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P-23

Cation-Exchange Chromatography for the Separation of Monoclonal Antibodies

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1. Introduction

Cation exchange chromatography (CEX) is a widely used non-denaturing approach for the characterization of acidic and basic variants of therapeutic monoclonal antibodies (mAb). Beside the classical salt gradient separation mAbs can be eluted applying pH gradient or salt-mediated pH gradient. In order to achieve good separation the combination of the mobile and the stationary phases can be an important aspect.

The aim of our work was to find alternative mobile phases for the pH gradient and salt-mediated pH gradient separation of monoclonal antibodies (1) and to study the effect of different stationary phases on the pH of the effluent when performing linear pH gradient separation (2).

2. Materials and methods

Equipment and Software

The experiments were performed on a Waters Acquity UPLC™ I-Class and H-Class Bio systems equipped with a fluorescent detector (FL) and an online pH meter. Empower Pro 3 software was used for data acquisition and instrument control. Calculations were performed using Python programming language.

Mobile phases

The studied mobile phase systems were combinations of 2-(N-morpholino)ethanesulfonic acid (MES), 1,3-diamino-2-propanol (DAP), citric acid (CA), 2-(cyclohexylamino)ethanesulfonic acid (CHES), imidazole and ammonium acetate. The pH was adjusted by ammonium hydroxide, sodium hydroxide or by acetic acid. The alternative mobile phases were compared to the commercially available Thermo Fisher CX-1 and to the Waters BioResolve CX pH buffer systems.

Columns

Among the five studied CEX column the ProPac Elite WCX (150 x 4 mm, 5 µm) and the Supelco Antibodix WCX-NP3 (150 x 4.6 mm, 3 µm) were weak cation exchangers, while YMC BioPro SP-F (100 x 4.6 mm, 5 µm) column, Waters Protein-Pak Hi Res SP (100×4.6 mm, 7 µm) and Waters BioResolve SCX mAb (100 x 4.6 mm, 3 µm) columns were strong cation exchangers.

Samples

Bevacizumab, daratumumab, panitumumab, pertuzumab and rituximab in intact and IdeS digested form were prepared in 1 mg/ml concentration.

3. Results

Alternative mobile phases

During pH gradient CEX separations the net charge of the mAbs is changing due to the deprotonation of functional groups. At the pH equal to the isoelectric point (pI) of the individual mAbs they elute from the column. Different compounds used as biological buffers were candidate as alternative chromatographic buffer components. According to the preliminary calculations pH response of various buffer pairs (MES/piperazine, citric adic/DAP, MES/DAP, citric acid/imidazole, MES/imidazole, citric acid/CHES+NaOH, ammonium acetate) was measured as a function of base content (*Figure 1*).

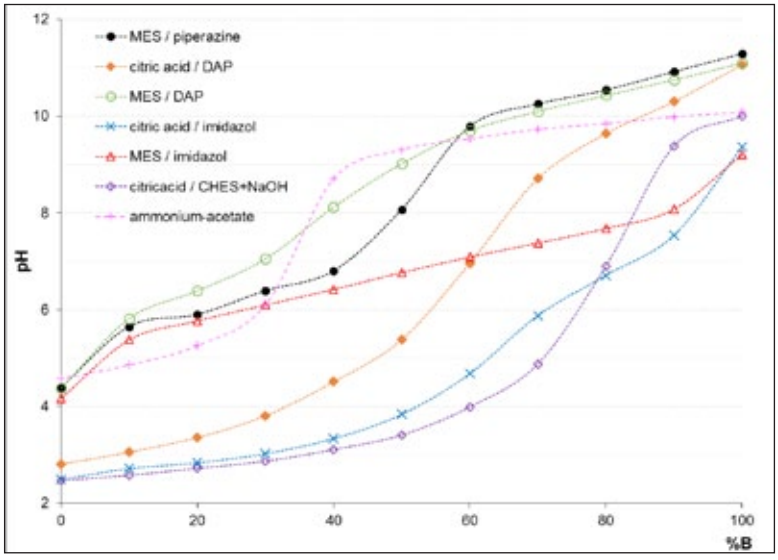

Figure 1 *Measured pH responses of various buffer systems as a function of base content*

Linear pH response was found only for some buffer pairs (MES/imidazole, MES/DAP, MES/piperazine). Even though the pH response is expected to be linear, all the 7 buffer systems were tested as chromatographic eluent for the analysis of monoclonal antibodies. The chromatograms of bevacizumab obtained with MES/DAP, citric acid/CHES and ammonium acetate (simple pH gradient and salt mediated pH gradient) are presented on ***Figure 2***, compared with the chromatograms of classical salt gradient and the commercially available pH gradient buffer.

It can be seen, that different retention times and peak shape is given on the two columns with different mobile phases. When studying different mAbs (daratumumab, panitumumab, pertuzumab and rituximab) different selectivity and retention order can be observed.

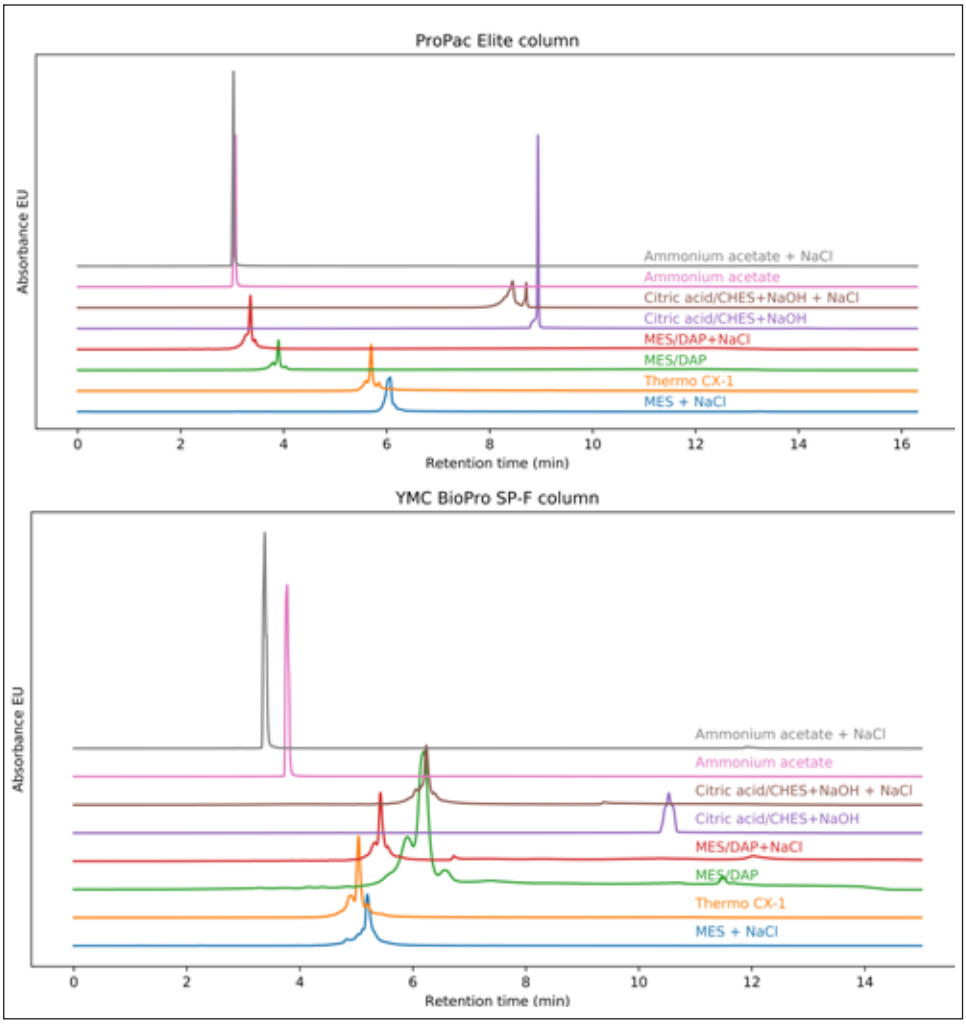

Figure 2 *Cation-exchange chromatograms of intact bevacizumab with different mobile phases on a weak (upper) and strong (lower) CEX column .Flow rate: 0.8 ml/min, 0-100%B in 10 min, temperature= 25°C, injected volume=1 µl, detection: FL(280-350nm)*

4. On-column pH response

For further examination of the mobile phases the pH of the effluent was measured for different combination of mobile and stationary phases. It was found that the commercially available Thermo CX-1 buffer provides linear pH response on the studied columns, while the in-house developed alternative mobile phases (MES/DAP and citric acid/CHES + NaOH) have a break point in the pH response. The pH of the effluent on different columns can be seen in ***Figure 3*** for citric acid/CHES + NaOH buffer system. Using this mo-

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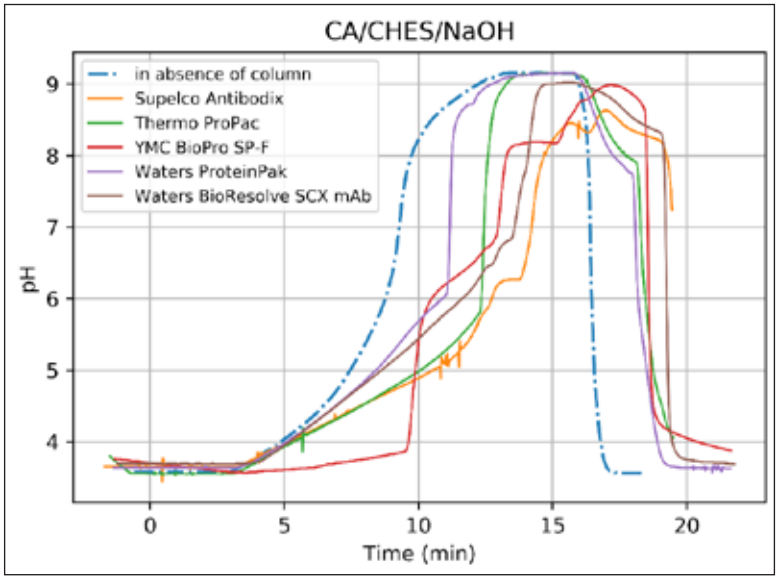

Figure 3 *pH response of citric acid/CHES + NaOH buffer system using different columns (the dead volume of the columns were taken into account).*

bile phase system a jump at pH~6 can be observed for each columns.

5. Conclusion

The goal of our work was to find alternative mobile phases for the cation exchange separation of monoclonal antibodies. Different biologically applied buffer compounds were used for studying the generated and the actually emerging pH response. It was found that the MES/DAP buffer system provides a linear pH response in the range of 5 ≤ pH ≤ 10 in pure pH and in salt mediated pH gradient modes, while the pH response of citric acid/CHES + NaOH is linear in 2.5 ≤ pH ≤ 10 range, but the observed peak shape is only acceptable, when the pH gradient is combined with a salt gradient.

It was also demonstrated that the stationary phase has an impact on the pH gradient forming in the columns. Therefore during method development for characterization of mAbs not only the mobile phase but the stationary phase has to be taken into consideration.

6. Acknowledgements

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