ION EXCHANGE COLUMNS
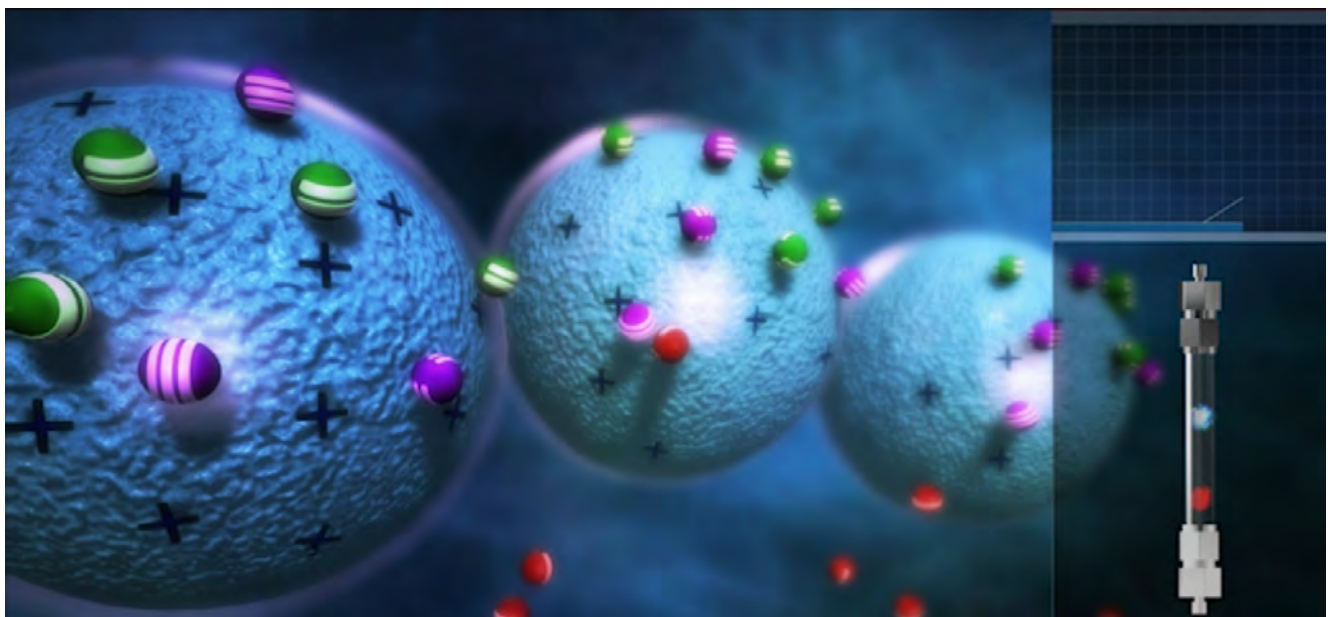
ION EXCHANGE CHROMATOGRAPHY

TOSOH BIOSCIENCE

PRINCIPLES OF CHROMATOGRAPHY

The analysis, isolation, and purification of biomolecules can be accomplished by a number of chromatographic modes. Each mode is based on specific physical, chemical, or biological interactions between the sample biomolecule and packing material.

Tosoh Bioscience offers a comprehensive line of TSKgel and TOYOPEARL media and pre-packed TSKgel columns for all common modes of liquid chromatography.

The various modes of chromatography involve separations that are based on specific features of the target or sample, like size, charge, hydrophobicity, function or specific content of the molecule. To find out more about general principles of liquid chromatography and on how each of them works, visit us on our YouTube channel.

ION EXCHANGE CHROMATOGRAPHY

ABOUT US

WITH A GLOBAL PERSPECTIVE.

Tosoh Bioscience is a leading manufacturer in the field of liquid chromatography. The portfolio of over 500 specialty products encompasses instruments for size exclusion/gel permeation chromatography and a comprehensive line of media and prepacked (U)HPLC columns for all common modes of liquid chromatography. Over the last 40 years, TSKgel SW columns have become the worldwide industry standard for size exclusion chromatography of biomolecules.

Tosoh manufacturing sites in Japan provide products to the sales and support subsidiaries in the U.S. and Europe, ensuring full global coverage. Our technical specialists in the European Headquarters provide assistance in developing HPLC applications or purification methods, in up-scaling, or packing process columns. We offer chromatographic workshops, on-site training, and are the sole sponsor of the HIC/RPC Bioseparation Conference series.

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TOSOH HISTORY

1935	FOUNDING OF TOYO SODA MANUFACTURING CO., LTD.
1936	OPERATION OF NANYO MANUFACTURING COMPLEX BEGINS
1971	SCIENTIFIC INSTRUMENTS DIVISION FORMED, FIRST GPC COLUMN USING TSKgel DEVELOPED BY TOSOH
1974	HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COLUMN PLANT IS COMPLETED
1979	TOSOH DEVELOPS TOYOPEARL MEDIA
1983	TOSOH DEVELOPS HYDROPHOBIC INTERACTION MEDIA
1987	TOSOHAAS US OPERATIONS FORMED IN MONTGOMERYVILLE
1989	TOSOHAAS GMBH OPERATIONS FORMED IN STUTTGART
1995	TOSOH NANYO GEL FACILITY RECEIVES ISO 9001
2002/2003	ALL TOSOH AFFILIATED SCIENTIFIC & DIAGNOSTIC SYSTEM RELATED COMPANIES IN EUROPE ARE UNIFIED UNDER THE NAME TOSOH BIOSCIENCE.
2008	EcoSEC, THE 7TH GENERATION GPC SYSTEM IS INTRODUCED GLOBALLY
2010	TOSOH CELEBRATES ITS 75TH YEAR IN BUSINESS WITH THE OPENING OF FIVE NEW PLANTS, AND CONTINUED RAPID EXPANSION IN CHINA
2011	TOSOH BIOSCIENCE CELEBRATES 40 YEARS OF OPERATION
2012	TOSOH RELEASES FIRST TOYOPEARL MIXED-MODE RESIN TOYOPEARL MX-Trp-650M
2013	TOSOH RELEASES A HIGH CAPACITY PROTEIN A CHROMATOGRAPHY RESIN
2014	TOSOH BIOSCIENCE GMBH CELEBRATES ITS 25TH ANNIVERSARY IN STUTTGART
2015	TOSOH BIOSCIENCE SUCCESSFULLY MOVES ITS SALES & MARKETING OFFICES TO GRIESHEIM, DARMSTADT

IEC
ION EXCHANGE CHROMATOGRAPHY

Ion exchange chromatography (IEC) is one of the most frequently used chromatographic modes in the separation and purification of biomolecules. It is a non-denaturing technique that is used for analysis and at all stages and scales of purification: from micro scale purification to industrial scale downstream processing.

Tosoh Bioscience offers analytical and semi preparative ion exchange HPLC columns as well as ion exchange resins for large scale biopurification.

TSKgel HPLC columns are packed with silica or polymer based porous or nonporous beads. They are well suited for a broad range of applications in R&D, quality control or reaction monitoring.

For TSKgel bulk and TOYOPEARL bulk please refer to our Chromatographic Process Media Catalogue.

IEC
HOW IT WORKS

IEC retains molecules based on ionic interactions. The stationary phase surface displays ionic functional groups that interact with analyte ions of opposite charge. IEC is further subdivided into cation exchange and anion exchange chromatography.
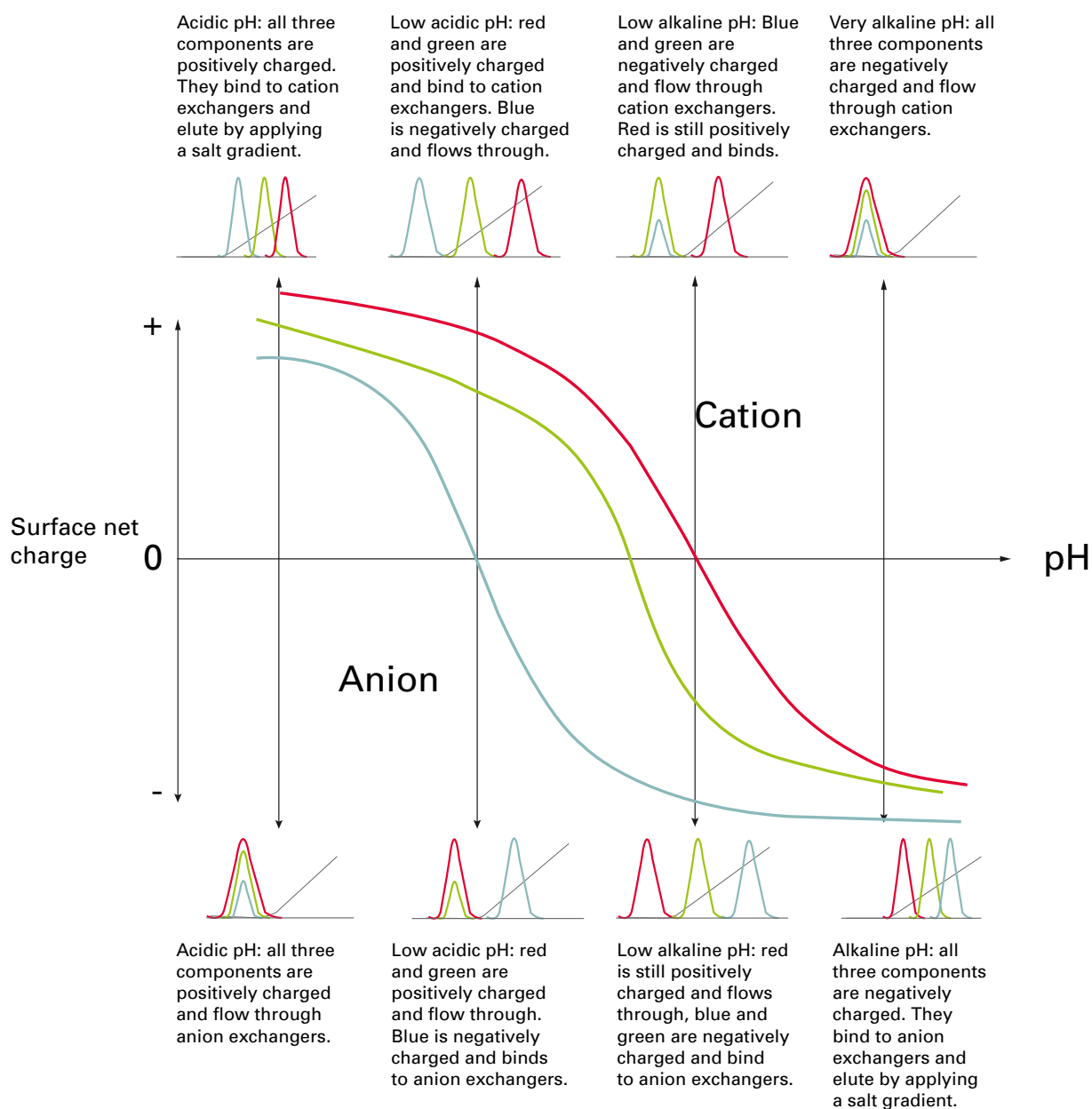
Anion exchange media carry positively charged groups that attract negatively charged anions. Cation exchange resins display negatively charged groups which attract positively charged cations. Charged target molecules are retained on the stationary phase but can be eluted by increasing the concentration of a similarly charged ion that will displace the analyte or target ions from the stationary phase.

Proteins have numerous functional groups that can have both positive and negative charges. IEC separates proteins according to their net surface charge, which is dependent

on the pH and ionic strength of the mobile phase. According to differences in their overall charge and surface charge distribution, proteins can be separated by IEC.

IEC takes advantage of the fact that the relationship between net surface charge and pH is unique for a specific protein. At a pH, equivalent to its isoelectric point, a protein has no net charge and will not interact with the charged stationary phase. At a pH above the pI the protein will have a negative net charge and will therefore bind to a positively charged anion exchanger. At a pH below its pI it will have a positive net charge and will consequently interact with a negatively charged cation exchanger. By adjusting the pH or the salt concentration of the mobile phase, separation can be optimized. For loading, the pH and ionic strength are selected in a way that the targets or analytes bind to the stationary phase (Figure 1).

FIGURE 1

Acidic pH: all three components are positively charged. They bind to cation exchangers and elute by applying a salt gradient.

Low acidic pH: red and green are positively charged and bind to cation exchangers. Blue is negatively charged and flows through.

Low alkaline pH: Blue and green are negatively charged and flow through cation exchangers. Red is still positively charged and binds.

Very alkaline pH: all three components are negatively charged and flow through cation exchangers.

Cation

Anion

Surface net charge

0

pH

+

-

Acidic pH: all three components are positively charged and flow through anion exchangers.

Low acidic pH: red and green are positively charged and flow through. Blue is negatively charged and binds to anion exchangers.

Low alkaline pH: red is still positively charged and flows through, blue and green are negatively charged and bind to anion exchangers.

Alkaline pH: all three components are negatively charged. They bind to anion exchangers and elute by applying a salt gradient.

IEC
INTRODUCTION TO TSKgel IEC COLUMNS

Elution is usually performed by changing the ionic strength of the mobile phase by applying a salt gradient. As the salt concentration of the mobile phase increases, the salt ions compete with the bound molecules for the functional groups of the stationary phase. The higher the net charge of the molecule, the higher the salt concentration needed for elution. Very tightly bound compounds are removed at the end of the elution by a wash step with very high salt buffer.

Ion exchange resins are classified as weak or strong ion exchangers. The terms strong and weak do not refer to the performance of the resins or to the strength of interaction between resin and target.

'Strong', respectively 'weak' refers to the extent that the ion exchange capacity varies with change in pH. Strong ion exchange groups have a steep titration curve. They show no variation of their ionization state with the pH and remain fully charged over a broad pH range.

Typical strong anion exchange groups are quaternary amine groups (Q & QAE type), typical strong cation exchange groups are sulfo or sulfopropyl groups (S & SP type). Carboxymethyl (CM, cationic) and diethylaminoethyl (DEAE, anionic) are weak ion exchange groups. Figure 2 shows the pKa values for these ligands.

FIGURE 2

PK$_a$ VALUES FOR ION EXCHANGE GROUPS

Q, SuperQ, QAE   pK$_a$ = 12.2
DEAE   pK$_a$ = 11.5

Anion

7.0

Cation

Decreasing resin pK$_a$

CM   pK$_a$ = 4.7*
S, SP   pK$_a$ = 1.2

*pK$_a$ Toyopearl GigaCap CM-650M = 3.6
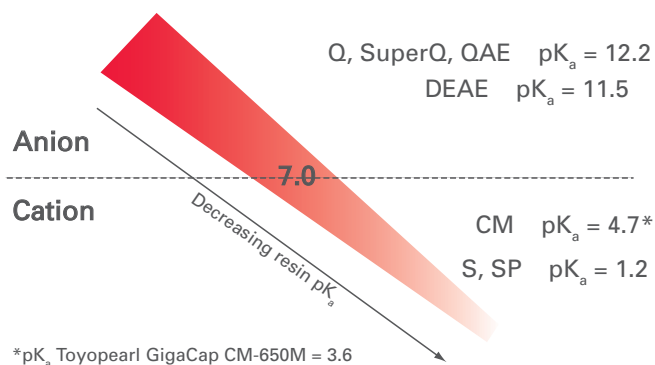
The binding buffer pH is selected between the pI of the protein and the pKa of the stationary phase.

TABLE 1

CATION EXCHANGE GROUPS		STRUCTURE
Carboxymethyl (CM)	Weak	$-O-CH_2COO^-$
Sulfopropyl (SP)	Strong	$-O-R-O-CH_2-CH_2-CH_2-SO_3^-$
ANION EXCHANGE GROUPS		
Diethylaminoethyl (DEAE)	Weak	$-O-CH_2-CH_2-N^+-(C_2H_5)_2$
Quarternary Ammonium (Q)	Strong	$-O-R-N^+-(CH_3)_3$
Quarternary Aminoethyl (QAE)	Strong	$-O-CH_2-CH_2-N^+H-(CH_3)_3$

IEC
INTRODUCTION TO TSKgel IEC COLUMNS

Tosoh Bioscience offers a broad line of high efficiency columns for analysis and isolation of biomolecules by anion and cation exchange chromatography. In either mode of IEC the product line contains methacrylate and silica based columns. Most of the available chemistries are offered in analytical as well as semi-preparative formats. Particle sizes range from 2.5 µm for fast analysis to 20 µm for preparative purposes. Proteins, peptides, oligonucleotides and nucleic acids are typical samples that are analyzed or isolated on TSKgel IEC columns.

PACKING MATERIALS AND CHEMISTRIES

Methacrylate, silica and polysterene are used as matrices for the TSKgel line of ion exchange columns. The base resins are derivatized either with diethylaminoethyl (DEAE), quaternary ammonium (Q), sulfopropyl (SP) or carboxymehtyl (CM) functionalities to provide weak anion, strong anion, strong cation and weak cation exchangers, respectively.

Columns from the silica-based TSKgel SW series are typically used in the separation of low molecular weight com pounds such as pharmaceuticals, nucleotides or small peptides.

The methacrylate backbone chemistry provides a robust, hydrophilic particle that is suitable as a support for high performance analytical and preparative separations of biomolecules. The G5000 PW base resin of the TSKgel 5PW series is a spherical particle with a mean pore size of 100 nanometer (1.000 Angström).

TSKgel BioAssist columns are also based on methacrylate particles with larger pores (400 nanometer for BioAssist Q and 130 nanometer for BioAssist S) providing better access to the functional groups for large proteins.

The methacrylate chemistry also forms the backbone of non-porous resin columns such as the TSKgel NPR and STAT series. Since rate-limiting pore diffusion is eliminated with nonporous particles, analysis time is often reduced by as much as 80% without loss in resolution. Also, recoveries are routinely greater than 90%.

TSKgel STAT columns are the latest addition to the IEC column line. An innovative bonding chemistry results in columns that show a reasonable sample capacity while traditional non-porous resins usually show limited capacity due to lower surface area. Specific application needs are addressed by offering various column formats and particle sizes.

For special applications polystyrene based columns are offered as well. They are most suitable for analyzing small molecular weight sugars, amino acids, individual nucleic acids and small drug candidates. For detailed information on these special columns please refer to the chromatography catalog.

➤ **FEATURES**

BioAssist Columns

➤ High capacity even for larger proteins (1 million Da)

➤ Unique pore structure provides fast mass transfer

➤ Biocompatible PEEK column hardware

➤ Available in analytical and semi-prep formats

Polymer-Based Ion Exchange Columns

➤ Methacrylate backbone

➤ Large pore size (100 nm) (excl. limit for proteins ~ 5,000,000 Da)

➤ Non porous resin-based (STAT and NPR) columns

➤ Several columns available in 2 mm ID format

Silica-Based Ion Exchange Columns

➤ Smaller pore size (2SW = 12.5 nm and 3SW = 25 nm)

➤ **BENEFITS**

➤ Fewer runs to collect required sample amounts

➤ Sharper peaks improve analysis and isolation

➤ Less sample loss due to adsorption

➤ Easy scale-up

➤ Mechanically and chemically stable (pH 2.0-12.0)

➤ Withstands repeated cleaning with base, and use of organic solvents, denaturants and surfactants

➤ Use same column for most biopolymers

➤ Fast QC analysis and process monitoring

➤ Reduced solvent consumption and analysis time

➤ Most suitable for analysing smaller MW samples such as nucleotides, drug candidates, catecholamines and small peptides or proteins

IEC
PROPERTIES OF TSKgel IEC COLUMNS

PROPERTIES OF TSKgel ION EXCHANGE COLUMNS

TSKgel ANION EXCHANGE COLUMNS

TSKgel	Matrix*	Particle size (µm)	Pore size (nm)	Functional group	Counter ion	Excl. limit, PEG** (Da)	Capacity (mg BSA/mL)	Small ion capacity meq/mL	pKa	Column hard-ware***
BioAssist Q	pMA	10, 13	~400	Polyamine	Cl^-	>5,000,000	70	0.1	9.4	PEEK
SuperQ-5PW	pMA	10,13	100	Trimethyl-amino	Cl^-	1,000,000	100	> 0.13	12.2	S, G
DEAE-5PW	pMA	10,13, 20	100	DEAE	Cl^-	1,000,000	30	0.1	11.5	S, G
Q-STAT	pMA	7,10	~ 0	Trimethyl-amino	Cl^-	500	20	0.27	10.5	S
DNA-STAT	pMA	5	~ 0	Trimethyl-amino	Cl^-	500	35	0.27	10.5	S
DEAE-NPR	pMA	2.5	~ 0	DEAE	Cl^-	500	5	> 0.1	11.2	S
DNA-NPR	pMA	2.5	~ 0	Proprietary	ClO_4^-	500	5	> 0.1	11.2	S
DEAE-2SW	Silica	5	12.5	DEAE	$H_2PO_4^-$	10,000	ND	> 0.3	11.2	S
DEAE-3SW	Silica	10	25	DEAE	Cl^-	30,000	ND	> 0.3	11.2	S
Sugar AXI	PS-DVB	8	6	Trimethyl-amino	HBO_3^-		ND	> 1.2	12.5	S
Sugar AXG	PS-DVB	10	6	Trimethyl-amino	HBO_3^-		ND	> 1.2	12.5	S
SAX	PS-DVB	5	6	Trimethyl-amino	Cl^-		ND	> 1.0	12.5	S

TSKgel CATION EXCHANGE COLUMNS

TSKgel	Matrix*	Particle size (µm)	Pore size (nm)	Functional group	Counter ion	Excl. limit, PEG** (Da)	Capacity (mg/mL)	Small ion capacity meq/mL	pKa	Column hard-ware***
BioAssist S	pMA	7, 13	~130	Sulfopropyl	Na^+	~4,000,000	70[1]	0.1	2.4	PEEK
SP-5PW	pMA	10, 13, 20	100	Sulfopropyl	Na^+	1,000,000	40[2]	> 0.1	2.3	S, G
CM-5PW	pMA	10, 13	100	Carboxymethyl	Na^+	1,000,000	45[2]	> 0.1	4.2	S, G
SP-STAT	pMA	7, 10	~ 0	Sulfopropyl	Na^+	500	10[3]	> 0.023	2.6	S
CM-STAT	pMA	7, 10	~ 0	Carboxymethyl	Na^+	500	15[3]	> 0.1	4.9	S
SP-NPR	pMA	2.5	~ 0	Sulfopropyl	Na^+	500	5[2]	> 0.1	2.3	S
SP-2SW	Silica	5	12.5	Sulfopropyl	Na^+	10,000	ND	0.3	2.2	S
CM-2SW	Silica	5	12.5	Carboxymethyl	Na^+	10,000	110[2]	> 0.3	4.2	S
CM-3SW	Silica	10	25	Carboxymethyl	Na^+	30,000	ND	> 0.3	4.2	S
SCX	PS-DVB	5	6	Sulfonic acid	Na^+, H^+		ND	> 1.5		S

*pMA = poly methacrylate; PS-DVB = polystyrene-divinylbenzene   **Polyethylene glycol

***PEEK = polyethyletherketone, S = stainless steel, G = glass (1) γ-globulin;   (2) hemoglobin; (3) lysozyme

IEC
TSKgel IEC COLUMN SELECTION

SAMPLE TYPE	MW RANGE (Da)	TSKgel COLUMN	pH RANGE
Amino Acids, Peptides and Proteins			
Amino acids	< 2,000	SAX	1.0 - 14.0
		SCX	1.0 - 14.0
Peptides and small proteins	< 10,000	Q-STAT	3.0 - 10.0
		SP-STAT	3.0 - 10.0
		CM-STAT	3.0 - 10.0
		SCX	1.0 - 14.0
		SP-2SW	2.0 - 7.5
		CM-2SW	2.0 - 7.5
		DEAE-2SW	2.0 - 7.5
Proteins	> 10,000 up to ~ 5,000,000	BioAssist S	2.0 - 12.0
		BioAssist Q	2.0 - 12.0
		Q-STAT	3.0 - 10.0
		SP-5PW	2.0 - 12.0
		DEAE-5PW	2.0 - 12.0
		CM-5PW	2.0 - 12.0
		SP-STAT	3.0 - 10.0
		CM-STAT	3.0 - 10.0
		SP-NPR	2.0 - 12.0
		DEAE-NPR	2.0 - 12.0
		SuperQ-5PW	2.0 - 12.0
Nucleic Acids			
Purines and pyrimidines		DEAE-2SW	2.0 - 7.5
		SP-2SW	2.0 - 7.5
Nucleosides		SP-2SW	2.0 - 7.5
		DEAE-2SW	2.0 - 7.5
Nucleotides		Q-/DNA-STAT	3.0 - 10.0
		DEAE-2SW	2.0 - 7.5
Oligonucleotides		Q-/DNA-STAT	3.0 - 10.0
		DEAE-5PW	2.0 - 12.0
		DEAE-NPR	2.0 - 12.0
		DNA-NPR	2.0 - 12.0
		SuperQ-5PW	2.0 - 12.0
DNA, RNA, and PCR products		Q-/DNA-STAT	3.0 - 10.0
		DNA-NPR	2.0 - 12.0
		DEAE-NPR	2.0 - 12.0
		DEAE-5PW	2.0 - 12.0
		DEAE-3SW	2.0 - 7.5
Other Molecules			
Mono and disaccharides		Sugar AXI, AXG	1.0 - 14.0
		SCX	1.0 - 14.0
		SAX	1.0 - 14.0

IEC
TSKgel STAT SERIES

TSKgel STAT columns are designed for high efficiency separation of biomolecules and low molecular weight compounds. They provide superior performance at reduced analysis time. STAT columns are available in various formats and sizes of the mono-disperse particles (5, 7 and 10 μm) to perfectly match specific application needs.

The surface of the hydrophilic non-porous particles consists of an open access network of multi-layered ion exchange groups (carboxymethyl, sulfopropyl or quaternary ammonium groups; see Figure 3). The innovative bonding chemistry results in columns that show a reasonable sample capacity while traditional non-porous resins usually show limited capacity due to lower surface area.

For fast and ultrafast IEC analysis short columns (3 mm ID x 3.5 cm length) are packed with large 10 μm particles. They are ideally suited for rapid candidate screening or process monitoring. Longer columns (4.6 mm ID x 10 cm length) packed with 7 μm particles were designed for high resolution IEC separation. They are perfect for the analysis of nucleic acids, mAb variants, protein aggregates or PEGylated proteins. The DNA-STAT columns (4.6 mm ID x 10 cm length) packed with 5 μm Q-type anion exchange resin are optimized for the analysis of nucleic acids.

The relatively large particle sizes support fast separation at moderate pressure while at the same time the proprietary surface modification technology ensures a high density of functional groups going along with high sample capacity.

PRODUCT HIGHLIGHTS TSKgel STAT SERIES

➤ Very efficient chromatography for high as well as low MW solutes made possible by novel bonding chemistry and the absence of micro-pores

➤ High speed and high resolution analysis of Biomolecules in HPLC and UHPLC

➤ Higher adsorption capacities and lower pressures compared with smaller particle sized TSKgel NPR columns

➤ 7 or 10 μm particles (TSKgel Q-STAT) and 5 μm particles (TSKgel DNA-STAT)

➤ 7 or 10 μm particles for SP and CM chemistries

APPLICATIONS WITH TSKgel STAT ANION EXCHANGE COLUMNS

Nucleotides

Mono-, di-, and tri-nucleotides were separated with excellent peak shape on a TSKgel DNA-STAT column. The narrow, symmetrical peaks, as shown in Figure 4, demonstrate the absence of micropores on this new generation of non-porous resin columns. TSKgel DNA-STAT columns are also, as the name implies, first choice for large nucleic acid fragments.

➤ **FIGURE 3**

SCHEMATIC DIAGRAM OF TSKgel STAT SERIES

Protein

Ionic group

Hydrophilic chain

Non porous material

➤ **FIGURE 4**

HIGH RESOLUTION SEPARATIONS OF NUCELOTIDES
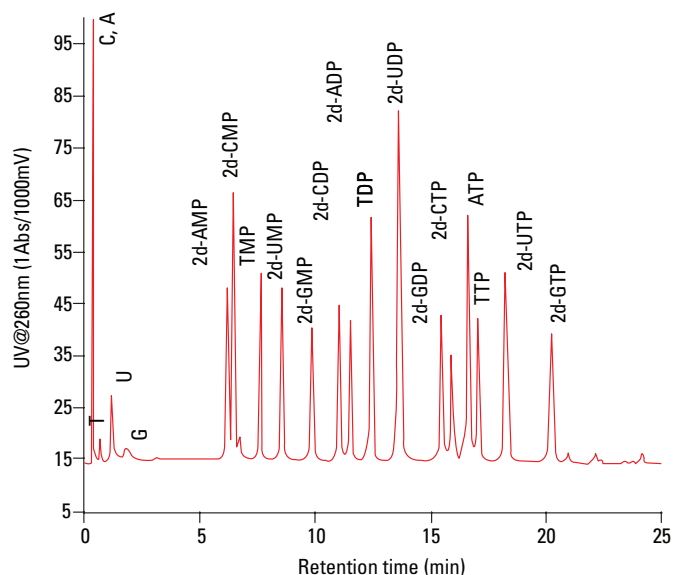
Column: TSKgel DNA-STAT, 5 μm, 4.6 mm ID x 10.0 cm L;
Eluent: A: 20 mmol/L Tris-HCl (pH 8.5); B: 0.75 mol/L NaCl in buffer A;
Gradient: 50% B (0 min), 75% B (25 min); Flow rate: 0.8 mL/min;
Detection: UV @ 260 nm

TOSOH BIOSCIENCE

IEC
TSKgel STAT SERIES

APPLICATIONS WITH TSKgel STAT ANION EXCHANGE COLUMNS

Monoclonal Antibodies

The monoclonal antibody was digested using pepsin and separated on a TSKgel Q-STAT column and a competitive non-porous WAX column. As shown in Figure 5, three peaks were isolated from the TSKgel Q-STAT column and assigned as F(ab')2, pFc and intact IgG by SDS-PAGE. There was no correlation between the peaks obtained on the competitive WAX column and SDS-PAGE.

High Resolution versus high Throughput Analysis of Nucleotides

The separation of nucleotides on a 10 cm length Q-STAT column was compared to the separation on a 3.5 cm length column. While the longer column provides a high resolution separation of the nucleotides within five minutes the same sample can be analyzed within one minute on the shorter column allowing high throughput. The chromatograms are shown in Figure 6.

FIGURE 5

ANALYSIS OF IgG FRAGMENTS

Non-reduced SDS-PAGE
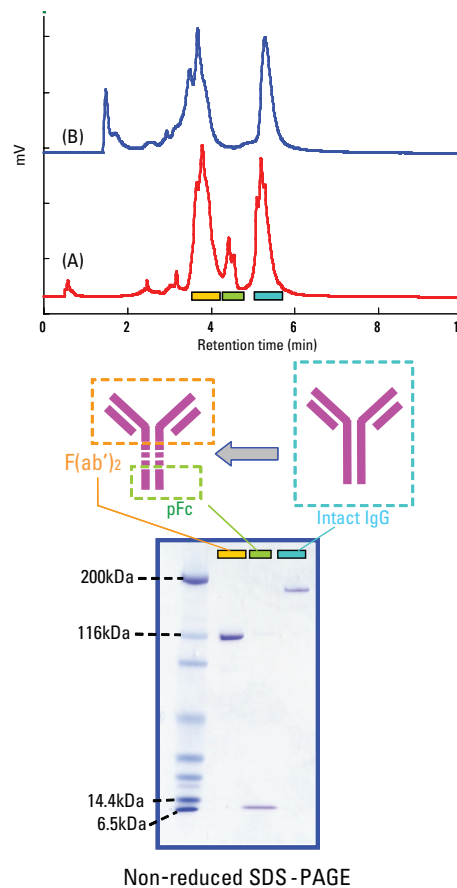
Column: A: TSKgel Q-STAT, 7 μm, 4.6 mm ID x 10 cm L: B: Competitor WAX, 10 μm, 4 mm ID x 25 cm L; Eluent: A: 20 mmol/l Tris-HCl (pH 8.5); B: 0.5 mol/L NaCl in buffer A; Gradient: 0% B (0 min), 100% B (10 min); Flow rate: 1.0 mL/min; Detection: UV @ 280 nm; Samples: pepsin digested mAb

FIGURE 6

HIGH RESOLUTION VERSUS HIGH THROUGHPUT ANALYSIS OF NUCLEOTIDES
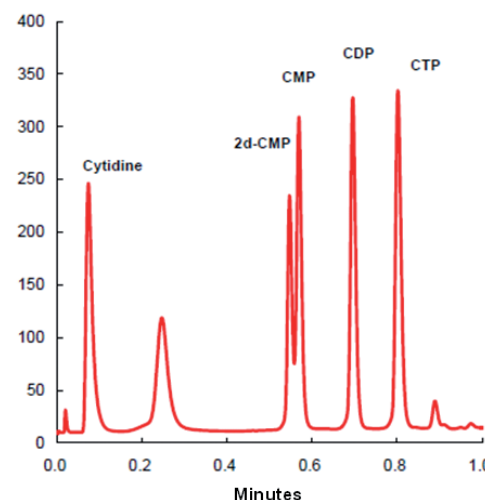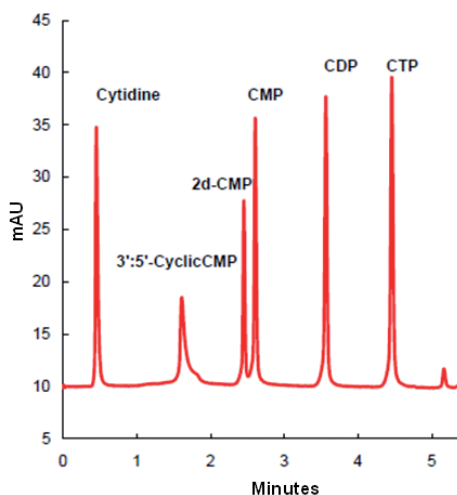
High resolution:
Column: Q-STAT, 4.6 mm ID x 10 cm L (7 μm); Eluent: A) 20 mmol/L Tris-HCl (pH 8.5) B) 0.5 mol/L NaCl in A (pH 8.5); Gradient: 0 to 100% B (10 min.); Flow rate: 1.5 mL/min.; Detection: UV @ 260 nm

High throughput:
Column: Q-STAT, 4.6 mm ID x 3.5 cm L (10 μm); Eluent: A) 20 mmol/L Tris-HCl (pH 8.5), B) 0.5 mol/L NaCl in A (pH 8.5); Gradient: 0 to 100% B (1 min.); Flow rate: 4.0 mL/min.; Detection: UV @ 260 nm
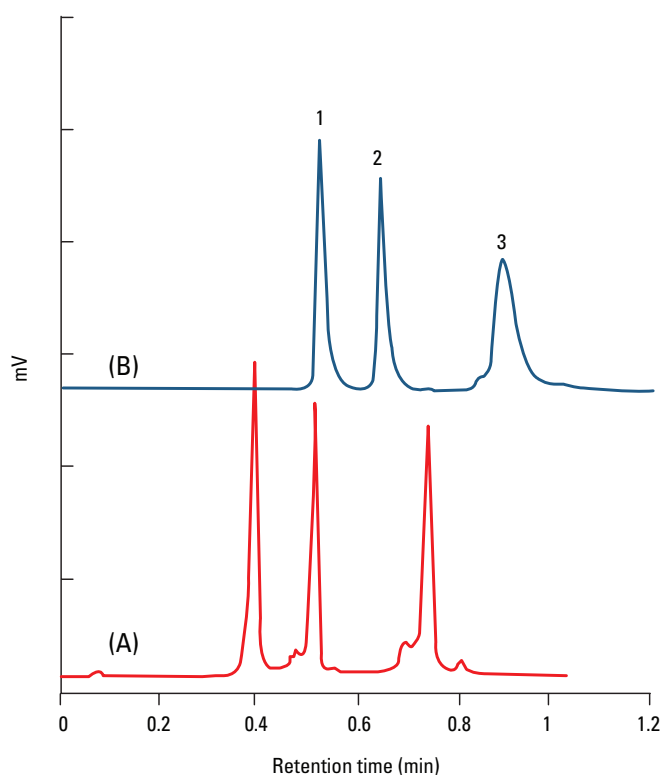
IEC
TSKgel STAT SERIES

APPLICATIONS WITH TSKgel STAT CATION EXCHANGE COLUMNS

Fast Separations

The fast separation of protein standards was investigated using short cation exchange columns (see Figure 7). A TSKgel SP-STAT column shows superior resolution, better peak shape, and shorter analysis time (< 60 seconds) compared to a competitive monolithic SP-type column.

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 L TSKgel SP-STAT column. As demonstrated in Figure 8, peak areas of mono-, di-, and tri- PEGylated β-lactoglobulin increased with reaction time, while the area of unreacted β-lactoglobulin declined.

▶ **FIGURE 7**

FAST PROTEIN SEPARATION

Column: A: TSKgel SP-STAT, 10 µm, 3.0 mm ID x 3.5 cm L;
B: Competitor column 4.6 mm ID x 5.0 cm L
Eluent: A: 20 mmol/L sodium acetate (pH 5.0); B: 1.0 mol/L NaCl in buffer A (pH 5.0) for column A; 1.5 mol/l NaCl in buffer A (pH 5.0) for column B;
Gradient: 0% B (0 min), 100% B (1 min);
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

▶ **FIGURE 8**

PEGYLATION MONITORING
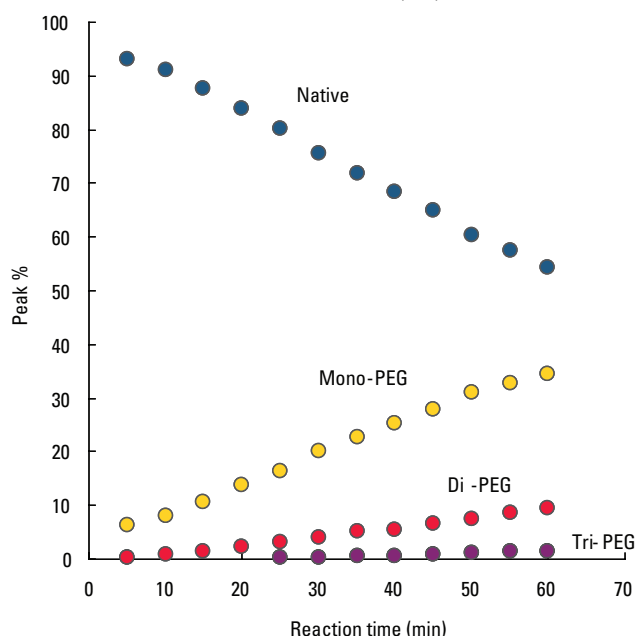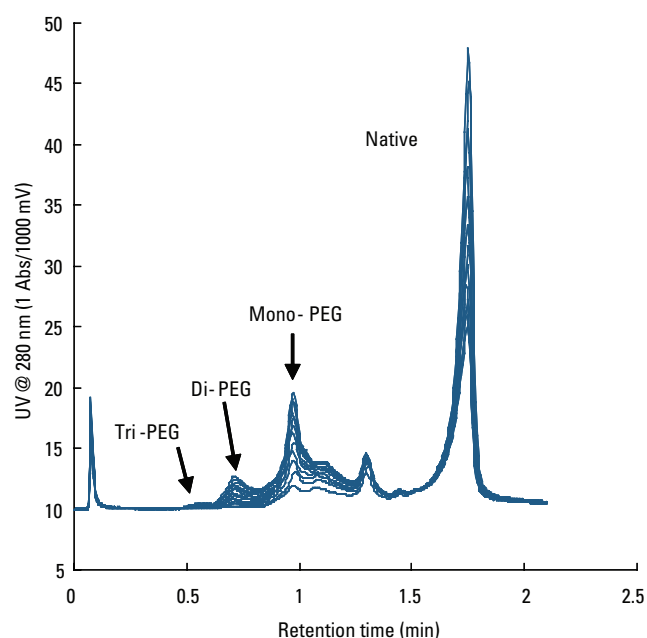
Column: TSKgel SP-STAT, 10 µm, 3.0 mm ID x 3.5 cm L; Eluent: A: 20 mmol/L sodium acetate (pH 5.0); B: 1.0 mol/L NaCl in buffer A (pH 5.0);
Gradient: 0% B (0 min), 100% B (2 min); Flow rate: 2.0 mL/min;
Detection: UV @ 280 nm; Samples: PEGylated β-lactoglobulin

IEC
TSKgel STAT SERIES

Antibody Analysis

The analysis profiles for five antibodies separated on a TSKgel CM-STAT column were compared with the profiles obtained on a competitive WCX column (Figure 9). Similar or higher resolution profiles were obtained on TSKgel CM-STAT in approximately half the time.

Column Selection for Antibody Analysis

Different antibodies were analyzed on a CM-STAT and SP-STAT column at pH 7. Figure 10 shows that it is dependent on the antibody which column provides the better resolution. The strong cation exchange column TSKgel SP-STAT shows a better separation of charge variants of mAb A (upper chromatograms) while the weak cation exchange column TSKgel CM-STAT delivers a better separation of a basic variant from the main peak for mAb B.
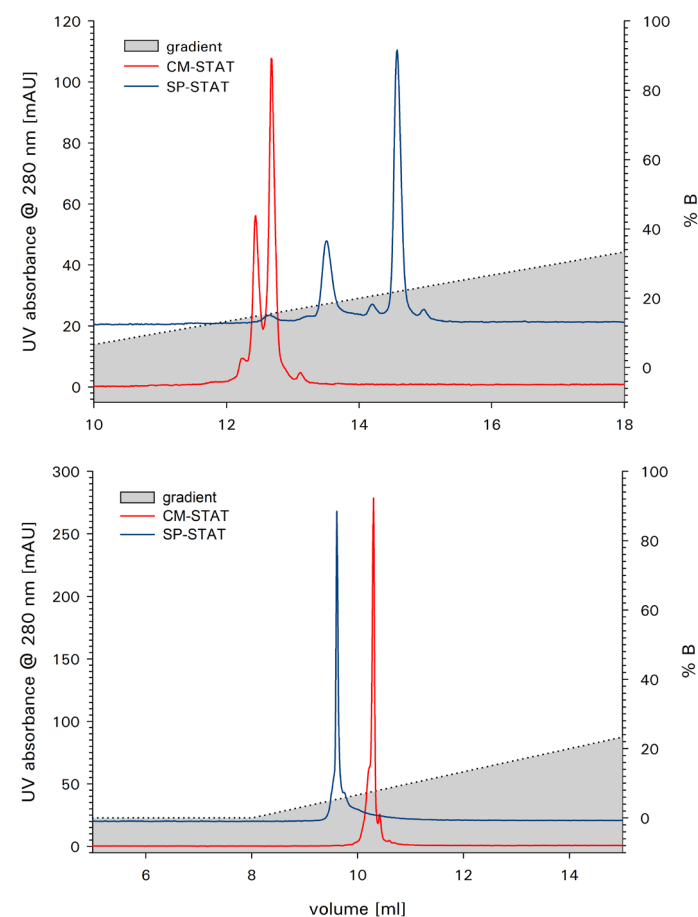
FIGURE 9

ANALYSIS OF CHARGE HETEROGENEITY

FIGURE 10

COMPARISON OF TSKgel CM- AND SP-STAT COLUMNS
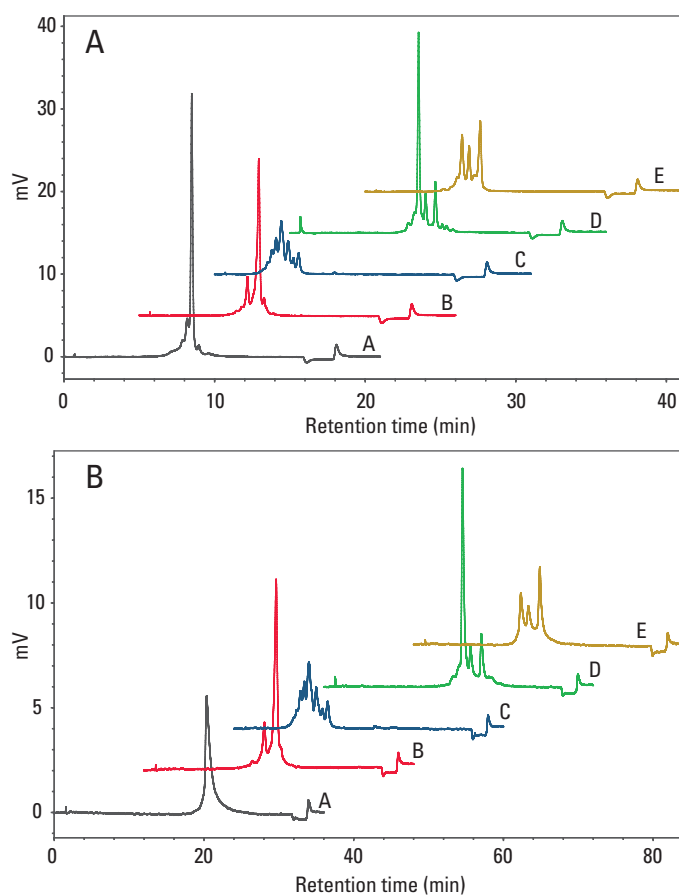
Column: A: TSKgel CM-STAT, 7 µm, 4.6 mm ID x 10 cm L; B:
Competitor WCX, 10 µm, 4.0 mm ID x 25 cm L;
Eluent: A: 20 mmol/L MES (pH 6.0); B: 20 mmol/L MES + 0.5 mol/L NaCl (pH 6.0)
Gradient: A: 10% B (0 min), 30% B (15 min), 100% B (15 min), 100% B (17 min), 10% B (17 min), 10% B (21 min); B: 10% B (0 min), 30% B (30 min), 100% B (30 min), 100% B (32 min), 10% B (32 min), 10% B (36 min)
Flow rate: A: 1.0 mL/min B: 2.0 mL/min; Temp.: Ambient; Detection: UV @ 280 nm; Inj. Vol.: 20 µL; Sample: monoclonal antibodies (mAb A through E)

Columns: TSKgel SP-STAT (7 µm, 4.6 mm ID x 10 cm);
TSKgel CM-STAT (7 µm, 4.6 mm ID x 10 cm)
Mobile phase A: 10 mmol/L sodium phosphate buffer pH 7.0
Mobile phase B: 100 mmol/L sodium phosphate pH 7.0 + 500 mmol/L NaCl
Gradient: 0-100 % B in 30 min; Flow rate: 1 mL/min; Detection: UV @ 280 nm
Injection vol.: 10 µL; Sample: mAb A (2 g/L); mAb B (2 g/L)

IEC
TSKgel BioAssist SERIES

TSKgel BioAssist columns are based on methacrylate particle design technology. TSKgel BioAssist Q contains particles with very large pores (~400 nm) that are derivatized with a network of polyamine groups. The capacity of TSKgel BioAssist Q has been shown to be high over a wide molecular weight range (up to 1,000,000 Da). TSKgel BioAssist S is packed with particles possessing 130 nm pores functionalized with sulfopropyl groups.

TSKgel BioAssist analytical IEC columns are provided in a 4.6 mm ID x 5 cm L PEEK housing with 7 µm or 10 µm particles for the respective S and Q functionalities.

Semipreparative TSKgel BioAssist columns are also available with a 13 µm particle size packed in a 10 mm ID x 10 cm L housing. The longer length of the semi-preparative column compensates for the increased particle size, resulting in similar resolution to the analytical column.

PRODUCT HIGHLIGHTS TSKgel BioAssist SERIES

 Pore structure and bonding chemistry of TSKgel BioAssist Q/S columns provide high capacity for small to very large MW proteins and nucleic acids.

 TSKgel BioAssist Q/S is suitable for use in systems that are designed for HPLC, laboratory or semi-preparative applications

 TSKgel BioAssist columns are packed in 4.6 mm ID or 10 mm ID PEEK hardware. Other columns are available in glass and stainless steel for analytical, semi-preparative and preparative applications.

Especially designed for the separation of large biomolecules the very large pores of the TSKgel BioAssist columns offer high capacity and resolution at a low column pressure drop. The polymerization technique used to construct these columns results in a homogenous distribution of ion exchange groups without significantly reducing pore size.

APPLICATIONS WITH TSKgel BioAsisst ANION EXCHANGE COLUMNS

Performance Enhancement on FPLC System

TSKgel BioAssist Q is suitable for use in systems that are designed for laboratory or semi-preparative applications. Figure 11 demonstrates the performance enhancement of TSKgel BioAssist Q over a competitive product when operated side-by-side on an FPLC system.

 FIGURE 11

PERFORMANCE ENHANCEMENT ON FPLC SYSTEM
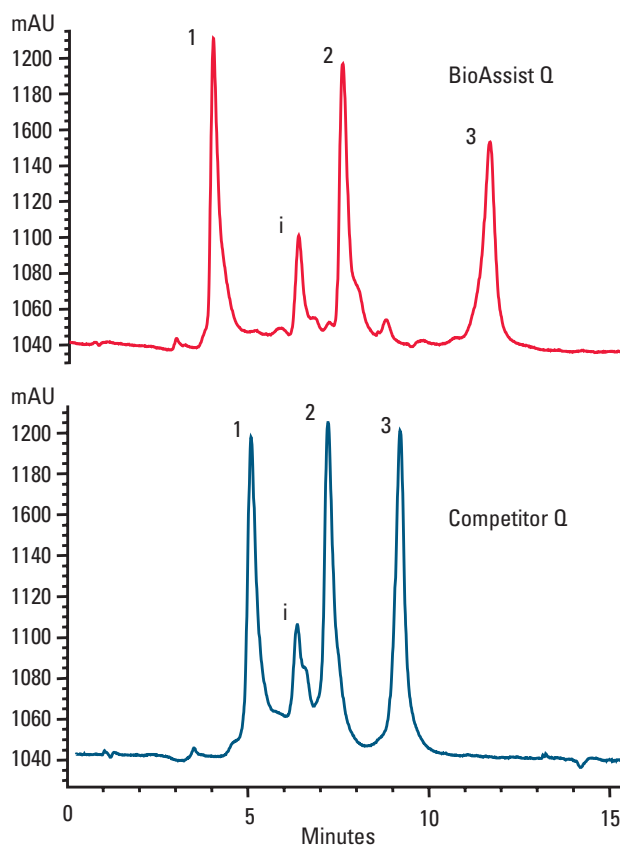
Column: TSKgel BioAssist Q, 4.6 mm ID x 5 cm L (PEEK),
Competitor Q, 5.0 mm ID x 5 cm L; Elution: 30 min linear gradient from 0 to 1 mol/L NaCl in 20 mmol/L sodium phosphate pH 8.0; Flow Rate: 1.0 mL/min; Detection: UV@ 280 nm; Sample: 1) conalbumin, i) ovalbumin impurity, 2) ovalbumin, 3) trypsin inhibitor

IEC
TSKgel BioAssist SERIES

APPLICATIONS WITH TSKgel BioAsisst ANION EXCHANGE COLUMNS

Comparison of Dynamic Binding Capacity

Table 2 shows typical dynamic binding capacities on Bio-Assist Q relative to competitive products.

➤ **TABLE II**

COMPARISON OF DYNAMIC BINDING CAPACITIES

	Binding capacity (mg/mL)			
Protein	BioAssist Q	SuperQ -5PW	Conv. Q type prod. A	Conv. Q type prod. B
Thyroglobulin	77.4	22.9	20.2	1.8
Monoclonal IgG$_1$	57.8	43.3	46.7	47.7
Human Serum Albumin	83.1	78.9	48.2	48.8
Trypsin Inhibitor	84.3	92.8	51.8	57.8

Columns: TSKgel BioAssist Q and TSKgel SuperQ-5PW (4.6 mm ID x 1 cm); Conventional Q type product A and B (4.6 mm ID x 1 cm)

Solvent: 20 mmol/L Tris-HCl buffer, pH 8.0;

Flow rate: 0.38 mL/min; Det.: UV @ 280 nm

*Capacity was determined at 10% height of the breakthrough curve; UV 280 nm.

APPLICATIONS WITH TSKgel BioAssist CATION EXCHANGE COLUMNS

### Bromelain Anaylsis on TSKgel BioAssist S and Competitor S Column

Figure 12 shows the analysis of bromelain, a proteolytic enzyme that is used as a nutritional supplement. Bromelain is a basic glycoprotein with a MW of 33 kDa and a pI of 9.55.

Analysis of 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 (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. Figure 13 shows the baseline separation of IgM from other contaminants using a 0.3 mol/L NaCl step gradient after elution of albumin.

➤ **FIGURE 12**

BROMELAIN ANALYSIS ON TSKgel BioAssist S & COMPETITOR S COLUMNS
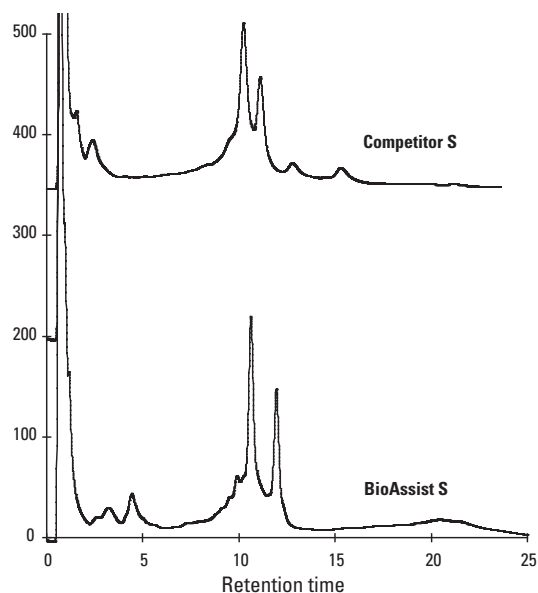
Columns: TSKgel BioAssist S, 4.6 mm ID x 5 cm L, PEEK
Competitor S 5 mm ID x 5 cm L; Elution: 20 min (TSKgel) or 30 min (Competitor S) linear gradient of NaCl from 0 to 0.5 mol/L in 20 mmol/L sodium phosphate buffer, pH 7.0; Flow rate: 0.8 mL/min for TSKgel; 1.0 mL/min for Competitor S
Detection: UV @ 280 nm; Temperature: 25°C;
Sample: crude bromelain (C4882, Sigma), 1 mg in 100 µL

➤ **FIGURE 13**

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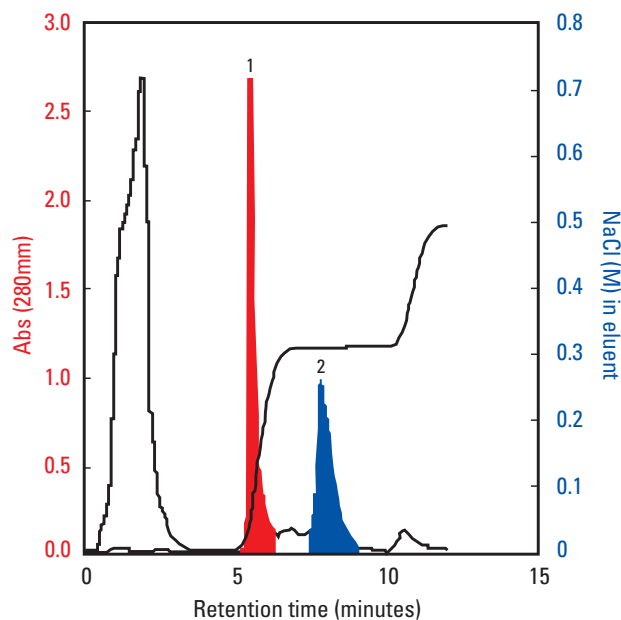
Column: TSKgel BioAssist S, 7 µm, 4.6 mm ID x 5 cm L;
Mobile phase: 20 mmol/L sodium phosphate buffer, pH 6.0;
Gradient: 0 mol/L - 0.3 mol/L NaCl (5 min), 0.3 mol/L - 0.5 mol/L NaCl (10 min);
Flow rate: 1 mL/min; Detection: UV @ 280 nm; Sample: 500 µL of 9.5 mg/mL IgM in mouse ascites fluid; shaded peaks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

IEC
TSKgel 5PW SERIES

The G5000 PW base resin of the TSKgel 5PW series is a spherical particle with a mean pore size of 100 nanometer. The chemistries of DEAE, SP and CM functionalities result in standard ion exchangers while the chemistry employed in the manufacturing of TSKgel SuperQ-5PW results in a higher capacity strong anion exchanger by introducing polyamine functional groups. Due to the higher density of anion exchange sites TSKgel SuperQ-5PW has a smaller effective pore size and a higher binding capacity than TSKgel DEAE-5PW.

APPLICATIONS WITH TSKgel 5PW ANION EXCHANGE COLUMNS

**Analysis of a synthetic Oligonucleotide on TSKgel SuperQ-5PW**

Figure 14 shows the analysis of a 16-mer morpholine oligo-nucleotide on TSKgel SuperQ-5PW column using a NaCl gradient in a 10 mmol/L sodium hydroxide mobile phase.

Analysis of high MW RNA on TSKgel DEAE-5PW

Figure 15 shows the fractionation of high molecular weight E. coli RNA on TSKgel DEAE-5PW, effectively utilizing the large 100 nm pores of this base resin.

➤ **FIGURE 14**

ANALYSIS OF SYNTHETIC OLIGONUCLEOTIDE ON TSKgel SuperQ-5PW
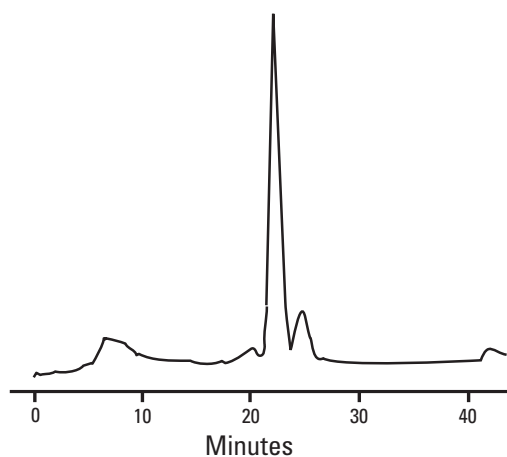
Column: TSKgel SuperQ-5PW, 7.5 mm ID x 7.5 cm L;
Sample: 16-mer morpholine oligonucleotide, AAG AAG AAG AGG GGA G;
Sample load: 0.5 O.D. (optical density); Mobile phase: A: 10 mmol/L NaOH;
B: 10 mmol/L NaOH with 1 mol/L NaCl; Gradient: Initial: 0 % B, 40 min: 50 % B,
41 min: 100 % B, 46 min: 100% B; Flow rate: 1 mL/min; Detection: UV @ 254 nm

➤ **FIGURE 15**

LARGE PORE TSKgel DEAE-5PW RESOLVES HIGH MW RNA
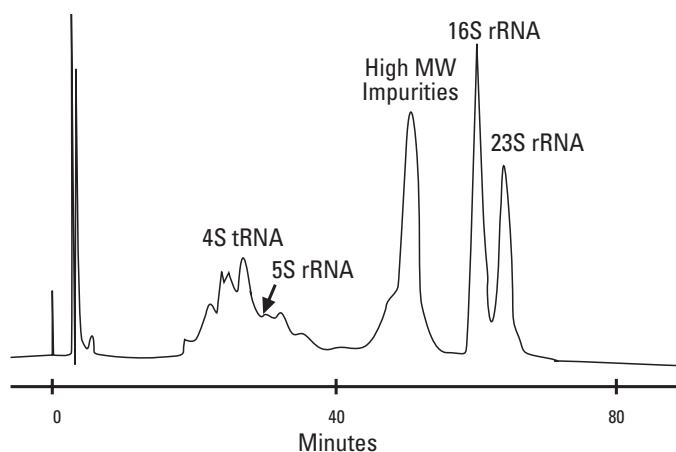
Column: TSKgel DEAE-5PW, 6 mm ID x 15 cm L; Sample: total E. coli RNA; Elution: 300 min linear gradient from 0.3 mol/L NaCl in 0.1 mol/L Tris-HCl, pH 7.6; Flow rate:1.0 mL/min; Detection: UV @ 260 nm

IEC
TSKgel 5PW SERIES

APPLICATIONS WITH TSKgel 5PW CATION EXCHANGE COLUMNS

Comparison of strong and weak Cation Exchangers

Differences in selectivity between strong (TSKgel SP-5PW) and weak (TSKgel CM-5PW) cation exchangers are demonstrated in Figure 16 which is a separation of globular proteins.

Semi-preparative Purification of Lipoxidase

The purification of 200 mg of crude lipoxidase on a 21.5 mm ID TSKgel SP-5PW column is illustrated in Figure 17. 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) colum.

FIGURE 16

SELECTIVITY ON TSKgel STRONG AND WEAK CATION EXCHANGERS
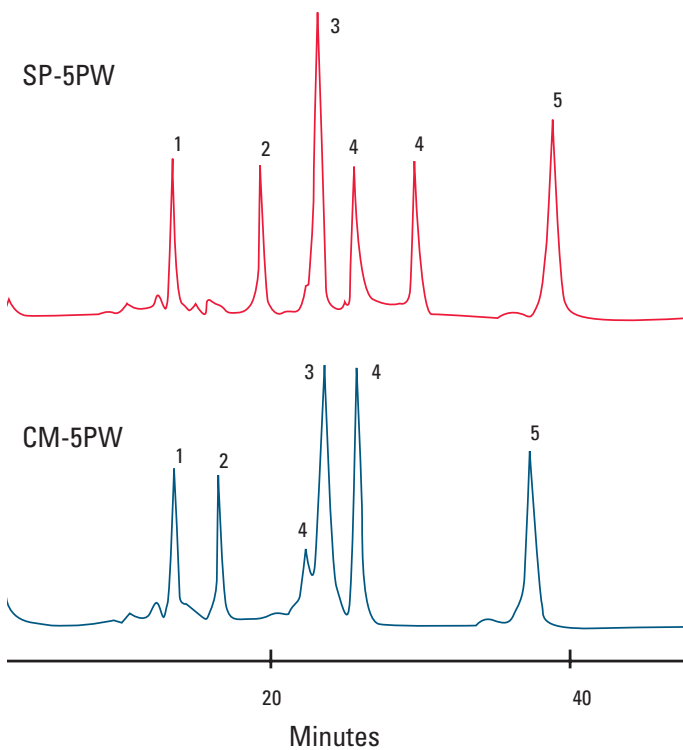
Columns: TSKgel SP-5PW and TSKgel CM-5PW, 7.5 mm ID x 7.5 cm L; Sample: 1. trypsinogen, 2. ribonuclease A, 3. α-chymotrypsinogen, 4. cytochrome C, 5. lysozyme;
Elution: 60 min linear gradient from 0 mol/L to 0.5 mol/L NaCl in 0.02 mol/L phosphate, pH 7.0; Flow rate: 1.0 mL/min; Detection: UV @ 280 nm

FIGURE 17

SEMI-PREPARATIVE PURIFICATION OF LIPOXIDASE
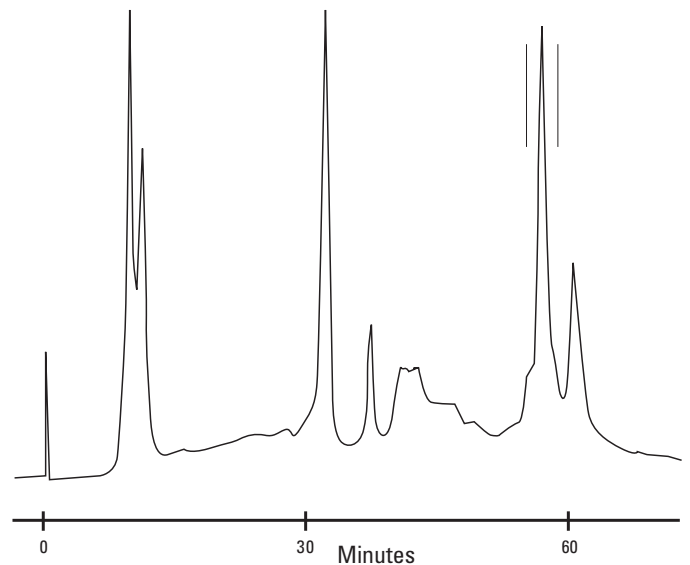
Column: TSKgel SP-5PW, 21.5 mm ID x 15 cm L; Sample: crude lipoxidase, 200 mg; Elution: 120 min linear gradient from 0 mol/L to 0.5 mol/L Na_2SO_4 in 0.02 mol/L acetate, pH 4.5; Flow rate: 4.0 mL/min; Detection: UV @ 280 nm; Recovery: Lipoxidase activity collected between the two vertical lines was 84%.

IEC
TSKgel NPR SERIES

TSKgel DEAE-NPR, DNA-NPR and SP-NPR are packed with 2.5 µm particles. High column efficiency coupled with low sample capacity restricts the application of these columns to fast analysis and micro-scale preparative isolation.

The DNA-NPR column is a longer version of the DEAE-NPR column that allows improved resolution of oligonucle-otides, including those amplified by PCR. Small guard columns are available to protect the DNA-NPR and DEAE-NPR columns.

APPLICATIONS OF TSKgel NPR ION EXCHANGE COLUMNS

TSKgel DEAE-NPR and DNA-NPR Anion Exchangers

Because of their small particle size, non-porous resin (NPR) columns excel in rapid separations of large biomolecules such as DNA digests. A chromatogram of a standard Hae III digest of pBR322 DNA on TSKgel DEAE-NPR, protected by a guard column, is shown in Figure 18.

To achieve better resolution for PCR fragment analysis we recommend the use of TSKgel DNA-NPR columns, which are 7.5 cm long and 4.6 mm wide, providing higher efficiency in a longer column.

TSKgel SP-NPR Cation Exchanger

TSKgel SP-NPR columns provide fast separations due to their small spherical particles. A purity check of adeno-associated virus, commonly used in gene therapy research, on a TSKgel SP-NPR column is shown in Figure 19. This 10 minute HPLC method replaces an existing assay that took two days.

FIGURE 18

HIGHER RESOLUTION AND FASTER ANALYSIS ON TSKgel DEAE-NPR
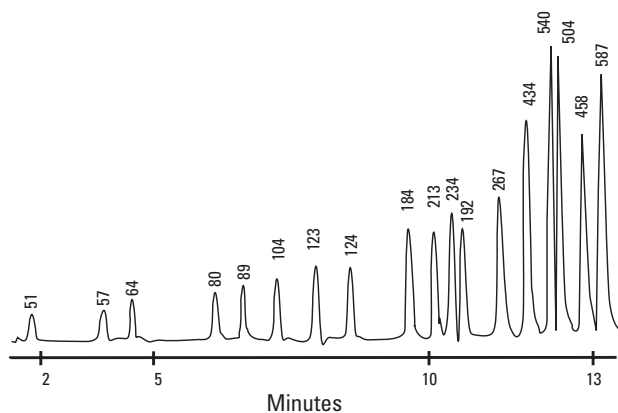
Column: TSKgel DEAE-NPR, 4.6 mm ID x 3.5 cm L, with guard column, 4.6 mm ID x 0.5 cm L; Sample: Hae III digest of pBR322 DNA, (base pair number for each peak is indicated); Buffer A: 0.02 mol/L Tris-HCl, pH 9.0; Buffer B: Buffer A plus 1.0 mol/L NaCl; Elution: 15 min linear gradient from 48 % to 65 % buffer B; Flow rate:1.5 mL/min; Pressure: 14 Mpa; Temp.: 40 C; Detection: UV @ 260 nm

FIGURE 19

ANALYSIS OF PURIFIED AAV WITH TSKgel SP-NPR
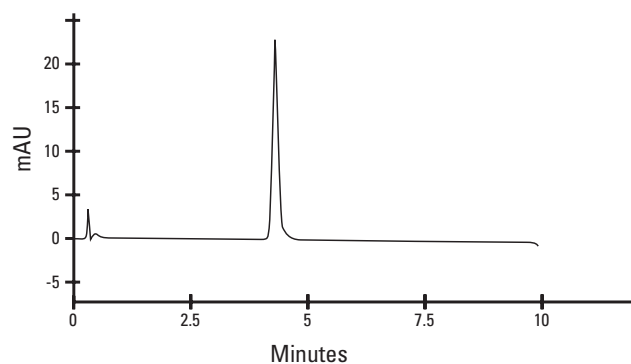
Column: TSKgel SP-NPR, 4.6 mm ID x 3.5 cm L; Sample: purified adeno-associated virus; Elution: A. 50 mmol/L HEPES, 1 mmol/L EDTA, 5 mmol/L MgCl, pH 7.5; B. 50 mmol/L HEPES, 1 mmol/L EDTA, 5 mmol/L MgCl, pH 7.5 with 0.5 mol/L NaCl; linear gradient from 20 % to 100 % B in 10 column volumes; Flow rate: 1 mL/min; Detection: UV @ 280 nm

IEC
TSKgel SW SERIES

The silica-based ion exchange columns are typically used in the separation of low molecular weight compounds such as pharmaceuticals, nucleotides or small peptides. Silica-based particles are used in pore sizes of 125 nanometer (2SW) and 250 nanometer (3SW) with either diethylaminoethyl (DEAE) or carboxymethyl (CM) functionality. Binding capacity for small to medium size proteins on TSKgel DEAE-3SW is roughly double that of the DEAE-5PW due to the smaller pore size and larger surface area.

The increased solubility of the silica backbone at pH above pH 7.5 limits the use of silica based ion exchange columns to acidic or neutral mobile phases.

APPLICATIONS OF TSKgel SW ION EXCHANGE COLUMNS

Silica-based Anion Exchange Columns

TSKgel 2SW-type columns provide high performance separations of small ionic solutes. High performance analyses of small anionic species are best performed on small pore silica-based anion exchangers, such as TSKgel DEAE-2SW. This is demonstrated in Figure 20. The 250 nanometer pore size TSKgel DEAE-3SW column is used for separating peptides, low MW proteins and DNA fragments.

Silica-based Cation Exchange Columns

Silica-based cation exchangers are typically used in the separation of low molecular weight compounds such as pharmaceuticals, nucleotides, catecholamines, and small peptides. For example, Figure 21 shows the separation of nucleosides on TSKgel SP-2SW.
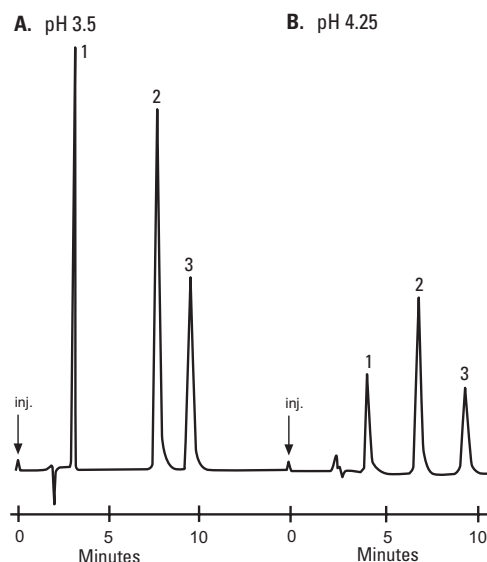
FIGURE 20

SEPARATION OF NUCLEOTIDES ON TSKgel DEAE-2SW
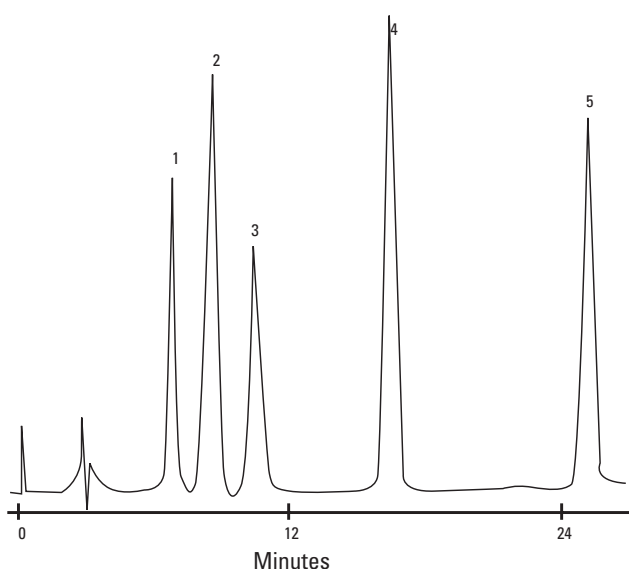
Column: TSKgel DEAE-2SW, 4.6 mm ID x 25 cm L; Sample: 1. AMP, 2. IMP, 3. GMP, 4.ADP, 5. ATP; Buffer A: ACN in 0.1 mol/L phosphate, pH 3.0, 20/80; Buffer B: ACN in 0.5 mol/L phosphate, pH 3.0, 20/80; Elution: 30 min linear gradient from buffer A to B; Flow rate: 1.0 mL/min; Detection: UV @ 260 nm

FIGURE 21

SEPARATION OF NUCLEOSIDES BY IEC ON TSKgel SP-2SW

Column: TSKgel SP-2SW 4.6 mm ID x 25 cm L
Sample: Nucleoside Standards: 1) Guanosine, 2) Cytidine, 3) Adenosine
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; Temperature 23 °C; Detection: UV @ 260 nm

TSKgel ANION EXCHANGE COLUMNS ORDERING INFORMATION

➤ **ORDERING INFORMATION**

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Flow rate (mL/min) Range	Maximum pressure drop (MPa)
TSKgel GLASS COLUMNS: POLYMER-BASED							
0013061	DEAE-5PW Glass, 100 nm	5.0	5.0	10	≥ 700	0.5 - 0.8	1.5
0008802	DEAE-5PW Glass, 100 nm	8.0	7.5	10	≥ 1,300	0.5 - 1.0	1.0
0014016	DEAE-5PW Glass, 100 nm	20.0	15.0	13	≥ 3,000	4.0 - 6.0	1.5
0018386	SuperQ-5PW Glass, 100 nm	8.0	7.5	10	≥ 1,300	0.5 - 1.0	2.0
TSKgel PEEK COLUMNS: POLYMER-BASED							
0019685	BioAssist Q, 400 nm	4.6	5.0	10	≥ 500	0.3 - 1.0	2.5
0021410	BioAssist Q, 400 nm	10.0	10.0	13	≥ 500	1.0 - 5.0	2.5
TSKgel STAINLESS STEEL COLUMNS: POLYMER-BASED							
0021960	Q-STAT, nonporous	3.0	3.5	10	> 200	1.0 - 2.0	10.0
0021961	Q-STAT, nonporous	4.6	10.0	7	> 4,000	0.5 - 1.4	10.0
0021962	DNA-STAT, nonporous	4.6	10.0	5	> 4,000	0.3 - 0.6	15.0
0013075	DEAE-NPR, nonporous	4.6	3.5	2.5	≥ 1,300	1.0 - 1.5	20.0
0018249	DNA-NPR, nonporous	4.6	7.5	2.5	≥ 6,000	0.5 - 1.0	30.0
0018757	DEAE-5PW, 100 nm	2.0	7.5	10	≥ 1,300	0.05 - 0.10	1.5
0007164	DEAE-5PW, 100 nm	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.5
0007574	DEAE-5PW, 100 nm	21.5	15.0	13	≥ 3,000	4.0 - 6.0	2.5
0007930	DEAE-5PW, 100 nm	55.0	20.0	20	≥ 1,500	20.0 - 40.0	0.4
0018257	SuperQ-5PW, 100 nm	7.5	7.5	10	≥ 1,300	0.5 - 1.0	2.0
0018387	SuperQ-5PW, 100 nm	21.5	15.0	13	≥ 3,000	4.0 - 6.0	2.0
0008639	Sugar AXI, 6 nm	4.6	15.0	8	≥ 3,700	0.2 - 0.4	3.0
0008640	Sugar AXG, 6 nm	4.6	15.0	10	≥ 2,700	0.2 - 0.5	2.0
0007157	SAX, 6 nm	6.0	15.0	5	≥ 2,000	0.5 - 1.0	15.0
TSKgel STAINLESS STEEL COLUMNS: SILICA-BASED							
0018761	DEAE-2SW, 12.5 nm	2.0	25.0	5	≥ 5,000	0.12 - 0.17	13.0
0007168	DEAE-2SW, 12.5 nm	4.6	25.0	5	≥ 5,000	0.6 - 0.8	15.0
0007163	DEAE-3SW, 25 nm	7.5	7.5	10	≥ 1,300	0.5 - 1.0	2.0
TSKgel GUARD COLUMN PRODUCTS							
0017088	DEAE-NPR Guard column	4.6	0.5	5	For P/N 0013075		
0018253	DNA-NPR Guard column	4.6	0.5	2.5	For P/N 0018249		
0018388	SuperQ-5PW Guardgel Kit			20	For P/N 0018257		
0007210	DEAE-5PW Guardgel Kit			20	For P/N 0007164		
0008806	DEAE-5PW Guardgel Kit, Glass			20	For P/Ns 0013061 and 0008802		
0014466	DEAE-5PW Guardcol., Glass	20.0	2.0	13	For P/N 0014016		
0016092	DEAE-5PW Prep Guardgel Kit			20	For P/N 0007574		
0007928	DEAE-5PW Guard column	45.0	5.0	20	For P/N 0007930		
0007648	DEAE-SW Guardgel Kit			10	For P/Ns 0007168 and 0007163		
0019308	Guard cartridge holder	2.0	1.5		For all 2 mm ID guard cartridges		

TSKgel CATION EXCHANGE COLUMNS ORDERING INFORMATION

➤ **ORDERING INFORMATION**

Part #	Description	ID (mm)	Length (cm)	Particle size (μm)	Number theoretical plates	Flow rate (mL/min) Range	Maximum pressure drop (MPa)
TSKgel GLASS COLUMNS: POLYMER-BASED							
0014012	CM-5PW Glass, 100 nm	20.0	15.0	13	≥ 2,500	4.0 - 6.0	1.5
0013062	SP-5PW Glass, 100 nm	5.0	5.0	10	≥ 700	0.5 - 0.8	1.5
0008803	SP-5PW Glass, 100 nm	8.0	7.5	10	≥ 1,300	0.5 - 1.0	1.0
0014017	SP-5PW Glass, 100 nm	20.0	15.0	13	≥ 3,000	4.0 - 6.0	1.5
TSKgel PEEK COLUMNS: POLYMER-BASED							
0019686	BioAssist S, 130 nm	4.6	5.0	7	≥ 1,500	0.3 - 0.8	2.5
0021411	BioAssist S, 130 nm	10.0	10.0	13	≥ 3,000	1.0 - 5.0	2.5
TSKgel STAINLESS STEEL COLUMNS: POLYMER-BASED							
0021965	CM-STAT, nonporous	3.0	3.5	10	≥ 200	1.0 - 2.0	10.0
0021966	CM-STAT, nonporous	4.6	10.0	7	≥ 2,000	0.5 - 1.0	10.0
0021963	SP-STAT, nonporous	3.0	3.5	10	≥ 200	1.0 - 2.0	10.0
0021964	SP-STAT, nonporous	4.6	10.0	7	≥ 200	0.5 - 1.4	10.0
0013068	CM-5PW, 100 nm	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.5
0018758	SP-5PW, 100 nm	2.0	7.5	10	≥ 1,300	0.05 - 0.10	1.0
0007161	SP-5PW, 100 nm	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.5
0007575	SP-5PW, 100 nm	21.5	15.0	13	≥ 3,000	4.0 - 6.0	2.5
0007934	SP-5PW, 100 nm	55.0	20.0	20	≥ 1,500	20.0 - 40.0	0.4
0013076	SP-NPR, nonporous	4.6	3.5	2.5	≥ 1,300	1.0 - 1.5	20.0
0007156	SCX (Na$^+$), 6 nm	6.0	15.0	5	≥ 2,000	0.5 - 1.0	15.0
0007158	SCX (H$^+$) 6 nm	7.8	30.0	5	≥ 12,000	0.5 - 1.0	5.0
TSKgel STAINLESS STEEL COLUMNS: SILICA-BASED							
0007165	SP-2SW, 12.5 nm	4.6	25.0	5	≥ 5,000	0.6 - 0.8	15.0
0007167	CM-2SW, 12.5 nm	4.6	25.0	5	≥ 5,000	0.6 - 0.8	15.0
0007162	CM-3SW, 25 nm	7.5	7.5	10	≥ 1,300	0.5 - 1.0	2.0
GUARD COLUMN PRODUCTS							
0013069	CM-5PW Guardgel Kit			10	For P/N 0013068		
0007211	SP-5PW Guardgel Kit			20	For P/N 0007161		
0008807	SP-5PW Guardgel Kit, Glass			20	For P/Ns 0013062 and 0008803		
0016093	SP-5PW Prep Guardgel Kit			20	For P/N 0007575		
0007932	SP-5PW Guard column	45.0	5.0	20	For P/N 0007934		
0007650	CM-SW Guardgel Kit			20	For P/Ns 0007167 and 0007162		

FURTHER INFORMATION

To get an overview about the whole range of TSKgel columns and small TOYOPEARL and TSKgel bulk media, please request our **Chromatography Catalog**.

For a complete overview of all TOYOPEARL and TSKgel bulk media, refer to our **Chromatographic Process Media Catalog**.
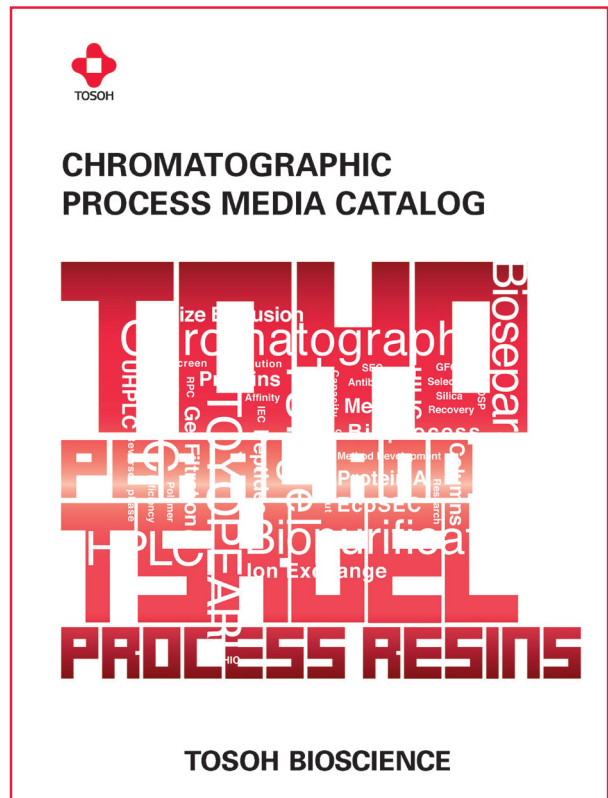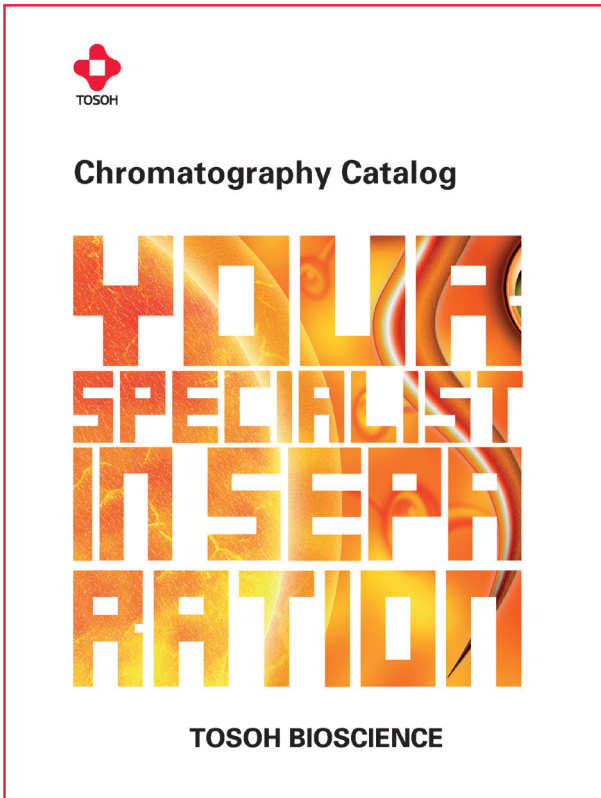
For a deeper insight into applications and questions related to the practical use of TSKgel and TOYOPEARL, check out the website **www.tosohbioscience.de**

Our technical experts are happy to discuss your specific separation needs by phone: **+49 (0)6155-70437-36** or mail: **techsupport.tbg@tosoh.com**