Optimizing a separation of IgG charge variants using weak cation-exchanging analytical monolithic column

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INTRODUCTION

The upstream and downstream monoclonal antibody (mAb) bioprocessing makes them susceptible to physical and chemical modifications. In the biotechnological production process of mAbs, structural variations may arise due to some enzymatic activity. Antibody charge variants have gained considerable attention in the biotechnology industry due to their potential influence on stability and biological activity and cation-exchange chromatography (CEX) is one of the typical approaches for mAb charge variant analyses. We tested several CEX columns under different conditions and the best column for isotype separation was weak cation-exchanging CIMac COOH chromatographic monolith in pH gradient. We have proven a flow independent separation of mAb charge variants and in this way, a resolution comparable to classical CEX particulate-based analytical columns was achieved in only 6 min analysis time.

RESULTS

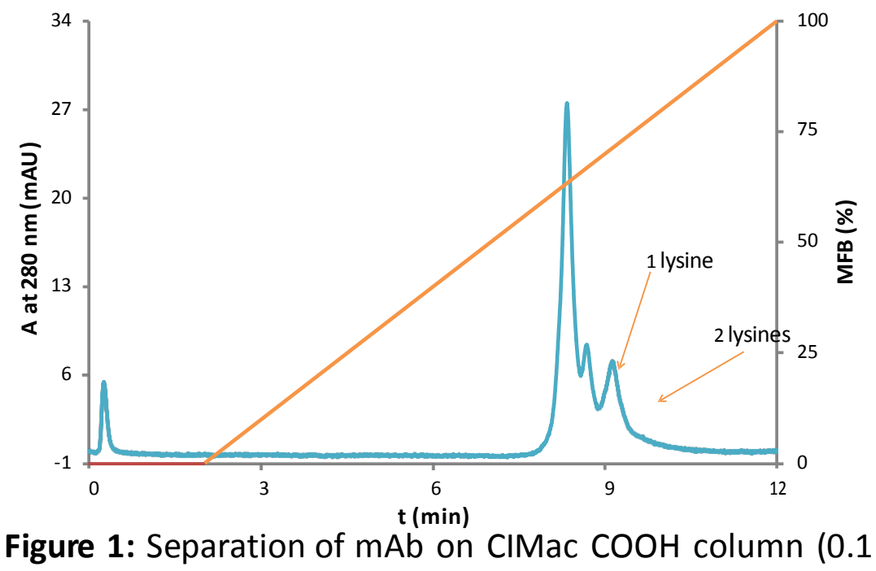

Figure 1: Separation of mAb on CIMac COOH column (0.1 ml) in phosphate-citrate pH gradient. Antibody separates in three resolved peaks in pH gradient from pH 4.6 to 7.3. We applied 10 minute linear gradient from 2 to 12 minutes (0-100% MFB).

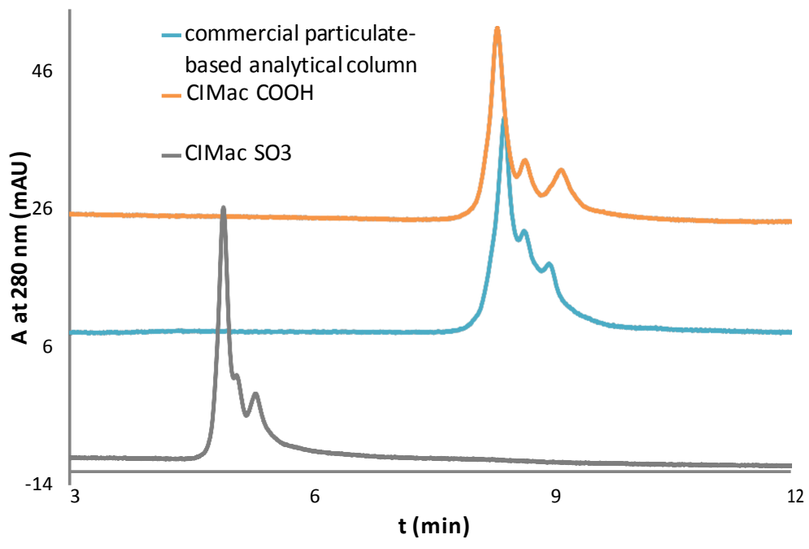

Figure 2: Separation of tested mAb on different ion exchangers: COOH column gives the highest resolution between the chromatographic peaks. Commercial particulate-based analytical column separates charge variants worse compared to weak ion exchanging chromatographic monolith.

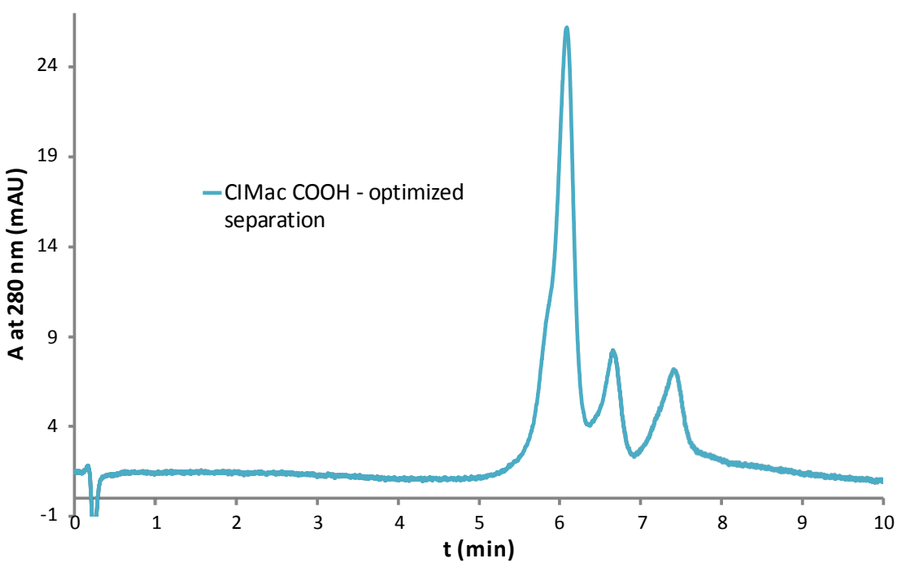

Figure 4: Separation of tested mAb in shortened (optimised) citrate-phosphate pH gradient. Peaks are slightly better separated, but the protein gets more diluted.

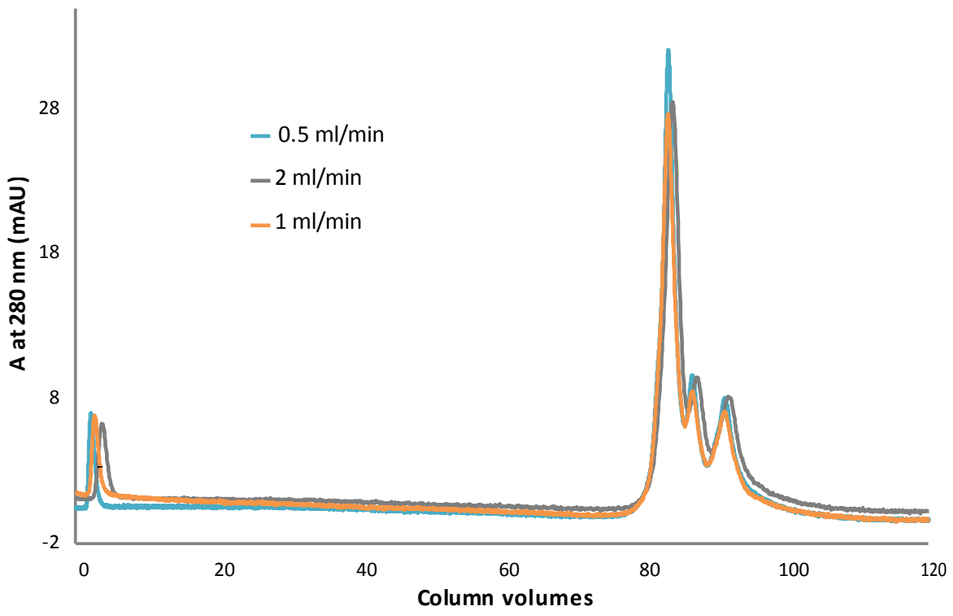

Figure 3: Separation of tested mAb at different flow rates. The flow rate itself does not interfere with the separation properties due to monolith propertiers. The efficient separation can be performed in 6 minutes at 2 ml/min.

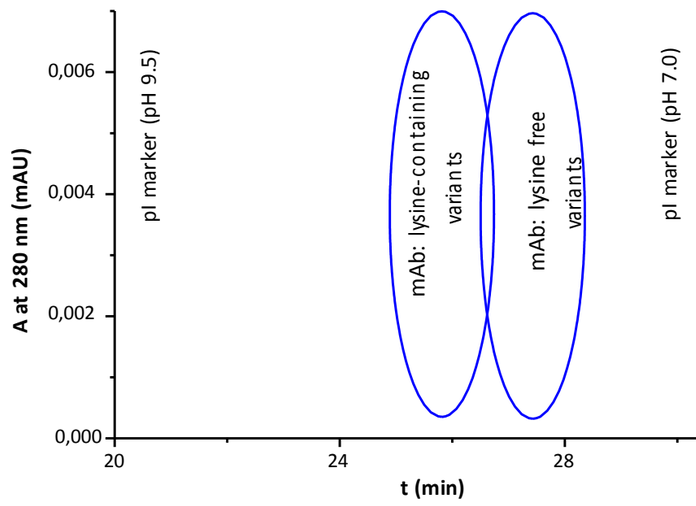

Figure 5: Capillary isoelectrical focusing (cIEF) analysis of tested mAb. Despite a sophisticated method and long analysis time the separation of lysine-containing isoforms from lysine-free is not on a base line.

CONCLUSIONS

- CIMac COOH column was shown to be an excellent choice for fast analytics of mAbs charge variants in pH gradient.
- A screening test for a specific antibody was done in linear pH gradient from pH 4.6 to 7.3, followed by the optimisation of the gradient using the narrower pH window.
- We have proven a flow independent separation of mAb charge variants and in this way, a resolution comparable to classical CEX particulate-based analytical columns was achieved in only 6 min analysis time.

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