About: TSKgel BioAssist S Cation Exchange Columns

Specially designed for the separation of large biomolecules such as antibodies, the large pores of the TSKgel BioAssist S cation exchange column offer superior capacity and resolution at a low column pressure drop. Constructed via a polymerization technique that allows an equivalent density of ionic exchange groups to be incorporated into the particle without reducing pore size, the TSKgel BioAssist S column is unlike other ion exchange columns that use graft polymerization for polymer chain introduction. The TSKgel BioAssist S columns' large pores are very accessible even for high molar mass proteins. This leads to higher chromatographic efficiency and binding capacity for purification.

TSKgel BioAssist S cation exchange columns are offered in a 4.6 mm ID × 5 cm format and a 10 mm ID × 10 cm semi-preparative column for scale up. Both columns are made of PEEK to reduce protein adsorption.

Attributes and Applications:

Table 10 lists the product attributes of TSKgel BioAssist S columns. The pore structure and bonding chemistry of TSKgel BioAssist S columns provide high capacity for medium to large molar mass proteins.

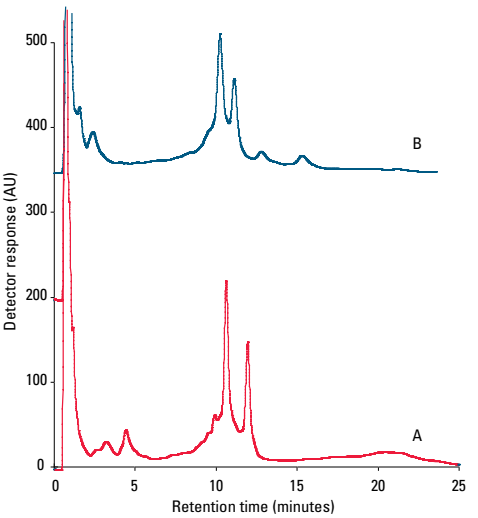
Table 10: Product attributes

Attribute	Value
Matrix	polymethacrylate
Particle size (mean)	7 µm and 13 µm
Pore size (mean)	130 nm
Functional group	sulfopropyl
Counter ion	Na^+
pH stability	2.0-12.0
Capacity (gamma globulin)	70
Small ion capacity	0.1 eq/L
pKa	2.4

Bromelain

The application in Figure 25 shows the analysis of bromelain, a proteolytic enzyme that is used as a nutritional supplement. Bromelain is a basic glycoprotein with a molar mass of 33 kDa and pI of 9.55.

Figure 25: Analysis of bromelain



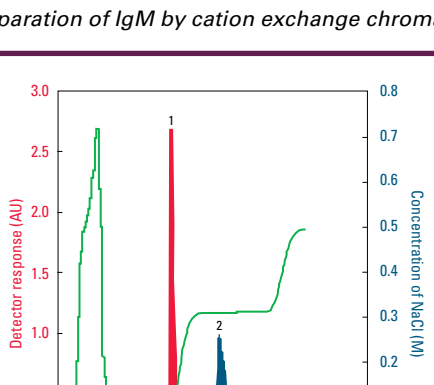
Columns:	**A: TSKgel BioAssist S, 7 µm, 4.6 mm ID × 5 cm**
	B: Competitor S, 5 mm ID × 5 cm
Mobile phase:	20 min (TSKgel) or 30 min (Competitor S) linear gradient of NaCl from 0 to 0.5 mol/L in 20 mmol/L sodium phosphate buffer, pH 7.0
Flow Rate:	0.8 mL/min for TSKgel; 1.0 mL/min for Competitor S
Detection:	UV @ 280 nm
Temperature:	25 °C
Sample:	crude bromelain (C4882, Sigma), 1 mg in 100 L

Immunoglobulin M (IgM)

IgM is known to possess unique and beneficial characteristics relative to other immunoglobulin classes; it is a large molecule comprised of five IgG subunits, resulting in a relatively unstable and difficult to purify protein. Unlike single chain antibodies, IgM cannot be purified by Protein A (an affinity material commonly used for its high binding capacity and excellent selectivity for antibodies) due to steric hindrance. Alternative affinity methods have been developed with thiophillic absorbents but these methods often result in low binding capacity.

An alternative purification method of IgM by ion exchange chromatography using a TSKgel BioAssist S column was developed. As shown in Figure 26, baseline separation of IgM from other contaminants is achieved using a 0.3 mol/L NaCl step gradient after elution of albumin.

Figure 26: Separation of IgM by cation exchange chromatography

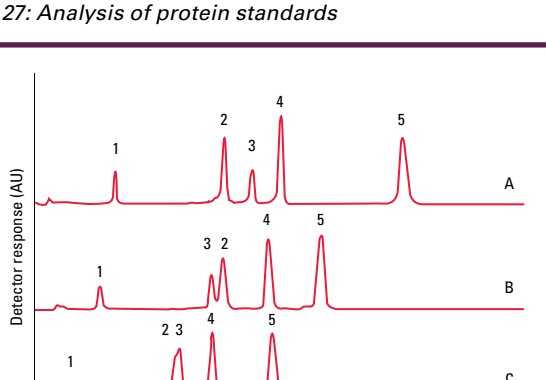


Column:	**TSKgel BioAssist S, 7 µm, 4.6 mm ID × 5 cm**
Mobile phase:	20 mmol/L sodium phosphate buffer, pH 6.0
Gradient	0 mol/L - 0.3 mol/L NaCl (5 min) 0.3 mol/L - 0.5 mol/L NaCl (10 min)
Flow Rate:	1 mL/min
Detection:	UV @ 280 nm
Sample:	500 µL of 9.5 mg/mL IgM in mouse ascites fluid; shaded peaks represent albumin and IgM respectively

Protein Standards

Figure 27 shows a comparison of a standard protein separation on a TSKgel BioAssist S column and conventional ion exchange columns. It is clear that the TSKgel BioAssist S column is more retentive and provides a higher resolution of the sample proteins compared to the conventional products.

Figure 27: Analysis of protein standards

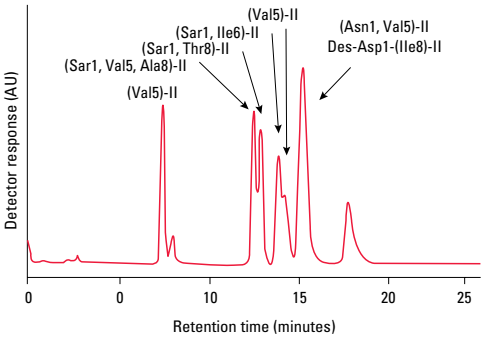


Columns:	**A: TSKgel BioAssist S, 7 µm, 4.6 mm ID × 5 cm** B: Conventional S type product C, 5.0 mm ID × 5 cm C: Conventional S type product D, 4.6 mm ID × 5 cm
Mobile phase:	A: 20 mmol/L sodium phosphate buffer, pH 6.5 B: 20 mmol/L sodium phosphate buffer containing 1.0 mol/L NaCl, pH 6.5
Gradient:	32 min (A-B)
Flow rate:	0.8 mL/min
Detection:	UV @ 280 nm
Temperature:	10 °C
Injection vol.:	20 µL
Samples:	1. myoglobin, 1 g/L 2. α-chymotrypsinogen A, 2 g/L 3. ribonuclease A, 4 g/L 4. cytochrome C, 2 g/L 5. lysozyme, 2 g/L

Peptides

Figure 28 shows chromatograms of peptides on a TSKgel BioAssist S column. It is generally known that an accurate quantification is difficult to obtain when peptides are analyzed on a column with a styrene-type base material, due to secondary interaction with the hydrophobic packing material. However, a TSKgel BioAssist S column is capable of analyzing such peptides as angiotensins without the need to add an organic solvent to the mobile phase since the acrylate packing material is hydrophilic.

Figure 28: Analysis of peptides



Column:	**TSKgel BioAssist S, 7 µm, 4.6 mm ID × 5 cm**
Mobile phase:	A: 20 mmol/L sodium acetate buffer, pH 5.0 B: 20 mmol/L sodium acetate buffer containing 1.0 mol/L NaCl, pH 5.0
Gradient:	A → B linear gradient (20 min)
Detection:	UV @ 280 nm
Temperature:	25 °C

About: TSKgel SP-2SW, CM-2SW, and CM-3SW Cation Exchange Columns

The TSKgel SP-2SW, TSKgel CM-2SW, and TSKgel CM-3SW columns are silica-based columns derivatized with sulfopropyl (SP) and carboxymethyl (CM) ligands to provide a strong cation and weak cation exchange column, respectively. They are used for the separation and analysis of small proteins, peptides, and other biologically active molecules. TSKgel CM-2SW has a smaller pore size than TSKgel CM-3SW.

Attributes and Applications:

Table 11 shows the product attributes of the TSKgel SP-2SW, TSKgel CM-2SW, and TSKgel CM-3SW columns. These columns are typically used for analyzing smaller molar mass samples such as nucleotides, drug candidates, catecholamines, and small peptides or proteins.

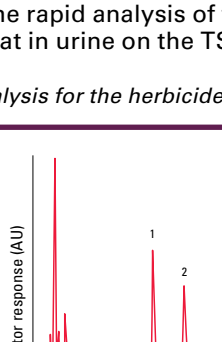
Table 11: Product attributes

TSKgel column	SP-2SW	CM-2SW	CM-3SW
Matrix	silica		
Particle size (mean)	5 µm	5 µm	10 µm
Pore size (mean)	12.5 nm	12.5 nm	25 nm
Functional group	sulfopropyl	$-CH_2COO^-$	$-CH_2COO^-$
Counter ion	Na^+		
pH stability	2.0-7.5		
Capacity (mg Hb/mL)	ND	110	ND
Small ion capacity	0.3 eq/L	>0.3 eq/L	>0.3 eq/L
pKa	2.2	4.2	4.2

Herbicides

Figure 29 shows the rapid analysis of the herbicides paraquat and diquat in urine on the TSKgel SP-2SW column.

Figure 29: Rapid analysis for the herbicides paraquat and diquat

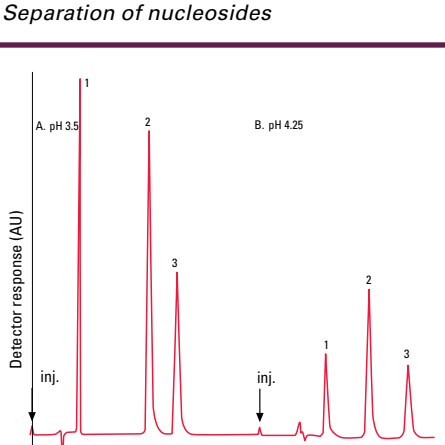


Column: **TSKgel SP-2SW, 5 µm, 4.6 mm ID × 25 cm**
Mobile phase: 20% CH_3CN in 0.2 mol/L phosphate, pH 3.0
Flow rate: 1.0 mL/min
Detection: UV @ 290 nm
Samples: 1. paraquat, 5 g/mL
2. diquat, 5 g/mL

Nucleosides

Silica-based cation exchange columns are typically used in the separation of low molar mass compounds, such as pharmaceuticals, nucleotides, and small peptides. For example, Figure 30 shows the separation of nucleosides on the TSKgel SP-2SW column.

Figure 30: Separation of nucleosides



Column: **TSKgel SP-2SW, 5 µm, 4.6 mm ID × 25 cm**
Mobile phase: A: 0.1 mol/L sodium citrate - phosphoric acid buffer, pH 3.5
B: 0.1 mol/L sodium citrate - acetic acid buffer, pH 4.25
Flow rate: 0.75 mL/min
Detection: UV @ 260 nm
Temperature: 23 °C
Samples: nucleoside standards:
1. guanosine 2. cytidine 3. adenosine

About: TSKgel SP-5PW and CM-5PW Cation Exchange Columns

The polymethacrylate-based resin, TSKgel 5PW, is a spherical 10 µm particle with approximately 100 nm pores. It is derivatized with sulfopropyl (SP) ligands to provide the strong cation exchange column, TSKgel SP-5PW, and with carboxymethyl (CM) ligands to provide the weak cation exchange column, TSKgel CM-5PW.

TSKgel CM-5PW columns are used for the separation and analysis of proteins, peptides, and other biologically active molecules. These columns are offered in dimensions of 7.5 mm ID × 7.5 cm in stainless steel housing.

TSKgel SP-5PW columns are also used for the separation and analysis of proteins, peptides, and other biologically active molecules. These columns are available in internal diameters varying from 2 mm to 21.5 mm and in column housings of either glass or stainless steel.

Attributes and Applications:

Table 12 lists the product attributes of TSKgel SP-5PW and CM-5PW columns. These columns are an excellent choice for analyzing biologically active molecules. TSKgel SP-5PW and CM-5PW columns are stable over the pH range of 2.0 – 12.0 and the porous particles have a mean pore size of 100 nm.

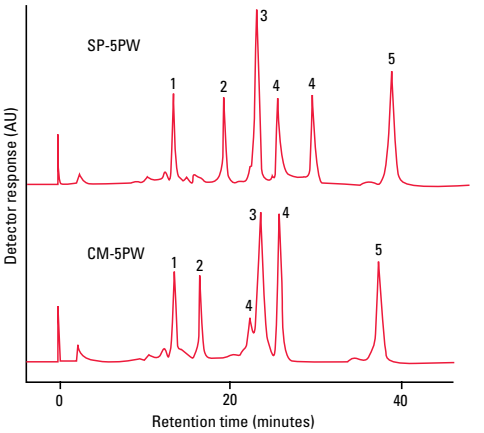
Table 12: Product attributes

TSKgel column	SP-5PW	CM-5PW
Matrix	polymethacrylate	
Particle size (mean)	10 µm, 13 µm, and 20 µm	10 µm and 13 µm
Pore size (mean)	100 nm	
Functional group	$-(CH_2)_3SO_3^-$	$-CH_2COO^-$
Counter ion	Na^+	
pH stability	2.0-12.0	
Capacity (mg Hb/mL):	40	45
Small ion capacity	>0.1 eq/L	
pKa	2.3	4.2

Differences in Selectivity

Differences in selectivity between strong (TSKgel SP-5PW) and weak (TSKgel CM-5PW) cation exchange columns are demonstrated in Figure 31, which is a separation of globular proteins.

Figure 31: Selectivity of strong and weak TSKgel cation exchange columns

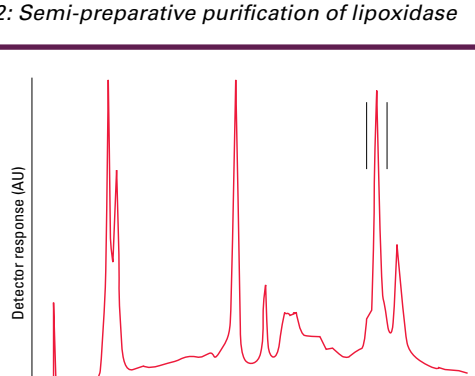


Columns: **TSKgel SP-5PW and TSKgel CM-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase: 60 min linear gradient from 0 mol/L to 0.5 mol/L NaCl in 0.02 mol/L phosphate, pH 7.0
Flow rate: 1.0 mL/min
Detection: UV @ 280 nm
Samples:
1. trypsinogen
2. ribonuclease A
3. α-chymotrypsinogen
4. cytochrome C
5. lysozyme

Crude Lipoxidase

The purification of 200 mg of crude lipoxidase on a 21.5 mm ID TSKgel SP-5PW column is illustrated in Figure 32. Scale up is simplified as only the particle size changes from 10 µm (7.5 mm ID) to 13 µm (21.5 mm ID) or 20 µm (55 mm ID) columns.

Figure 32: Semi-preparative purification of lipoxidase

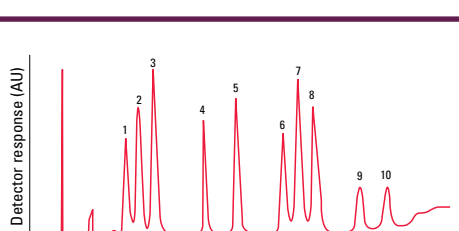


Column:	**TSKgel SP-5PW, 13 µm, 21.5 mm ID × 15 cm**
Mobile phase:	120 min linear gradient from 0 mol/L to 0.5 mol/L Na_2SO_4 in 0.02 mol/L acetate, pH 4.5
Flow rate:	4.0 mL/min
Detection:	UV @ 280 nm
Recovery:	lipoxidase activity collected between the two vertical lines was 84%
Sample:	crude lipoxidase, 200 mg

Peptides

One of the common HPLC modes for analysis and separation of peptides is cation exchange. Figure 33 shows that separations of peptides can be efficiently separated on the strong cation exchange column TSKgel SP-5PW.

Figure 33: Separation of peptide mixture



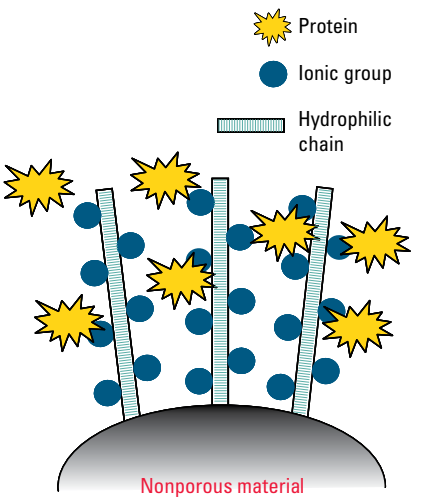
Column:	**TSKgel SP-5PW, 10 µm, 7.5 mm ID × 7.5 cm**
Mobile phase:	30 min linear gradient from 0.02 mol/L to 0.5 mol/L phosphate, pH 3.0, in 30% acetonitrile
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Injection vol.:	50 µL
Samples:	2 µg each of: 1. oxytocin 2. met-enkephalin 3. TRH 4. α-MSH 5. LH-RH (1 µg) 6. neurotensin 7. α-MSH 8. angiotensin II 9. substance P 10. β-endorphin

About: TSKgel SP-STAT and CM-STAT Cation Exchange Columns

TSKgel CM-STAT and SP-STAT columns are packed with 7 or 10 µm hydrophilic nonporous resin particles of which the surface consists of an open access network of multi-layered weak cation exchange groups (see Figure 34). The innovative bonding chemistry, combined with a relatively large particle size, results in a respectable loading capacity, low operating pressure, and rapid analysis.

Applications for the TSKgel CM-STAT and SP-STAT columns include the separation of proteins, protein aggregates, charge variants of monoclonal antibodies, PEGylated proteins, and peptide digests.

Figure 34: Schematic diagram of TSKgel STAT columns



Attributes and Applications:

Table 13 lists the product attributes of TSKgel CM-STAT and SP-STAT columns. These columns are an excellent choice for high throughput protein separations. Nonporous TSKgel CM-STAT and SP-STAT columns are supplied in stainless steel (SS) housing with SS fittings and PEEK frits and are stable in a pH range from 3.0 – 10.0.

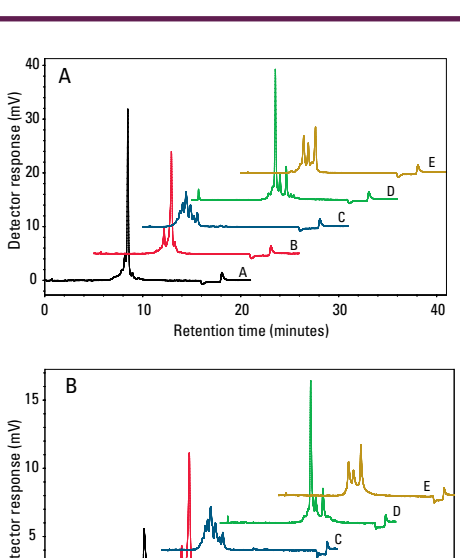
Table 13: Product attributes

TSKgel column	TSKgel CM-STAT	TSKgel SP-STAT
Particle size (mean)	7 µm and 10 µm	
Pore size (mean)	nonporous	
Functional group	carboxymethyl	sulfopropyl
Counter ion	Na^+	
pH stability	3.0-10.0	
Static binding capacity (mg lysozyme/g dry gel)	ca. 20 (7 µm) ca. 15 (10 µm)	ca. 15 (7 µm) ca. 10 (10 µm)
Small ion capacity	100 µeq/g dry gel	23 µeq/g dry gel
pKa	4.9	2.6

Antibody Analysis

The analysis profiles for five antibodies separated on a TSKgel CM-STAT column (Figure 35A) were compared with the profiles obtained on a competitive WCX-10 column (Figure 35B). Similar or higher resolution profiles were obtained on the TSKgel CM-STAT column in approximately half the time.

Figures 35A & 35B: Antibody analysis

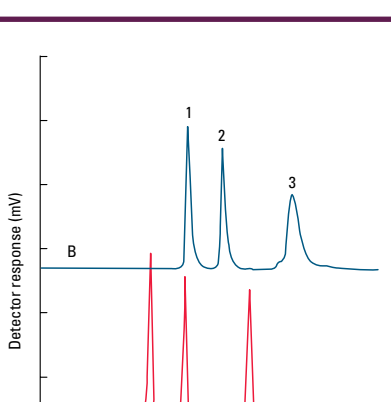


Columns:	**A: TSKgel CM-STAT, 7 µm, 4.6 mm ID × 10 cm**
	B: ProPac WCX-10, 10 µm, 4 mm ID × 25 cm
Mobile phase:	A: 20 mmol/L MES, pH 6.0
	B: 20 mmol/L MES + 0.5 mol/L NaCl, pH 6.0
Gradient:	A: 0 min (10% B) 15 min (30% B) 15 min (100% B) 17 min (0% B) 17 min (10% B) 21 min (10% B)
	B: 0 min (10% B) 30 min (30% B) 30 min (100% B) 32 min (100% B) 32 min (10% B) 36 min (10% B)
Flow rate:	A: 1.0 mL/min B: 2.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	20 µL
Samples:	monoclonal antibodies (mAb A through E)

Protein Standards

The fast separation of protein standards was investigated using short cation exchange columns (see Figure 36). A TSKgel SP-STAT column shows superior resolution, better peak shape, and a shorter analysis time (<60 seconds) compared to a competitive monolithic SP-type column.

Figure 36: Fast separation of protein standards

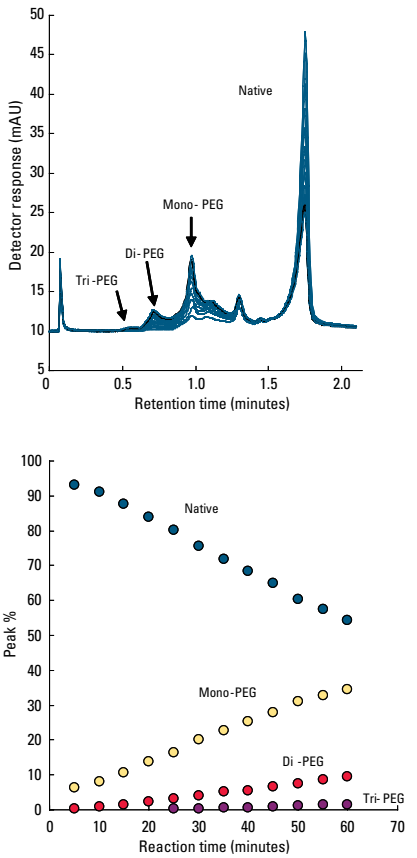


Columns:	**A: TSKgel SP-STAT, 10 µm, 3.0 mm ID × 3.5 cm**
	B: ProSwift® SCX-1S Monolith, 4.6 mm ID × 5 cm
Mobile phase:	A: 20 mmol/L sodium acetate, pH 5.0
	B: 1.0 mol/L NaCl in mobile phase A, pH 5.0 for column A
	1.5 mol/L NaCl in mobile phase A, pH 5.0 for column B
Gradient:	0 min (0% B) 1 min (100% B)
Flow rate:	A: 2.0 mL/min
	B: 4.73 mL/min
Detection:	UV @ 280 nm
Samples:	1. α-chymotrypsinogen A
	2. cytochrome C
	3. lysozyme

Reaction Monitoring

A sample of β-lactoglobulin (5 mg/mL) was reacted with polyethylene glycol (5 kDa) in a pH 6.5 phosphate buffer. The formation of pegylated protein reaction products was monitored in 5 minute intervals on a 3.5 cm TSKgel SP-STAT column. As demonstrated in Figure 37, peak areas of mono-, di-, and tri-pegylated β-lactoglobulin increased with reaction time, while the area of unreacted β-lactoglobulin declined.

Figure 37: Monitoring of reaction products

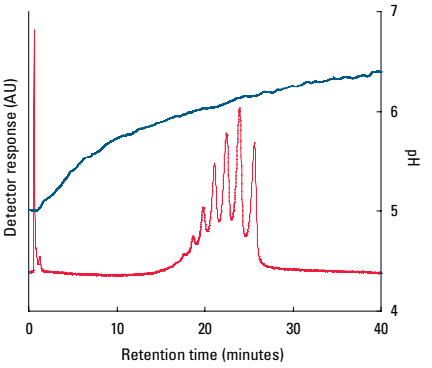


Column:	**TSKgel SP-STAT, 10 µm, 3 mm ID × 3.5 cm**
Mobile phase:	A: 20 mmol/L sodium acetate buffer, pH 5.0
	B: 1.0 mol/L NaCl in mobile phase A, pH 5.0
Gradient:	0 min (0%B) 2 min (100%B)
Flow rate:	2.0 mL/min
Detection:	UV @ 280 nm
Sample:	pegylated β-lactoglobulin

Charge Isomers

As shown in Figure 38, the TSKgel CM-STAT column can also be used to separate charge isomers of a purified monoclonal antibody by pH gradient.

Figure 38: Separation of charge isomers



Column:	**TSKgel CM-STAT, 7 µm, 4.6 mm ID × 10 cm**
Mobile phase:	A: 50 mmol/L sodium acetate buffer, pH 5.0
	B: 30 mmol/L sodium acetate buffer (pH not adjusted)
	Column equilibrated with mobile phase A, the sample is injected, then eluted stepwise to 100% mobile phase B
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	10 µL
Sample:	purified mAb
Sample concentration:	1 g/L

About: TSKgel SCX and OApak-A Cation Exchange Columns

The TSKgel SCX column is packed with porous polystyrene divinylbenzene polymer beads of which the surface has been modified with strong cation exchange groups that are surrounded by Na^+ counterions. This column is optimized for the separation and analysis of organic acids, saccharides, and alcohols. The TSKgel SCX column is also available in the H^+ form for the separation of isomerized sugars, alcohols, and lower organic acids.

A TSKgel OApak-A column is packed with porous hydrophilic polymer beads which have been chemically modified with a weak cation exchange group. This column is optimized for the separation and analysis of organic acids by an ion exclusion mechanism. Applications include: organic acids in fruit juices, wine, beer, coffee, and salt solutions.

The TSKgel OApak-A column is to be used in conjunction with the TSKgel OApak-P guard column which has a strong cation exchange group for the removal of dissociated strong acids under the isocratic mobile phase conditions of 0.75 mmol/L H_2SO_4.

Attributes and Applications:

Table 14 shows the product attributes of the TSKgel SCX column and the TSKgel OApak-A column. Both of these columns are composed of 5 µm particles and are stable in the pH range of 2.0 – 12.0.

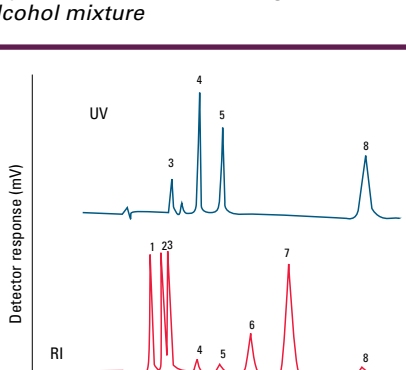
Table 14: Product attributes

TSKgel column	SCX	OApak-A
Matrix	PS-DVB	hydrophilic polymer
Particle size (mean)	5 µm	
Pore size (mean)	6 nm	ND
Functional group	sulfonic acid	proprietary
Counter ion	H^+ and Na^+	H^+
pH stability	2.0-12.0	
Small ion capacity	>1.5 eq/L	

Saccharide, Organic Acid, and Alcohol Mixture

Ion exclusion chromatography can be used as an effective method for separating alcohols. An example of saccharide, organic acid, and alcohol separation is shown in Figure 39 on two TSKgel SCX (H^+) columns in series.

Figure 39: Separation of saccharide, organic acid, and alcohol mixture

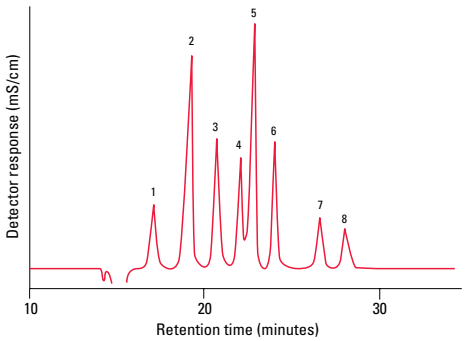


Column: **TSKgel SCX (H^+), 5 µm, 7.8 mm ID × 30 cm × 2**
Mobile phase: 0.05 mol/L $HClO_4$
Flow rate: 0.8 mL/min
Detection: UV @ 210 nm, RI
Samples:
1. maltose
2. glucose
3. fructose
4. lactic acid
5. acetic acid
6. methanol
7. ethanol
8. butyric acid

Organic Acids in Wine and Beer

Figure 40 demonstrates the separation of organic acids commonly found in wines and beers on the TSKgel OApak-A column.

Figure 40: Separation of organic acids commonly found in beer and wine

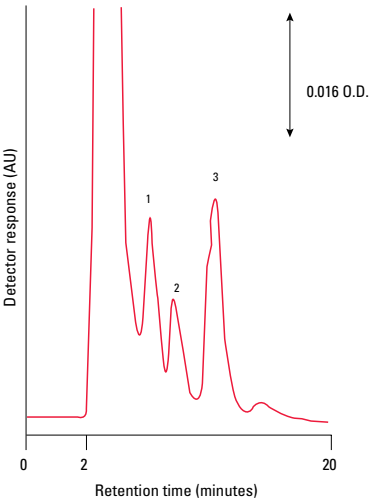


Column:	**TSKgel OApak-A, 5 µm, 7.8 mm ID × 30 cm** **TSKgel OApak-P guard, 6 mm ID × 4 cm**
Mobile phase:	0.75 mmol/L sulfuric acid
Flow rate:	0.8 mL/min
Detection:	conductivity
Temperature:	60 °C
Injection vol.:	20 µL
Samples:	1. pyruvic acid (50 ppm) 2. tartaric acid (500 ppm) 3. citric acid (500 ppm) 4. malic acid (500 ppm) 5. pyroglutamic acid (500 ppm) 6. lactic acid (1,000 ppm) 7. acetic acid (2,000 ppm) 8. succinic acid (1,000 ppm)

Column Stability

An example of the stability of the TSKgel SCX column is demonstrated in Figure 41 where 1 mol/L NaOH is used as the mobile phase for the separation of organic acids.

Figure 41: Separation of acids



Column:	**TSKgel SCX (Na^+), 5 µm, 8 mm ID × 10 cm**
Mobile phase:	1 mol/L NaOH
Flow rate:	0.8 mL/min
Detection:	UV @ 210 nm
Samples:	1. formic acid (50 ppm) 2. acetic acid (50 ppm) 3. propionic acid (100 ppm)

About: TSKgel SP-NPR Cation Exchange Columns

The TSKgel SP-NPR column is packed with spherical, nonporous (NPR) hydrophilic polymer beads of which the surface has been modified with a strong cation exchange group. Nonporous resin columns provide fast separations due to their small (2.5 µm) particle size. High column efficiency coupled with low sample capacity restricts the application of these columns to fast analysis and micro-scale preparative isolation.

The TSKgel SP-NPR column is used for the separation and analysis of proteins and peptides. This column is particularly useful for adeno-associated viruses and other large biopolymers.

Attributes and Applications

Table 15 shows the product attributes of the TSKgel SP-NPR column. Due to the absence of all but very small pores, protein recovery is generally high on TSKgel NPR columns.

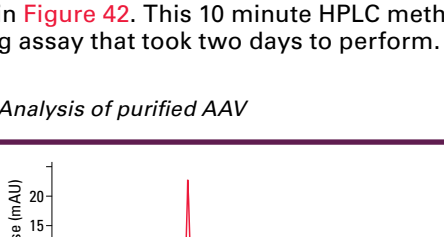
Table 15: Product attributes

TSKgel column	SP-NPR
Matrix	hydroxylated methacrylic polymer
Particle size (mean)	2.5 µm
Pore size (mean)	nonporous
Functional group	sulfopropyl
Counter ion	Na^+
pH stability	2.0-12.0
Capacity (mg Hb/mL):	5
Small ion capacity	>0.1 eq/L
pKa	2.3

Purified Adeno-Associated Virus

A purity check of adeno-associated virus (AAV), commonly used in gene therapy research, on a TSKgel SP-NPR column is shown in Figure 42. This 10 minute HPLC method replaces an existing assay that took two days to perform.

Figure 42: Analysis of purified AAV

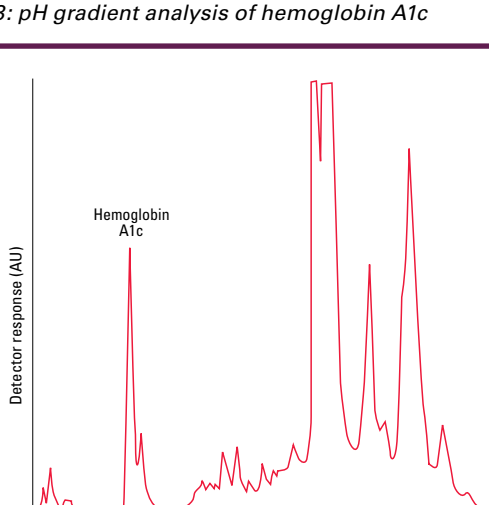


Column:	**TSKgel SP-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm**
Mobile phase:	A. 50 mmol/L HEPES, 1 mmol/L EDTA, 5 mmol/L MgCl, pH 7.5 B. 50 mmol/L HEPES, 1 mmol/L EDTA, 5 mmol/L MgCl, pH 7.5 with 0.5 mol/L NaCl; linear gradient from 20% to 100% B in 10 column volumes
Gradient:	0 min (0% B) 2 min (100% B)
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Sample:	purified adeno-associated virus

Hemoglobin A1c

The analysis of hemoglobin A1c levels in blood is used to monitor glucose levels in diabetic patients. Figure 43 shows that the HbA1c fraction can be separated from other human Hb variants on a TSKgel SP-NPR column by running a linear pH gradient in 10 minutes.

Figure 43: pH gradient analysis of hemoglobin A1c



Column:	**TSKgel SP-NPR, 2.5 µm, 4. 6 mm ID × 3.5 cm**
Mobile phase:	A: 0.02 mol/L MES, and 0.02 mol/L HEPES-NaOH, pH 6.0 B: 0.02 mol/L MES, and 0.02 mol/L HEPES- NaOH, pH 8.0
Gradient:	10 min linear gradient from 32% to 75% buffer B (pH 6.66 to pH 7.43)
Flow rate:	1.5 mL/min
Detection:	VIS @ 415 nm
Sample:	hemoglobin standard