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 changes in mAbs. It is important to characterize and storage also can cause structural and functional degradation reactions during manufacturing, formulation, processing can contribute to an antibody’s heterogeneity.

EXPERIMENTAL

Columns:
- Proteomix® SCX NPS 4.6 x 250 mm
- Antibodix™ WCX NPS 4.6 x 250 mm
- Proteomix® WXC NPS 4.6 x 250 mm

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

Functional groups:
- WCX: Carboxylate (—COOH), pKa 4.75
- SCX: Sulfonate (—SO3H), pKa < 1.0

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

Instrument: Agilent 1200 HPLC with quaternary pump

Analysis of mAb H comparison on Antibodix™ WXC NPS 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.

Analysis of mAb 321 on Antibodix™ WXC NPS 4.6 x 250 mm. Mobile phases were A: 20 mM sodium acetate pH 8.2 and B: A + 1 M NaCl. Flow rate was 0.7 mL/min and column temperature was at 30°C. 20 µL of Antibody Q (1 mg/mL) was injected.

Analysis of three lots of Antibody Q on Antibodix™ WXC NPS 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.

Analysis of a fresh and an aggregated mAb on Antibodix™ WXC NPS 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.

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

REFERENCES