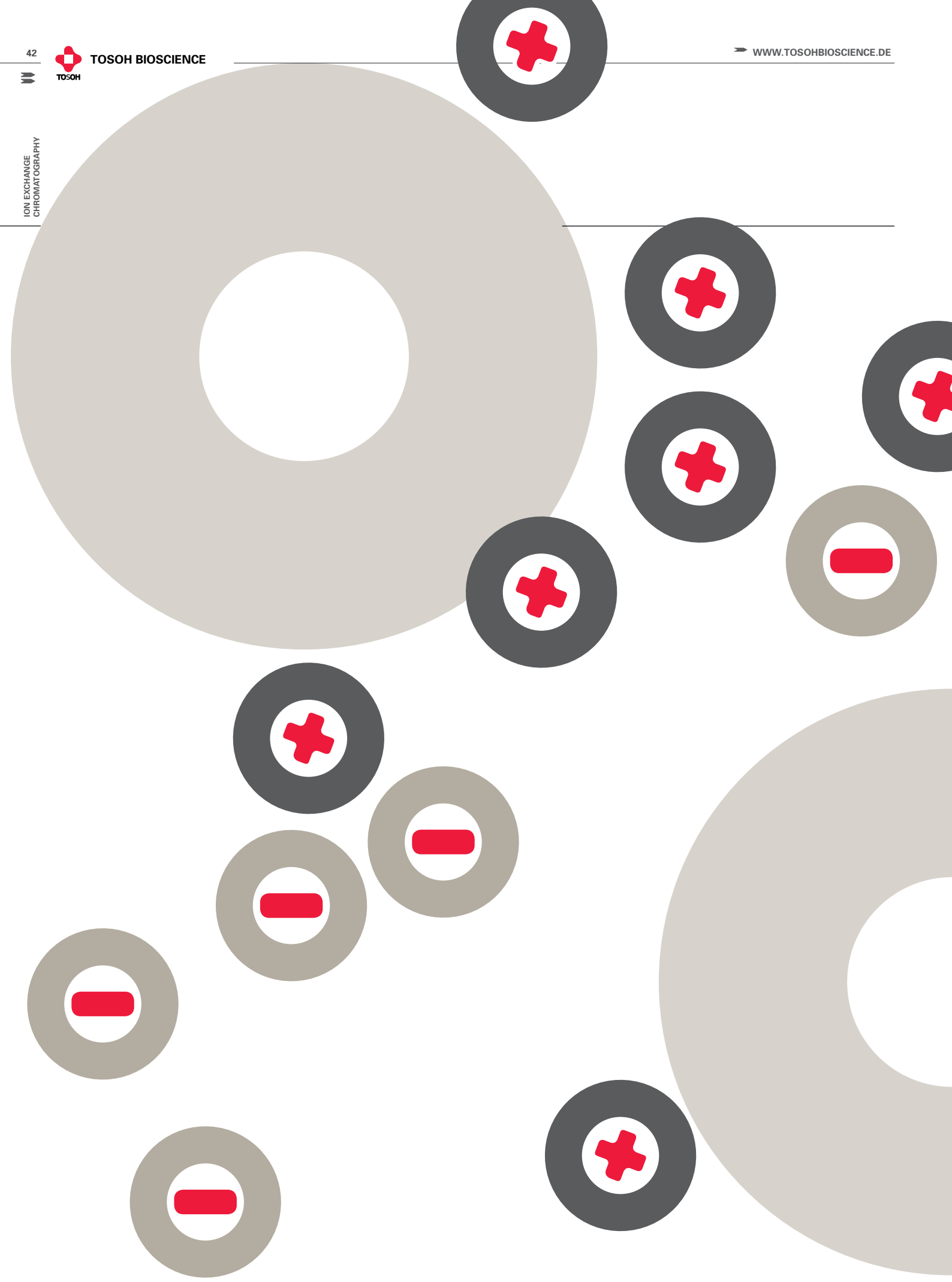


IEC
ION EXCHANGE CHROMATOGRAPHY

IEC PRODUCTS

ANION EXCHANGE

TSKgel Q-STAT
TSKgel DNA-STAT
TSKgel BioAssist Q
TSKgel SuperQ-5PW
TSKgel DEAE-5PW
TSKgel DEAE-NPR
TSKgel DNA-NPR
TSKgel DEAE-2SW
TSKgel DEAE-3SW
TSKgel Sugar AXI
TSKgel Sugar AXG
TSKgel SAX

CATION EXCHANGE

TSKgel SP-STAT
TSKgel CM-STAT
TSKgel BioAssist S
TSKgel SP-5PW
TSKgel CM-5PW
TSKgel SP-2SW
TSKgel SP-NPR
TSKgel CM-2SW
TSKgel CM-3SW
TSKgel SCX

TOSOH FACT

Tosoh Corporation maintains a large database of HPLC applications utilizing TSKgel columns. Sources for this database include articles in journals citing the use of TSKgel columns by Tosoh customers as well as technical papers and presentations created by Tosoh scientists.

Tosoh Bioscience offers a large literature library consisting of application notes, instruction manuals, product overviews and separation reports.

Both the literature library and the chromatogram database are available on the website at www.tosohbioscience.de.

INTRODUCTION TO TSKgel ION EXCHANGE 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 Ion Exchange Chromatography (IEC), the product line contains methacrylate-, silica- and polystyrene-based columns. Proteins, peptides, oligonucleotides and other nucleic acid fragments are typical samples that are analyzed or isolated on TSKgel ion exchange columns. Most of the available chemistries are offered in analytical as well as semi-preparative column formats. Particle sizes range from 2.5 µm, for fast quality control and process monitoring, to 20 µm and larger particle sizes utilized in process scale separations.

TSKgel STAT® columns are the latest addition to the IEC column line. They are designed for high effiency separation of biomolecules and low molecular weight compounds. TSKgel STAT columns provide superior performance at reduced analysis time. The STAT series encompasses a range of high efficiency anion and cation exchange columns, suitable for various applications from research to quality control.

Also available are a series of ion exchange columns based on a polystyrene matrix. They are most suitable for analyzing small molecular weight sugars, amino acids, individual nucleic acids, and small drug candidates.

PACKING MATERIALS AND CHEMISTRIES

Methacrylate, silica, and polystyrene are used as matrices for the TSKgel line of ion exchange columns. 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 polymethacrylate base resin, G5000PW (5PW), is a 10 µm spherical particle with approximately 100 nm pores. The base resin is derivatized either with diethylaminoethyl (DEAE), sulfopropyl (SP) or carboxymethyl (CM) functionalities to provide a weak anion, a strong cation, and a weak cation exchanger, respectively. While these chemistries result in standard ion exchangers, 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 than TSKgel DEAE-5PW.

For more detailed information, please refer to our ***TSKgel IEC brochure*** on www.tosohbioscience.de, or request a printed copy at sales-marketing.tbg@tosoh.com.

FEATURES	BENEFITS
BioAssist columns	
High capacity even for larger proteins (1 million Da)	Fewer runs to collect required sample amounts
Unique pore structure provides fast mass transfer	Sharper peaks improve analysis and isolation
Biocompatible PEEK column hardware	Less sample loss due to adsorption
Available in analytical and semi-prep formats	Easy scale-up
Polymer-Based Ion Exchange columns	
Methacrylate backbone	Mechanically and chemically stable (pH 2-12)
	Withstands repeated cleaning with base, and use of organic solvents, denaturants and surfactants
Large pore size (100 nm) (excl. limit for proteins ~ 5,000,000 Da)	Use same column for most biopolymers
Non porous resin-based (STAT and NPR) columns	Fast QC analysis and process monitoring
Several columns available in 2 mm ID format	Reduced solvent consumption and analysis time
Silica-Based Ion Exchange columns	
Smaller pore size (2SW = 12.5 nm and 3SW = 25 nm)	Most suitable for analysing smaller MW samples such as nucleotides, drug candidates, catecholamines and small peptides or proteins

IEC

INTRODUCTION TO TSKgel ION EXCHANGE COLUMNS

TSKgel BIOASSIST columns are also 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. Semi-preparative 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.

The methacrylate chemistry also forms the backbone of non-porous resin columns such as TSKgel STAT and NPR columns. Since rate-limiting pore difusion 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 ion exchange columns are packed with 5, 7 or 10 µm hydrophilic non-porous resin particles of which the surface consists of an open access network of multi-layered ion exchange groups (carboxymethyl, sulfopropyl, or quaternary ammonium groups; see FIGURE 1.

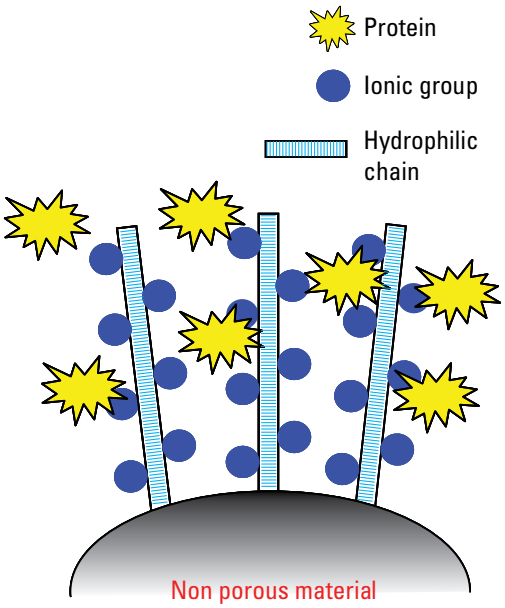
These relatively large particle sizes, combined with an innovative bonding chemistry, result in columns that enable fast equilibration and analysis of complex biomolecular samples, attributes not found in traditional mono-disperse, non-porous stationary phases.

Specific application needs are addressed by offering various column formats and particle sizes: For fast and ultra-fast analysis (e.g. screening or process monitoring) short 3 mm ID columns are packed with 10 µm particles. For high resolution separations longer columns with 4.6 mm ID are packed with 7 µm particles. The DNA-STAT column is packed with smaller particles (5 µm).

TSKgel DEAE-NPR, SP-NPR and DNA-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 oligonucleotides, including those amplified by PCR. Small guard columns are available to protect the DNA-NPR and DEAE-NPR columns.

In the development of new drug candidates, it is often desirable to use the same backbone chemistry throughout the development process. For that reason, the backbone of the 20 µm and 30 µm particle size TSKgel PW-type resins and the larger particle size TOYOPEARL process media are chemically similar to that used in prepacked TSKgel PW-type column lines. As a result, TSKgel SuperQ-5PW scales directly to TOYOPEARL SuperQ-650. Similarly, the TSKgel DEAE-5PW scales directly to TSKgel DEAE-5PW bulk resins, which in turn scales to TOYOPEARL DEAE-650. The same is true for CM and SP products in the cation exchange column line.

FIGURE 1
Schematic diagram of TSKgel STAT Series



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	25.0	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	4.0	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*

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TSKgel ION EXCHANGE COLUMN SELECTION

Sample type	MW range (Da)	TSKgel column	pH range
Amino acids, peptides and proteins			
Amino acids	< 2000	SAX	1 - 14
		SCX	1 - 14
Peptides and small proteins	< 10,000	Q-STAT	3 - 10
		SP-STAT	3 - 10
		CM-STAT	3 - 10
		SCX	1 - 14
		SP-2SW	2 - 7.5
		CM-2SW	2 - 7.5
		DEAE-2SW	2 - 7.5
Proteins	> 10,000 up to ~ 5,000,000	BioAssist S	2 - 12
		BioAssist Q	2 - 12
		Q-STAT	3 - 10
		SP-5PW	2 - 12
		DEAE-5PW	2 - 12
		CM-5PW	2 - 12
		SP-STAT	3 - 10
		CM-STAT	3 - 10
		SP-NPR	2 - 12
		DEAE-NPR	2 - 12
		SuperQ-5PW	2 - 12
Nucleic acids			
Purines and pyrimidines		DEAE-2SW	2 - 7.5
		SP-2SW	2 - 7.5
Nucleosides		SP-2SW	2 - 7.5
		DEAE-2SW	2 - 7.5
Nucleotides		Q-/DNA-STAT	3 - 10
		DEAE-2SW	2 - 7.5
Oligonucleotides		Q-/DNA-STAT	3 - 10
		DEAE-5PW	2 - 12
		DEAE-NPR	2 - 12
		DNA-NPR	2 - 12
		SuperQ-5PW	2 - 12
DNA, RNA, and PCR products		Q-/DNA-STAT	3 - 10
		DNA-NPR	2 - 12
		DEAE-NPR	2 - 12
		DEAE-5PW	2 - 12
		DEAE-3SW	2 - 7.5
Other molecules			
Mono and disaccharides		Sugar AXI, AXG	1 - 14
		SCX	1 - 14
		SAX	1 - 14

TSKgel ANION EXCHANGE COLUMNS

HIGHLIGHTS

- TSKgel Q- and DNA-STAT columns provide high efficiency separations at short analysis time.
- TSKgel DNA-NPR columns are ideal for PCR fragment analysis.
- TSKgel SuperQ-5PW columns have higher capacity than TSKgel DEAE-5PW due to novel bonding chemistry, effective pore size is smaller for SuperQ-5PW.
- Pore structure and bonding chemistry of TSKgel BioAssist Q columns provide high capacity for small to very large MW proteins and nucleic acids.
- 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.
- 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.
- Specialty columns for analysis of mono- and disaccharides and sugar alcohols are also available.

NON-POROUS TSKgel STAT ANION EXCHANGE COLUMNS

STAT columns are available in various column formats and particle sizes to perfectly match specific application needs. For fast and ultra-fast analysis anion and cation exchange columns in 3 mm ID and 3.5 cm length are packed with 10 µm particles. They are ideally suited for rapid candidate screening or process monitoring. 4.6 mm ID and 10 cm length columns packed with 7 µm particles are designed for high resolution IEC separation for example for the separation of nucleic acids, mAb variants, PEGylated protein or protein aggregates.

The DNA STAT column (4.6 mm ID x 10 cm L) packed with 5 µm Q-type anion exchange resin is ideally suited for the analysis of nucleic acids.

The basic properties of TSKgel STAT anion exchange columns are summerized in TABLE I.

APPLICATIONS OF TSKgel ANION EXCHANGE COLUMNS

TABLE I

Basic properties of TSKgel STAT Anion Exchange columns

Property	TSKgel Q-STAT		TSKgel DNA-STAT
Base material	Cross-linked hydrophilic polymer (mono-disperse particles)		
Pore size	Non-porous		
Functional group	Quaternary ammonium (same chemistry)		
Particle size	7 µm	10 µm	5 µm
Column size	4.6 mm ID x 10 cm L	3 mm ID x 3.5 cm L	4.6 mm ID x 10 cm L
Application	High resolution protein separation	High resolution protein separation	High resolution DNA separations

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OLIGONUCLEOTIDES

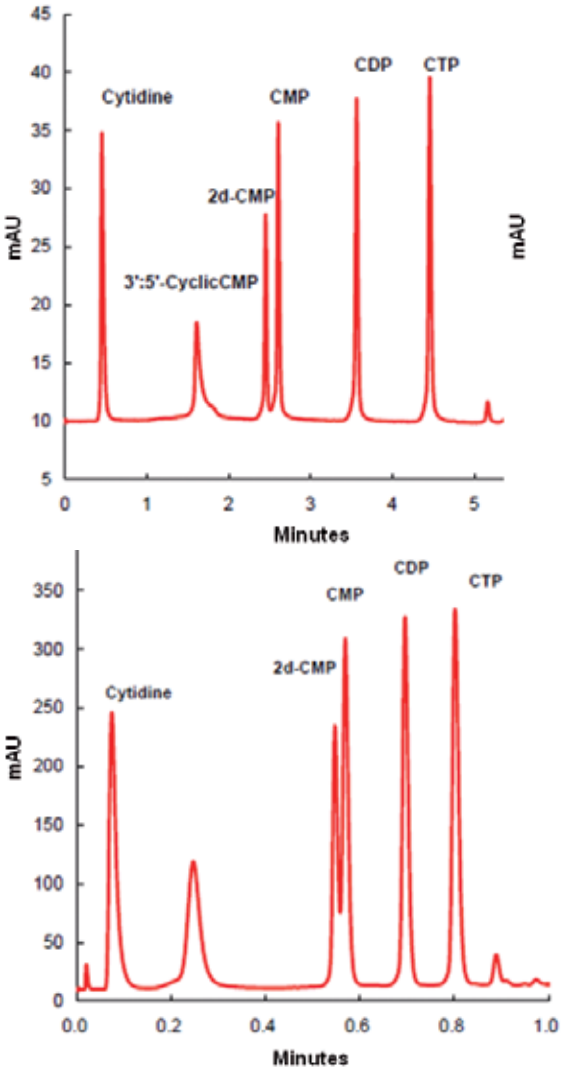
FIGURE 2 compares the high resolution separation of nucleotides on a 10 cm length column to the high throughput separation on a 3.5 cm length column.

POLYMER-BASED ANION EXCHANGE COLUMNS

TSKgel BioAssist Q is suitable for use in systems that are designed for laboratory or semi-preparative applications. FIGURE 3 demonstrates the performance enhancement of TSKgel BioAssist Q over a competitive product when operated side-by-side on an FPLC system.

FIGURE 2

High resolution versus high throughput analysis of nucleotides



High resolution:

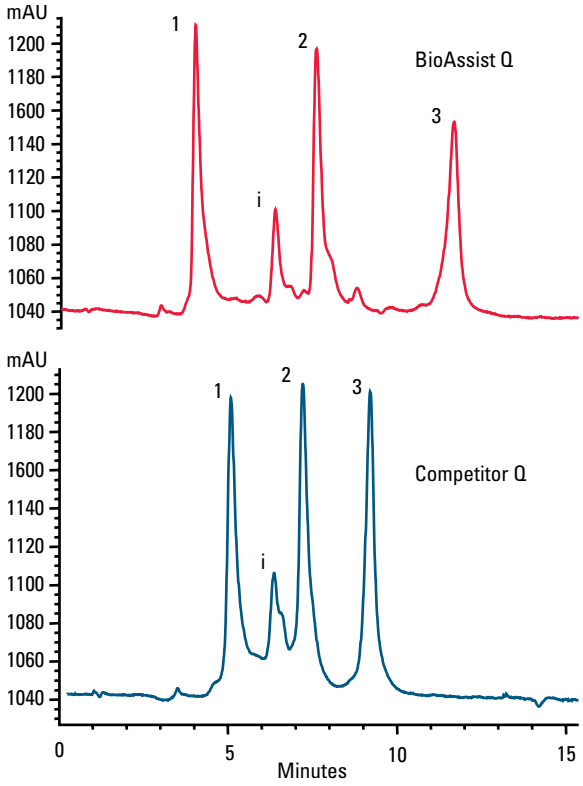
Column: TSKgel Q-STAT, 4.6 mm ID x 10 cm L (7 µm);
Eluent: A) 20 mmol/L Tris-HCl (pH8.5) B) 0.5 mol/L NaCl in A (pH8.5)
Gradient: 0 to 100% B (10 min.); Flow rate: 1.5 mL/min.
Detection: UV @ 260 nm

High throughput:

Column: TSKgel Q-STAT, 4.6 mm ID x 3.5 cm L (10 µm);
Eluent: A) 20 mmol/L Tris-HCl (pH8.5), B) 0.5mol/L NaCl in A (pH8.5)
Gradient: 0 to 100% B (1min.); Flow rate: 4.0 mL/min.
Detection: UV @ 260 nm

FIGURE 3

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

APPLICATIONS OF TSKgel ANION EXCHANGE COLUMNS

TSKgel SuperQ-5PW AND DEAE-5PW

FIGURE 4 shows the analysis of a 16-mer morpholine oligonucleotide on TSKgel SuperQ-5PW column using a NaCl gradient in a 10 mmol/L sodium hydroxide mobile phase.

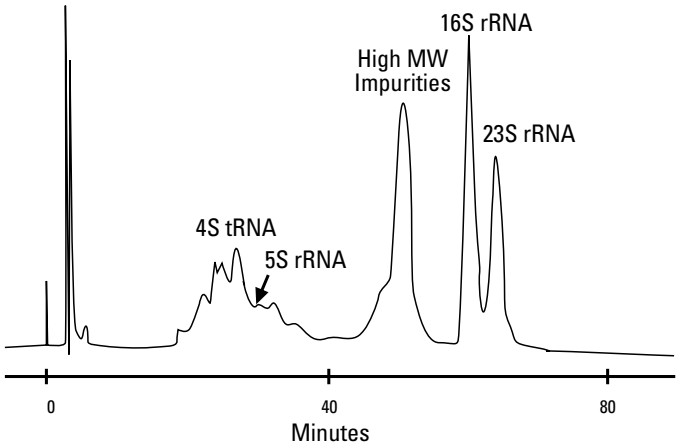
FIGURE 5 shows the fractionation of high molecular weight E. coli RNA on TSKgel DEAE-5PW, effectively utilizing the large 100 nm pores of this base resin.

TSKgel DEAE-NPR AND DNA-NPR

Because of their small (2.5 µm) 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 6. 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.

FIGURE 5

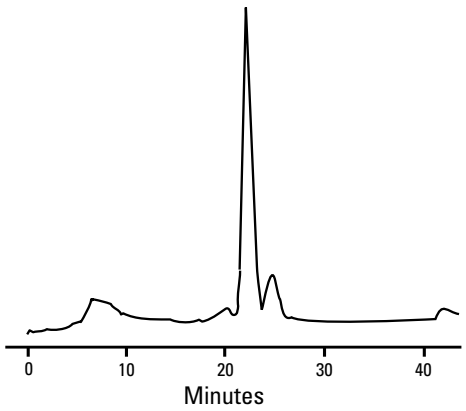
Large pore TSKgel DEAE-5PW resolves high MW RNA



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

FIGURE 4

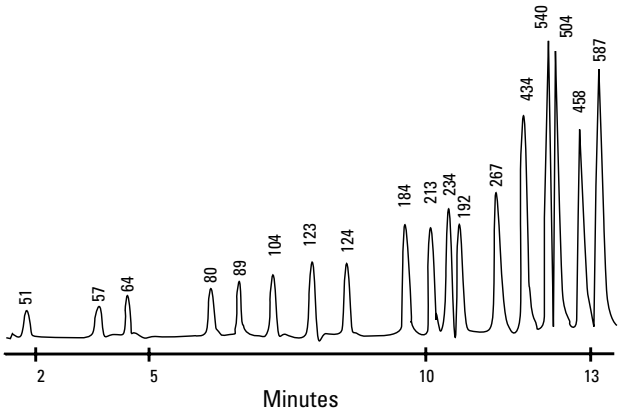
Analysis of synthetic oligonucleotide on TSKgel SuperQ-5PW



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, 40min: 50 % B, 41 min: 100 % B, 46min: 100% B; Flow rate: 1 mL/min; Detection: UV @ 254 nm

FIGURE 6

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: 2000 psi; Temp.: 40 °C; Detection: UV @ 260 nm

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APPLICATIONS OF TSKgel ANION EXCHANGE COLUMNS

SILICA-BASED ANION EXCHANGE COLUMNS

TSKgel 2SW-type columns provide high performance separations of small ionic solutes. The increased solubility of the silica backbone above pH 7 limits the use of the TSKgel 2SW-type columns to acidic or neutral mobile phases. This restricts method development and requires special cleaning procedures when compared to the more robust TSKgel 5PW-type polymer-based columns.

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 7. The 25 nm pore size TSKgel DEAE-3SW column is used for separating peptides, low MW proteins and DNA fragments.

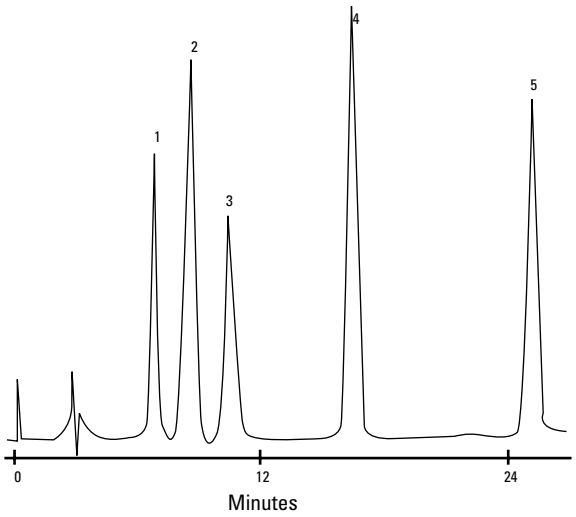
SPECIALTY COLUMNS

Analyses of monosaccharides, disaccharides, and sugar alcohols can be performed on PS-DVB columns, either by isocratic (TSKgel Sugar AXI) or by gradient (TSKgel Sugar AXG) analysis. Saccharides are retained on Sugar AX columns following the formation of negatively charged complexes with boric acid at alkaline pH. FIGURE 8 shows the separation of twelve mono- and di-saccharides.

The strong anion exchange TSKgel SAX column can be used for the separation of isomerized sugars, alcohols, and low molecular weight organic acids.

FIGURE 7

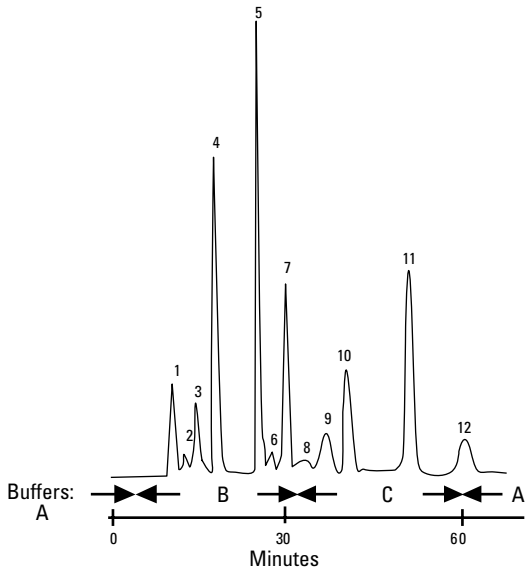
Separation of nucleotides on TSKgel DEAE-2SW



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 8

Separation of saccharide mixture on TSKgel Sugar AXG



Column: TSKgel Sugar AXG, 4.6 mm ID x 15 cm L; Sample: disaccharides, 25 mmol/L; monosaccharides, 50 mmol/L: 1. cellobiose, 2. maltose, 3. lactose, 4. rhamnose, 5. lyxose, 6. ribose, 7. mannose, 8. fructose, 9. arabinose, 10. galactose, 11. xylose, 12. glucose; Elution: step gradient: 6 min buffer A, 0.6 mol/L boric acid, pH 7.7; then 27 min buffer B, 0.7 mol/L boric acid, pH 7.25; then 30 min buffer C, 0.7 mol/L boric acid, pH 8.7; Flow rate: 0.4 mL/min (column and post column reagent solution); Pressure:16 kg/cm^2; Temperature: 70°C (column), 100 °C (post column reactor);
Detection: fluorescence excitation @331 nm, emission @383 nm;
PC reagent: 2.5 % 2-cyanoacetamide solution

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 - 0.1	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
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 Guard column, Glass	20.0	2.0	13	For P/N 0014016		
0016092	DEAE-5PW Prep Guardgel Kit			20	For P/N 0007574		
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		

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TSKgel CATION EXCHANGE COLUMNS

HIGHLIGHTS

- TSKgel SP-STAT and CM-STAT nonporous columns provide high efficiency separation at short analysis time.
- Pore structure and bonding chemistry of TSKgel BioAssist S provides high capacity for medium to large MW proteins.
- 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.
- Binding capacity for small to medium size proteins on TSKgel CM-3SW is approximately double that of TSKgel CM-5PW due to the smaller pore size and larger surface area.
- The TSKgel SP-5PW column is available in 2 mm ID format for LC-MS applications.

The basic properties of TSKgel STAT cation exchange columns are summarized in Table II

APPLICATIONS

TSKgel SP-STAT, CM-STAT

Nonporous TSKgel STAT columns provide fast, high resolution separations at moderate pressures. FIGURE 9 shows the monitoring of a PEGylation reaction of beta-lactoglobulin on a short SP-STAT column.

TSKgel CM-STAT columns are ideally suited to analyze the profile of charge isomers of proteins. FIGURE 10 shows the analysis profiles for five antibodies and their charge isomers separated on a TSKgel CM-STAT column.

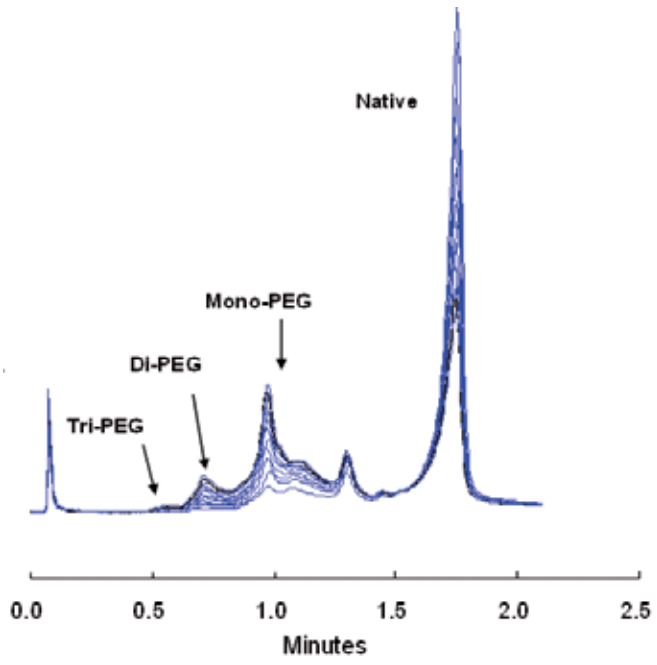
TABLE I I

Basic Properties of TSKgel STAT cation exchange Columns

Property	TSKgel SP-STAT		TSKgel CM-STAT	
Base material	Cross-linked hydrophilic polymer (mono-disperse particles)			
Pore size	Non-porous			
Functional group	Sulfonate		Carboxymethyl	
Particle size	7 µm	10 µm	7 µm	10 µm
Column size	4.6 mm ID x 10 cm L	3 mm ID x 3.5 cm L	4.6 mm ID x 10 cm L	3 mm ID x 3.5 cm L
Applica-tion	High resolu-tion protein separation	High throughput protein separation		

FIGURE 9

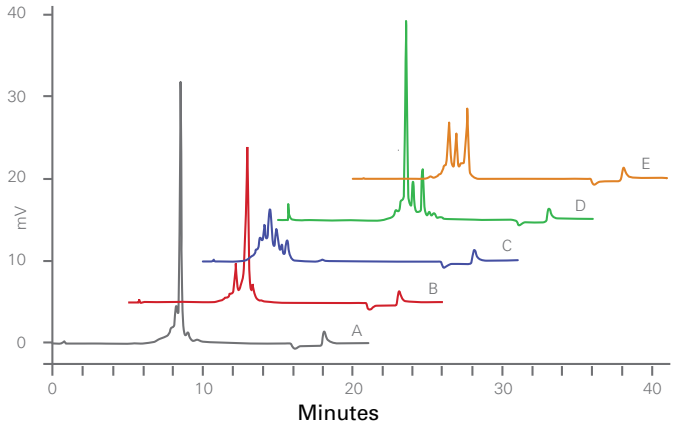
Monotoring of PEGylation of -lactoglobulin



Column: Prototype SP-STAT, 4.6 mm ID x 3.5 cm L, (10 um)
Eluent: A: 20 mmol/L Na acetate buffer pH 4.5, B: 0.8 mol/L NaCl in A pH 4.5; Gradient: 0 to 30% B (2 min); Flow rate: 4.0 mL/min; Detection: UV @ 280 nm
Real-time analysis of PEGylation reaction (PEG MW=5000) at 5-minutes intervals

FIGURE 10

Separation of MAB charge variants on TSKgel CM-STAT



Column: TSKgel CM-STAT column (7 µm, 4.6 mm ID x 10 cm L); Flow rate: 1 mL/min; Mobile phase: A: 20 mM MES (pH 6.0), B: 20 mM MES + 0.5 M NaCl (pH 6.0); Gradient 10% B to 15 % B in 15 minutes; Detection: UV @ 280 nm, Injection volume 20 µL

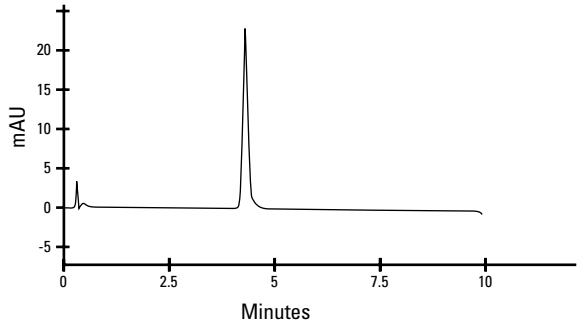
APPLICATIONS - TSKgel CATION EXCHANGE COLUMNS

TSKgel SP-NPR

TSKgel SP-NPR columns provide fast separations due to their small (2.5 µm) 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 11. This 10 minute HPLC method replaces an existing assay that took two days.

FIGURE 11

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. 50mmol/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

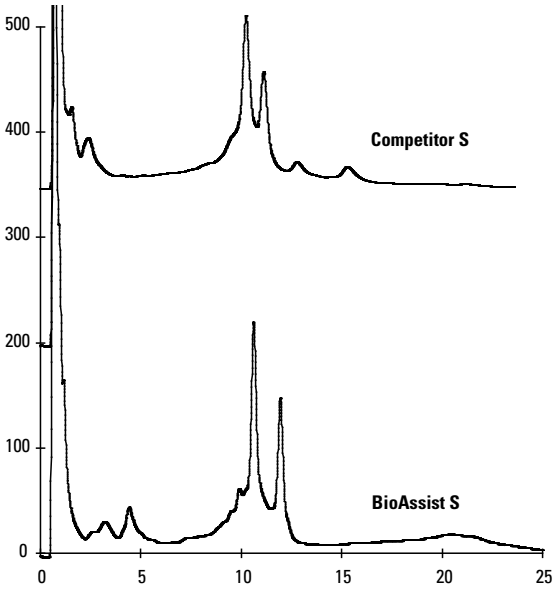
TSKgel Bioassist S

Especially designed for the separation of large biomolecules such as antibodies, 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. TSKgel BioAssist S is suitable for use in systems that are designed for HPLC, laboratory, or semi-preparative applications. The large pore size of the TSKgel BioAssist S resin provides high dynamic capacity due to novel bonded phase design. FIGURE 12 demonstrates these features for 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.

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 13 baseline separation of IgM from other contaminants is achieved using a 0.3 mol/L NaCl step gradient after elution of albumin.

FIGURE 12

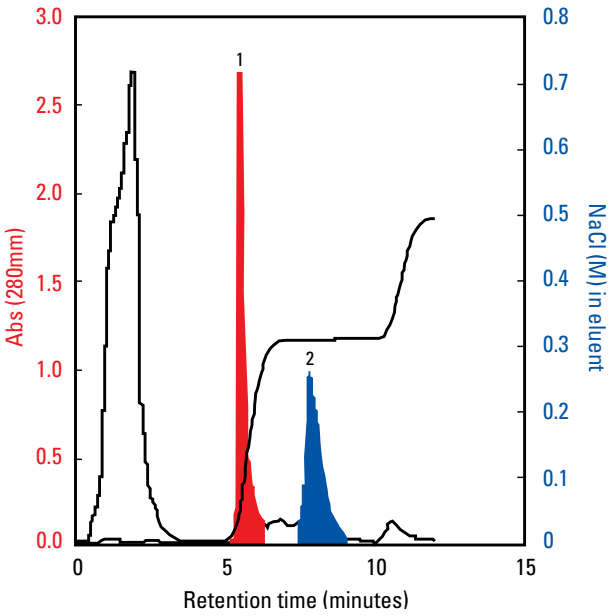
Bromelain Analysis on TSKgel Bioassist S and competitor S Columns



Columns: TSKgel BioAssist S, 4.6 mm ID x 5 cm L, PEEK
Competitor S 5mm ID x 5cm; 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

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TSKgel SP-5PW AND TSKgel CM-5PW

Differences in selectivity between strong (TSKgel SP-5PW) and weak (TSKgel CM-5PW) cation exchangers are demonstrated in FIGURE 14 which is a separation of globular proteins.

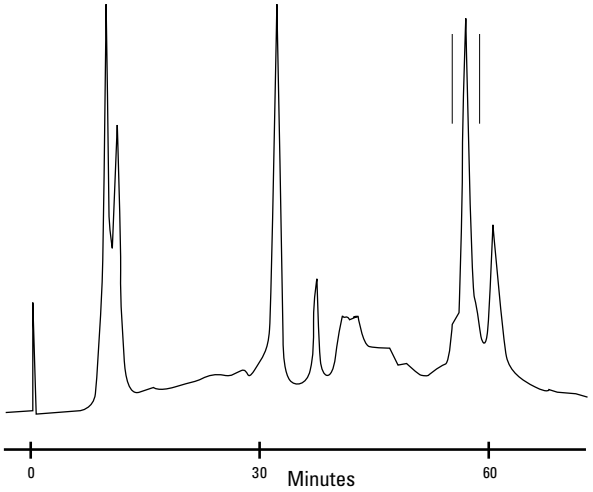
The purification of 200 mg of crude lipoxidase on a 21.5 mm ID TSKgel SP-5PW column is illustrated in FIGURE 15. Scale-up is simplified as only the particle size changes from 10 µm (7.5 mm ID) to 13 µm (21.5 mm ID) or 20 µm (55 mm ID) columns.

TSKgel SP-2SW, CM-2SW AND CM-3SW

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 16 shows the separation of nucleosides on TSKgel SP-2SW.

FIGURE 15

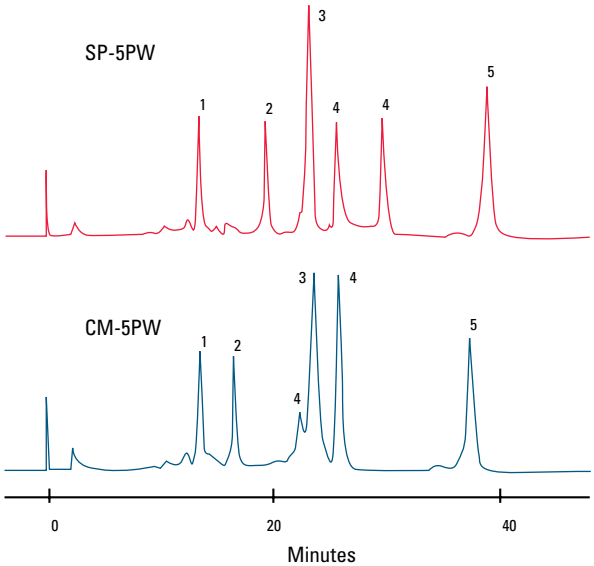
Semi-preparative purification of lipoxidase



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%.

FIGURE 14

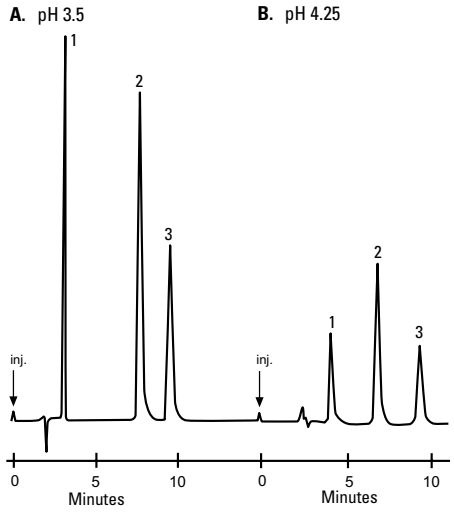
Selectivity on TSKgel strong and weak cation exchangers



Columns: TSKgel SP-5PW and TSKgel CM-5PW, 7.5 mm ID x 7.5 cm L; Sample: 1. trypsinogen, 2. ribonuclease A, 3. a-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 16

Separation of nucleosides by ion-exchange chromatography 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

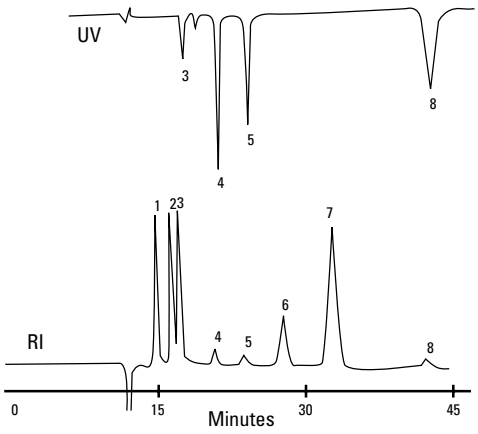
APPLICATIONS - TSKgel CATION EXCHANGE COLUMNS

SPECIALTY COLUMNS

Ion exclusion chromatography can be used as an effective method for separating alcohols. An example of a saccharide, organic acid, and alcohol separation is shown in FIGURE 17 on two TSKgel SCX (H^+) columns in series.

FIGURE 17

Separation of mixture of saccharides, organic acids and alcohols



Column: TSKgel SCX (H^+), two 7.8 mm ID x 30 cm L (in series);
Sample: 1. maltose, 2. glucose, 3. fructose, 4. lactic acid, 5. acetic acid, 6. methanol, 7. ethanol, 8. butyric acid; Elution: 0.05 mol/L $HClO_4$; Flow rate: 0.8 mL/min; Detection: UV @ 210 nm, Refractive Index

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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							
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
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
0007650	CM-SW Guardgel Kit	20	For P/Ns 0007167 and 0007162