

pH Gradient Based Cation Exchange Chromatography for Monoclonal Antibody Separations

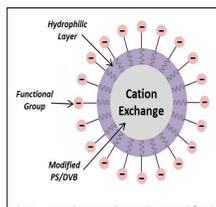
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INTRODUCTION

Recombinant monoclonal antibodies (mAbs) have become a very important segment of protein drug therapeutics. Due to chemical or enzymatic modifications, monoclonal antibodies exhibit great protein heterogeneity such as C-terminal processing, glycosylation, oxidation, deamidation, amino acid sequence variations. Monitoring and characterizing mAb heterogeneity is an important step in quality control for protein consistency and stability through the lifespan of the biomolecules. Cation exchange chromatography (CEX) has been widely used for mAb charge variant separations. Salt, ionic strength, based methods are commonly developed for mAb variant separations. In this study we present a pH gradient based method, an alternative to salt gradients, for mAb characterization using strong cation exchange (SCX) chromatography. Proteomix® SCX particle surface is grafted with a highly hydrophilic, neutral polymer layer with a thickness in the range of nanometers. The hydrophobic PS/DVB resin surface is completely covered by a hydrophilic coating which minimizes non-specific bindings with antibody proteins, leading to high efficiency and high recovery separations. Here we optimized a pH based gradient for a number of mAb variant separations. Proteomix® SCX columns exhibited high lot to lot consistency and great run to run reproducibility with the optimized pH gradient. This particular three component pH gradient can be a great addition to CEX method scouting for mAb variants study.

EXPERIMENTAL

Column: Proteomix® SCX NP5 4.6 x 250 mm



Material: Non-porous PS/DVB beads grafted with a highly hydrophilic, neutral polymer thin layer.

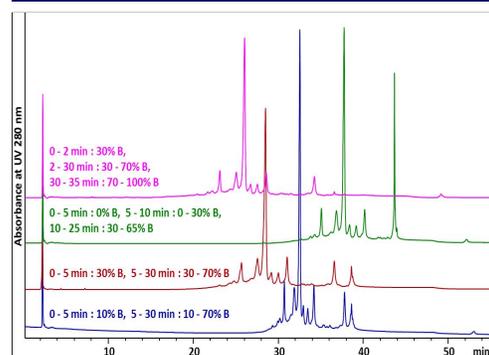
Functional group: Sulfonate ($-\text{SO}_3\text{H}$), $\text{pKa} < 1.0$

Mobile phase: A: 2.4 mM tris, 1.5 mM imidazole and 11.6 mM piperazine pH 6.0
B: A at pH 10.5

Sample: mAb 321 (5 mg/mL stock in tris)

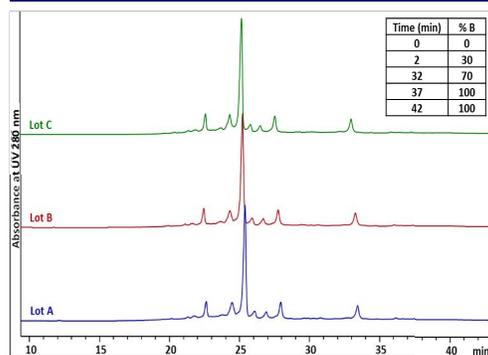
Instrument: Agilent 1200 HPLC with quaternary pump

OPTIMIZATION OF pH GRADIENT FOR MAB 321 ANALYSIS



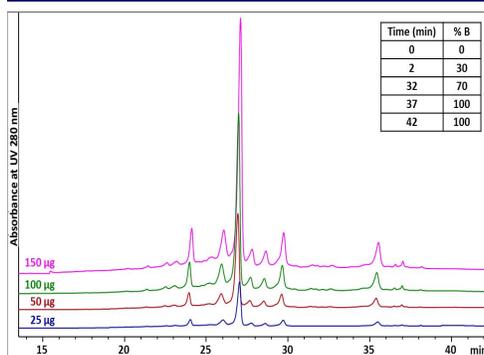
Optimizing the pH gradient for the analysis of mAb 321 on Proteomix® SCX NP5 4.6 x 250 mm. Mobile phases were A: 2.4 mM tris, 1.5 mM imidazole and 11.6 mM piperazine pH 6.0 and B: A at pH 10.5. Flow rate was 0.8 mL/min and 10 μL of mAb 321 at 5 mg/mL was injected.

LOT TO LOT COMPARISON OF PROTEOMIX® SCX



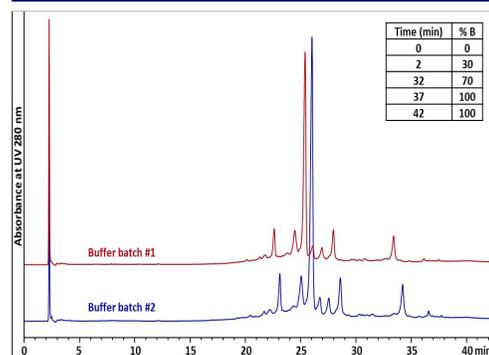
Analysis of mAb 321 on three different lots of Proteomix® SCX NP5 4.6 x 250 mm. Mobile phases were A: 2.4 mM tris, 1.5 mM imidazole and 11.6 mM piperazine pH 6.0 and B: A at pH 10.5. Flow rate was 0.8 mL/min and the column temperature was at 25°C. 10 μL mAb 321 (5 mg/mL) was injected.

LOADING TEST FOR MAB ON PROTEOMIX® SCX



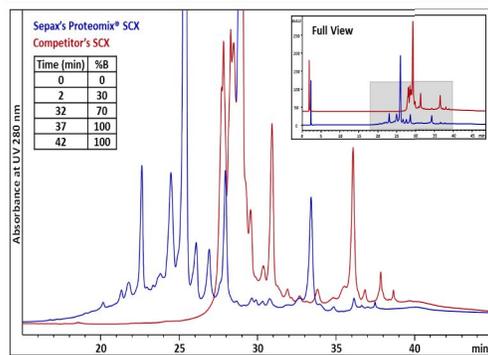
Loading test for mAb 321 on Proteomix® SCX NP5 4.6 x 250 mm. Mobile phases were A: 2.4 mM tris, 1.5 mM imidazole and 11.6 mM piperazine pH 6.0 and B: A at pH 10.5. Flow rate was 0.8 mL/min and the column temperature was at 25°C. 5, 10, 20 and 30 μL mAb 321 (5 mg/mL) was injected.

TWO BATCHES OF MOBILE PHASE COMPARED ON PROTEOMIX® SCX



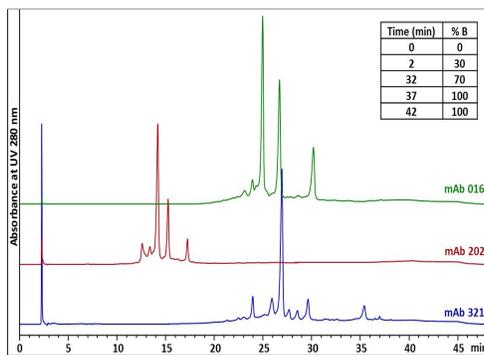
Analysis of mAb 321 using two different batches of mobile phase on the same Proteomix® SCX NP5 4.6 x 250 mm. Mobile phases were A: 2.4 mM tris, 1.5 mM imidazole and 11.6 mM piperazine pH 6.0 and B: A at pH 10.5. Flow rate was 0.8 mL/min and the column temperature was at 25°C. 10 μL mAb 321 (5 mg/mL) was injected.

MAB ON PROTEOMIX® SCX COMPARED TO COMPETITOR'S SCX



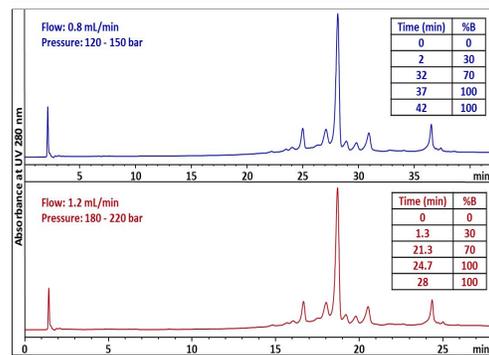
Analysis of mAb 321 on Proteomix® SCX NP5 (4.6 x 250 mm) and a Competitor's SCX (5 μm , 4.0 x 250 mm). Mobile phases were A: 2.4 mM tris, 1.5 mM imidazole and 11.6 mM piperazine pH 6.0 and B: A at pH 10.5. Flow rate was 0.8 mL/min for Proteomix® SCX and 0.6 mL/min for the Competitor's SCX. 10 μL mAb 321 (5.0 mg/mL) was injected on each column.

THREE DIFFERENT MABS WITH THE SAME pH GRADIENT



Analysis of three different mAbs on Proteomix® SCX NP5 4.6 x 250 mm. Mobile phases were A: 2.4 mM tris, 1.5 mM imidazole and 11.6 mM piperazine pH 6.0 and B: A at pH 10.5. Flow rate was 0.8 mL/min and the column temperature was at 30°C. 10 μL mAb 321 (5 mg/mL), 50 μL mAb 202 (1 mg/mL) and 10 μL mAb 016 (5.9 mg/mL) was injected.

FASTER MAB VARIANT ANALYSIS ON PROTEOMIX® SCX



Analysis of mAb 321 on Proteomix® SCX NP5 4.6 x 250 mm at two different flow rates. Mobile phases were A: 2.4 mM tris, 1.5 mM imidazole and 11.6 mM piperazine pH 6.0 and B: A at pH 10.5. Flow rates were 0.8 and 1.5 mL/min and 10 μL of mAb 321 at 5 mg/mL was injected.

CONCLUSION

- » Proteomix® SCX NP5 4.6 x 250 mm can successfully separate mAb variants using a pH gradient as an alternative method to a salt gradient.
- » The pH gradient introduced here with three components (mobile phase A: 2.4 mM Tris, 1.5 mM Imidazole, 11.6 mM piperazine pH 6.0 and B: A at pH 10.5) can be a great starting point for mAb analyses using cation exchange.
- » The low pressure and high stability of packing material allows faster run time with higher flow rate.
- » Proteomix® SCX exhibits high run to run and lot to lot reproducibility.

REFERENCES

1. Farnan D, Moreno GT. Multi-product high-resolution monoclonal antibody charge variant separations by pH gradient ion-exchange chromatography. *Anal Chem.* 2009; 81: 8846-57.
2. Liu, H., Gaza-Bulseco, G., Faldut, D., Chumsae, C. and Sun, J. Heterogeneity of monoclonal antibodies. *J. Pharm. Sci.* 2008; 97: 2426-2447.