The Elution Dilemma in Protein A Chromatography

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The Elution Dilemma

Protein A chromatography is the standard capture step for monoclonal antibody (mAb) purification due to its high selectivity and scalability. However, elution requires acidic conditions, which can destabilize antibodies and promote aggregation. This creates a key process challenge: balancing efficient elution with preservation of antibody stability.

- pH < 4.0 is required for sufficient antibody elution
- The required elution conditions promote aggregate formation

pH 3.5 produced narrower peaks than pH 2.7, indicating improved elution efficiency despite less acidic conditions.

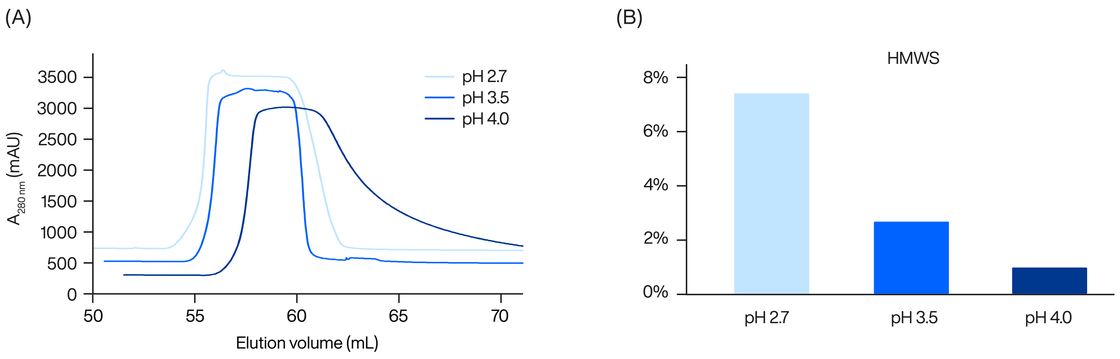

Figure 1. pH dependence of mAb elution and higher molecular weight species (HMWS) levels from the corresponding elutions. at pH 2.7, 3.5, and 4.0.

Study design

To investigate how buffer composition and pH influence elution two different elution buffers at three pH were tested during purification of an aggregation-prone monoclonal antibody (Antibody X). Sample source was a CHO supernatant (~5 mg/mL). HMWS levels were analyzed by analytical size exclusion chromatography (Superdex 200 10/300 Increase, Cytiva).

Column:	WorkBeads™ affimAb Edge, 3.4 mL MabSelect™ PrismA, 3.4 mL MabSelect SuRe 70, 3.4 mL
Running buffer:	PBS pH 7.4
Elution buffer:	100 mM Glycine-HCl pH 3.0, 3.2, 3.5 50 mM Citrate pH 3.0, 3.2, 3.5
Sample:	35 ml CHO cell supernatant (~175 mg IgG)
Flow rate:	0.855 ml/min (150 cm/h) (4 min RT)
Gradient:	100% Elution buffer in 10 CV

Performance Comparison of Protein A Resins

Using citrate at pH 3.5, WorkBeads affimAb Edge was compared with commercial Protein A resins.

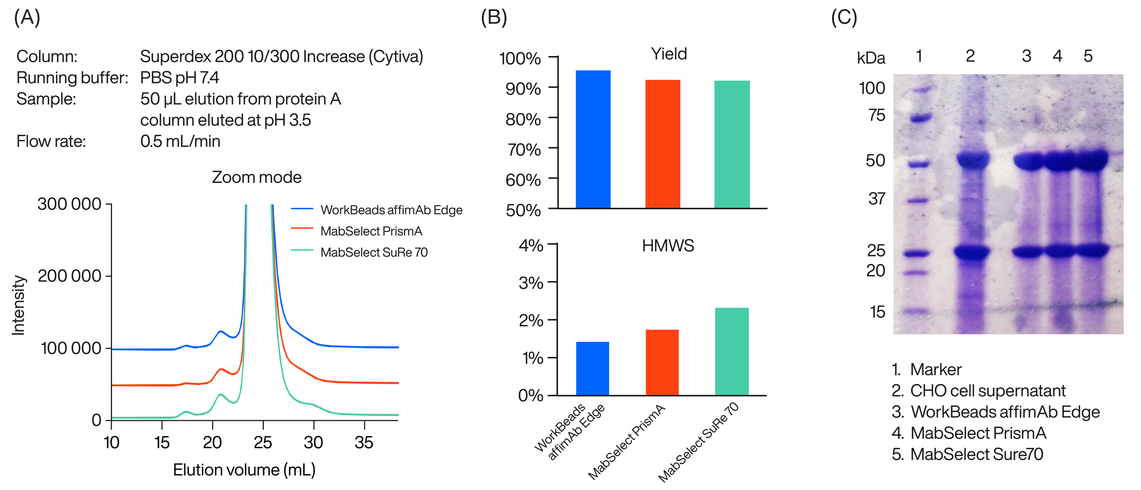

Figure 5. Comparative evaluation of WorkBeads affimAb Edge (blue), MabSelect PrismA (red), and MabSelect SuRe 70 (green) during purification of Antibody X using citrate pH 3.5 as the elution buffer. (A) analytical SEC profile, (B) yield and HMWS analysis, and (C) SDS-PAGE of eluted mAb pool.

This is WorkBeads™ affimAb Edge

WorkBeads affimAb Edge is a next-generation Protein A resin designed for efficient mAb purification from lab to process scale.

- **High DBC**, with >60 mg/mL at 4 min and >70 mg/mL at 6 min residence time, enabling efficient purification and lower process costs.
- **Superior pressure flow properties** supporting high flow velocities at low back pressure, enabling faster cycle times and robust scale-up.
- **Strong alkali stability** enables repeated cleaning and sanitization using 0.5 M NaOH for extended resin lifetime and improved process economy.
- **Cost-effective performance**, competitive with leading Protein A resins while delivering improved process economics.

Buffer composition significantly influence elution behavior

Differences in elution profile and peak shape arise from how each buffer propagates through the column and establishes pH conditions.

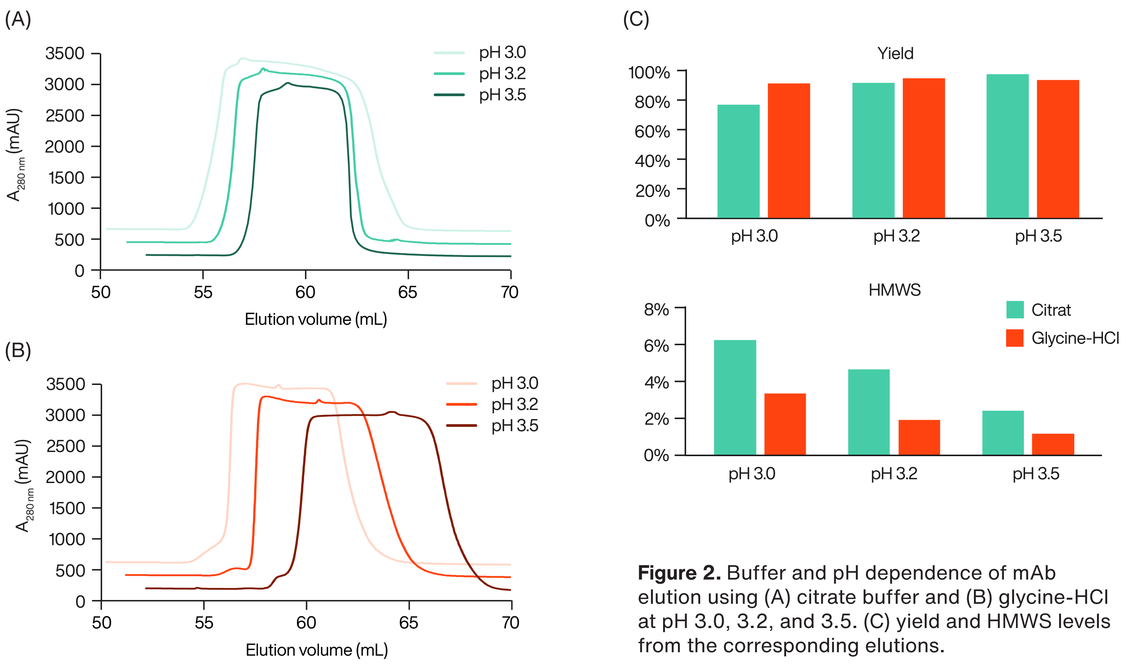

Figure 2. Buffer and pH dependence of mAb elution using (A) citrate buffer and (B) glycine-HCl at pH 3.0, 3.2, and 3.5. (C) yield and HMWS levels from the corresponding elutions.

Conclusions

Elution pH and buffer chemistry are critical parameters in protein A chromatography.

- Lower pH increases aggregation
- Buffer chemistry controls pH transition and peak shape
- Citrate enables more efficient and controlled elution

Optimal condition: Citrate buffer at pH 3.5

Mechanistic Insight

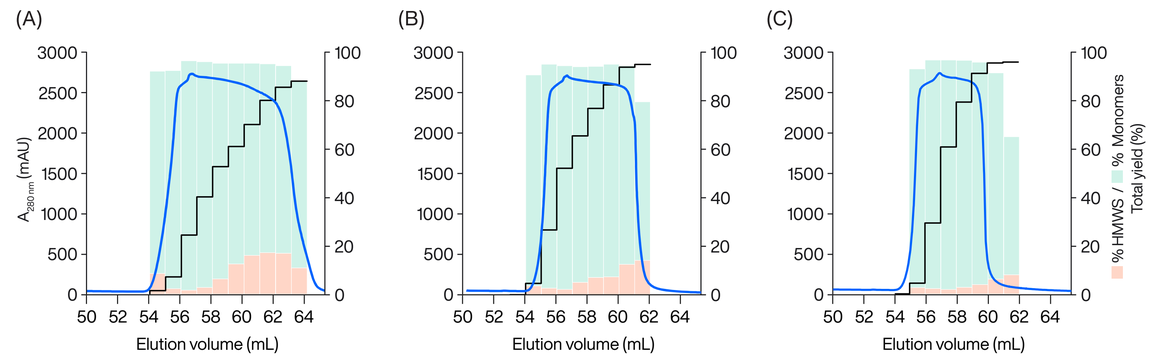

Figure 3. Fraction-wise analysis of mAb elution using citrate buffer at pH (A) 3.0, (B) 3.2, and (C) 3.5. The blue line represents the UV signal, the red and green bars represent the level of HMWS and monomers, respectively and the black line represents the total yield.

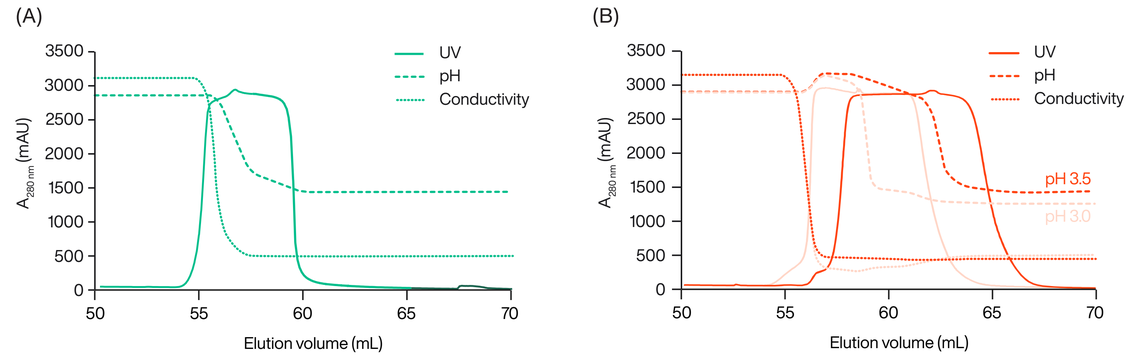

Figure 4. Buffer and pH dependence of mAb elution using (A) citrate buffer at pH 3.5 and (B) glycine-HCl at pH 3.0 (light red) and 3.5 (red). Monitoring UV (solid line), pH (dashed line), and conductivity (dotted line).

Citrate enables a smooth and predictable pH transition, resulting in efficient antibody desorption.

Glycine-HCl exhibits a pH overshoot due to ion-exchange interactions that delays elution and broadens peak profiles[1].

[1] Hahn et al., Biotechnol. Prog., 41.3 (2025) e3534
MabSelect is a trademark of Cytiva.

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