A047 CIMac COOH monolith for separation of monoclonal antibodies charge variants in pH gradient

Monoclonal antibody (mAb) charge variants have gained considerable attention in the biotechnology industry, due to their potential influence on stability and biological activity of the active pharmaceutical. Cation-exchange chromatography (CEX) is historically one of the typical approaches for mAb purification and characterization, especially charge variant analysis. **We have proven the flow independent separation of mAb charge variants using CIMac COOH monolithic column in a pH gradient. In this way, a resolution comparable to classical CEX particulate-based analytical columns was achieved in only 6 min analysis time.** Therefore a CIMac COOH column is a perfect choice for fast Process Analytical Control (PAT) of purified mAb samples.

Charge variants separation of monoclonal antibodies

Mouse mAb, isotype IgG1, was firstly purified and concentrated from cell supernatant using CIM protein G chromatography. The buffer exchange was done into phosphate/citrate buffer, pH 4.6.

The separation of charge variants was performed using CIMac COOH column (volume 0.1 mL) in citrate-phosphate pH gradient from pH 4.6 to pH 7.3. 25 mM NaCl was added in both mobile phases. As one can see from Figure 1, three chromatographic peaks belonging to mAb are well resolved by CIMac COOH column even without the optimization of the gradient. The last two eluting peaks belong to mAbs with one or two additional lysine molecules attached to a mAb (Figure 1).

METHOD for Figures 1 and 2

Column:	CIMac™ COOH column
Load:	50 µL of mAb sample, 10x diluted with phosphate-citrate buffer, 25 mM NaCl, pH 4.6
Flow rate:	1.0 mL/min
Mobile phases:	Solvent A: 45 mM Na_2HPO_4, 27.4 mM citric acid, 25 mM NaCl, pH 4.6 Solvent B: 87.2 mM Na_2HPO_4, 6.5 mM citric acid, 25 mM NaCl, pH 7.3
Gradient elution method:	Solvent A (2 min), 0-100 % solvent B in 10 min
Wash:	50 mM carbonate buffer, pH 11
Regeneration:	Additionally regenerated between 5 runs with 1 M NaOH

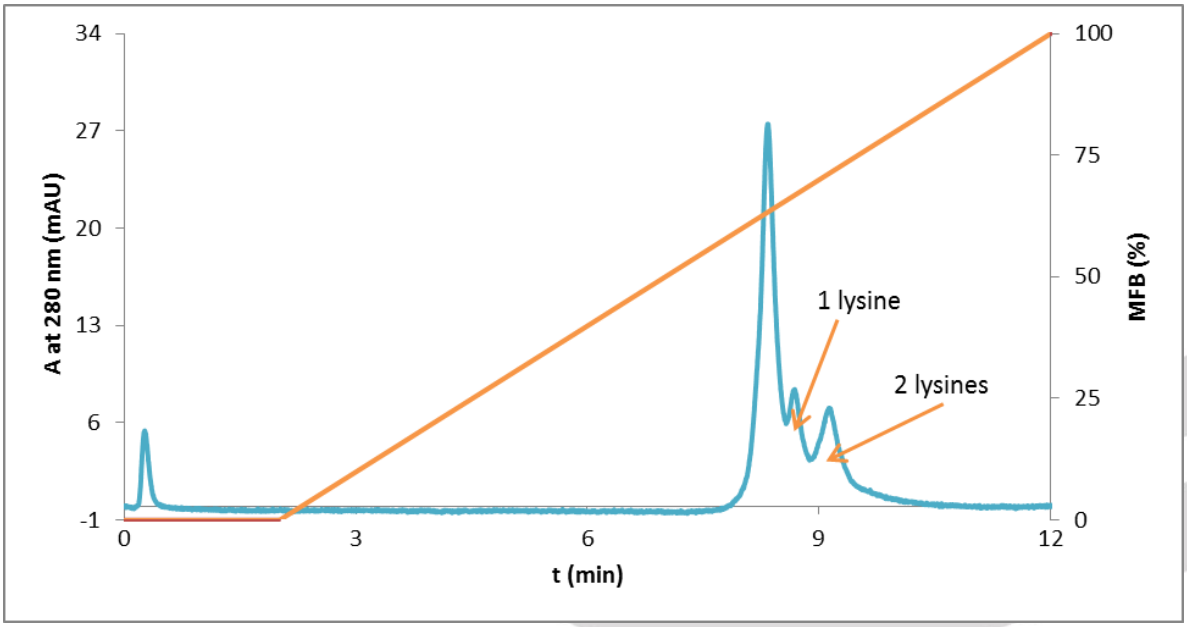

Figure 1: *Separation of mAb on CIMac COOH column (0.1 ml) in simple pH gradient. Antibody separates in three resolved peaks in pH gradient from pH 4.6 to 7.3. We applied 10 minute linear gradient from2 to 12 minutes (0-100% MFB).*

The same sample was injected on commercially available particulate-based CEX column, advertised as suitable for the same application. The third column tested was CIMac SO3, a representative of strong cation exchangers. The Figure 2 shows the superiority of CIMac COOH in mAb charge variants separation. It is clearly evident that the separation of the peaks on COOH matrix is far better compared to separation on commercially available product or on CIMac SO3 column.

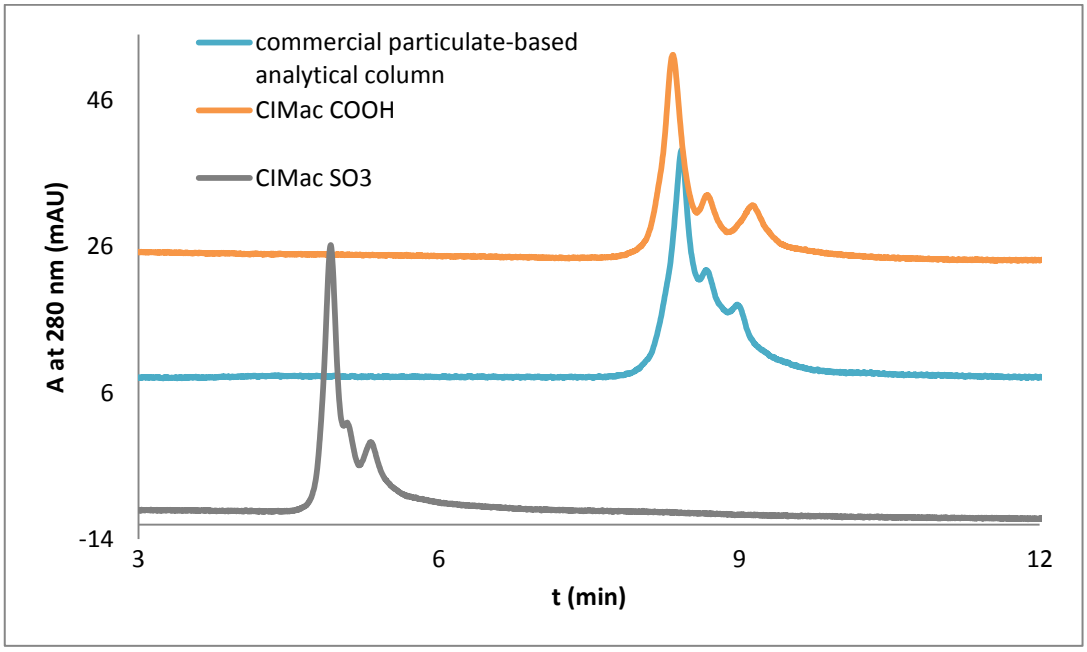

Figure 2: *Comparison of mAb charge variants separation on three different columns in simple pH gradient from pH 4.6 to 7.3.*

The flow independent chromatographic properties

Chromatographic monoliths are stationary phases cast in a single piece with highly interconnected large channels, enabling convective mass transport that results in flow independent chromatographic properties. We tested three different flow rates, while keeping the same steepness of the gradient (always in 100 column volumes (CV). As expected different flow rates do not influence separation properties of CIMac COOH column (Figure 3). The chromatographic analysis at 2 ml/min was performed in only 6 min.

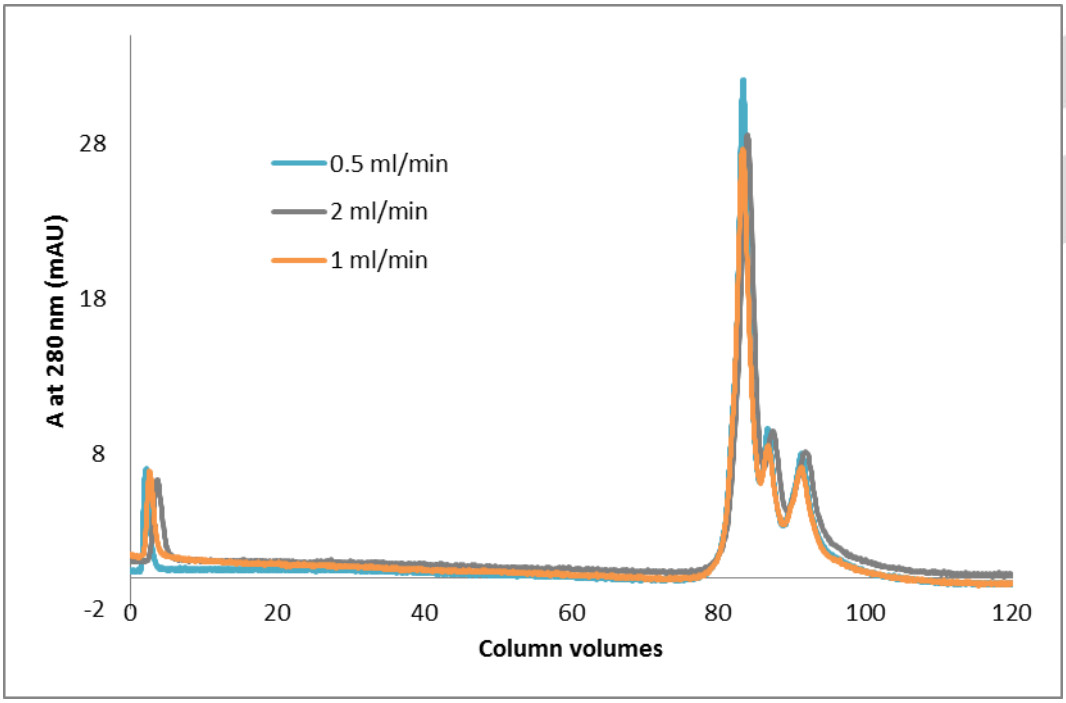

Figure 3: Separation of mAb charge variants on 0.1 mL CIMac COOH column in 100 CV gradient slope at different flow rates.

Optimization of the analytical chromatographic method

NOTE: Optimization of the method is mAb specific and the method described below cannot be directly transferred to different mAb

In order to show the superiority of the column, we optimized the analytical method for the specific mAb by narrowing the pH gradient from 0-100 % of elution mobile phase to 0-40 % and finally to 15-40 % of elution mobile phase. The length of the gradient was kept 10 min. The results are gathered in Figure 4. Under the optimized conditions, almost baseline separation of the charge variants was indeed possible. Another peak started resolving in front of the main chromatographic peak, which potentially could be another acidic isoform of the mAb.

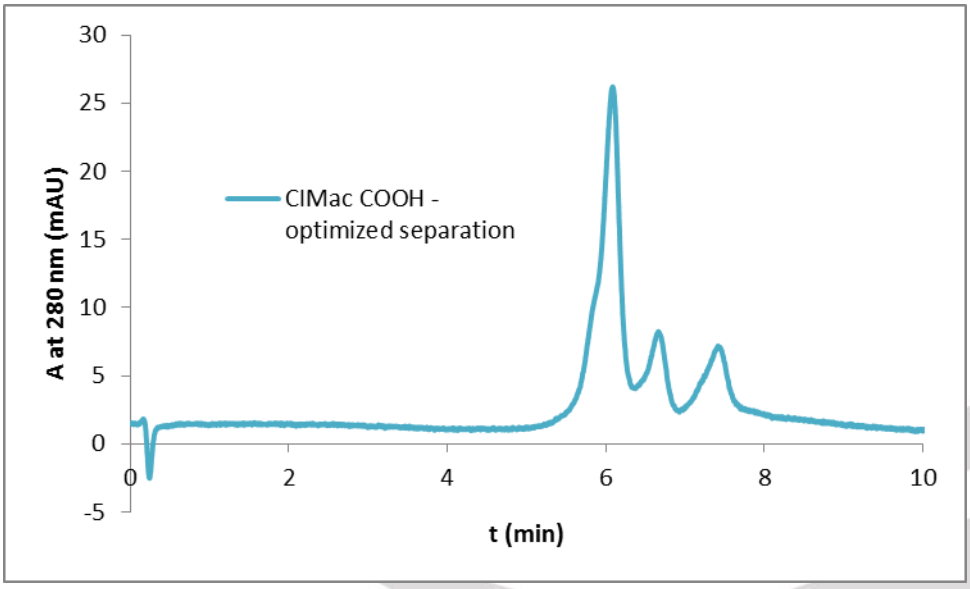

***Figure 4:** Separation of mAb charge variants in shallower 10 minute gradients (15-40 % MFB). Flow rate was set to 1 mL/min.*

NOTE: CIMac COOH column withstood 10 reproducible injections of mAb sample. To prolong the reproducibility of the analyses, it is strongly recommended to wash the column after each injection with with 20 CV of 50 mM carbonate buffer, pH 11.

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 is done in linear pH gradient from pH 4.6 to 7.3, followed by the optimization of the gradient using a narrower pH window. It was confirmed that the flow rate does not influence the separation resolution, thus enabling fast analyses in only 6 min. Flushing the column with carbonate buffer, pH 11, between the runs, significantly improves the reproducibility of the analytical chromatograms.

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