Cation Exchange Chromatography for Monoclonal Antibody (mAb) Separations

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INTRODUCTION

Recombinant monoclonal antibodies (mAbs) have become a very important segment of protein drug therapeutics. Post-translational modifications including glycosylation, oxidation, deamidation and incomplete C-terminal processing can contribute to an antibody's heterogeneity. Degradation reactions during manufacturing, formulation, and storage also can cause structural and functional changes in mAbs. It is important to characterize and evaluate the heterogeneities and monitor the stabilities of mAbs for shelf-life studies. Antibodix™ weak cation exchange (WCX) and Proteomix® strong cation exchange (SCX) are designed to separate antibodies with minor forms. Both the Antibodix™ WCX and Proteomix® SCX particle surfaces are grafted with a highly hydrophilic and neutral polymer layer. The hydrophobic PS/DVB resin surface is completely covered by this hydrophilic coating which minimizes non-specific bindings with antibody proteins, leading to high efficiency and high recovery separations. In this study, we applied Antibodix™ WCX and Proteomix® SCX for separations of variants and stability studies with a number of mAbs. Different mobile phase systems such as pH and salt gradients were applied to the separations.

EXPERIMENTAL

Columns:

Proteomix® SCX NP5 4.6 x 250 mm
Antibodix™ WCX NP5 4.6 x 250 mm
Proteomix® WCX NP5 4.6 x 250 mm

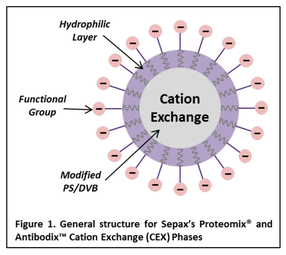


Figure 1. General structure for Sepax's Proteomix® and Antibodix™ Cation Exchange (CEX) Phases

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

Functional groups:

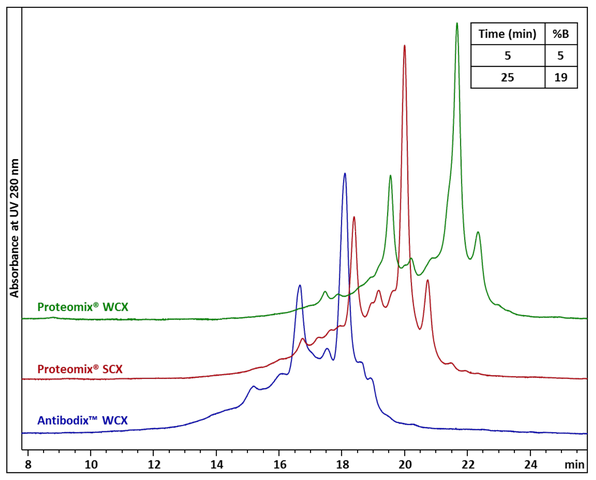
SCX: Sulfonate (—SO_3H), pKa < 1.0
WCX: Carboxylate (—COOH), pKa 4.75

Linkers:

Proteomix® is a tentacle linker
Antibodix™ is a random polymer linker, more hydrophilic

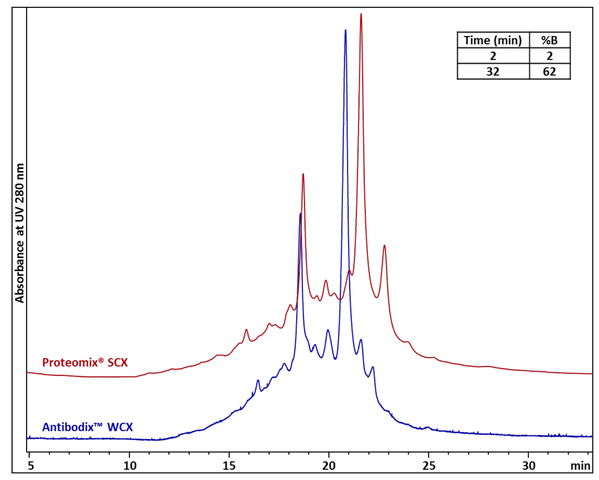
Instrument: Agilent 1200 HPLC with quaternary pump

OVERLAY OF ANTIBODY ANALYSES ON THREE DIFFERENT CATION EXCHANGE PHASES



Comparison of Antibodix™ WCX NP5, Proteomix® SCX NP5 and Proteomix® WCX NP5 (all 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 + 0.5 M NaCl pH 10.5. Flow rate was 0.8 mL/min and column temperature was at 30°C. 20 µL of Antibody Q (1 mg/mL) was injected.

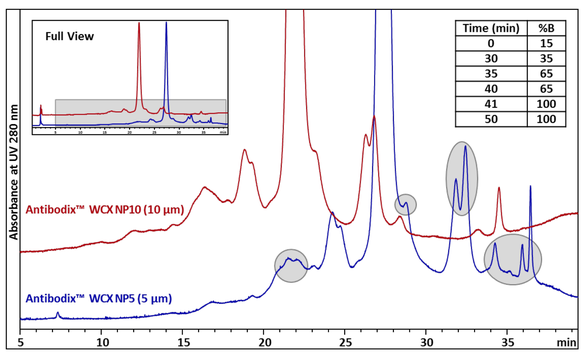
OVERLAY OF ANTIBODY ANALYSES ON PROTEOMIX SCX AND ANTIBODIX WCX



Analysis of Antibody Q on Antibodix™ WCX NP5 and Proteomix® SCX NP5, both 4.6 x 250 mm. Mobile phases were A: 20 mM tris pH 8.2 and B: A + 100 mM NaCl. Flow rate was 0.7 mL/min and column temperature was at 30°C. 20 µL of Antibody Q at 1 mg/mL was injected.

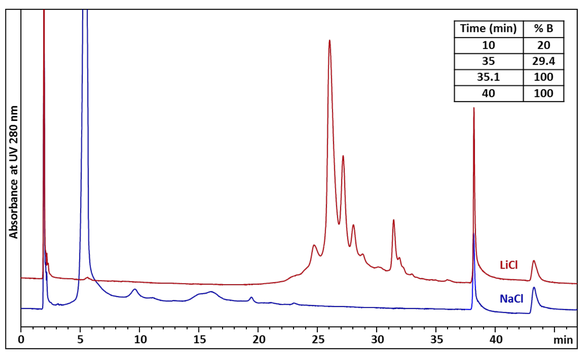
ANTIBODIX™ WCX

PARTICLE SIZE COMPARISON FOR ANTIBODIX™ WCX



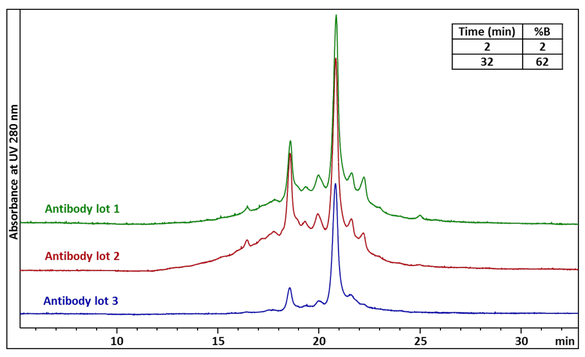
Analysis of mAb H comparison on Antibodix™ WCX NP5 and NP10, both 4.6 x 250 mm. Mobile phases were A: 10 mM sodium phosphate pH 7.5 and B: A + 0.1 M NaCl. Flow rate was 0.8 mL/min and the column temperature was at 30°C. 50 µL of mAb H at 1 mg/mL was injected.

COMPARISON OF LiCl AND NaCl ELUTION SALTS



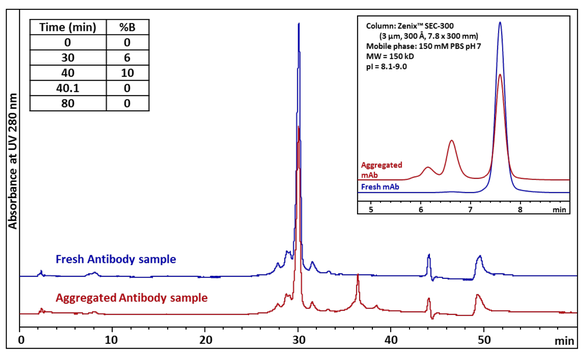
Analysis of mAb 321 on Antibodix™ WCX NP5 4.6 x 250 mm. Mobile phases were A: 20 mM sodium acetate pH 5.15 and B: A + 1 M of the indicated salt. Flow rate was 0.8 mL/min and the column temperature was at 30°C. 20 µL of mAb 321 at 5 mg/mL was injected.

LOT TO LOT ANALYSIS OF ANTIBODY Q ON ANTIBODIX™ WCX



Analysis of three lots of Antibody Q on Antibodix™ WCX NP5 4.6 x 250 mm. Mobile phases were A: 20 mM tris pH 8.2 and B: A + 100 mM NaCl. Flow rate was 0.7 mL/min and column temperature was at 30°C. 20 µL of each lot of Antibody Q at 1 mg/mL was injected.

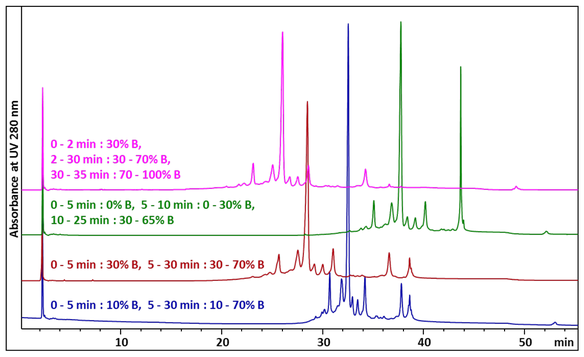
OVERLAY OF AGGREGATED AND FRESH MAB SAMPLES ON ANTIBODIX™ WCX



Analysis of a fresh and an aggregated mAb on Antibodix™ WCX NP5 4.6 x 250 mm. Mobile phases were A: 20 mM HEPES pH 7.0 and B: A + 1 M NaCl. Flow rate was 0.6 mL/min and column temperature was at 30°C. 3 µL of antibody (17 mg/mL) from inlaid SEC run was injected.

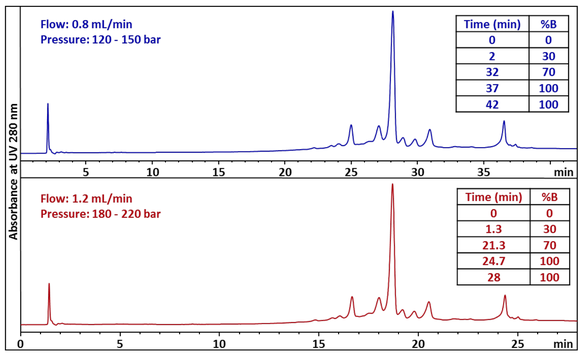
PROTEOMIX® SCX

OPTIMIZATION OF pH GRADIENT FOR MAB 321 ANALYSIS



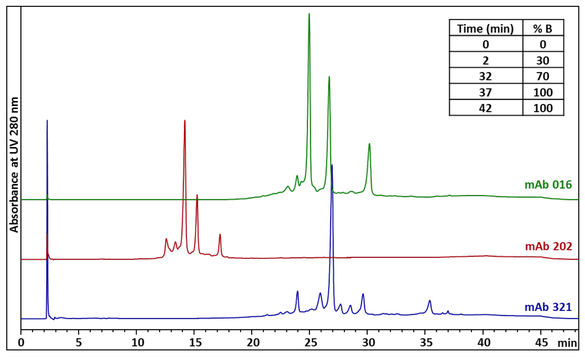
Optimizing the pH gradient for the analysis of mAb 321 on Proteomix® SCX NP5 4.6 x 250 mm. Mobile phases were A: 2.4 mM tris, 1.5 mM imidazole and 11.6 mM piperazine pH 6.0 and B: A at pH 10.5. Flow rate was 0.8 mL/min and 10 µL of mAb 321 at 5 mg/mL was injected.

FASTER MAB VARIANT ANALYSIS ON PROTEOMIX® SCX



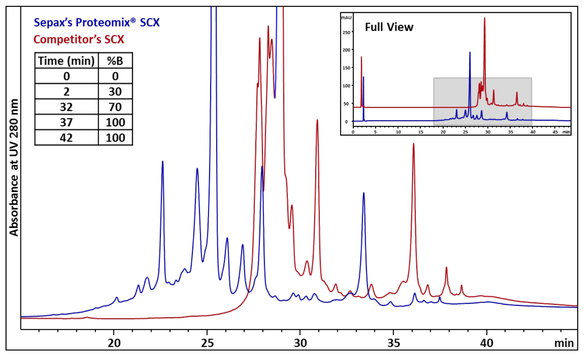
Analysis of mAb 321 on Proteomix® SCX NP5 4.6 x 250 mm at two different flow rates. Mobile phases were A: 2.4 mM tris, 1.5 mM imidazole and 11.6 mM piperazine pH 6.0 and B: A at pH 10.5. Flow rates were 0.8 and 1.5 mL/min and 10 µL of mAb 321 at 5 mg/mL was injected.

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.

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.

CONCLUSION

- A wide range of running conditions can be applied to both Antibodix™ WCX and Proteomix® CEX for mAb analysis. The method development can be shortened by varying different parameters.
- Antibodix™ WCX and Proteomix® CEX columns can successfully separate mAb variants using an optimized salt gradient and/or pH gradient.
- Monoclonal antibody purity, heterogenity and stability can all be monitored using Sepax's CEX phases.

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., Faldu, D., Chumsae, C. and Sun, J. Heterogeneity of monoclonal antibodies. J. Pharm. Sci. 2008; 97: 2426–2447.