Take Full Advantage of Caprylic Acid-Induced Impurity Precipitation in MAb Purification

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Improved host cell protein clearance

- Low pH treatment in a stirred system enables simple integration or replacement by caprylic acid (CA) precipitation
- Host cell protein (HCP) clearance was improved from <0.3 LRV in conventional low pH treatment (0 mmol L^{-1} CA) to up to 2 LRV by adding 10-25 mmol L^{-1} CA (Fig. 1). Thus, HCP values <100 ppm can be achieved.
- pH range of 4.5-5.5 is optimal to precipitate HCP at high mAb yields of > 90%

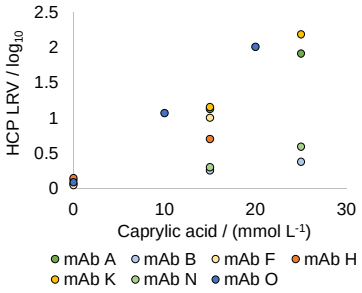


Figure 1: Improvement of HCP log reduction by CA

Precipitating mAb high molecular weight species

- Besides host cell proteins, mAb high molecular weight species (HMWS) can be prone to caprylic acid (CA)-induced precipitation
- We showed HMWS removal of 1-1.5 % for different mAbs (5 g L^{-1}) using 10-20 mmol L^{-1} CA at pH 5.0. HMWS clearance stayed constant at higher CA concentrations presumably due to the limited solubility of CA (Fig. 2).
- Most important parameters to be considered are pH value and CA concentration

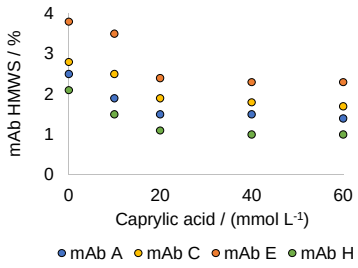
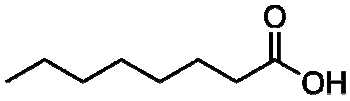


Figure 2: CA-induced mAb HMWS removal at pH 5



Caprylic acid (CA)

- Octanoic acid
- Short-chain saturated fatty acid
- pKa value: 4.89
- Low solubility in water: 4.85 mmol L^{-1}
- Used to precipitate proteins
- Standard method in plasma fractionation
- Recently applied in mAb purification

Tackling enveloped viruses

- Undissociated form of caprylic acid (CA) acts as virus inactivation agent
- Penetrates the viral membrane, disrupting the lipid coat of enveloped viruses[1]
- Complete inactivation at CA concentrations of 2.4-5.8 mmol L^{-1} (Tab. 1)
- No significant inactivation even after 300 min incubation at lower protonated CA concentrations (≤1 mmol L^{-1}), which can be triggered by pH adjustment (Tab. 1)
- Inactivation by protonated CA allows an additional virus safety margin to low pH inactivation
- Replacement of S/D treatment for pH-sensitive mAbs possible

Table 1: Inactivation of MuLV by protonated CA

pH	total CA (mmol L^{-1})	protonated CA (mmol L^{-1})	MuLV LRV (log_{10})
5.1	6.2	**2.40**	≥ 5.53#1
5.1	15.0	**5.80**	≥ 5.18#1
5.6	3.0	**0.50**	0.76#2
5.6	6.0	**1.00**	0.94#2
6.5	3.0	**0.10**	0.04#2
6.5	6.0	**0.15**	0.22#2

#1 incubation time 60 min #2 incubation time 300 min

Precipitation mechanism

- At acidic pH, hydrophobicity of the caprylic acid (CA) octyl moiety dominates, enabling penetration of protein hydration shells[2]
- As isoelectric point (pI) affects solubility, acidic HCPs can be best precipiated at pH 4.5-5.5
- We figured out that the pI of the mAb light chain (LC) significantly affects mAb recovery[3]. MAbs with mild acidic LC are prone to precipitation within pH range of their LC pI (Fig. 3).

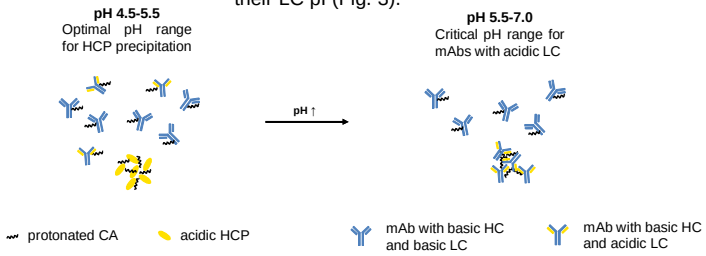


Figure 3: Mechanism of caprylic acid-induced precipitation, adapted from Trapp *et al.*[3]

Conclusion

Caprylic acid (CA) precipitation provides excellent purification performance regarding HCPs, mAb HMWS and enveloped viruses

Studies on mAb stability demonstrate the importance of the charge of the mAb chains to counteract the hydrophobicity of CA

Using CA impurity precipitation enables the design of a next-generation intensified two-column mAb purification platform

[1] Lundblad *et al*. 1991. Vox Sang 60(2): 75–81
[2] Singh *et al.* 2016. Biotechnol Bioeng 113: 698-716
[3] Trapp *et al.* 2018. J Biotechnol 279: 13-21