Biowaivers, Biologics and Biosimilars

Hyderabad, India

October 26-28, 2014

Shalini Sharma, Intas

Jennifer Campbell, Merck Millipore

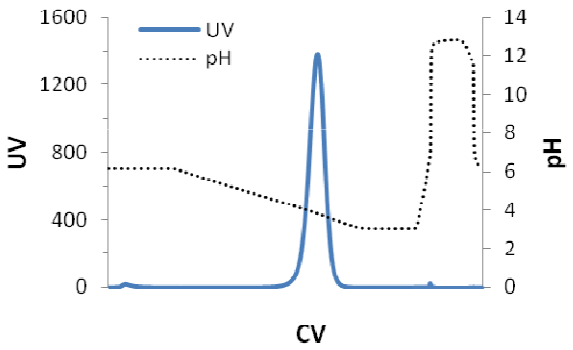
Aggregate removal

- Aggregates – purity and clinical safety challenges
- Aggregate removal often relies heavily on cation exchange chromatography
  - Optimization of resin chemistry, binding/elution conditions, additives, peak collection, column loading
- Other approaches include hydrophobic interaction chromatography.
- Aggregation can be monitored by size exclusion chromatography, dynamic light scattering, analytical ultracentrifugation, asymmetrical field flow fractionation, etc.

Background – Protein A Aggregate Removal

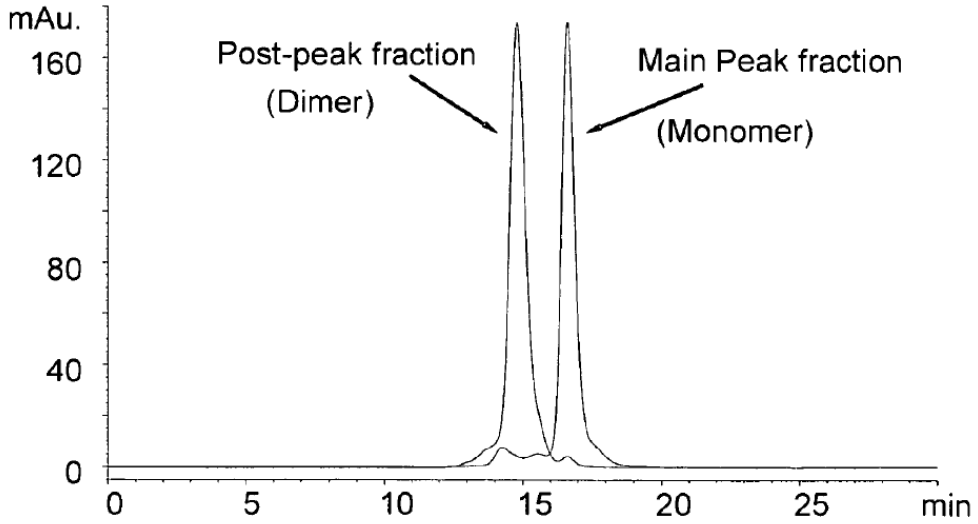
- Aggregates are concentrated at the tail end of the Protein A elution peak

pH gradient elution



Collect fractions, analyze by size exclusion chromatography

Corresponding SEC profiles

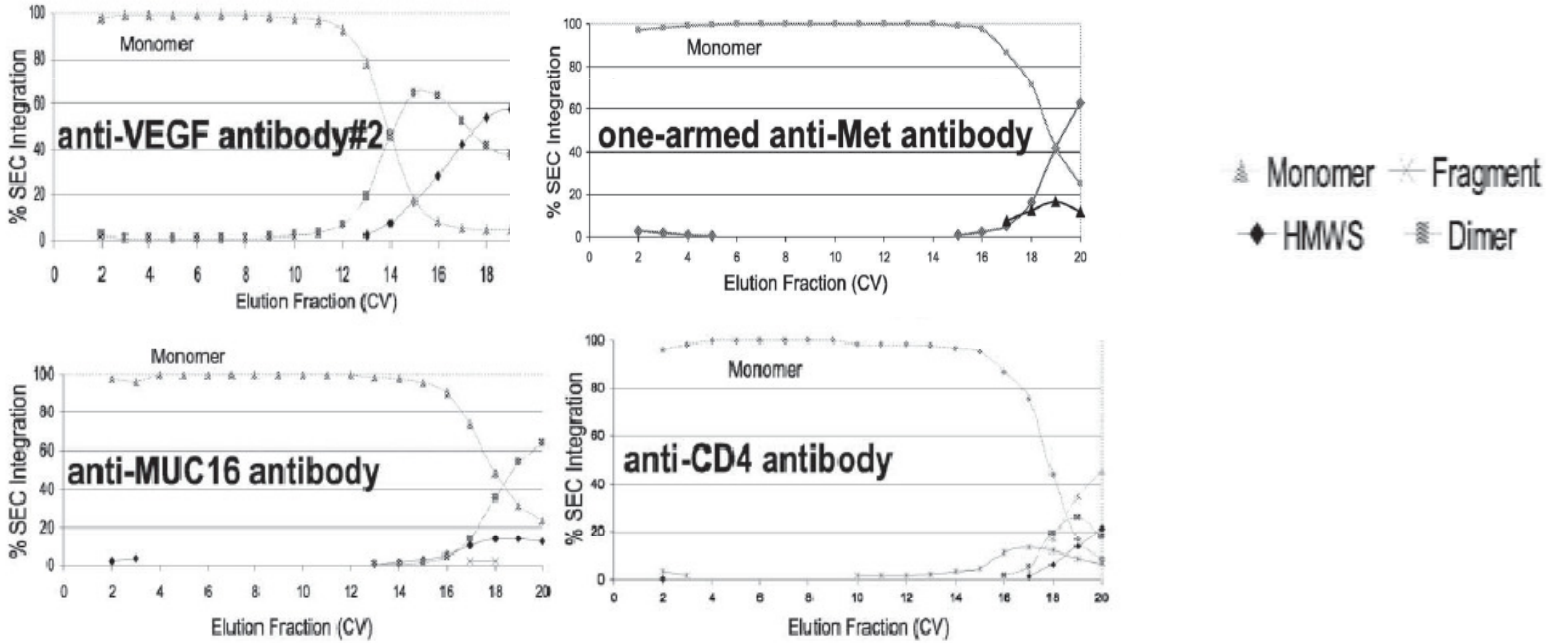


Pan H., Amgen, US 2008/0167450 A1

Pan, H. et al., Analytical Biochemistry, 388 (2009) 273-278.

Background – Protein A Aggregate Removal

- Aggregates are at the tail end of the elution peak
- Demonstrated for MabSelect®, MabSelect SuRe®, ProSep® Ultra Plus, and POROS® MabCapture™ A (WO2011/028753)
- Observed with multiple molecules

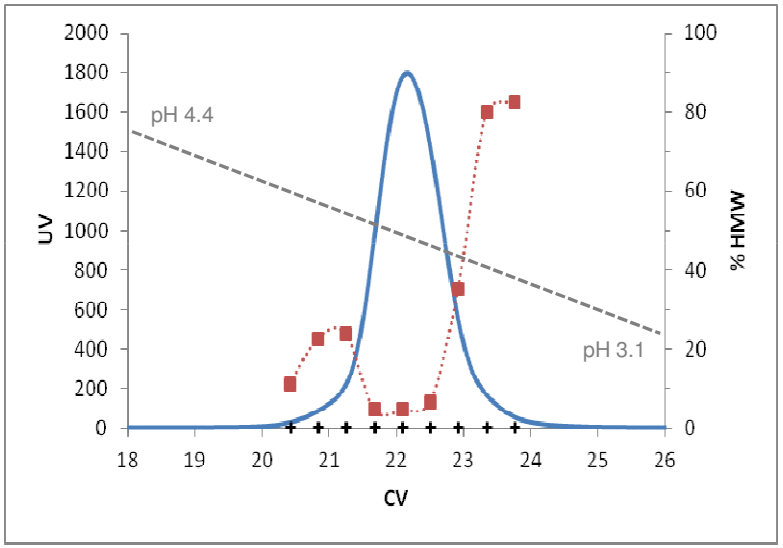


WO2011/028753 A1: Enhanced protein purification through a modified protein A elution. (Genentech)

mAb-A pH Gradient Elution

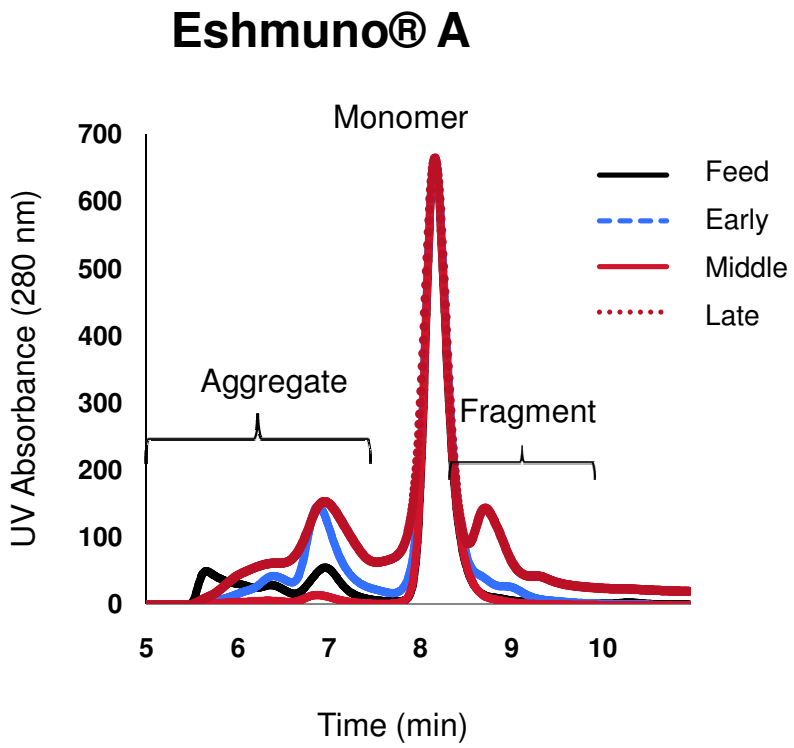
- Unique behavior observed during **pH gradient elution** when demonstrating the reduced Fab binding feature of Eshmuno® A

Loading density: 12 mg/mL;
Feed: 13.5% aggregate (generated by pH cycling method – Bill Cataldo, ACS 2012)



Resin	Monomer in Pool (%)	Overall mAb Yield (%)	Pre-Peak		Post-Peak		Total Aggregate Removed (%)
			% Aggregate Removed	% of Total	% Aggregate Removed	% of Total	
Eshmuno® A	94.6	79.4	25.7	41.1	36.9	58.9	62.6

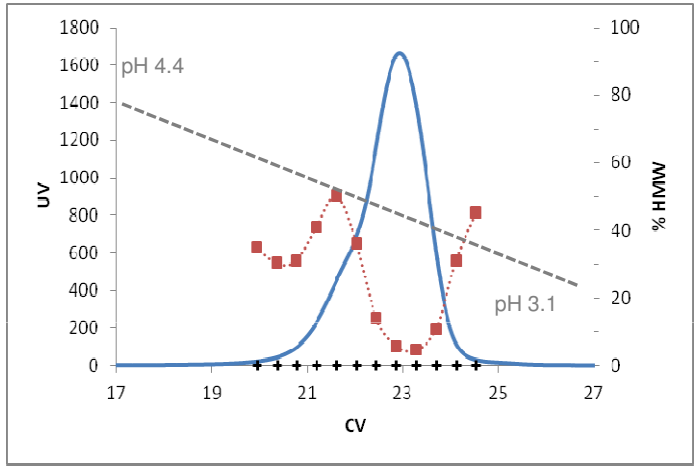
SEC Profiles – mAb-A



mAb-B: pH Gradient Elution

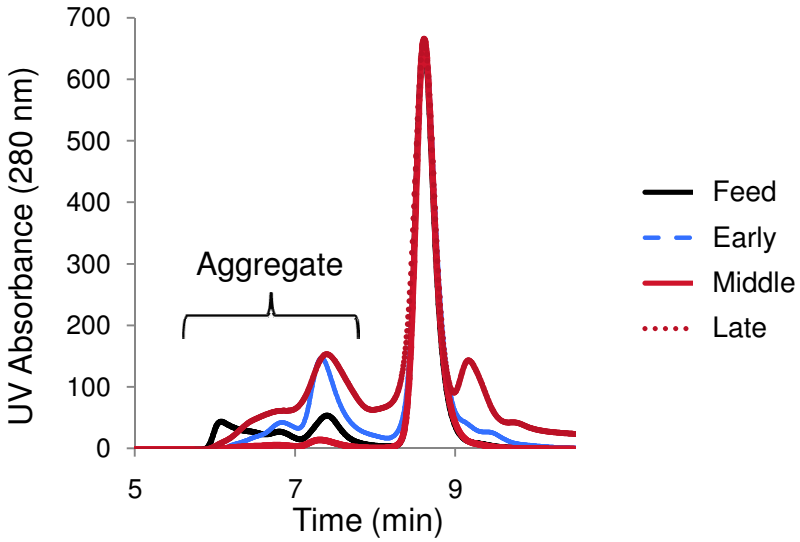
Feed: 21.7% aggregate

Load density: 12 mg/ml



Resin	Monomer in Pool (%)	Overall mAb Yield (%)	Pre-Peak		Post-Peak		Total Aggregate Removed (%)
			% Aggregate Removed	% of Total	% Aggregate Removed	% of Total	
Eshmuno® A	86.6	82.2	40.5	97.6	1.0	2.4	41.5

SEC Profiles – mAb-B



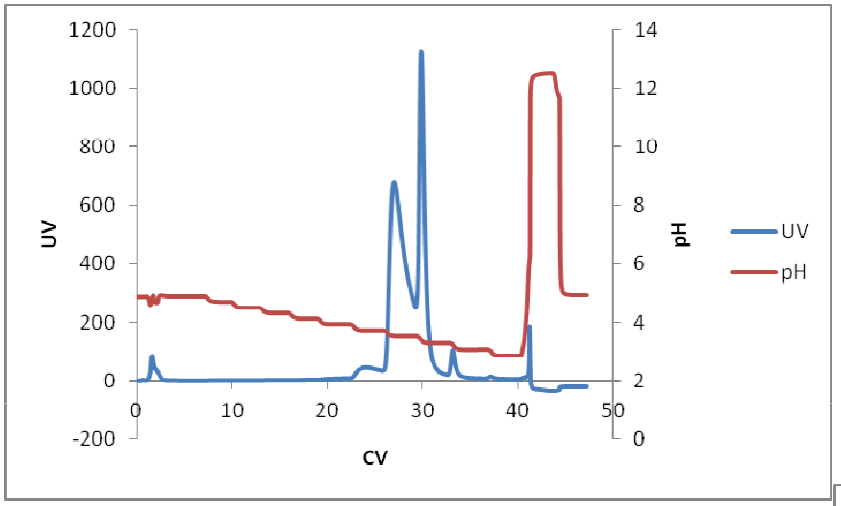
Feed: 21.7% aggregate

How to implement this approach

- Gradient elution considerations:
  - Gradient pH elution is not easily practiced in production; difficult to control pH
  - Gradient pH elution usually leads to larger elution pool, larger tank volumes

- Step elution method development and considerations:
  - Can select pH steps such that the early eluting aggregates are eluted, then decrease the pH to collect the main monomer pool, then decrease the pH again to elute the strongly bound aggregates
  - Can use gradient elution as a guide to determine pH levels
  - Can run a series of small pH steps to identify desired pH levels
  - Could use this to remove the early eluting aggregates as part of a wash step prior to elution and the late eluting aggregates as part of the cleaning step after elution

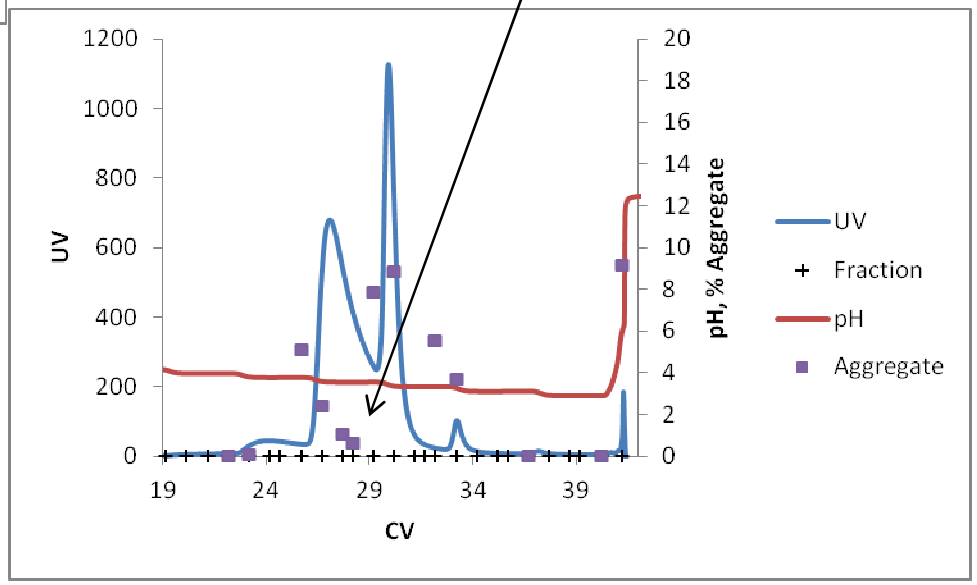
mAb-C Step Elution - every 0.2 pH unit



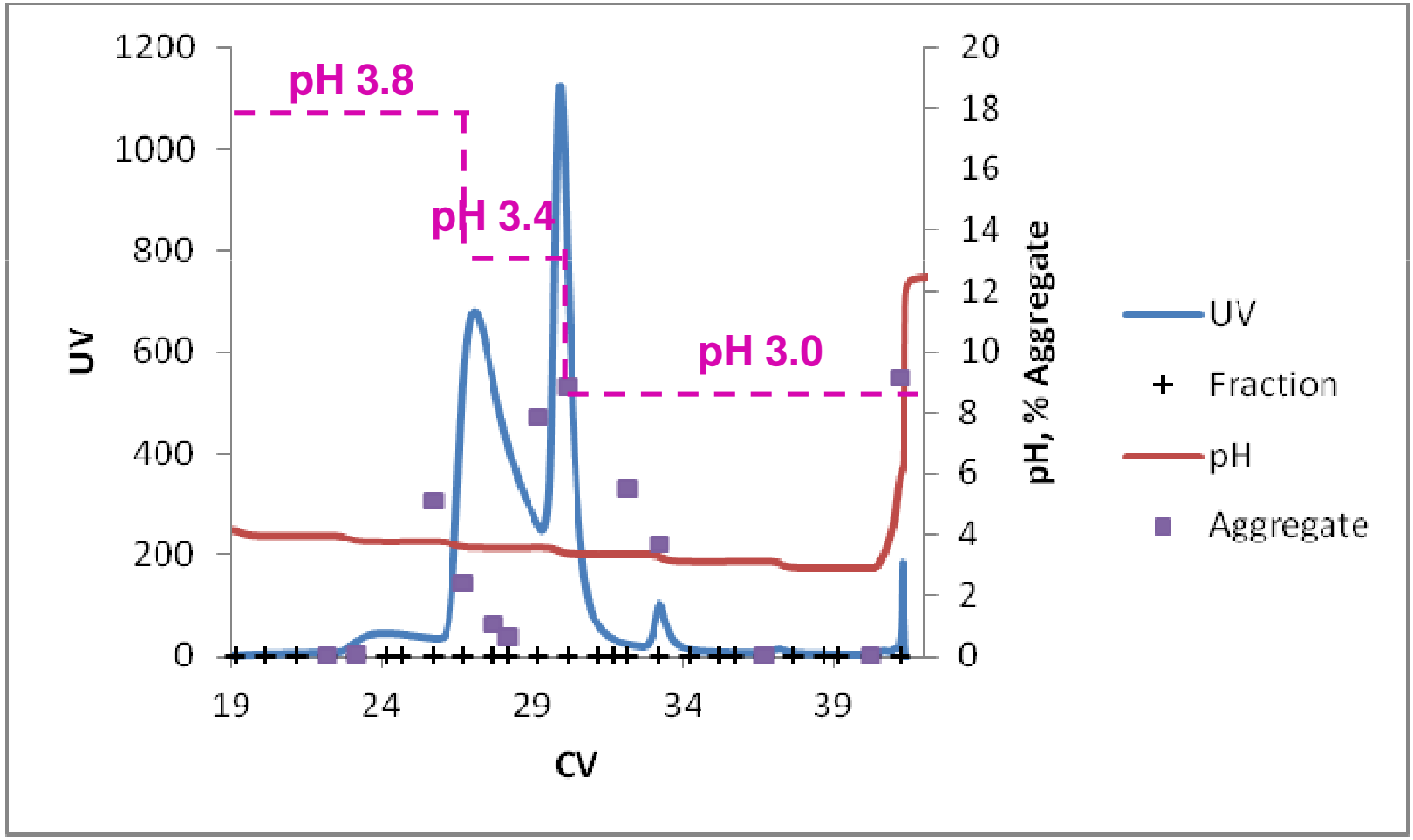
	Yield (%)	Purity (%)
Middle 5 fractions	89.7	93.5
Middle 4 fractions (cut 5th)	70.0	95.2
Middle 4 fractions (cut 1st)	76.8	93.7
Middle 3 fractions	57.1	96.0

Feed: 10.7% aggregate

96.1% monomer



Aggregate Removal
Proposed pH step elution



Technical Summary

- Protein A Chromatography was successfully used to reduce high aggregate levels, therefore lowering the aggregate burden for further steps in the DSP
- Product recovery was acceptable, as well as productivity of the unit operation
- The resulting product meets the desired impurity profile
- The media has good ease of use for the plant, as step elution can be implemented, saving buffer volume, tankage, footprint

Collaboration Outcome

- The use of Eshmuno A media with peak shaving and pH control resulted in acceptable aggregate levels and yield from the Protein A chromatography step
- This work helped to overcome challenges such as:
  - exploring options for protein A chromatography
  - creating cost effective manufacturing operations with ease of use
  - meeting regulatory standards for acceptable impurity profile in a biosimilar
- Future work is ongoing on clarification and other chromatography operations

Acknowledgements

Intas Pharmaceuticals Ltd.:

Himanshu Gadgil, Yogendra V. V. Singh, Vikram Kadam, Mayank Thakkar, Abijar Bhori, Vivek Farkade, Dipti Khanna

Merck Millipore:

Subhasis Banerjee, Pritam Rajkule, Nikhil Shaligram, Venkatesh Natarajan, Alejandro Becerra-Arteaga