Protein A Chromatography:

Important Features in Purification to Reduce mAb Aggregation and Increase Recovery

Jukka Kervinen, Ph.D., William Evans, J. Kevin O'Donnell, Ph.D., Phu Duong, Ph.D., Atis Chakrabarti, Ph.D.

Tosoh Bioscience LLC

Introduction

- Protein A affinity chromatography is widely used for the capture of monoclonal antibodies (mAbs) and other Fc-containing biotherapeutic drugs.
- In recent years more mAbs have been developed and produced to address the high demand for treating diseases.
- These modern mAbs, due to their genetically modified forms, have substantially varied affinity to protein A media.
- Here data are presented and discussed to increase recovery and reduce mAb aggregation during the usage of protein A chromatography.

Materials and Methods

Media and column characteristics

Media	TOYOPEARL® AF-rProtein A HC-650F
Column	Minichrom
Bed size	0.8 cm ID ×10 cm length (5 mL)
Particle size	45 µm
Pore diameter	100 nm
DBC* (5 min)	70 g/L
DBC* (2 min)	50 g/L
Caustic stability	>200 CIP cycles (0.1 mol/L NaOH)
Max. pressure	0.3 MPa

*DBC = Dynamic Binding Capacity

Materials and Methods (cont.)

mAbs used in this study

Name	Expression Source	**pI
Trastuzumab (Herceptin® biosimilar)	CHO*	8.7
Adalimumab (Humira® biosimilar)	CHO*	8.4

*CHO = Chinese hamster ovary cells
**pI = approximate of main isoform

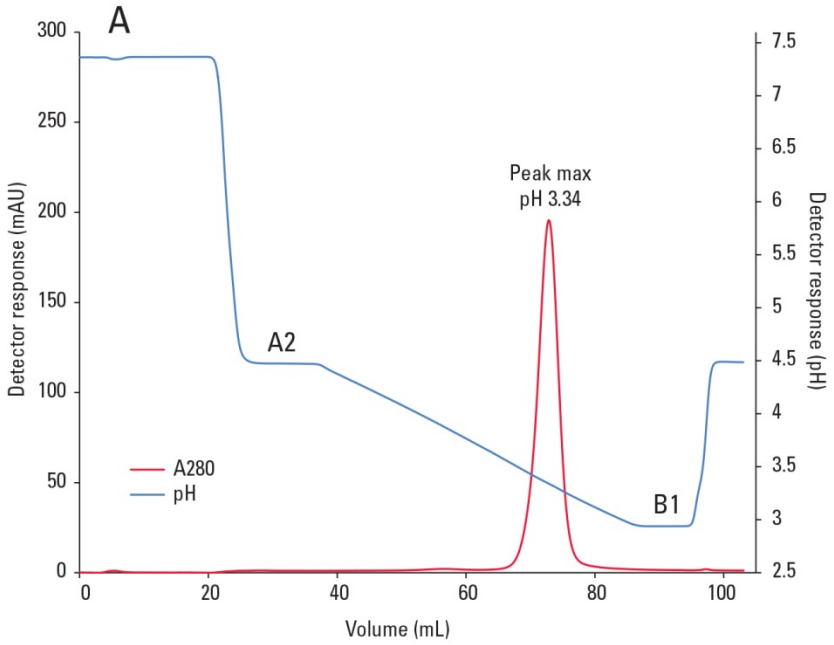
Materials and Methods (cont.)

Linear pH gradient scouting method

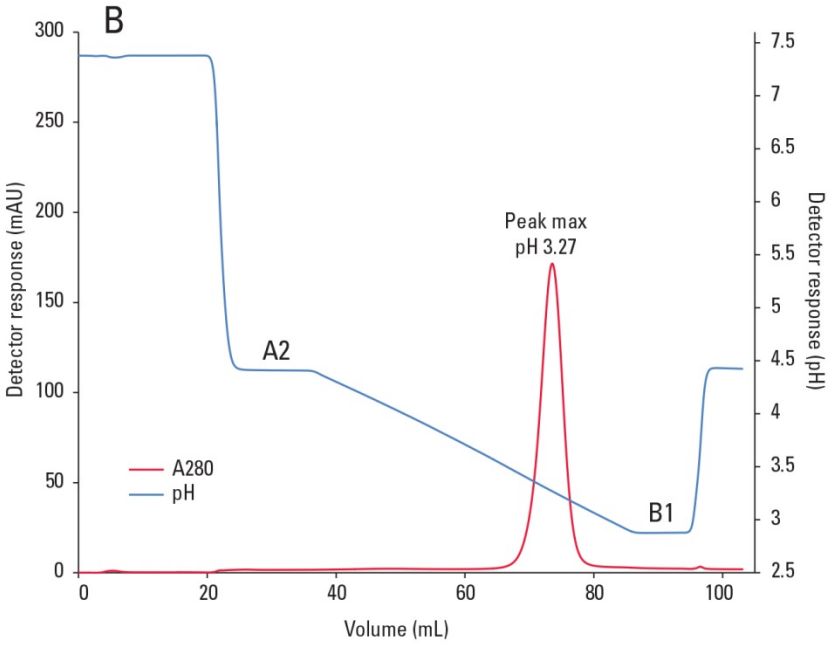
Instrument:	ÄKTA™ avant 25
Equilibration buffer:	100 mmol/L Na_2HPO_4/NaH_2PO_4, 150 mmol/L NaCl, pH 7.4
2nd wash/gradient start:	buffer as indicated (see figures), pH 4.5
Gradient end/strip:	buffer as indicated (see figures), pH ~3.0
Elution gradient:	linear from pH 4.5 to ~3 in 10 CV
Flow (load):	150 cm/hr (1.25 mL/min), 4 min residence time
Flow (wash/gradient):	240 cm/hr (2.0 mL/min)
Samples:	partially purified mAb (previously purified on protein A) or CHO cell culture supernatant

Results: Linear pH Elution Gradient with Citrate

Determination of trastuzumab elution pH at different citrate concentrations



Injection: 2.5 mg partially purified mAb
Mobile phase A: 25 mmol/L citrate (NaOH), pH 4.5
Mobile phase B: 25 mmol/L citrate (NaOH), pH 3.0

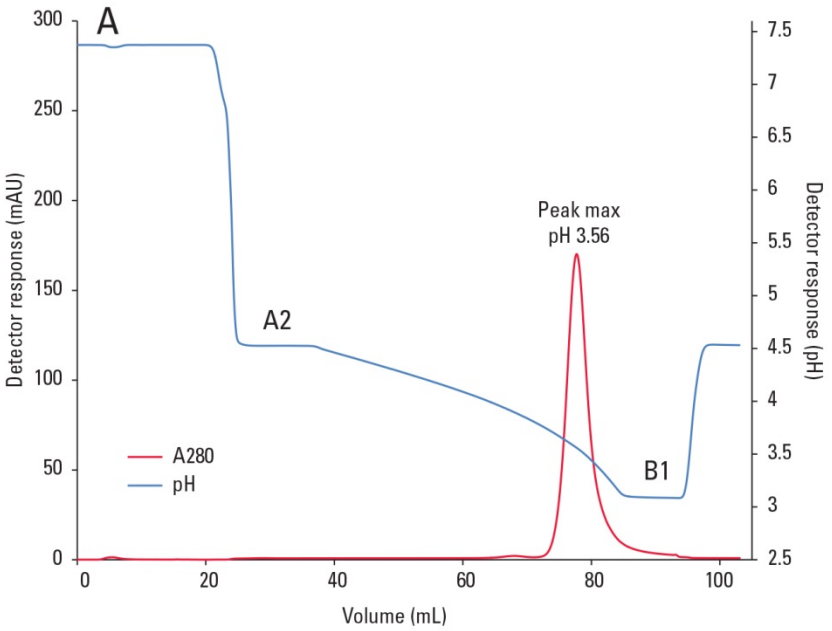


Injection: 2.5 mg partially purified mAb
Mobile phase A: 75 mmol/L citrate (NaOH), pH 4.5
Mobile phase B: 75 mmol/L citrate (NaOH), pH 3.0

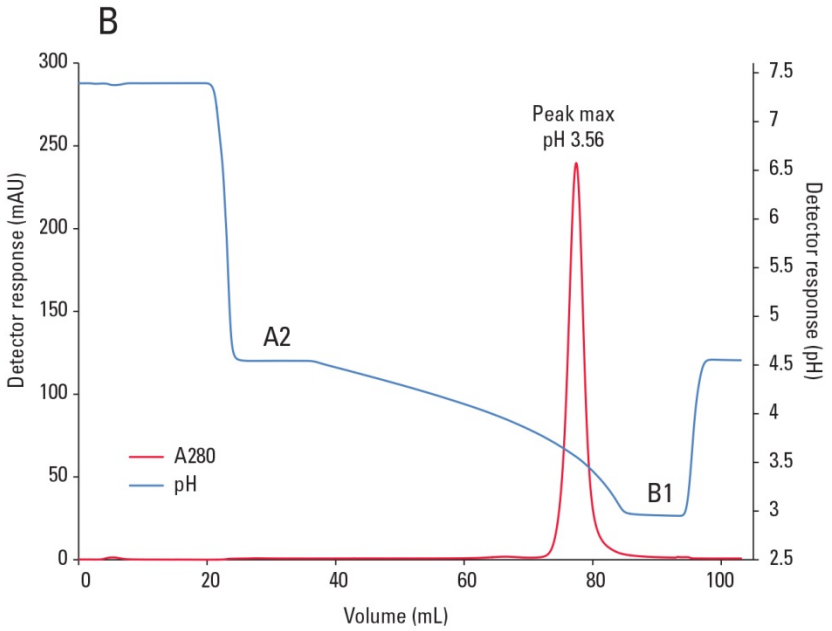
- Citrate was tested with a pH gradient from 4.5 to 3.0 over 10 CV.
- Trastuzumab elution pH was ~3.3 with both buffer molarities.
- A sharp elution peak was obtained with 25 mmol/L citrate (panel A).

Results: Linear pH Elution Gradient with Acetate

Determination of trastuzumab elution pH at different acetate concentrations



Injection: 2.5 mg partially purified mAb
Mobile phase A: 50 mmol/L acetate (NaOH), pH 4.5
Mobile phase B: 50 mmol/L acetic acid, pH 2.9



Injection: 2.5 mg partially purified mAb
Mobile phase A: 100 mmol/L acetate (NaOH), pH 4.5
Mobile phase B: 100 mmol/L acetic acid, pH 2.9

- Acetate was tested with a pH gradient from 4.5 to 2.9 over 10 CV.
- mAb elution pH was 3.56 with both buffer molarities.
- A sharp elution peak was obtained with 100 mmol/L acetate (panel B).

Results: Summary of mAb Elution pH with Linear Citrate vs. Acetate Gradients

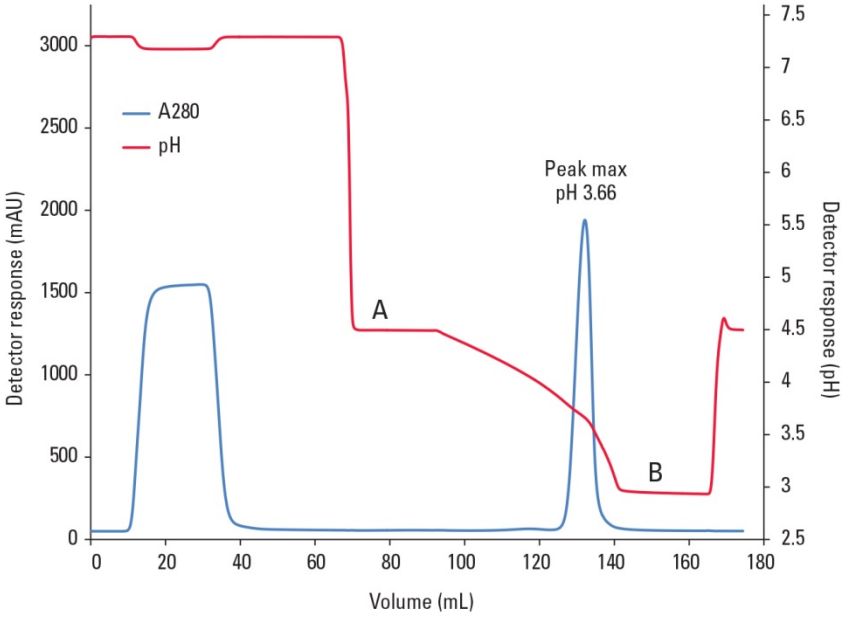
mAb Name	Buffer and pH gradient, pH at elution max and (±10% range)*	
	25 mmol/L citrate (NaOH) pH 4.5 → 3.0	100 mmol/L acetate (NaOH) pH 4.5 → 2.9
Trastuzumab	**3.37** (3.50 - 3.26)	**3.56** (3.68 - 3.39)
Adalimumab	**3.39** (3.58 - 3.28)	**3.57** (3.73 - 3.41)

*pH range measured at ±10% (ascending and descending) of peak max

- Both mAbs eluted at ~0.2 pH units higher in acetate as compared to citrate.
- Acetate (100 mmol/L) was selected for further experiments due to higher pH for elution and sharper elution peak.
- Further process optimization was carried out with CHO cell culture supernatant containing trastuzumab.

Results: Process Optimization

Trastuzumab capture from CHO cell culture supernatant (feedstock)

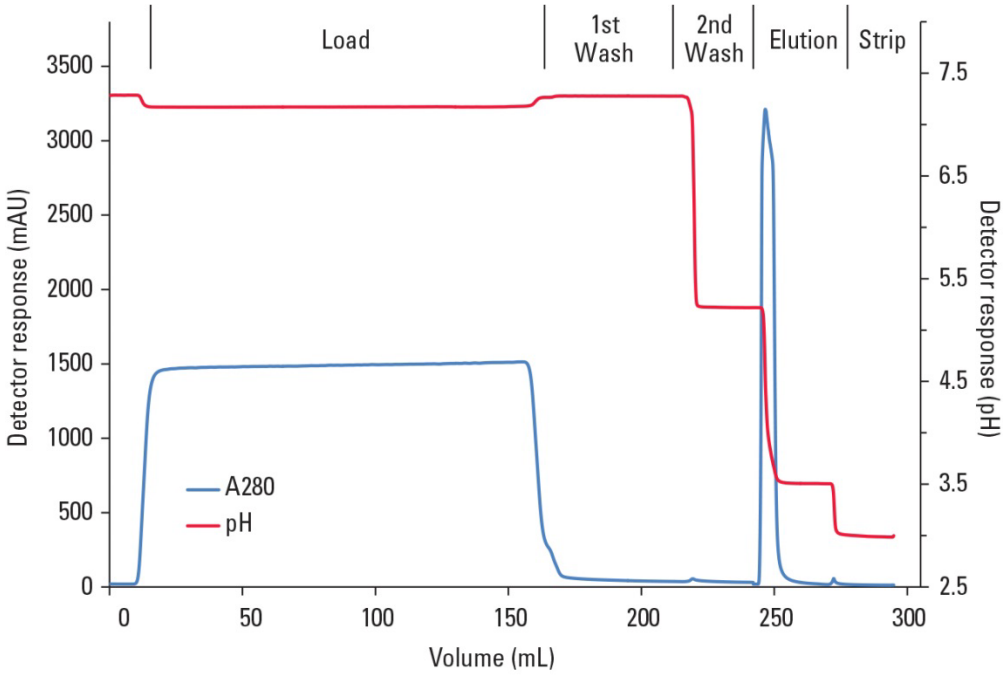


Injection: 11 mL trastuzumab-containing CHO cell culture supernatant (adjusted to pH 7.3)
Mobile phase A: 100 mmol/L acetate (NaOH), pH 4.5
Mobile phase B: 100 mmol/L acetic acid, pH 2.9

- Trastuzumab-containing feedstock was tested with a acetate pH gradient from 4.5 to 2.9 over 10 CV.
- Elution pH was 3.66, thus confirming that the pH scouting method is accurate also for cell culture samples.
- Elution at pH 3.5 was selected for further experiments in step gradient.

Results: Process Optimization

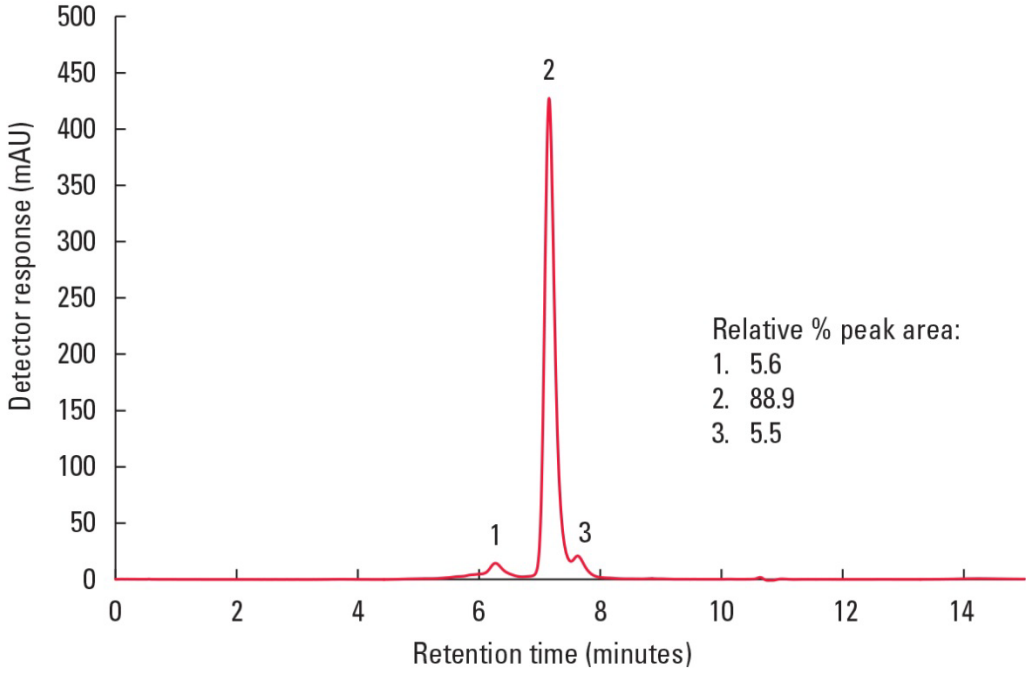
Optimized protein A capture step for trastuzumab



Instrument:	AKTA avant 25 (room temperature)
Eq. & 1st wash:	100 mmol/L Na_2HPO_4/NaH_2PO_4, 150 mmol/L NaCl, pH 7.4
2nd wash:	100 mmol/L acetate (NaOH), pH 5.2
Elution:	100 mmol/L acetate (NaOH), pH 3.5
Strip:	100 mmol/L acetic acid, pH 2.9
Flow (load):	150 cm/hr (1.25 mL/min), 4 min residence time in load
Flow (wash):	240 cm/hr (2.0 mL/min)
Sample:	87 mL CHO cell culture supernatant (trastuzumab titer 2.7 g/L, total 235 mg)

- No target leak was detected during pH 5.2 wash.
- Sharp and complete elution peak was obtained at the start of pH 3.5 elution.
- No residual protein in strip step.

Results: Analytical SEC of the Eluate at pH 3.5 from Optimized Method



Instrument:	Agilent 1100 HPLC (Chemstation® Rev. C.01.08)
Column:	TSKgel® UP-SW3000, 2 µm, 4.6 mm ID × 30 cm
Mobile phase:	0.1 mol/L KH_2PO_4/Na_2HPO_4, 0.1 mol/L Na_2SO_4, 0.05% NaN_3, pH 6.7
Flow rate:	0.35 mL/min
Temperature:	ambient (room temp.)
Detection:	UV @ 280 nm
Injection vol:	5 µL
Sample:	pH 3.5 eluate, diluted to 5 mg/mL prior to injection

Elution of the main peak (#2) occurred at ~7.5 min, indicating a largely monomeric (88.9%) mAb in the pH 3.5 eluate from protein A.

Results: Compilation of Trastuzumab Eluate Analysis for the Optimized Method

Eluate	11.3 mL
Concentration (A_{280})	19.3 mg/mL
Eluate pH*	4.29
Yield	218 mg
Recovery	93%
% Monomer (SEC)	88.9%

*Eluate at pH 4.29 of optimized method was titrated to 5.8 with 5 mol/L NaOH and the eluate was aseptically filtered, aliquoted and stored at -20 °C if not used immediately.

Conclusions

- A polymethacrylate, high binding capacity protein A media was used for a trastuzumab and adalimumab capture step.
- A linear pH gradient was used to determine the highest pH that gives complete mAb elution, which would reduce the risk of aggregation.
- The final process for trastuzumab capture from CHO cell culture supernatant yielded >90% mAb recovery at 80% loading resin capacity.
- The process optimization (buffers, pH, etc.) protocol described here is recommended for other mAbs, or their derivatives, that have affinity to protein A.
- The information obtained enables protein A column scale-up of the capture process for both batch and continuous chromatography platforms.