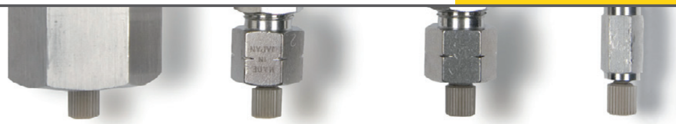

TOSOH

TSKgel® Butyl-NPR

INTRODUCTION

Hydrophobic Interaction chromatography (HIC) is based on the interaction between hydrophobic patches on the protein surface and a hydrophobic ligand on the solid support. HIC sorts biomolecules by degree of their surface hydrophobicity. Samples are adsorbed to the stationary phase at relatively high salt concentrations and eluted by applying a decreasing salt gradient. The mild conditions used in HIC separation of peptides and proteins typically maintain protein structure and biologic activity.

HIGHLIGHTS

- Small, non-porous 2.5 µm micron particles
- Dimensions optimized for high speed or high resolution analysis, respectively
- Hydrophilic polymer base matrix enables high recovery of proteins and trace analysis
- High chemical stability and wide pH range (pH 2 – pH 10)

FEATURES

Non-porous resins (NPR) are typically used for high-speed analytical applications. The base material of TSKgel Butyl-NPR consists of spherical 2.5 µm non-porous polymethacrylate particles. TSKgel Butyl-NPR columns provide fast and quantitative HIC, because smaller particles provide higher efficiency. By packing the 2.5 µm non-porous resin particles into short columns, typical analysis times are reduced to less than 10 minutes. TSKgel Butyl-NPR columns are available in 3.5 cm length for fast analysis and 10 cm length for high resolution.

Pore diffusion is often the rate-limiting step in the overall mass transport of large biomolecules through a porous stationary phase. Eliminating the pores provides higher resolution at higher flow rates.

Another benefit of NPR media is excellent mass recovery, allowing quantitation down to nanogram levels. Table 1 shows the recovery of some standard proteins with TSKgel Butyl-NPR. These properties make TSKgel Butyl-NPR the preferred choice for process monitoring and quality control.

RECOVERY OF PROTEINS

Proteins	Recovery (%)
Myoglobin	96
Ribonuclease	90
Lysozyme	102
Alpha-Chymotrypsin	95
Alpha-Chymotrypsinogen A	98
Trypsin inhibitor	83
Ovalbumin	92

Table 1

Column: TSKgel Butyl-NPR (4.6 mm ID x 3.5 cm);
Mobile phase: A: 0.1 mol/L phosphate buffer (pH 7.0) + 2.3 mol/L ammonium sulfate, B: 0.1 mol/L phosphate buffer (pH 7.0); Gradient: 0 - 100% mobile phase B, 12 min, linear; Flow rate: 1.5 mL/min;
Detection: UV @ 280 nm; Sample load: 5 µm

APPLICATIONS

TSKgel Butyl-NPR can be applied in a broad range of applications ranging from the separation of the open circular and supercoiled forms of plasmid DNA to the analysis of modified or aggregated peptides and proteins. Figure 1 shows the separation of five standard proteins on a short TSKgel Butyl-NPR column within ten minutes.

FAST SEPARATION OF PROTEINS

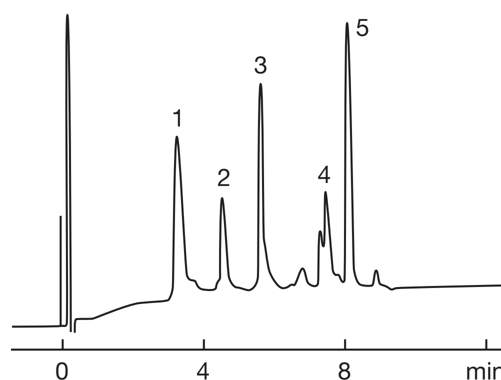

Figure 1

Column: TSKgel Butyl-NPR (4.6 mm ID × 3.5 cm);
Mobile phase: A: 0.1 mol/L phosphate buffer (pH 7.0) + 2.3 mol/L ammonium sulfate; B: 0.1 mol/L phosphate buffer (pH 7.0); Gradient: 0 - 100 % mobile phase B, 12 min, linear; Flow rate: 1.0 mL/min; Detection: UV @ 280 nm; Samples: 1. myoglobin (4 µg) 2. ribonuclease (4 µg) 3. lysozyme (1.5 µg) 4. α-chymotrypsin (3 µg) 5. α-chymotrypsinogen A (2 µg)

ANALYSIS OF PROTEIN OXIDATION

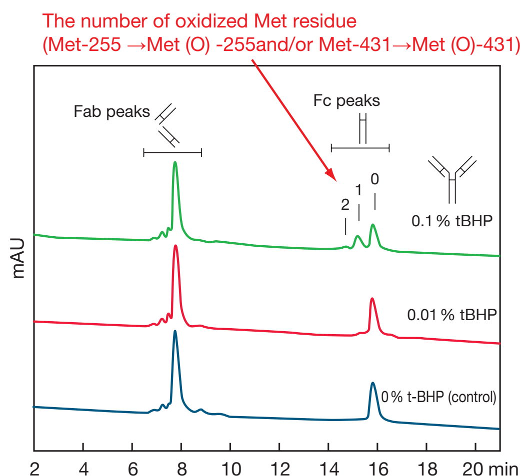

Figure 2

Column: TSKgel Butyl-NPR (4.6 mm ID × 10 cm)
Mobile phase A: 20 mmol/L phosphate buffer + 2 mol/L ammonium sulfate (pH 7.0); B: 20 mmol/L phosphate buffer (pH 7.0); Gradient: 25 to 60 % mobile phase B, 20 min, linear; Flow rate: 1.0 mL/min;
Detection: UV @ 215 nm; Temp.: 35 °C; Inj. volume: 2 µL;
Sample conc.: 2 g/L; Sample: enzymatic digest of antibody therapeutics;
Oxidation: 0.01 % / 0.1 % tert-butylhydroperoxide (tBHP) was added to antibody solution. After digestion by papain aliquots of reacted solution were subjected to HPLC.

ANALYSIS OF PROTEIN DEAMIDATION

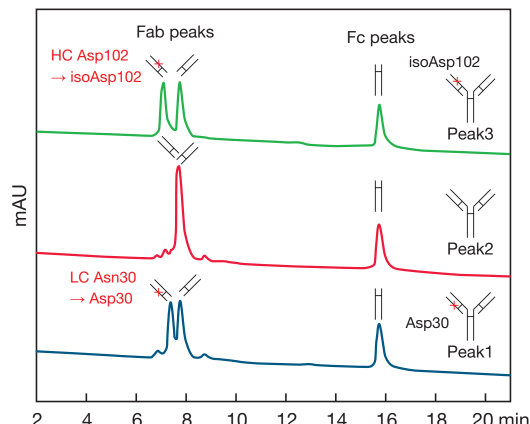

Figure 3

Column: TSKgel Butyl-NPR (4.6 mm ID × 10cm)
Mobile phase A: 20 mmol/L phosphate buffer + 2 mol/L ammonium sulfate (pH 7.0); B: 20 mmol/L phosphate buffer (pH 7.0)
Gradient: 25 to 60 % mobile phase B, 20 min, linear; Inj. volume: 2 µL
Flow rate: 1.0 mL/min; Detection: UV @ 214 nm; Temp.: 35°C;
Sample conc.: 2 g/L Sample: enzymatic digest of therapeutic antibody
The antibody was fractionated by cation-exchange chromatography (TSKgel BioAssist® S). Each fraction was digested by papain and aliquots were subjected to HPLC.

Figure 2 shows the analysis of oxidation variants of monoclonal antibodies. The antibody was cleaved by papain and subjected to HPLC analysis. The number of oxidized methionine residues can be easily detected.

Figure 3 shows the analysis of structural differences of a therapeutic antibody. Charge variants were fractionated by cation-exchange chromatography (CEC), cleaved with papain and fragments were analyzed by HIC. The main CEC peak (Peak 2) represents the main form of the antibody without any modification. Deamidation of the light chain asparagine 30 to aspartate in one light chain is responsible for an acidic form (Peak 1). The variant collected in Peak 3 is based on isomerization of one heavy chain aspartate 102.

Figure 4 shows the application of the short TSKgel Butyl-NPR column for the separation of an aggregated mAb sample. As aggregates are more hydrophobic than the corresponding mAb monomers, aggregates, monomers and fragments can easily be identified. The total aggregate content of this sample is about 11 %, which was also confirmed by SEC on TSKgel G3000SWXL, the current industrial standard for mAb aggregate analysis.

SEPARATION OF mAb AGGREGATES AND FRAGMENTS

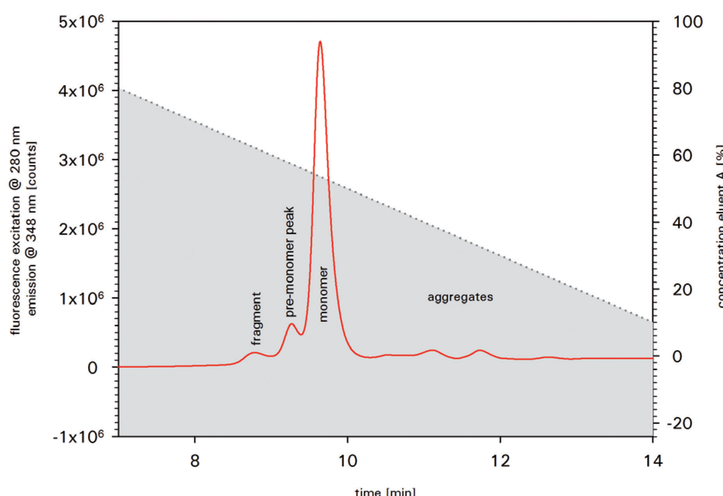

Figure 4

Column: TSKgel Butyl-NPR (4.6 mm ID x 3.5 cm)
Mobile phase A: 10 mmol/L sodium phosphate buffer (pH 7.0) + 3 mol/L NaCl; B: 10 mmol/L sodium phosphate buffer (pH 7.0)
Gradient: 0 - 100 % mobile phase B, 25 min, linear; Flow rate: 1.0 mL/min
Detection: Fluorescence; Sample: aggregated monoclonal antibody

Ordering information

Part-No	Description	Particle Size (µm)	Dimensions
0014947	TSKgel Butyl-NPR	2.5	4.6 mm ID x 3.5 cm L
0042168	TSKgel Butyl-NPR	2.5	4.6 mm ID x 10.0 cm L