columns

ProPac® Ion Exchange Columns for Protein Analysis

Ion-Exchange Columns for Characterization and Quality Control Assessment of Closely Related Protein Variants:

- *Sequence*
- *Truncation*
- *Phosphorylation*
- *Sialylation*
- *Deamidation*

Now sold under the Thermo Scientific brand

Thermo SCIENTIFIC

Unique Chemistry for High-Resolution Analysis of Proteins with Small Differences in Charge

Dionex developed the ProPac line of ion-exchange columns specifically to provide high-resolution, high-efficiency separations of proteins and glycoproteins (*pI* = 3–10; MW > 10,000). The rigid, nonporous pellicular resin provides exceptionally high resolving power, permitting the separation of proteins that differ by as little as one charged residue. Hydrophobic interactions with the resin are eliminated for very efficient peaks.

ProPac columns are designed to address the regulatory requirements for biopharmaceutical characterization. The reproducible resin chemistry and manufacturing processes eliminate column variability as a concern in methods development and data analysis.

ProPac columns are available with weak or strong anion-exchange or cation-exchange resins packed in 2-, 4-, 9-, and 22-mm i.d. formats. The selectivity differences between the various resin types provide flexibility in maximizing the resolution of closely related proteins.

DIONEX

HPLC | IC | EXTRACTION | PROCESS | AUTOMATION

Sequence Variants

Clinical assays of hemoglobin sequence variants are important in the diagnosis of hemoglobinopathies.[1] The strong cation-exchange resin of the ProPac SCX-10 produces a rapid, high-resolution separation of hemoglobin variants. Figure 1 shows several commonly observed hemopathic hemoglobins.[2] The high rate of mass transfer associated with the pellicular resin affords efficient peaks and high resolution.

Truncation Variants (MAbs)

The weak cation-exchange packing of the ProPac WCX-10 is a unique resin with a hydrophilic coating and carboxylate functional groups. The physicochemical properties of this support eliminate secondary interactions between the protein analytes and the stationary phase. This results in minimum band spreading and high selectivity, as illustrated in the baseline resolution of C-terminal variants of an IgG_1 monoclonal antibody (MAb) (Figure 2).[1]

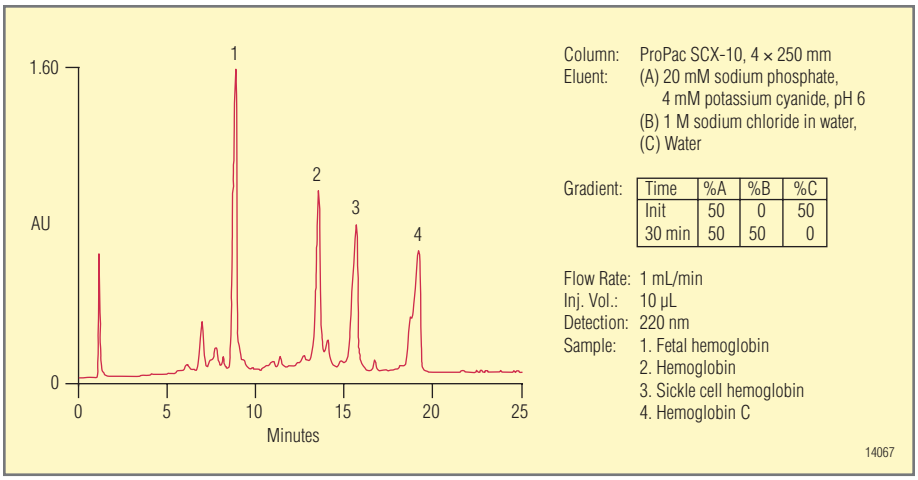

Figure 1. Separation of hemoglobin variants, including fetal (HbF), sickle cell (HbS), normal (HbAo), and hemoglobin C (HbC).

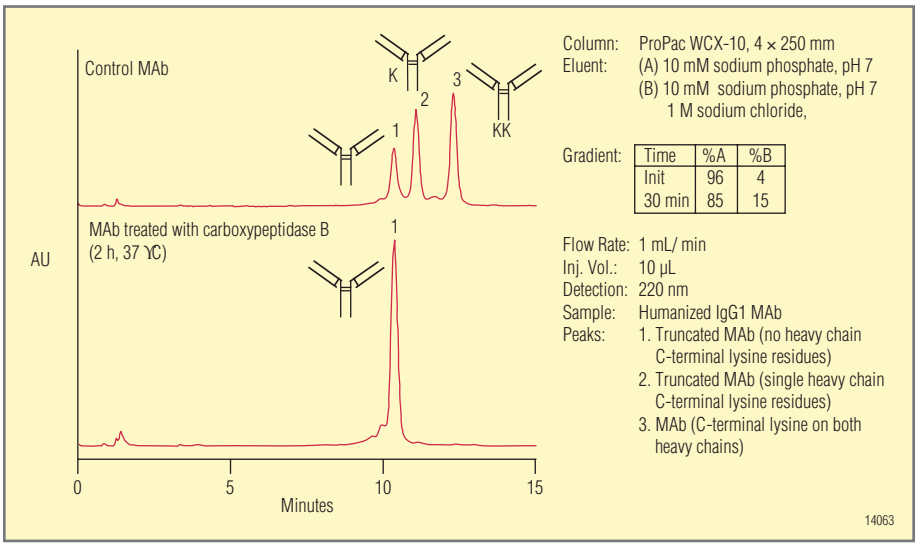

Figure 2. Analysis of an IgG_1 MAb before and after treatment with carboxypeptidase B for two hours at 37 ºC.

Phosphorylation Variants

The resolution of phosphorylation variants is important in the characterization of biomacromolecules.[3] The high efficiency of the strong anion-exchange resin of the ProPac SAX-10 allows for the resolution of several phosphorylation isoforms of ovalbumin using a simple linear gradient (Figure 3, bottom trace). After treatment with alkaline phosphatase, the eight resolved isoforms simplify to one major peak and three minor peaks (Figure 3, top trace).

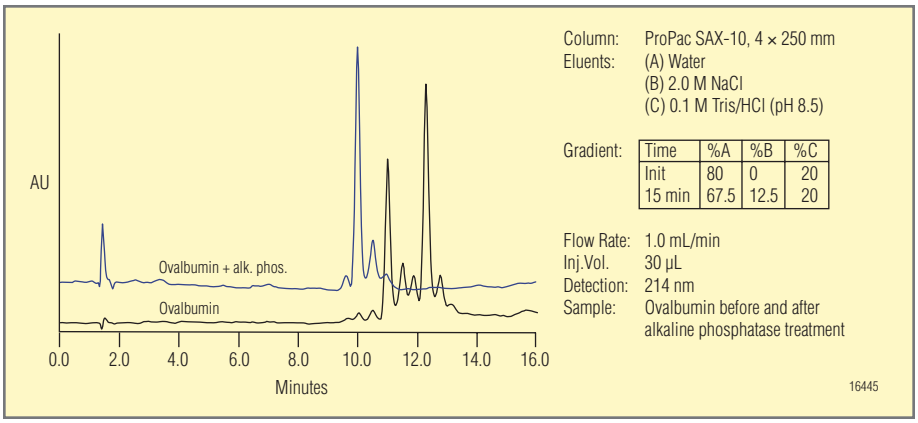

Figure 3. Resolution of phosphorylation variants of ovalbumin.

Sialylation Variants

Published data suggest that different isoform profiles of transferrin are diagnostic of different clinical conditions.[4] Using the ProPac SAX-10, the differences in elution profile resulting from differences in sialylation can be seen (Figure 4).[5] When the different transferrin samples are digested with neuraminidase to remove sialic acid, the profiles collapse into a similar pattern.

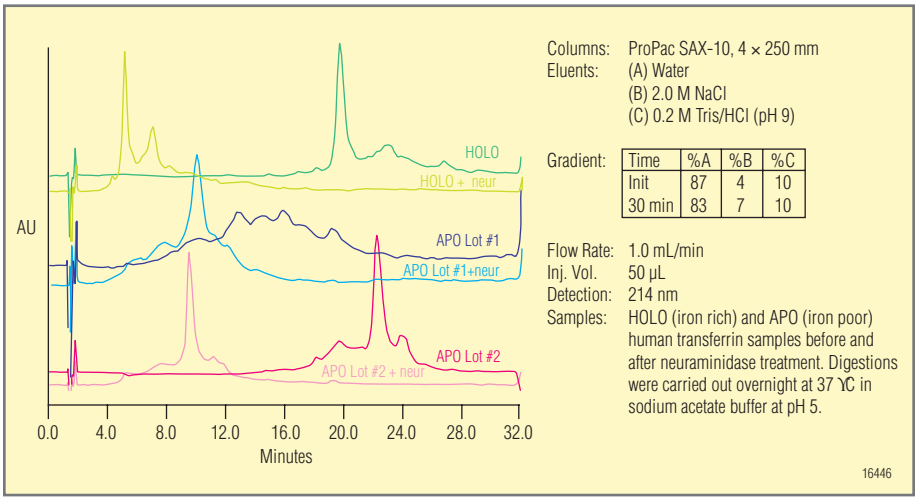

Figure 4. Effect of sialylation on transferrin chromatography.

Deamidation Variants

Determining the deamidation of asparagine (Asn) residues is a significant challenge for analytical and protein chemists in the QA and process departments at biotechnology and pharmaceutical companies.[6] The unique selectivity of the ProPac columns allows the rapid analysis of protein deamidation with baseline resolution in a single step, as illustrated in Figure 5 for the separation of ribonuclease A and its two deamidation products.[1] The baseline resolution allows quantification of each form of the protein as a function of time.

Unique Resin Technology

The ProPac resin technology is the basis for the superior performance of these ion-exchange columns. The thin, hydrophilic layer grafted to the particle core eliminates hydrophobic interactions with proteins (Figure 6). To this layer are grafted the unique polymer chains (containing the anion- or cation-exchange groups) that impart selectivity and reproducible capacity.

References

1. Basset, P.; Braconnier, F.; Rosa, J. *J. Chromatogr.* **1982,** *227*, 267–304.
2. Weitzhandler, M.; Farnan, D.; Horvath, J; Rohrer, J.; Slingsby, R.; Avdalovic, N.; Pohl, C. *J. Chromatogr. A* **1998,** *828*, 365–372.
3. Frenz, J.; Quan, C. P.; Cacia, J.; Democko, C.; Bridenbaugh, R.; McNerney, T. *Anal. Chem.* **1994,** *66*, 335–340.
4. Stibler, H. *Clin. Chem.* **1991,** *371*, 2029–2039.
5. Rohrer, J. S.; Avdalovic, N. *Protein Expression and Purification*, **1994,** *7*, 39–44.
6. Aswad, D. W. *Deamidation and Isoaspartate Formation in Peptides and Proteins;* CRC Series in Analytical Biotechnology; CRC Press: Boca Raton, FL, **1995**.

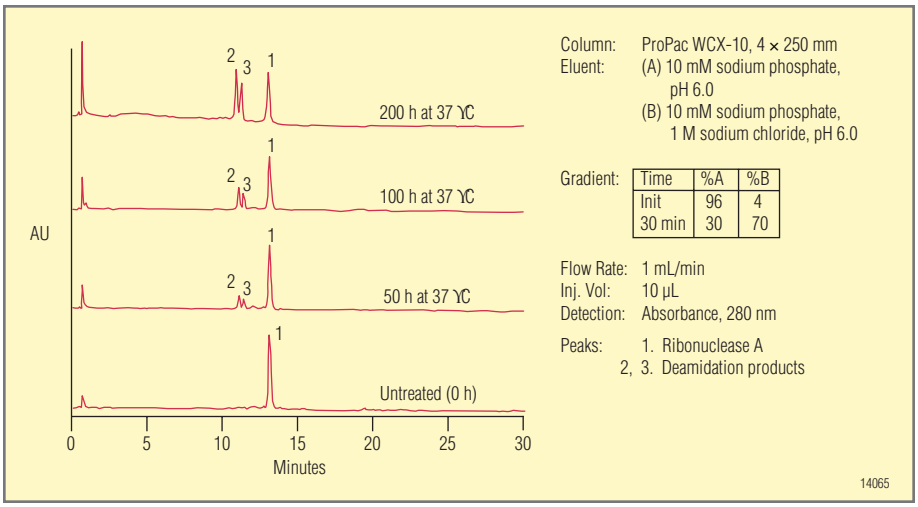

Figure 5. Separation of ribonuclease A and its two deamidation products during the course of forced deamidation. Ribonuclease A (3 mg/mL) was incubated in 1% ammonium carbonate buffer, pH 8.2, at 37°C.

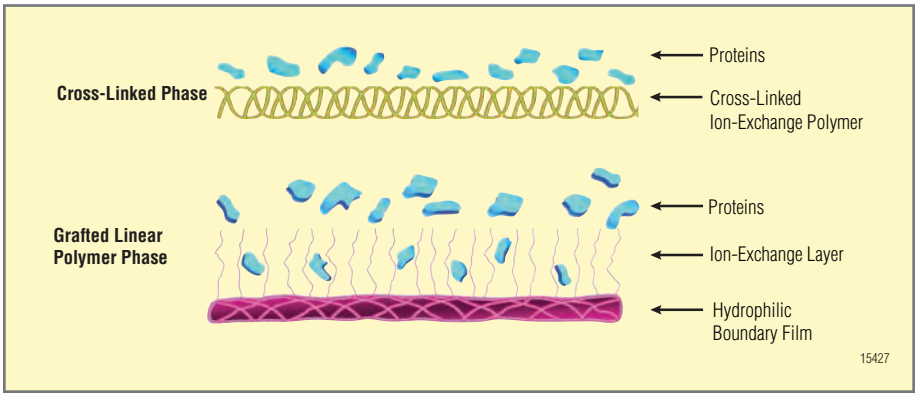

Figure 6. Protein interaction with cross-linked phase compared to grafted linear polymer phase.

SPECIFICATIONS

Maximum Operating Pressure:
21 MPa (3,000 psi)

Chemical Compatibility:
Solvents: 80% MeCN, acetone for all ProPac columns (SAX, WAX, SCX, WCX); 80% methanol for SAX, WAX, and SCX only. Alcohols are incompatible with the WCX-10.

Detergents: Nonionic and zwitterionic are compatible. Cationic detergents are incompatible with cation-exchange columns (WCX-10, SCX-10); anionic detergents are incompatible with anion-exchange columns (WAX-10, SAX-10).

pH Range: 2–12

Typical Buffers: Phosphate, acetate, Tris

Temperature:
≤40 °C

Column Construction:
PEEK with 10–32 threaded, ferrule-style end fittings. All components are nonmetallic.

Resin Composition:
Substrate Bead Diameter: 10 µm

Substrate Composition: Ethylvinyl-benzene 55% cross-linked with divinylbenzene

Pellicular Layer: Proprietary, neutral, hydrophilic

Functional Groups:
WCX-10: Carboxylate

SCX-10: Sulfonate

WAX-10: Tertiary amine

SAX-10: Quaternary ammonium

Column Capacity:
Depending on the specific proteins and resolution of the protein peaks, >100 µg of protein can be injected on a 4 × 250 mm column without loss of resolution.

ORDERING INFORMATION

In the U.S., call 1-800-346-6390 or contact the Dionex regional office nearest you. Outside the U.S., order through your local Dionex office or distributor.
Refer to the following part numbers. Dionex can also make special-order ProPac columns to your specifications; call for more information.

Description	Part Number
ProPac Weak Cation-Exchange Columns (Carboxylate Functionality)	
ProPac WCX-10 Analytical Column (4 × 250 mm)	054993
ProPac WCX-10G Guard Column (4 × 50 mm)	054994
ProPac WCX-10 Analytical Column (2 × 250 mm)	SP5249
ProPac WCX-10G Guard Column (2 × 50 mm)	SP5251
ProPac WCX-10 Semipreparative Column (9 × 250 mm)	SP5480
ProPac WCX-10 Semipreparative Column (22 × 250 mm)	SP5482
Lot select column set—Three columns from one resin lot (4 × 250 mm)	SP5512
Lot select column set—One column from each of three resin lots (4 × 250 mm)	SP5513
ProPac Strong Cation-Exchange Columns (Sulfonate Functionality)	
ProPac SCX-10 Analytical Column (2 × 250 mm)	SP5572
ProPac SCX-10G Guard Column (2 × 50 mm)	SP5574
ProPac SCX-10 Analytical Column (4 × 250 mm)	054995
ProPac SCX-10G Guard Column (4 × 50 mm)	079930
ProPac SCX-10 Semipreparative Column (9 × 250 mm)	SP5520
ProPac SCX-10 Semipreparative Column (22 × 250 mm)	SP5522
Lot select column set—Three columns from one resin lot (4 × 250 mm)	SP5727
Lot select column set—One column from each of three resin lots (4 × 250 mm)	SP5728
ProPac Weak Anion-Exchange Columns (Tertiary Amine Functionality)	
ProPac WAX-10 Analytical Column (2 × 250 mm)	SP5558
ProPac WAX-10G Guard Column (2 × 50 mm)	SP5560
ProPac WAX-10 Analytical Column (4 × 250 mm)	054999
ProPac WAX-10G Guard Column (4 × 50 mm)	055150
ProPac WAX-10 Semipreparative Column (9 × 250 mm)	SP5596
ProPac WAX-10 Semipreparative Column (22 × 250 mm)	SP5598
Lot select column set—Three columns from one resin lot (4 × 250 mm)	SP5729
Lot select column set—One column from each of three resin lots (4 × 250 mm)	SP5730

ORDERING INFORMATION

ProPac Strong Anion-Exchange Columns (Quaternary Ammonium Functionality)

ProPac SAX-10 Analytical Column (2 × 250 mm)..... SP5554

ProPac SAX-10G Guard Column (2 × 50 mm) SP5556

ProPac SAX-10 Analytical Column (4 × 250 mm).....054997

ProPac SAX-10G Guard Column (4 × 50 mm) 054998

ProPac SAX-10 Semipreparative Column (9 × 250 mm) SP5592

ProPac SAX-10 Semipreparative Column (22 × 250 mm) SP5594

Lot select column set—Three columns from one resin lot
(4 × 250 mm)..... SP5731

Lot select column set—One column from each of three resin lots
(4 × 250 mm)..... SP5730

Passion. Power. Productivity.

DIONEX

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