Separation of Monoclonal Antibodies by Weak Cation-Exchange Chromatography Using ProPac and ProSwift Columns

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ABSTRACT

Monoclonal antibodies (MAbs) are currently developed by pharmaceutical and biotechnology companies for various therapeutic applications. MAbs undergo several post-translational modifications including oxidations, deamidations, lysine truncations, and glycan modifications. Manufacturing of MAbs and subsequent stability testing procedures involve routine analysis and monitoring of the impurities resulting from asparagine deamidation, aspartic acid isomerization, disulfide interchange, peptide bond cleavage, and oxidation. ProPac® WCX and ProSwift™ WCX columns are widely used to characterize MAb heterogeneity.

ProPac WCX columns are packed with particles that are well suited for analytical separations for monoclonal antibody variants. We have separated MAbs using various gradients, pH, and temperatures and have suggested optimal conditions for separation of monoclonal antibodies. We also suggested a conditioning step to recover under-performing columns. The conditioning is done by treating the column at 50 °C for 48 h in presence of 20 mM MES pH 5.6 (2-[*N*-Morpholino] ethanesulfonic acid) or, alternatively, treating the column at 70 °C for 7 h. When MAbs are separated on these heat-conditioned columns, they exhibited restored efficiency, peak shapes, and improved overall performance as compared to unconditioned under performing control columns.

ProSwift monolith columns offer significant advantages over conventional porous columns, including fast mass transfer, high loading capacity, and wide pH stability. This exclusive combination of characteristics supports versatile performance in a wide range of biomolecule separations, including monoclonal antibody separations. ProSwift WCX columns offer excellent capacity with high resolution, making them suitable for the first dimension of multi-dimensional chromatography.

INTRODUCTION

Monoclonal antibody (MAb) microheterogeneity can be attributed to glycosylation, oxidation, mutation, phosphorylation, amino terminal modifications (e.g., to pyroglutamate), incomplete processing of the C-terminus, and asparagine (Asn) deamidation. These variations in protein composition occur in many types of proteins and can impact their activity and stability of biotherapeutics. Monitoring stability of therapeutic proteins and peptides is essential for demonstrating safety and efficacy of these drugs and is required by the FDA and other regulatory agencies. These variations are routinely monitored preferably by cation-exchange chromatography.

Dionex cation-exchange ProPac WCX[1-3] and ProSwift WCX columns are ideal for resolving closely related protein charge variants. ProPac packings are pellicular polymeric supports with hydrophilic coatings and grafted surface chemistry which exhibit minimal hydrophobic character. In addition, these particles exhibit a wide range of pH stability with high selectivity and minimal band spreading. ProSwift monolithic columns are specifically designed to provide high-resolution and high-efficiency separations of proteins. ProSwift media are based on polymeric monoliths prepared by an in situ polymerization process. They are a new generation of separation media, which are uniquely designed and engineered for separation of biomolecules. The monolith is a single cylindrical polymer rod containing an uninterrupted, interconnected network of through pores, which are also called channels. The ProSwift columns have excellent resolution and also high capacity. They are available with reversed-phase and ion-exchange surface chemistries, and provide fast protein separations using conventional HPLC.

In this study we present data using ProPac WCX and ProSwift WCX columns. Excellent separations are achieved for the immunoglobulin G (IgG) MAb charge variants. We test the effect of pH, temperature, and NaCl gradients on MAb separation. The authors suggest a simple column conditioning step for ProPac WCX column to improve the resolution of MAbs. In this procedure, the column is heat treated in presence of 20 mM MES pH 5.6 or 6.5 (2-[*N*-Morpholino] ethanesulfonic acid) for 48 h at 50 °C or, for 7–8 h at 70 °C. When MAbs are separated on the heat conditioned columns, they exhibited higher efficiency, better peak shapes and improved overall performance as compared to separations performed on unconditioned control columns. The authors used the ProSwift WCX column for characterizing MAb heterogeneity. Due to high capacity of the ProSwift WCX, milligram quantities can be loaded on to the column for separation and fractionation of variants for further characterization. The authors compared the separation of MAb variants using different buffer components at various pH conditions. Also, we compared the ProSwift WCX column with a leading WCX biocolumn for MAb separations and present the results.

MATERIALS

Chromatographic Components

MAb Separations: All PEEK™ System

ICS-3000 DP gradient pump, VWD absorbance detector, AS autosampler and TCC-10 thermostatted column compartment (Dionex Corporation)

Chromatography was controlled by Chromeleon® Chromatography Data System software (Dionex Corporation).

Chemicals

Protein Standards, MES, HEPES and all other analytical grade chemicals were obtained from Sigma. MAb is a gift from a biotech company.

Columns

ProPac WCX-10 (P/N 054993) analytical columns are from Dionex Corporation. These columns consist of a polymer support coated with a hydrophilic polymer.

ProSwift WCX-1S 4.6 × 50 mm (P/N 064295) and 1 × 50 mm (P/N 066643) are polymer monoliths from Dionex Corporation.

Competitor A: Leading weak cation-exchange column, 10 µm, 1000 Å, 5 × 50 mm

ProPac WCX

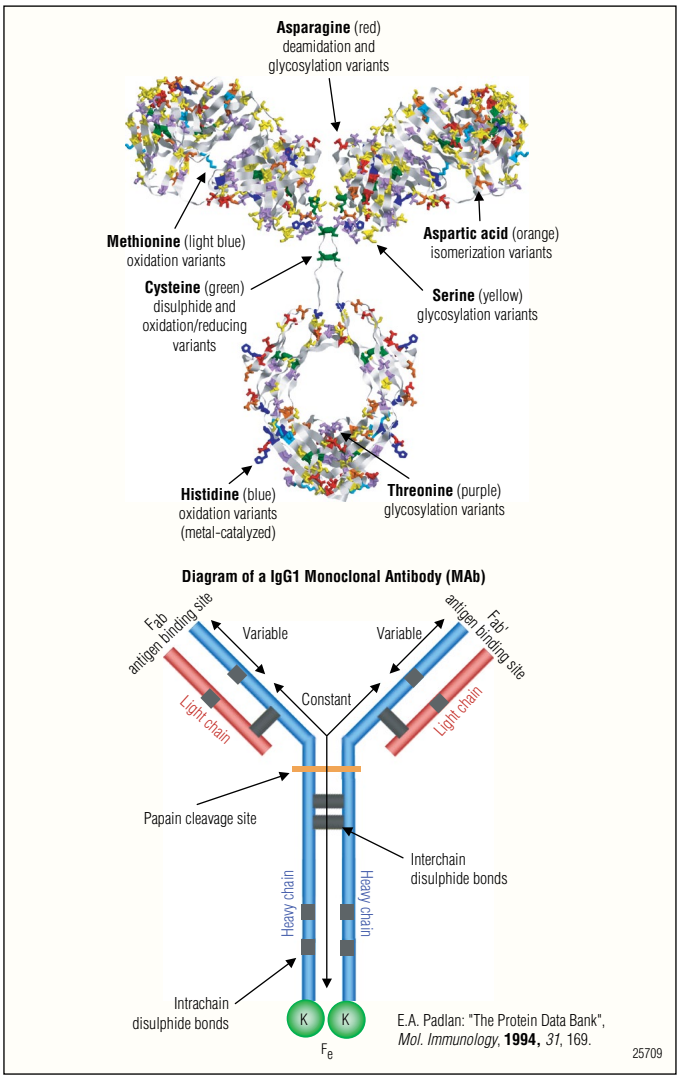

Figure 1. Crystal structure of a human IgG1 MAb.

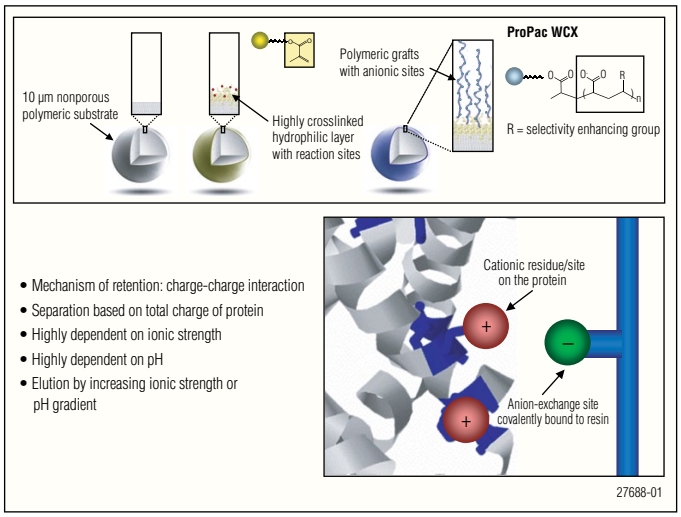

Figure 2. ProPac WCX: phase design and mechanism of retention.

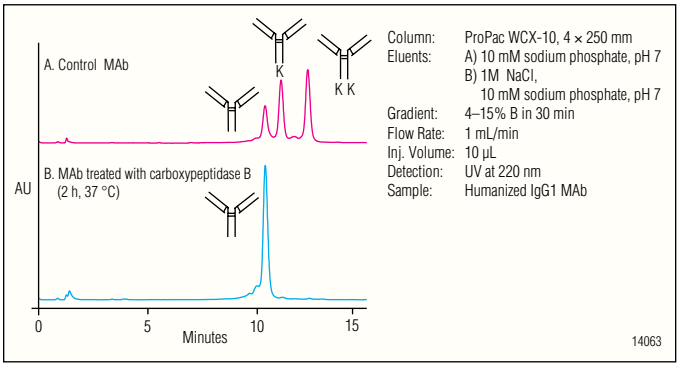

Figure 3. Identification of MAb variants. The humanized IgG1 MAb variants differing in heavy chain C-terminal lysine content were resolved using a shallow NaCl gradient. Differences in C-terminal lysine were verified by treatment of the MAb with carboxypeptidase B, an enzyme that cleaves C-terminal lysine residues.

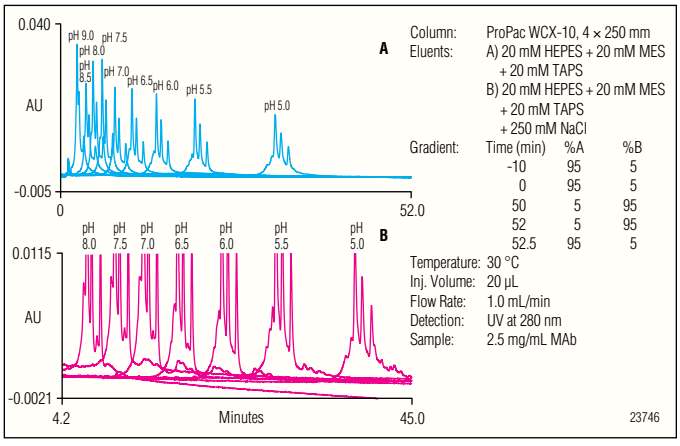

Figure 4. Effect of pH on MAb separation. Panel A displays from pH 5.0 to 9.0. Panel B shows a zoomed version to point out variants separation from pH 5.0 to 8.0. Excellent separation was achieved at pH 5.5.

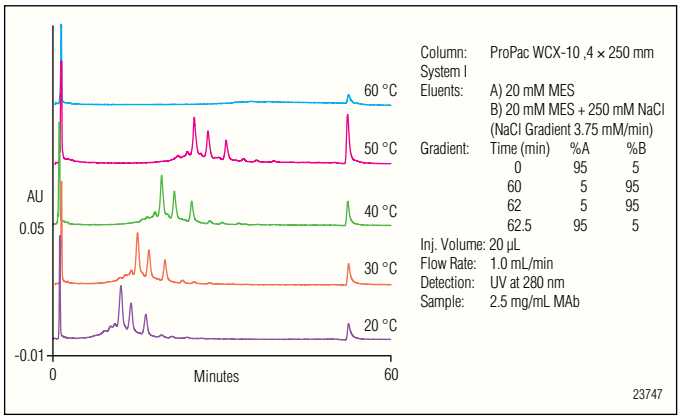

Figure 5. Effect of temperature (20 °C to 60 °C) on MAb separation at pH 5.5.

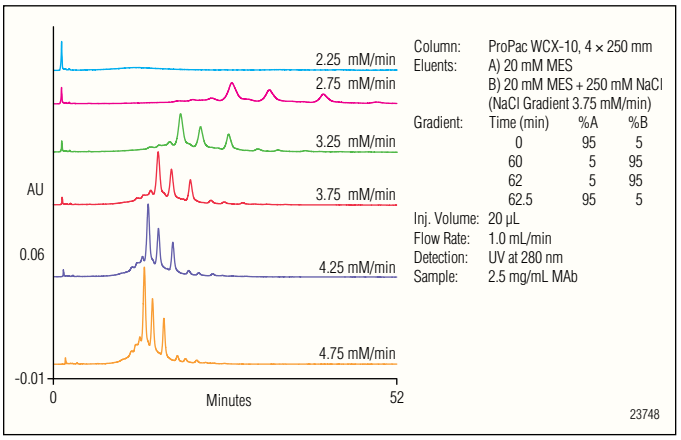

Figure 6. Effect of salt gradients (4.75 mM/min to 2.25 mM/min) on MAb separation at pH 5.5.

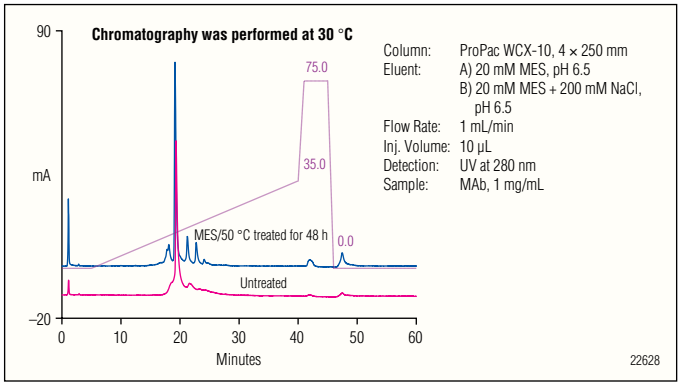

Figure 7. Effect of MES/50 °C treatment on WCX-MAb separation. The ProPac WCX-10 column was treated with 20 mM MES pH 6.5 at 50 °C for 48 h. The treated column was used to separate MAb and compared with an untreated column. The chromatography was performed at 30 °C.

ProSwift WCX

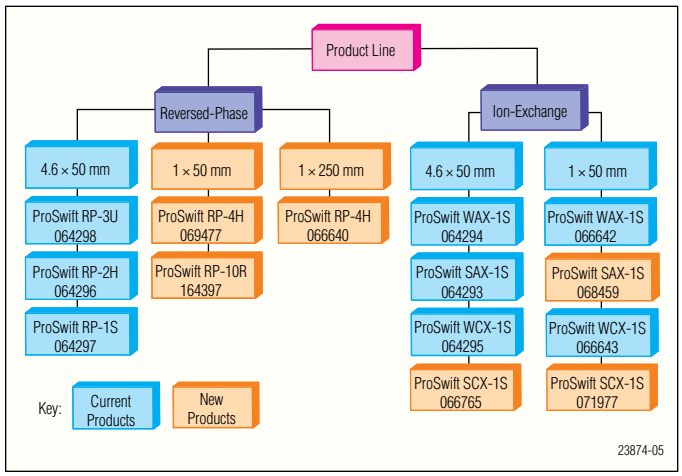

Figure 8. Dionex ProSwift family columns.

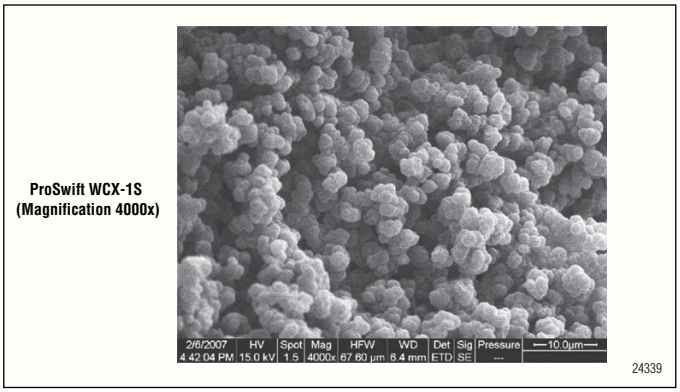

Figure 9. Morphology of ProSwift WCX-1S: SEM image.

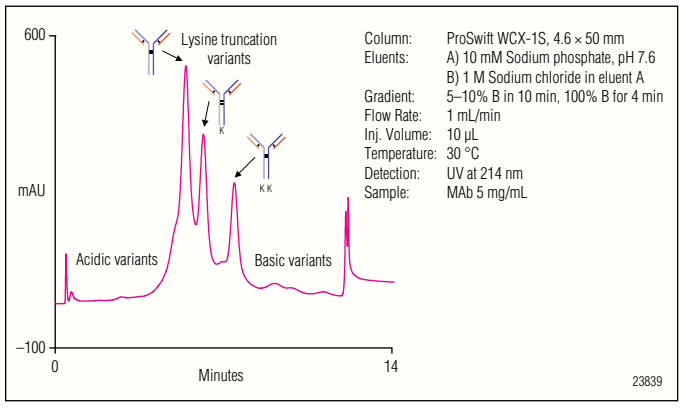

Figure 10. Separation of MAb on ProSwift WCX-1S.

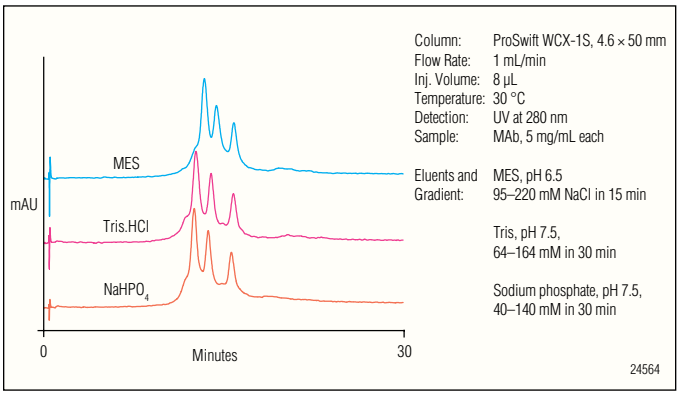

Figure 11. MAb separated on the ProSwift WCX-1S using different buffer conditions.

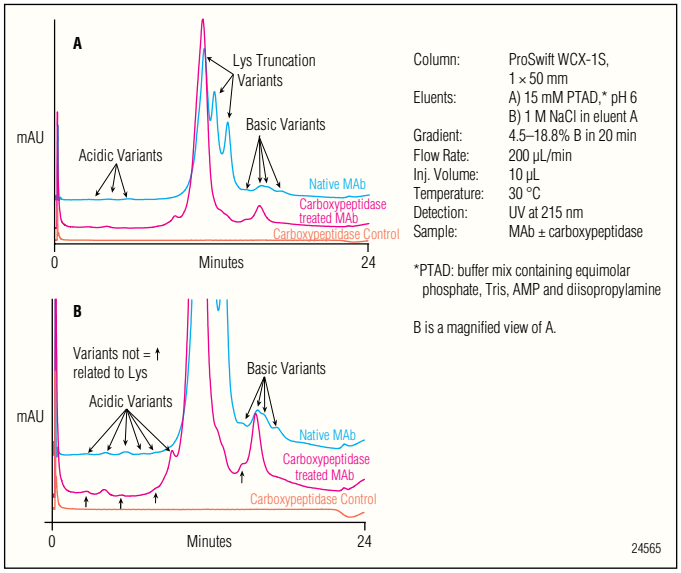

Figure 12. Assay of MAb variants (PTAD buffer) with and without carboxypeptidase.

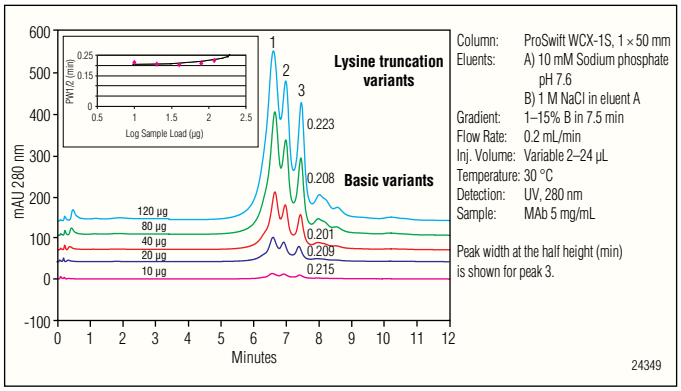

Figure 13. Loading capacity of ProSwift WCX-1S.

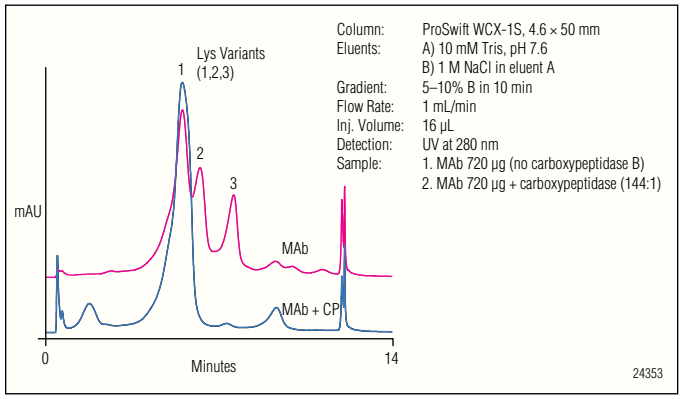

Figure 14. Assay of MAb variants with and without carboxypeptidase on WCX-1S.

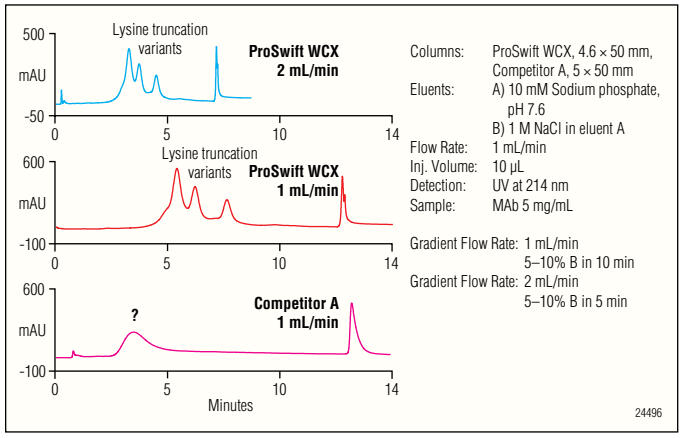

Figure 15. Comparison of ProSwift WCX-1S with competitor A: separation of MAb.

ProSwift WCX—
RUGGEDNESS AND STABILITY

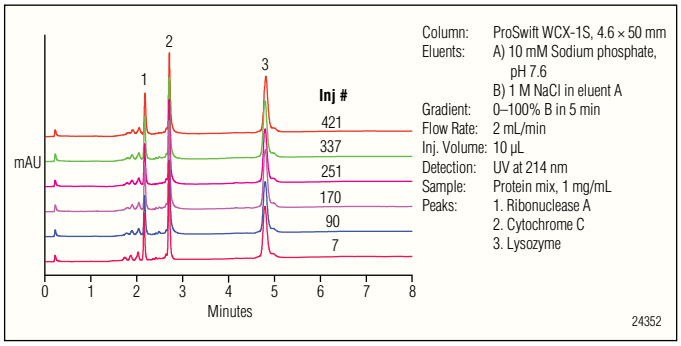

Figure 16. Ruggedness of ProSwift WCX-1S.

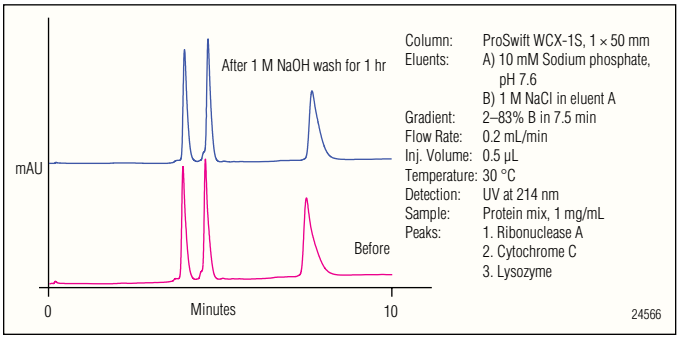

Figure 17. ProSwift WCX-1S: Base stability.

CONCLUSION

The ProPac WCX-10 column is used routinely for separation of acidic and basic MAb variants, papain digested MAb components, and for other protein applications.

The authors have studied effect of pH, temperature, and different salt gradients on MAb separation using ProPac WCX-10 column. All these parameters appear to influence MAb separation and therefore play an important role in method development.

The authors have suggested a simple conditioning step to improve and obtain the maximum MAb resolution: treating the column at 50 °C for 48 h in a 20 mM MES buffer at pH 5.6 or 6.5 or, alternatively, treating the column at 70–80 °C for 7–8 h. When MAbs are separated on these MES/heat conditioned columns, they exhibited higher efficiency, better peak shapes, and improved overall performance compared to unconditioned control columns. Once the column is heat treated, there is no need to repeat this step for subsequent runs. The authors assume that this change is permanent, although the authors have not yet evaluated how long this change may last. The authors hypothesize that change in the conformation of the polymer grafts may be responsible for this improved performance.

ProSwift media are based on polymeric monoliths prepared by an in situ polymerization process. They are a new generation of separation media, uniquely designed and engineered for fast separation of biomolecules.

ProSwift monolith columns offer significant advantages over conventional porous columns. They include fast mass transfer, high loading capacity, and wide pH stability. These exclusive characteristics support versatile performance in a wide range of biomolecule separations including monoclonal antibodies.

ProSwift WCX columns are available in different dimensions: 4.6 × 50 mm and 1.0 × 50 mm. Columns with 1 mm i.d. offer improved sensitivity and reduced solvent consumption.

ProSwift WCX columns are rugged and offer high capacity with excellent resolution making them suitable to be used in the first dimension of multidimensional chromatography.

REFERENCES

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