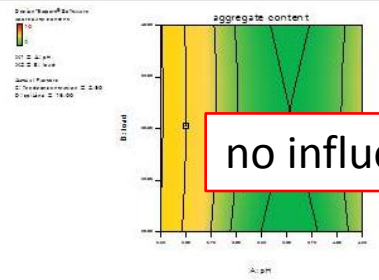
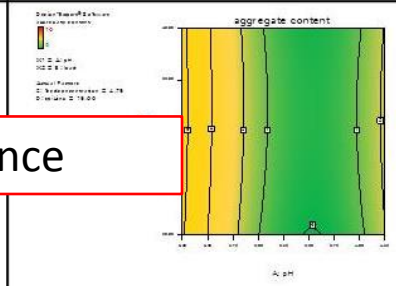
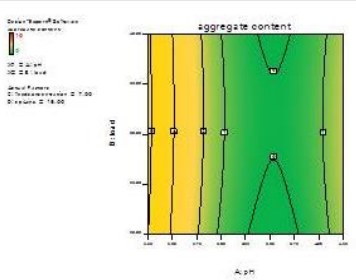
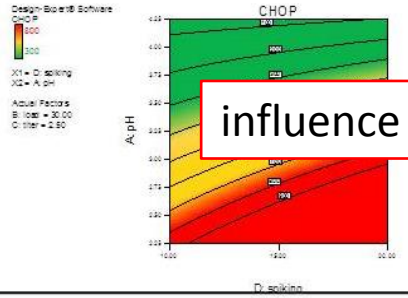
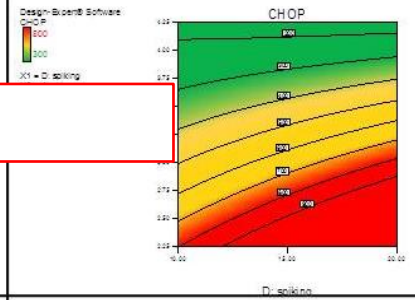
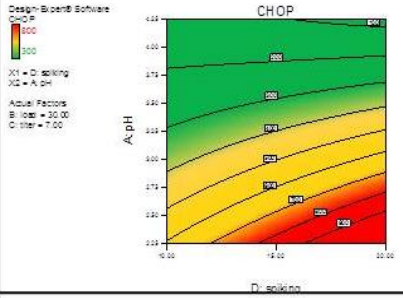
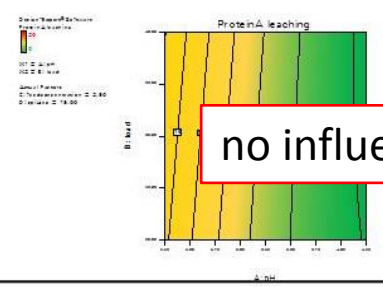
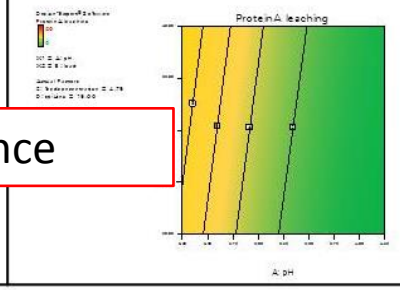
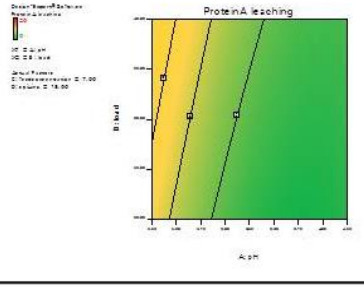
Little influence

no influence

influence

little influence

Dependency of feed concentration
example: TP AF-rProteinA HC 650F, Citrate Elution

antibody titer	2.5 g/L	4.75 g/L	7 g/L
Recovery [%]			
Aggregate content [%]			
CHOP [ng/mL]			
Protein A [ng/mL]			

Little influence

no influence

influence

no influence

TOSOH

results

- Recovery
- Aggregate content
- Reduction of CHO proteins
- Ligand leaching
- Dependency of antibody feed titer
- Calculation of optimal working range
- Longterm stability data

Optimization of process parameters

specifications – model 1

Name	Goal	Lower Limit	Upper Limit	Importance
Design factors				
A: pH	In range	2.75	3.75	3
B: load	In range	20	40	3
C: titer	In range	2.5	7	3
D: spiking	In range	10	20	3
Responses				
Recovery [%]	Maximize	90	100	3
Aggregate content [%]	Minimize	0	5	1
LOG (CHOP reduction)	Maximize	2.8	3.2	3
Protein A leaching [ng/mL]	Minimize	1	50	1

Table: Input values to calculate the optimized values
(A-D are given from the experimental schedule, responses were set according to applicable goals and boarders, i.e. recovery >90%, aggregates <5%, CHOP reduction >log 2.8, protein A leaching <50ng/mL

Optimization of process parameters

results – model 1

	pH	Load [mg/mL resin]	Titer [g/L]	spiking [%]	Recovery [%]	aggregate content [%]	LOG(CHOP reduction)	ProteinA leaching [ng/mL]	Desirability
MabSelect SuRe LX (Citrate)	3.17	24.26	7.00	20.00	100.0	0.38	3.27	40.73	0,823
MabSelect SuRe LX (Acetate)	3.02	40.00	2.50	20.00	104.9	0.59	3.21	36.86	0,863
Toyopearl AF rProteinA 650F (Citrate)	2.89	28.66	7.00	20.00	99.9	1.41	3.07	165.70	0,754
Toyopearl AF rProteinA 650F (Acetate)	2.77	22.04	7.00	20.00	100.0	1.31	3.20	94.37	0,918
Toyopearl AF rProteinA HC 650F (Citrate)	2.89	20.76	7.00	18.95	97.4	1.22	2.90	1.98	0,678
Toyopearl AF rProteinA HC 650F (Acetate)	2.75	20.00	2.50	20.00	103.5	1.48	3.04	9.54	0,759

Optimization of process parameters

specifications model 2

Name	Goal	Lower Limit	Upper Limit	Importance
Design factors				
A: pH	In range	2.75	3.75	3
B: load	In range	20	40	3
C: titer	In range	2.5	7	3
D: spiking	In range	10	20	3
Responses				
Recovery [%]	Maximize	90	100	5
CHOP concentration [ng/mL]	Minimize	0	2000	3
Protein A leaching [ng/mL]	Minimize	0	200	3

Table: Input values to calculate the optimized values ; model 2
(A-D are given from the experimental schedule, responses were set according to applicable goals and boarders, i.e. recovery >90%, CHOP < 2000 ng/mL, protein A leaching <200ng/mL

Optimization of process parameters

results

	pH	Load [mg/mL resin]	Titer [g/L]	spiking [%]	Recovery [%]	ProteinA leaching [ng/mL]	CHOP concentration [ng/mL]	Desirability
MabSelect SuRe LX (Citrate)	3.27	30.36	2.50	10.00	100	36.42	247.46	0,897
MabSelect SuRe LX (Acetate)	3.18	29.23	7.00	10.00	100	32.36	207.71	0,911
Toyopearl AF rProteinA 650F (Citrate)	2.98	27.42	5.14	20.00	100	149.16	424.63	0,645
Toyopearl AF rProteinA 650F (Acetate)	2.89	22.73	5.50	18.77	100	79.31	377.77	0,823
Toyopearl AF rProteinA HC 650F (Citrate)	3.01	33.11	7.00	10.00	100	2.51	373.69	0,942
Toyopearl AF rProteinA HC 650F (Acetate)	3.12	31.17	2.50	10.00	100	6.05	651.10	0,891

TOSOH

results

- Recovery
- Aggregate content
- Reduction of CHO proteins
- Ligand leaching
- Dependency of antibody feed titer
- Calculation of optimal parameters
- Longterm stability data

Longterm alkali-stability of TP AF rProteinA HC-650F

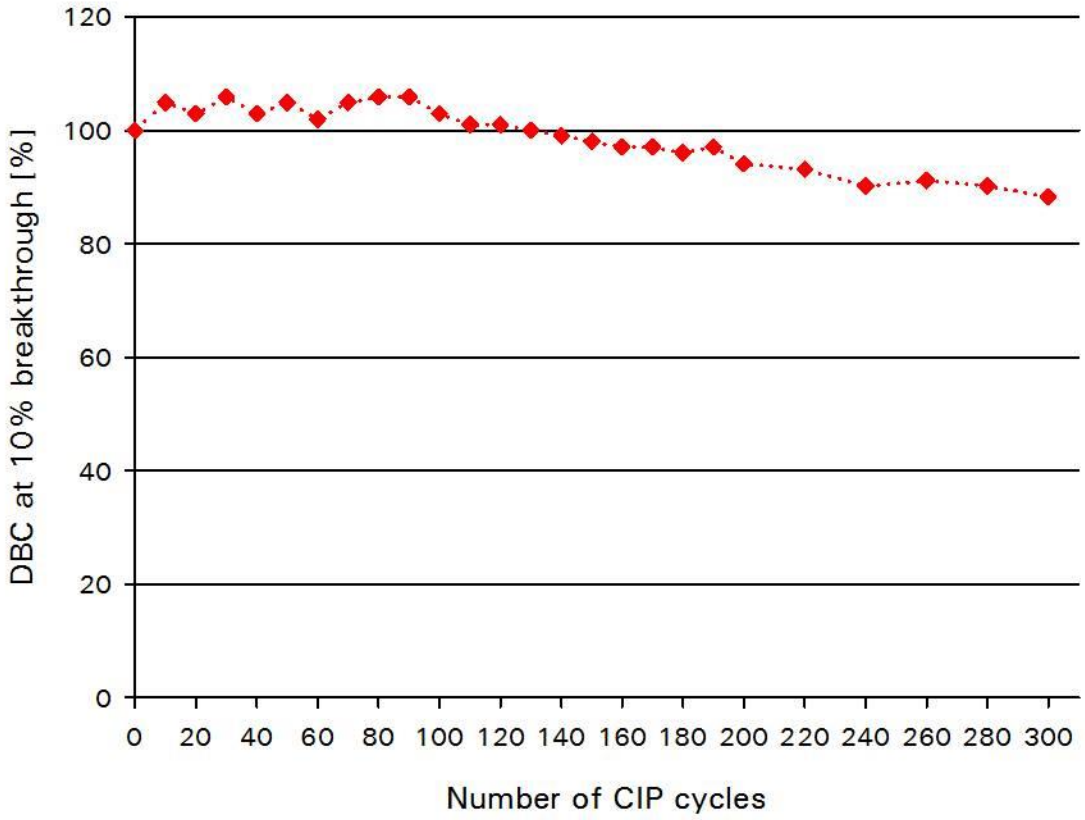


Figure: DBC at 10% breakthrough is stable over 300 CIP cycles using 0.2 M NaOH with 15 min contact time per cycle. The binding capacity begins to decline after 200 cycles.
DBC is measured with monoclonal IgG with a titer of 5 g/L
and a residence time of 2 minutes and reaches values of 102 mg/mL (=100%)

Comparison of longterm alkali-stability of TP AF rProteinA HC-650F with MabSelect SuRE LX

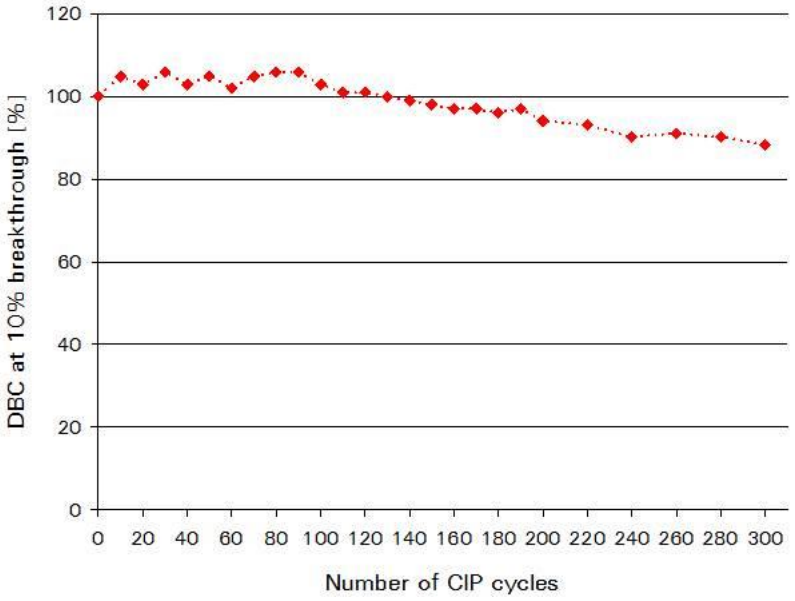


Figure: DBC at 10 % breakthrough is stable over 300 CIP cycles using 0.2 M NaOH with 15 min contact time per cycle. The binding capacity begins to decline after 200 cycles. DBC is measured with monoclonal IgG at 5 g/l and a residence time of 2 min. 100 % are equal to 102 mg/ml

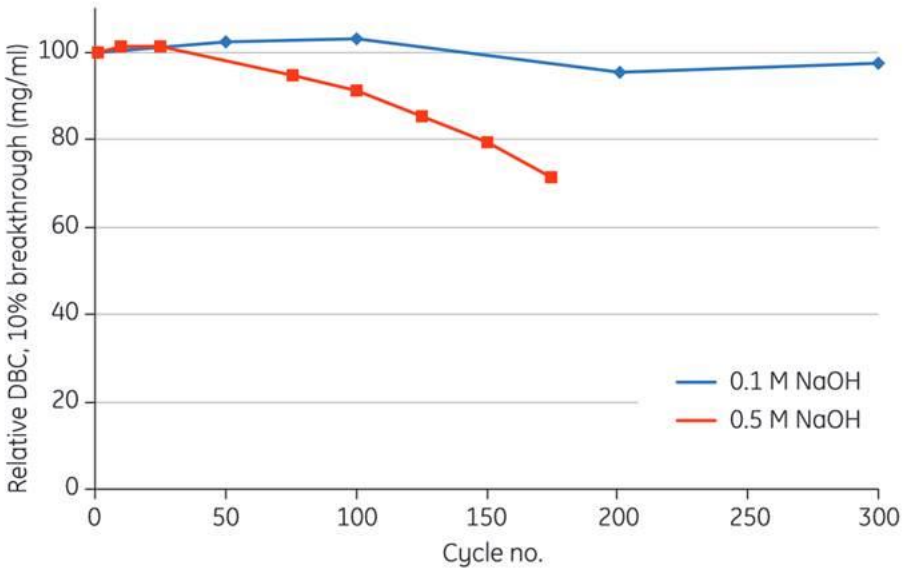


Figure: DBC of MabSelect SuRe LX is stable at 0.1 M NaOH for up to 300 purification cycles. At 0.5 M NaOH, DBC begins to decline earlier.

Longterm stability of TP AF rProteinA HC-650F

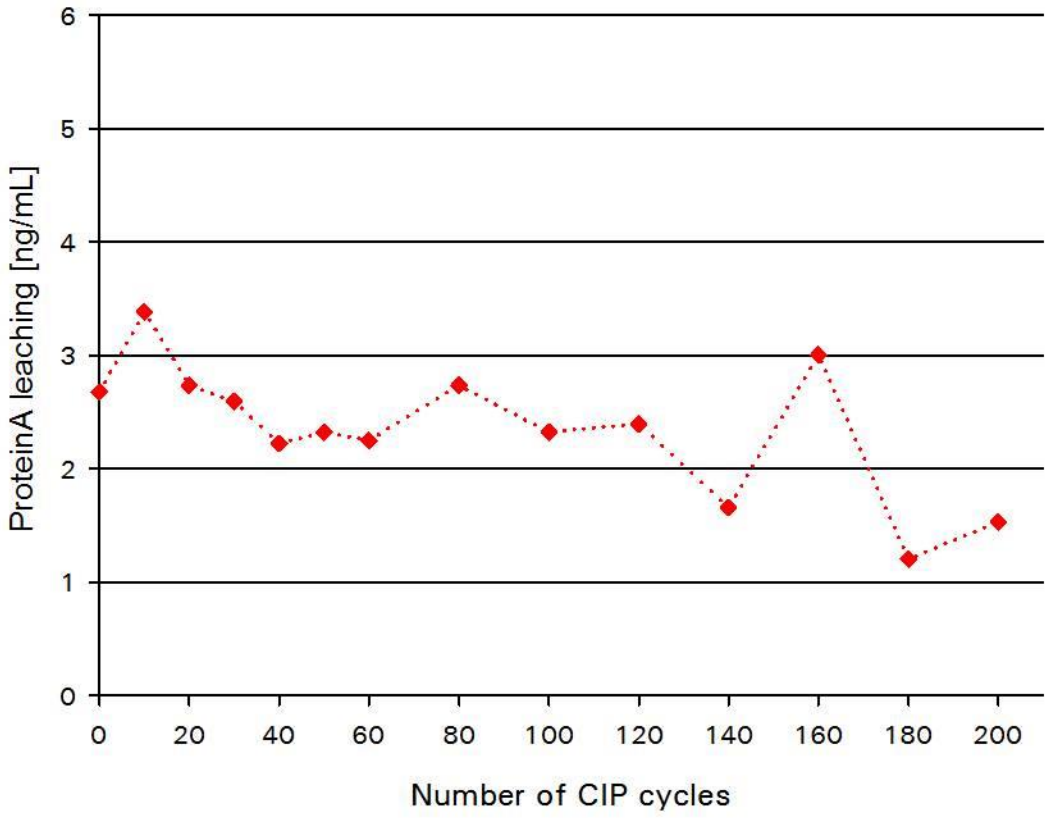


Figure: Ligand leakage is stable over 200 CIP cycles using 0.2 M NaOH with 15 min contact time per cycle.

Longterm stability of TP AF rProteinA HC-650F

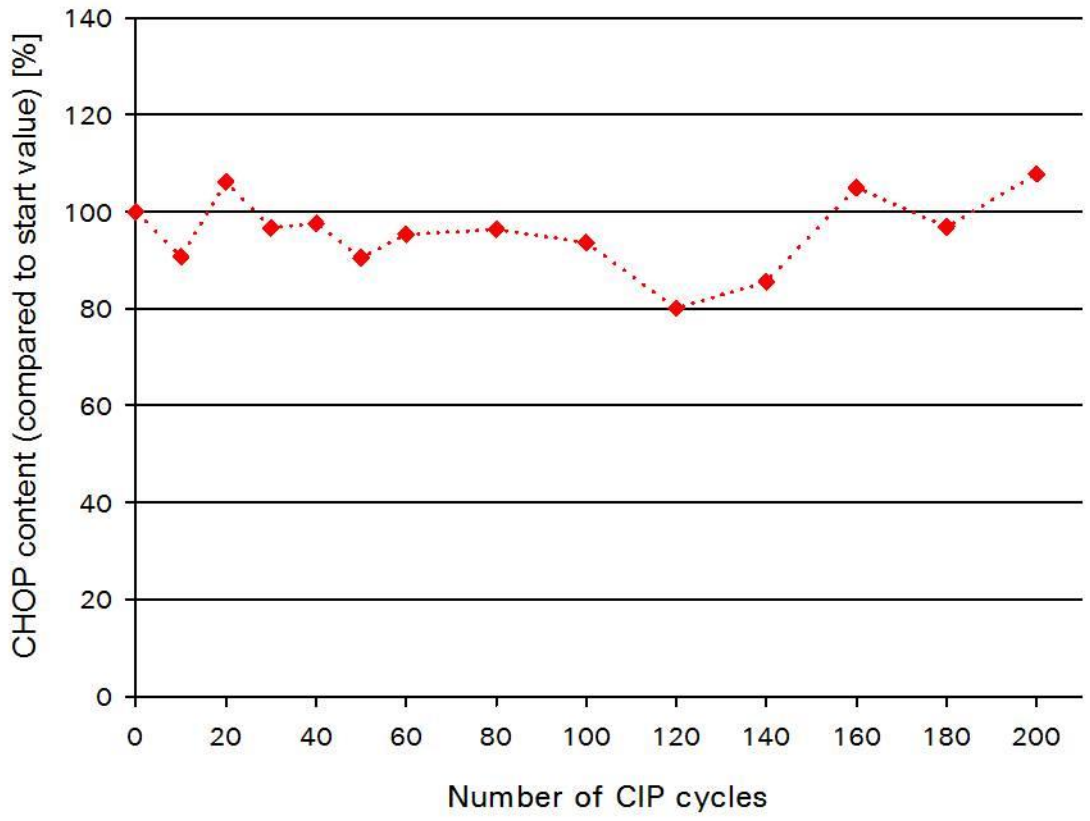


Figure: Purity is stable over 200 CIP cycles using 0.2 M NaOH with 15 min contact time per cycle.

Overall Conclusion

1. Recovery is higher and recovery window is larger for both Toyopearl Protein A resins compared to MAbSure LX (acetate slightly better then citrate), optimal elution pH is slightly higher for Mab Sure LX (about 0.1 pH units)

2. Aggregate content is below 4 % for all resins and increases with decreasing pH (acetate is better then citrate)

3. CHO log reduction is for all resin in the very acceptable range of 2.5 to 3.5 log stages (MAbSure LX is some centipoints better than our Toyopearl AF rProtein A HC-650F, in absolut values in mean is the final purity about 200 ng/ml for Mab Sure LX and 500-600 ng/ml for our resin)

4. Very low Protein A ligand leaching for our Toyopearl Protein A HC.
   We found a relativly high leaching value for our „old“ Toyopearl AF - rProteinA

5. For all three Protein A resins we found an increase in purity with increasing Mab titer

Overall Conclusion

6. In the CIP by using 0.2 M NaOH for cleaning we found a slightly capacity decline after 200 cycles for our new Protein A. The data are very comparable to the data presented for Mab Sure LX (Unfortunatly only 0.1 M and 0.5 M was used)

7. CHO Purity and ligand leaching remains stable for 200 cycles

Summary

Our „new“ Toyopearl AF rProtein A HC 650F is a very competitive products with some advantages but also some minor disadvantages compared to GE‘s best product Mab Sure LX