Intensification of mAb downstream processes using modular innovative purification technologies

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Process intensification

New innovative strategies improving performance and flexibility of monoclonal antibody (mAb) purification processes are necessary for further bioprocess intensification.

Caprylic acid (CA)-induced precipitation can be combined with low pH treatment to enhance precipitation of host cell proteins (HCPs). By adding CA, HCP clearance of 1-2.5 log_{10} can be achieved while leaving the mAb in solution[1].

Using a cation exchange (CEX) in overload mode enables efficient aggregate removal at high mAb load. Instead of column chromatography, this approach can be performed as a single-use static batch adsorption after low pH treatment prior to resin separation by filtration.

We aim to combine both innovative strategies within one mAb downstream process.

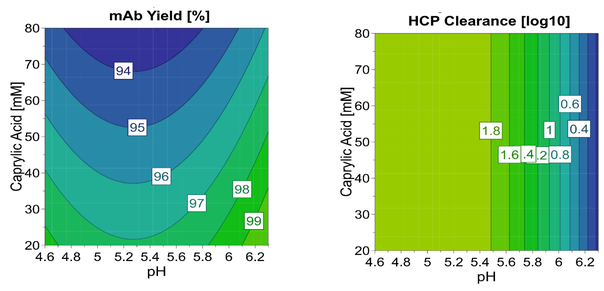
Caprylic acid precipitation

HCP clearance by CA precipitation was investigated after Protein A chromatography. Influence of pH value and CA concentration was evaluated in a design of experiments approach (Fig. 1).

Figure 1:

Contour plots of DoE experiments to determine best CA precipitation conditions.

Tested CA concentrations of 20 mM to 80 mM at pH 4.6 to 5.5 delivered highest HCP reduction. No significant HCP clearance was observed at pH 6.3, even at highest concentration of 80 mM CA. mAb yields ranged from 94-100%.



CEX in overload mode

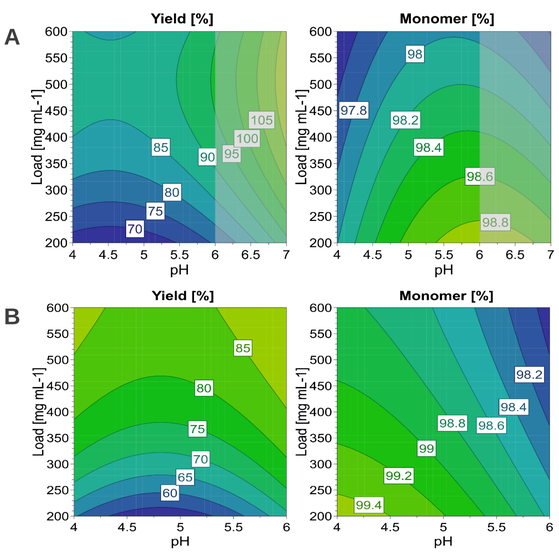
Batch screenings in 96-well format were performed to investigate CEX overload conditions for highest mAb aggregate clearance (Fig. 2).

Figure 2:

Contour plots of DoE experiments aiming for optimal CEX conditions in overload mode.

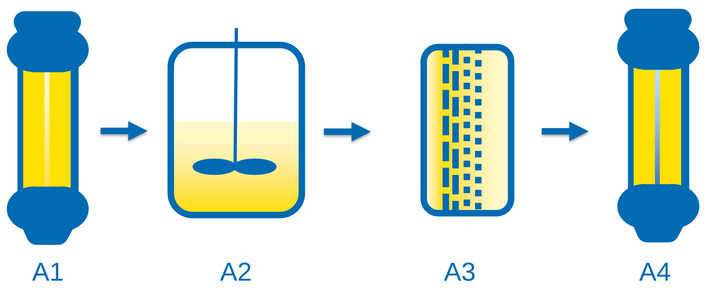
Poros 50 HS (A) showed highest aggregate removal at pH 6-7.
In contrast, Eshmuno CP-FT (B) was more suitable to be used at pH < 5.

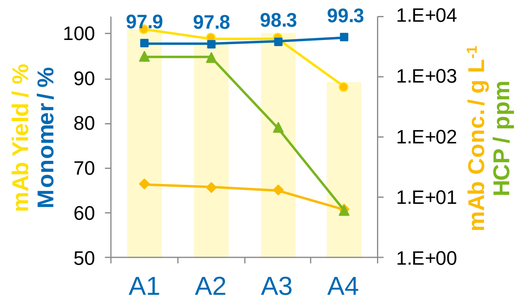
Monomer content of starting material: 97.5%.



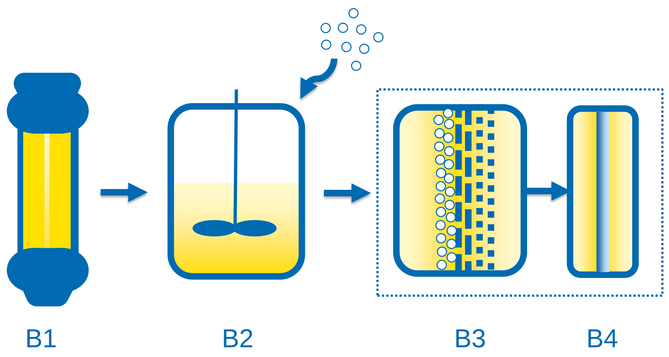
Intensified mAb downstream processes

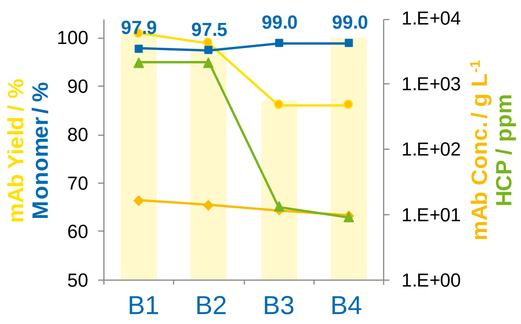
A Two-column purification process



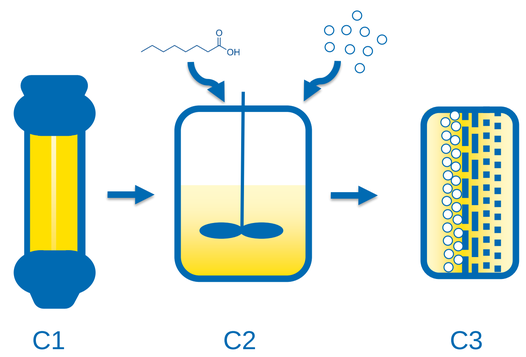


B Static batch adsorption (overloaded CEX) + AEX Adsorber





C Caprylic acid treatment + static batch adsorption (overloaded CEX)



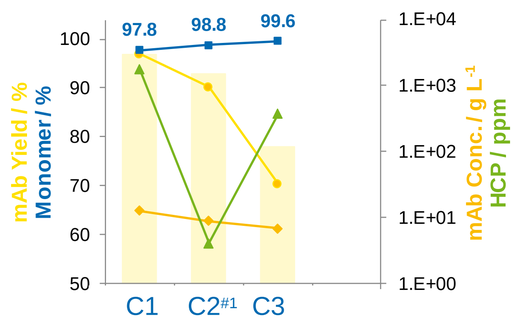


Figure 3: Different mAb downstream process variants prior to virus filtration and final ultra- and diafiltration.

A1, B1, C1	Toyopearl® AF-rProtein A HC-650F	-
A2	Virus inactication	75 ± 15 min at pH 3.6, pH↑ 5.0
A3	ZetaPlus™ 90ZB05A	pH 5.0, 200 L m^{-2}, 150 LMH
A4	Capto™ Adhere	pH 7.5, 60 g L_{resin}^{-1}, 4 min residence time
B2	Virus inactivation	75 ± 15 min at pH 3.6, pH↑ 6.5
B3	CEX Batch Poros™ 50 HS Purafix® Bio SD CH 145ZP	pH 6.5, 400 g L_{resin}^{-1}, 60 ± 30 min 218 L m^{-2}, 270 LMH
B4	NatriFlo® HD-Q	pH 6.5, 6.7 kg m^{-2}, 10 MV min^{-1}
C2	Virus inactivation Caprylic acid precipitation	pH 3.6, 75 ± 15 min 40 mM CA, pH 4.5
C3	CEX Batch Eshmuno® CP-FT ZetaPlus™ 90ZB05A	pH 4.5, 450 g L_{resin}^{-1}, 60 ± 30 min pH 4.5 108 L m^{-2}, 360 LMH

[#1] Sample preparation: Millex® Syringe filter 0.22 µm, PVDF

Our well-known mAb downstream process (Fig. 3, A) using Protein A and Capto™ Adhere in flowthrough mode enables very high purity and high monomer yield. However, this two-column process generates high costs in late stage process characterization studies and process validation (e.g. resin lifetime studies) as well as buffer consumption (EQ, CIP, SIP, S).

The innovative single-use static batch adsorption chromatography using a CEX resin (Poros™ HS) in overload mode (Fig. 3, B) replaces the Capto™ Adhere column of process A. The static batch adsorption can be easily combined with low pH treatment followed by resin separation using a body feed depth filter (B3). A NatriFlo® HD-Q adsorber in flowthrough mode is added in order to maintain sufficient virus safety (B4). The step can be performed in-line to the depth filtration. This short and simple process enables high purity and similar process yield. Monomer content is only slightly lower compared to the two column process (A).

Moreover, single-use static batch adsorption chromatography with CEX resin (Eshmuno® CP-FT) in overload mode was combined with caprylic acid (CA) precipitation at mild acidic pH (Fig. 3, C). Besides HCP clearance, the precipitation step (C2) provides an orthogonal virus inactivation method by the protonated CA[1] to ensure virus safety. The CEX resin was added subsequently, followed by resin and precipitate removal via depth filtration (C3).
As already proposed by the 96-well batch screenings (Fig. 2, B) highest monomer purity of 99.6 % was achieved after CEX adsorption. Moreover, In-process-control (IPC) sample after CA precipitation (C2) confirmed HCP clearance of >2.5 logs. However, final specification for HCP content was not achieved after CEX resin separation by charged depth filter (C3).

Further experiments using a ZetaPlus™ Activated Carbon filter for resin separation were performed (data not shown). Compared to charged depth filtration (6 mM residual CA, determined by RP-HPLC), CA could be completely removed by this filter type. Nevertheless, HCP content of the filtrate was significantly higher compared to the sample after CA precipitation. Thus, possible interactions between caprylic acid, CEX resin and different functionalized filter types have to be investigated in more detail in future experiments.

Conclusion & Prospects

- Caprylic acid precipitation and overloaded CEX in static batch mode can be used in simplified mAb processes providing high HCP and mAb aggregate removal
- Key process advantages include elimination of column packing, cleaning, lifetime studies as well as shorter process time, reduced buffer consumption, and disposability

[1] Trapp *et al.* 2018. J Biotechnol 279: 13-21

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