TOSOH

Chromatography Catalog

YOUR SPECIALIST IN SEPARATION

TOSOH BIOSCIENCE

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 30 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.

1
TOSOH BIOSCIENCE GMBH
IM LEUSCHNERPARK 4
64347 GRIESHEIM
GERMANY

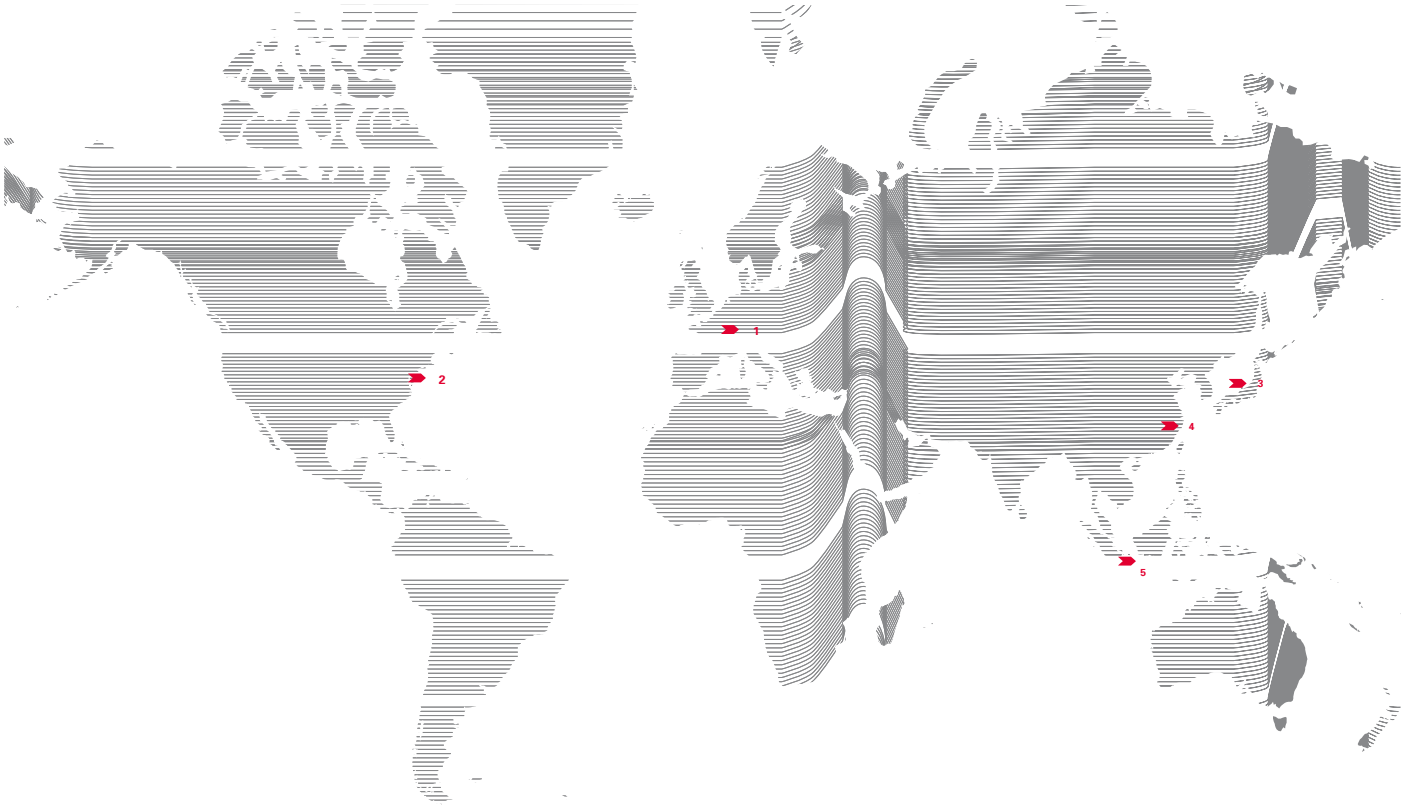
T + 49 (0) 6155 70437 00
F + 49 (0) 6155 83579 00
INFO.TBG@TOSOH.COM
WWW.TOSOHBIOSCIENCE.DE

2
TOSOH BIOSCIENCE LLC
3604 HORIZON DRIVE,
SUITE 100
KING OF PRUSSIA, PA 19406, USA

T +1 484 805 1219
F +1 610 272 3028
INFO.TBL@TOSOH.COM
WWW.SEPARATIONS.US.TOSOHBIOSCIENCE.COM

3
TOSOH CORPORATION
3-8-2 SHIBA, MINATO-KU
TOKYO 105-8623
JAPAN

T +81 3 5427 5118
F +81 3 5427 5198
INFO@TOSOH.CO.JP
WWW.TOSOHBIOSCIENCE.COM



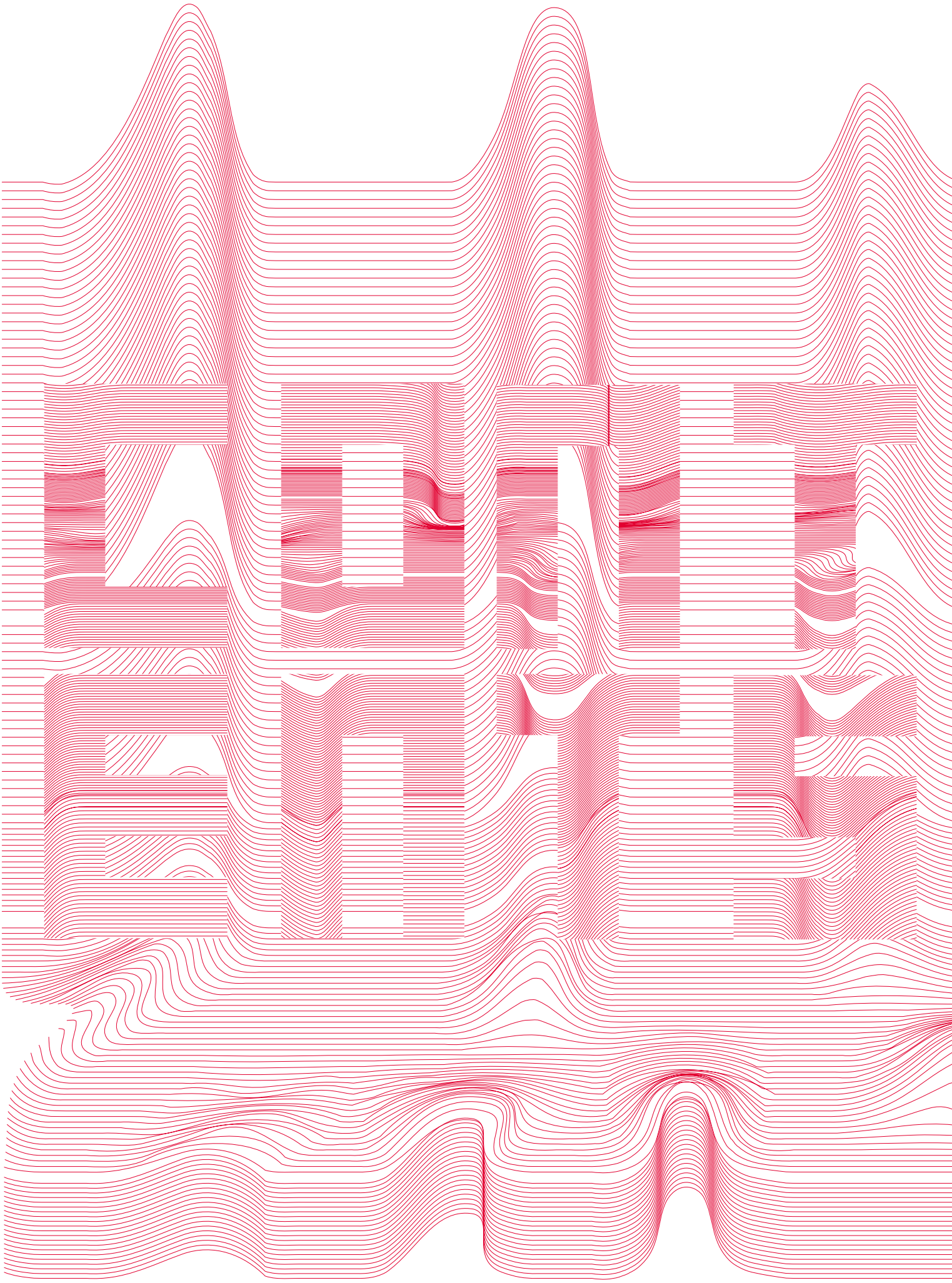
4
TOSOH BIOSCIENCE SHANGHAI CO. LTD.
ROOM 301, PLAZA B,
NO. 1289 YI SHAN ROAD
XU HUI DISTRICT
SHANGHAI, 200233, CHINA
T +86 21 3461 0856
F +86 21 3461 0858
INFO@TOSOH.COM.CN
WWW.SEPARATIONS.ASIA.TOSOHBIOSCIENCE.COM

5
TOSOH ASIA PTE. LTD.
63 MARKET STREET #10-03
BANK OF SINGAPORE CENTRE
SINGAPORE 048942, SINGAPORE

T +65 6226 5106
F +65 6226 5215
INFO.TSAS@TOSOH.COM
WWW.SEPARATIONS.ASIA.TOSOHBIOSCIENCE.COM

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



TOSOH BIOSCIENCE

YOUR SPECIALIST IN SEPARATION

INTRODUCTION

Tosoh Bioscience is a major supplier of liquid chromatography products worldwide, particularly to the pharmaceutical and biotechnology industries. The company distributes and supports products manufactured by our parent company, Tosoh Corporation, which has offices and manufacturing facilities in Japan. Located in Griesheim, Germany, Tosoh Bioscience GmbH provides sales and service to customers in Europe, the Middle East, South Asia and Africa.

This Chromatography Products Catalog describes analytical and semi-preparative TSKgel® prepacked columns for each of the major chromatography modes. It also gives a short overview of TOYOPEARL® and TSKgel bulk resins for laboratory scale purifications, as well as ToyoScreen process development columns.

TSKgel and TOYOPEARL products are used for the analysis, isolation and purification of proteins, peptides, DNA, oligonucleotides, antibiotics and other small molecular weight compounds. Our products are known for their ability to withstand high pressure, their robust chemical stability, and their superior performance for the recovery, concentration and purification of biomolecules.

WHAT'S NEW

➨ TSKgel SuperSW mAb/UltraSW mAb columns

TSKgel SuperSWmAb HTP columns support high throughput antibody analysis. Packed in 4.6 x 150 mm UHPLC column hardware they were engineered to enable an easy transfer of SEC methods, which have been developed on conventional gel filtration columns, to fast UHPLC analysis. TSKgel SuperSW mAb HR, designed for high resolution monoclonal analysis, is packed in columns featuring the conventional dimensions for HPLC gel filtration columns. This allows for superior resolution between fragments, monomers, and aggregates. TSKgel UltraSW Aggregate covers the range of higher molecular weights and provides a very good separation of antibody dimers and higher aggregates. It is based on smaller silica particles with slightly larger pore size than the SuperSW mAb columns.

➨ EcoSEC High Temperature Instrument

Tosoh Bioscience launched a compact high temperature (HT) system for GPC analysis. EcoSEC-HT, the all-in-one GPC/SEC system, provides stable thermostatization up to 220° C. Autoinjector, pumps, and a dual-flow RI detector are integrated in a compact design. The optional sample processing unit can process up to 24 samples. The TSKgel GMHHR- HT2 GPC columns were developed to ideally complement the EcoSEC-HT.

➨ TOYOPEARL AF-rProtein A HC-650F resin

Tosoh Bioscience introduces new high capacity Protein A affinity resin. It is specifically designed for efficient purifications of mAbs. It is a rigid, alkaline resistant protein A affinity resin that offers the largest binding capacity for IgG of all protein A media currently available on the market.

➨ TOYOPEARL MX-Trp-650 resin

TOYOPEARL MX-Trp-650M is a multimodal cation exchange resin with unique selectivity and high recovery. It provides high protein binding capacities and tolerates high conductivity feedstocks. Its ligand, the amino acid tryptophan, provides ionic and hydrophobic interactions. This new mixed-mode resin is well-suited for intermediate and polishing steps, such as aggregate removal in antibody purification, and for the purification of targets that are difficult to purify using common purification platforms.

TOSOH BIOSCIENCE CHROMATOGRAPHY CATALOG

TSKgel COLUMNS

TSKgel columns are known worldwide for their reliability and suitability for a variety of chromatographic applications. Applications using TSKgel columns are continuously published in the scientific journals and are listed in the current U.S. Pharmacopoeia (see Appendix C). Prepacked columns contain TSKgel media designed for the analysis of proteins, peptides, biopolymers and low molecular weight compounds by size exclusion, ion exchange, hydrophobic interaction, reversed phase, affinity and normal phase chromatography. The packings in the columns are either silica-based or polymeric-based material, in particle sizes ranging from 2 µm to 20 µm.

Columns are available in analytical to preparative sizes, in stainless steel, PEEK®, or glass. To ensure specified column performance and to maximize the longevity of your Tosoh columns, please note the guidelines found in Appendix A for the use, cleaning, rehydration, and storage of your TSKgel columns.

ToyoScreen PROCESS DEVELOPMENT COLUMNS

ToyoScreen Process Development columns are available as an additional tool for convenient scale-up. These are easy-to-use, pre-packed columns containing Tosoh Bioscience's most popular TOYOPEARL resins. They provide a convenient, low-cost product for the evaluation of TOYOPEARL ligand chemistries. The most popular resins are also available in RoboColumn (®) format for high throughput screening on robotic workstations and as 5 mL (8 mm ID x 10 cm) ready-to-use MiniChrom columns. Both formats are packed by Atoll GmbH, Weingarten.

TOYOPEARL AND TSKgel LABPAK MEDIA

LabPak products are small package sizes of TOYOPEARL and TSKgel bulk media products. Typically they contain three or four different ligand types offered for a particular chromatography mode. They are useful for developmental scientists who wish to familiarize themselves with resin physical properties in differing buffer systems. The larger resin amounts in LabPak products allow the packing of wider bore and longer columns than what is available in the ToyoScreen products. This helps the developmental scientist or engineer to measure more accurately, under actual packing conditions, the resin's dynamic binding capacity, and selectivity.

TSKgel RESINS

The same media used in TSKgel columns are also available in bulk. They are offered in particle sizes of 20 µm and 30 µm, for ion exchange and hydrophobic interaction chromatography. TSKgel media are the most efficient packing materials available from Tosoh Bioscience for process chromatography. In tandem with their high efficiency, high mechanical stability and permeability, TSKgel resins are an excellent choice under medium to high pressure conditions.

TOYOPEARL RESINS

TOYOPEARL resins are hydrophilic, macroporous, bulk bioprocessing media suitable for large-scale chromatographic applications. Because of their polymeric backbone structure, the rigid TOYOPEARL packings assure excellent pressure/flow characteristics. The media are stable over the pH range of 2-12 for normal operating conditions and pH 2-14 for cleaning conditions. The particle sizes are: 20-50 µm superfine grade for the highest performance, 40-90 µm medium grade for economical purification, and 50-150 µm coarse grade for capture chromatography. Large pore sizes ensure greater capacity for high molecular weight molecules, while allowing faster separation and recycling times. TOYOPEARL media are available for size exclusion, ion exchange, mixed-mode, hydrophobic interaction, and affinity chromatography.

For predictable results in scale-up, TOYOPEARL resins are based on the same chemistry as the prepacked TSKgel columns. This allows the seamless scale-up of methods developed on TSKgel columns to TOYOPEARL, without additional optimization at pilot scale. In addition, TOYOPEARL resins are also available in the ToyoScreen Process Development columns for convenient scouting and methods development.

ORDERING INFORMATION

Tosoh Bioscience chromatography products are sold directly or can be purchased from distributors. An up-to-date list of distributors is available on our website www.tosohbioscience.de. Orders may be placed by phone, fax or email. Tosoh Bioscience strives to ship all standard chromatography products within 24 hours of placing the order. Items that are not listed in the catalog may be provided as special (custom) products, which usually ship within four weeks. Contact your local Tosoh Bioscience office to discuss the availability of specialty products.

Contact us or a local chromatography products distributor for a copy of our terms and conditions of sale.

Tosoh Bioscience is fully committed to delivering quality products and service. TSKgel columns are accompanied by a chromatogram demonstrating the performance of a test mixture and by an OCS sheet that contains information about the Operating Conditions and Specifications for the column. Bulk TSKgel and TOYOPEARL media products are accompanied by a Certificate of Analysis. Despite our commitment to product quality, columns and resins occasionally perform differently than expected in a customer's application. Therefore, we ask you to inspect TSKgel and TOYOPEARL columns or media within 30 days of receipt by using the same conditions employed on the OCS sheet to ensure product performance. Let Tosoh Bioscience know within this 30-day period if the product does not meet the specifications on the OCS (Operating Conditions and Specifications) sheet and QC document, or as listed on the Certificate of Analysis. Subject to prior authorization, Tosoh Bioscience will accept the return of all products that do not perform according to specifications. If a product is authorized for return for reasons other than Tosoh Bioscience's error or because of a product defect, there will be a restocking charge of 10% of the list price or a minimum of 25 Euro.

FOR MORE INFORMATION

Full descriptions and example applications of Tosoh Bioscience chromatography products are provided in this catalog. Our website www.tosohbioscience.de provides complete product information as well as a literature library and chromatogram database.

For technical support, please call **+49 (0) 711 13257-57** or write an e-mail to: **techsupport.tbg@tosoh.com**

For pricing and availability, please contact our Customer Service department at **+49(0) 6155 70437-30** or **customerservice.tbg@tosoh.com**.

To receive a copy of our Process Chromatographic Media catalog or technical literature, please call **+49(0) 6155 70437-00** or contact **info.tbg@tosoh.com**.

A price list for Tosoh Bioscience Chromatography Products is published each December and may be requested by contacting the nearest Tosoh Bioscience office.

TOSOH BIOSCIENCE CHROMATOGRAPHY CATALOG

SAFETY DATA AND WARRANTY

Tosoh Bioscience provides Material Safety Data Sheets (MSDS) on all of its bulk resins. These sheets contain pertinent information that may be needed to protect employees and customers against any known health or safety hazards associated with our products. The end user is responsible for knowing all information and precautions disclosed in the MSDS and any other available materials provided by Tosoh Bioscience. The MSDS sets forth information concerning our products, describes precautions to be taken in the storage and handling of our products, and in the maintenance of the health and safety of persons exposed to our products, the public and the environment with respect to our products. The end user should convey such information and precautions to the persons who may be exposed to our products.

We also suggest contacting the supplier of other materials recommended for use with our products for appropriate health and safety precautions prior to their use.

Many of our bulk products are on file with the FDA in the form of Drug Master Files (DMF) or Chemistry Manufacturing and Controls (CMC) documents. Permission to reference these documents may be obtained upon written request. Direct inquiries can be send to our Technical Service, Tosoh Bioscience GmbH, Im Leuschner Park 4, 64347 Griesheim, Germany. Tosoh Bioscience warrants that at the time of delivery each of our products will conform to the specifications there of contained in the Certificate of Analysis (COA) or the Operating Conditions and Specifications (OCS) sheet, as relevant, as will be provided together with such products; provided, however, that the foregoing warranty applies only if the products have been properly handled, stored and used by Buyer.

THIS WARRANTY IS GIVEN AND ACCEPTED IN LIEU OF ANY OTHER WARRANTIES AND REPRESENTATIONS, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, OR NONINFRINGEMENT OF INTELLECTUAL PROPERTY RIGHTS. IN NO EVENT SHALL TOSOH BIOSCIENCE BE LIABLE FOR SPECIAL, INDIRECT, INCIDENTAL OR CONSEQUENTIAL DAMAGES OR DAMAGES FOR LOST PROFIT OR LOSS OF USE AS A RESULT OF ANY CLAIM BY BUYER OR ANY ACT OR OMISSION OF TOSOH BIOSCIENCE.

Please refer to Tosoh Bioscience's terms and conditions of sale for additional information on our warranty.

TECHNICAL DATA AND TRADEMARKS

The technical information and data herein contained (the Technical Data) are based on information Tosoh Bioscience believes to be reliable and are offered in good faith, but are given without warranty or representation, as the conditions of use and application by the end user of our products and the Technical Data are beyond the control of Tosoh Bioscience. The products should be tested against the Technical Data to determine if they will be suitable for the intended use and applications. Suggestions for the uses of our products should not be understood as recommending the use of our products in violation of any patent or other intellectual property right or as permission or license to use any patent or other intellectual property right.

SEC
SIZE EXCLUSION CHROMATOGRAPHY

SEC PRODUCTS

TSKgel SW-type

TSKgel SW
TSKgel SWXL
TSKgel SuperSW
TSKgel SuperSW mAb
TSKgel UltraSW Aggregate

TSKgel PW-type

TSKgel PW
TSKgel PWXL
TSKgel PWXL-CP
TSkgel SuperMultiporePW
TSkgel SuperOligo PW

TSKgel Alpha-type

TSKgel Alpha
TSKgel SuperAW
TSKgel VMpak

TSKgel H-type

TSKgel HXL
TSKgel HHR
TSKgel HHR-HT
TSKgel SuperH
TSKgel SuperHZ
TSKgel Super MultiporeHZ
TSKgel MultiporeHXL

TSKgel SEC Standards

TOSOH FACT

Tosoh has a long history in size exclusion chromatography (SEC). In 1978 Tosoh first introduced porous silica-based SW columns for the isolation of proteins using LC. These first gels had particle sizes from 10 to 13 µm and were quickly adopted and referred to as the standard for analytical SEC on FPLC and HPLC systems.

As new packing materials were discovered and new bonding chemistries developed, the SEC product line has grown into four major classes of SEC columns. The following pages will help you choose the best column for your application.

INTRODUCTION TO TSKgel SIZE EXCLUSION COLUMNS

Size Exclusion Chromatography (SEC) is the dominant mode of separation for polymers. SEC is the general name for the chromatographic mode in which components of a mixture are separated according to their molecular size, based on the flow of the sample through a column packed with porous particles. Large sample molecules cannot or can only partially penetrate the pores, whereas smaller molecules can access all or a larger number of pores. In SEC, large molecules elute from the column first followed by smaller molecules, and the smallest molecules that can access all the pores elute last from the column. Size exclusion chromatography is the only mode of chromatography that does not involve interaction with a stationary phase by means of adsorption or partitioning of the solutes.

The terms SEC, GFC (gel filtration chromatography) and GPC (gel permeation chromatography) all refer to the same chromatographic technique. In GFC, an aqueous mobile phase is used, while an organic mobile phase is employed in GPC. The general term SEC covers both uses. Available TSKgel products are classified by application area and particle composition.

GEL FILTRATION CHROMATOGRAPHY (GFC)

The principal feature of GFC is its gentle non-interaction with the sample, enabling retention of enzymatic activity while separating multimers that are not easily distinguished by other chromatographic methods. SEC has limited peak capacity, however, requiring that the molar mass of the biomolecules differ by at least twofold. GFC is popular among biochemists for the isolation of proteins, for the removal of aggregates, to desalt a protein sample, to separate nucleic acid fractions, or to characterize water soluble polymers used in food products, paints, pharmaceutical preparations, etc.

TSKgel columns for GFC analysis consist of the TSKgel SW and PW series column lines. The main criterion in choosing between these TSKgel columns is the molar mass of the sample and its solubility. The fact that the TSKgel SW columns are based on silica and the TSKgel PW columns are derived from a hydrophilic polymer network has less impact on the separation than the particle and pore size differences between the column lines. While a TSKgel SW column is typically the first column to try for biopolymers, TSKgel PW columns have demonstrated good results for smaller peptides (<1,000 Da), protein aggregates, DNA fragments, and viruses.

Application area: Proteins and other biopolymers

Base material: silica

- SW
- SW$_{XL}$
- SuperSW/SuperSW mAb
- UltraSW

Due to higher resolving power, the TSKgel SW series columns are ideal for proteins and nucleic acids using an aqueous buffer as mobile phase. The TSKgel SW mAb columns within the TSKgel SW series are designed specifically for the analysis of monoclonal antibodies.

Application area: Water soluble polymers

Base material: polymethacrylate

- PW
- SuperMultiporePW
- SuperOligoPW
- PW$_{XL}$
- PW$_{XL}$-CP

TSKgel PW series columns are commonly used for the separation of synthetic polymers, oligosaccharides, nucleic acids and small viruses using aqueous buffer or salt solutions as mobile phase. The TSKgel SuperMultiporePW semi-micro SEC columns provide near linear calibration curves and are ideally suited to analyze the MW distribution of water soluble polymers with a wide range of molecular weights. The SuperOligoPW semi-micro column featuring a small particle size has been designed for fast analysis of oligosaccharides and other oligomers. The PW$_{XL}$-CP columns are developed to facilitate SEC separation of cationic polymer under low salt conditions.

FEATURES

- Rigid hydrophilic and hydrophobic packings
- Four series of SEC columns with diferent ranges of solvent compatibility
- Easy scale up

BENEFITS

- Minimal swelling and excellent physical strength
- Low adsorption resulting in high mass recovery
- Suitable for both types of size exclusion, aqueous (GFC) and non-aqueous (GPC)
- Analytical and preparative pre-packed SEC column

SEC

GEL PERMEATION CHROMATOGRAPHY (GPC)

GPC plays an important role in the characterization of polar organic-soluble and organic-soluble polymers in consumer, chemical, and petrochemical industries. GPC is often used to determine the relative molar mass of polymer samples as well as the distribution of molar masses.

Application area: Water- and organic-soluble polymers

Base material: highly crosslinked polymethacrylate

- Alpha
- SuperAW

TSKgel Alpha and SuperAW columns are compatible with a wide range of solvents and were developed for the GPC analysis of polymers of intermediate polarity, soluble in water, buffers and many organic solvents. TSKgel SuperAW columns are based on the same chemistry as TSKgel Alpha columns but have smaller particle sizes and shorter, narrower column dimensions for high throughput applications.

For the GPC analysis of organic-soluble polymers, Tosoh developed TSKgel H series, filled with polystyrene/divinylbenzene polymer particles. Each line of columns within the TSKgel H series differs in degree of inertness and operating temperature range. The proprietary multi-pore particle technology applied in some linear GPC columns ensures a wide pore size distribution in each particle leading to calibration curves with excellent linearity.

Application area: Organic-soluble polymers

Base material: polystyrene

Ultra-low adsorption columns with limited solvent range

- SuperHZ (high throughput)
- SuperMultiporeHZ
- HXL (conventional)

Low adsorption columns with expanded solvent range

- SuperH (high throughput)
- HHR (conventional)

High temperature GPC columns

- GMHHR HT/HT2

SUMMARY OF TSKgel SIZE EXCLUSION COLUMN LINES

Column line	TSKgel SW / SWXL / SuperSW / UltraSW	TSKgel PW / PWXL	TSKgel Alpha / TSKgel SuperAW	TSKgel H
Particle composition	Silica	Polymethacrylate	highly crosslinked Poly-methacrylate	PS-DVB
No. of available pore sizes	3/2/1	7	5	6
pH stability	2.5 - 7.5	2.0 - 12.0	2.0 - 12.0	1.0 - 14.0
Solvent compatibility	100% polar	50% polar	100% polar and nonpolar	100% nonpolar, limited polar
Max. temperature	30°C	80°C*	80°C	60-80°C (HXL, SuperHZ) 140°C (HHR and SuperH) 220°C HHR HT2
Pressure (MPa)**	1.0-12.0	1.0 - 4.0	2.0 - 4.0	1.5-6.0
Application focus	proteins	water soluble polymers	intermediate polar polymers	organic-soluble polymers

* *Except for the TSKgel G-DNA-PW, which can be operated up to 50°C and the 55 mm ID TSKgel PW-type columns, which can be operated up to 60°C. When operating below 10°C, reduce the flow rate to ensure that the maximum pressure is not exceeded.*

** *Depends on column dimensions and particle size.*

Note: The operating conditions and specifications for each column are listed on the Operating Conditions and Specifications sheet (OCS) shipped with the column and in the Ordering Information section at the end of each section.

COLUMN SELECTION GUIDE FOR TSKgel GEL FILTRATION COLUMNS

SAMPLE			COLUMN SELECTION		SELECTION CRITERIA
			FIRST CHOICE	ALTERNATIVE	
Carbohydrates	polysaccharides		TSKgel GMPW$_{XL}$ TSKgel SuperMultiporePW	TSKgel G5000PW$_{XL}$ & TSKgel G3000PW$_{XL}$	large pore size, small particles, linear calibration curve, high resolving power
	oligosaccharides		TSKgel G-Oligo-PW TSKgel SuperOligoPW	TSKgel G2500PW$_{XL}$	small particles, high resolving power
Nucleic acids	DNA fragments	large	TSKgel G-DNA-PW or TSKgel G5000PW$_{XL}$		large pore size, small particles, high resolving power
		medium and small	TSKgel G4000SW$_{XL}$, TSKgel BioAssist G4SW$_{XL}$ TSKgel SuperSW3000 or TSKgel G3000SW$_{XL}$	 TSKgel BioAssist G3SW$_{XL}$	suitable pore sizes
	RNA		TSKgel G4000SW$_{XL}$ TSKgel SuperSW3000 or TSKgel G3000SW$_{XL}$	TSKgel BioAssist G4SW$_{XL}$ TSKgel BioAssist G3SW$_{XL}$	suitable pore sizes
	oligonucleotides		TSKgel G2500PW$_{XL}$		small pore size, ionic interaction
Proteins	small to medium sized proteins		TSKgel SuperSW3000 TSKgel G3000SW$_{XL}$ TSKgel BioAssist G3SW$_{XL}$ TSKgel G4000SW$_{XL}$ TSKgel SuperSW2000 or TSKgel G2000SW$_{XL}$	TSKgel G3000PW$_{XL}$ / G4000PW$_{XL}$ TSKgel BioAssist G4SW$_{XL}$ TSKgel BioAssist G2SW$_{XL}$	small particles small to medium range pore sizes
	antibodies		TSKgel SuperSW mAB HR/HTP TSKgel UltraSW Aggregate		fragments/monomer & dimer higher aggregates
	large proteins	low density lipoprotein	TSKgel G6000PW$_{XL}$ or TSKgel G5000PW$_{XL}$		large pore sizes
		gelatin	TSKgel GMPW$_{XL}$ TSKgel SuperMultiporePW-M TSKgel G3000SW$_{XL}$	TSKgel G5000PW$_{XL}$ & G3000PW$_{XL}$	large pore size, linear calibration curve
Peptides	large		TSKgel SuperSW3000 TSKgel G3000SW$_{XL}$ TSKgel BioAssist G3SW$_{XL}$ or TSKgel G2000SW$_{XL}$	TSKgel SuperSW2000 / TSKgel G3000PW$_{XL}$ TSKgel BioAssist G2SW$_{XL}$	small to medium range pore size, versatile
	small		TSKgel G2500PW$_{XL}$	TSKgel SuperSW2000 / TSKgel G2000SW$_{XL}$	linear calibration curve, high resolving power
Viruses			TSKgel G6000PW$_{XL}$ or TSKgel G5000PW$_{XL}$ TSKgel SuperMultiporePW-H		large pore size, high resolving power
Synthetic polymers			TSKgel GMPW$_{XL}$ or TSKgel Alpha-M TSKgel SuperMultiporePW	TSKgel G5000PW$_{XL}$ & G3000PW$_{XL}$ / TSKgel Alpha-5000 & Alpha-3000	large pore size, low adsorption, linear calibration curve
	cationic		TSKgel G3000PW$_{XL}$-CP TSKgel G5000PW$_{XL}$-CP TSKgel G6000PW$_{XL}$-CP		medium to large pore size, low adsorption, linear calibration curve
Synthetic oligomers	nonionic		TSKgel G-Oligo-PW TSKgel G2500PW$_{XL}$ or TSKgel Alpha-2500 TSKgel SuperOligoPW and TSKgel SuperMultiporePW-N	TSKgel G2500PW / TSKgel SuperAW2500	small pore size, high resolving power
	anionic		TSKgel G2500PW$_{XL}$ or TSKgel Alpha-2500	TSKgel G2500PW / TSKgel SuperAW2500	small pore size, ionic interaction

SEC

TSKgel SW, SWXL AND SuperSW GEL FILTRATION COLUMNS

HIGHLIGHTS

- Dedicated columns for the analysis of monoclonals available
- TSKgel SW-type columns are all based on spherical silica particles with very high internal pore volumes.
- Silica particles in SW-type columns are chemically bonded with polar diol groups.
- SW-type columns feature low residual adsorption, which is essential for gel filtration analysis.
- Various pore sizes ranges available.
- Stainless steel, glass and PEEK column hardware available.

Tosoh recently added three TSKgel SW mAb columns to the renowned line of TSKgel SW series SEC columns. The TSKgel SW mAb columns meet the growing demand for the higher resolution and high throughput separation of monoclonal antibody (mAb) monomer and dimer/fragment, as well as higher resolution of mAb aggregates. While mAbs can be analyzed using many different modes of HPLC, size exclusion is best for determination of aggregate and fragment content.

TSKgel SW series columns contain a large pore volume per unit column volume, which results in either higher MW selectivity or better resolution when analyzing proteins. They are based on highly porous silica particles, the surface of which has been shielded from interacting with proteins by derivatization with ligands containing diol functional groups. TSKgel SW series columns stand out from other silica- or polymer-based high performance size exclusion columns by virtue of their large pore volumes and low residual adsorption.

TSKgel SW mAb, SW, SuperSW and Ultra SW columns are stable from pH 2.5 to 7.5 and can be used in 100% aqueous conditions. The different pore sizes of the TSKgel SW series columns result in different exclusion limits for globular proteins, polyethylene oxides and dextrans, as summarized in TABLE I. Furthermore, different particle sizes, column dimensions and housing materials are available for each of the TSKgel SW series columns. When the protein analysis needs to be performed in a metal free environment, the BioAssistSW series offers TSKgel SW packings in PEEK housings, featuring the same performance as stainless steel columns.

RECOMMENDATIONS FOR TSKgel SW SERIES SELECTION

Samples of known molecular weight
Calibration curves for each TSKgel SW series column are provided in this catalog. Each curve represents a series of various standards (protein, PEO, or globular proteins, for example) with known molar masses. The molar mass range of the compound to be analyzed should be within the linear range of the calibration curve and similar to the chemical composition and architecture of the calibration standards.

Samples of unknown molecular weight
TSKgel G3000SWXL is the ideal scouting column. If the protein of interest elutes near the exclusion volume, then G4000SWXL is the logical next step. conversely, if the protein of interest elutes near the end of the chromatogram, try the G2000SWXL.

TABLE I
Properties and separation ranges for TSKgel SW-type packings

TSKgel packing	Particle size (µm)	Pore size (nm)	Molecular weight of sample (Da)		
			Globular proteins	Dextrans	Polyethylene glycols and oxides
SuperSW2000	4	12.5	$5 \times 10^3 – 1.5 \times 10^5$	$1 \times 10^3 – 3 \times 10^4$	$5 \times 10^2 – 15 \times 10^3$
G2000SWXL/BioAssist G2SWXL	5	12.5	$5 \times 10^3 – 1.5 \times 10^5$	$1 \times 10^3 – 3 \times 10^4$	$5 \times 10^2 – 15 \times 10^3$
QC-PAK TSK 200	5	12.5	$5 \times 10^3 – 1.5 \times 10^5$	$1 \times 10^3 – 3 \times 10^4$	$5 \times 10^2 – 15 \times 10^3$
G2000SW	10, 13, 20	12.5	$5 \times 10^3 – 1.5 \times 10^5$	$1 \times 10^3 – 3 \times 10^4$	$5 \times 10^2 – 15 \times 10^3$
SuperSW3000	4	25	$1 \times 10^4 – 5 \times 10^5$	$2 \times 10^3 – 7 \times 10^4$	$1 \times 10^3 – 3.5 \times 10^4$
SuperSW mAb	4	25	$1 \times 10^4 – 5 \times 10^5$		
G3000SWXL/BioAssist G3SWXL	5	25	$1 \times 10^4 – 5 \times 10^5$	$2 \times 10^3 – 7 \times 10^4$	$1 \times 10^3 – 3.5 \times 10^4$
QC-PAK TSK 300	5	25	$1 \times 10^4 – 5 \times 10^5$	$2 \times 10^3 – 7 \times 10^4$	$1 \times 10^3 – 3.5 \times 10^4$
G3000SW	10, 13, 20	25	$1 \times 10^4 – 5 \times 10^5$	$2 \times 10^3 – 7 \times 10^4$	$1 \times 10^3 – 3.5 \times 10^4$
UltraSW Aggregate	3	30	$1 \times 10^4 – 2 \times 10^6$		
G4000SWXL/BioAssist G4SWXL	8	45	$2 \times 10^4 – 7 \times 10^6$	$4 \times 10^3 – 5 \times 10^5$	$2 \times 10^3 – 2.5 \times 10^5$
G4000SW	13, 17	45	$2 \times 10^4 – 7 \times 10^6$	$4 \times 10^3 – 5 \times 10^5$	$2 \times 10^3 – 2.5 \times 10^5$

Data generated using the following conditions:

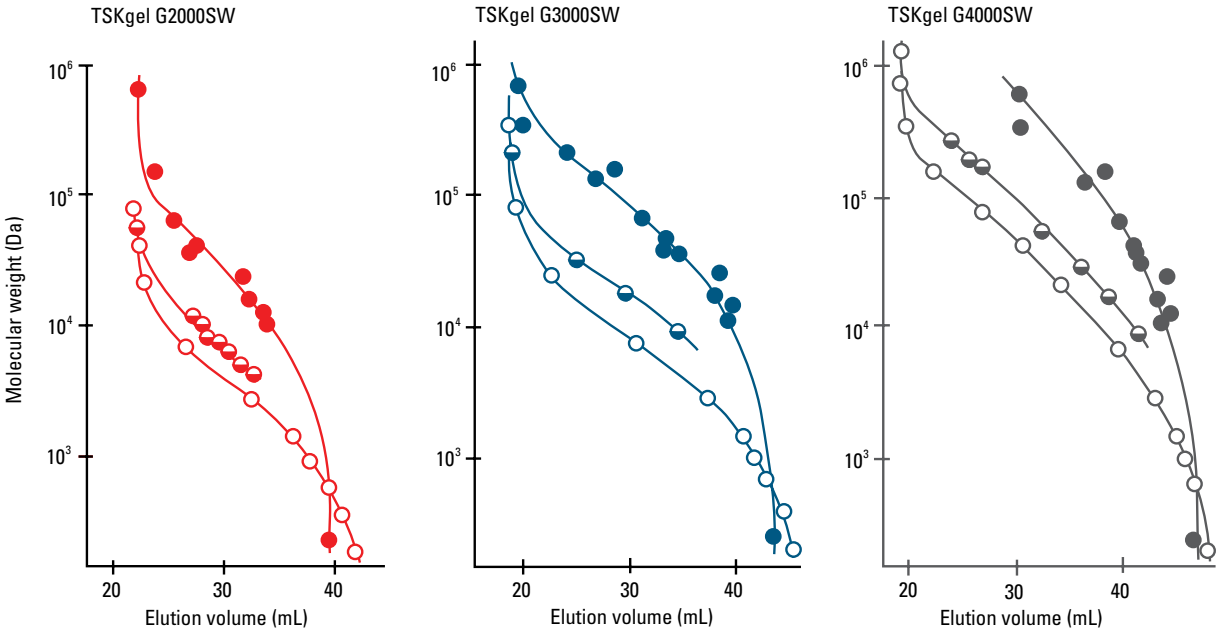
Columns: *Two 4 µm, 4.6 mm ID x 30 cm L TSKgel SuperSW columns in series; two 5 µm, 7.8 mm ID x 30 cm L TSKgel SWXL columns in series; two 10 µm, 7.5 mm ID x 60 cm L TSKgel SW columns in series*

Elution: *Globular proteins: 0.3 mol/L NaCl in 0.1 mol/L (0.05 mol/L for SWXL columns) phosphate buffer, pH 7.0*
Dextrans and polyethylene glycols and oxides (PEOs): distilled water

CALIBRATION CURVES FOR TSKgel SW-TYPE GEL FILTRATION COLUMNS

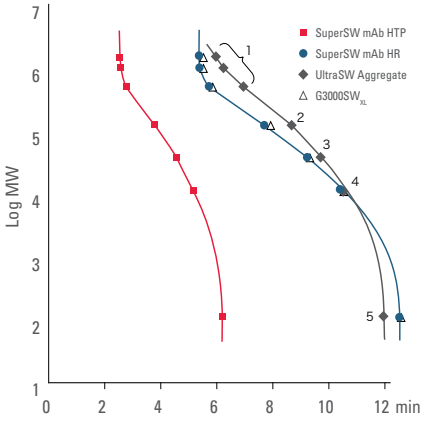
The best results are obtained when selecting a column with the sample's molecular weight in the linear portion of the calibration curve.

Polyethylene oxide, dextran and protein calibration curves for TSKgel SW columns

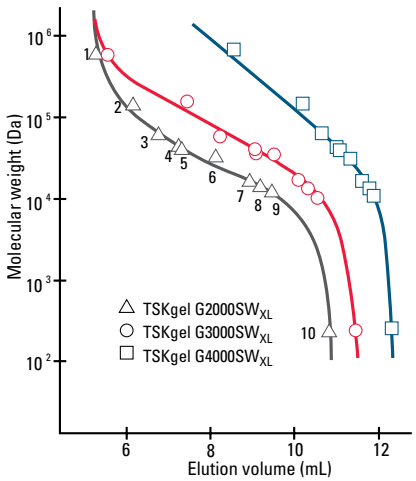


Column: TSK-GEL SW, two 7.5 mm ID x 60 cm L columns in series
Sample: ● proteins, ○ polyethylene oxides, ◒ dextrans
Elution: dextrans and polyethylene oxides: distilled water; proteins: 0.3 mol/L NaCl in 0.1 mol/L phosphate buffer, pH 7.0
Flow Rate: 1.0 mL/min
Detection: UV @ 220 nm and RI

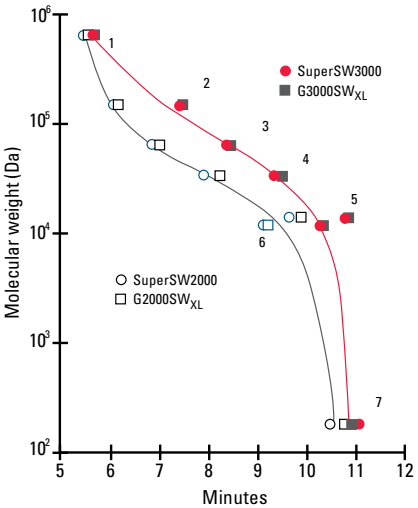
Calibration curves for TSKgel SW columns



Columns: TSKgel SuperSW mAb HTP, 4 µm, 4.6 mm ID × 15 cm
TSKgel SuperSW mAb HR, 4 µm
TSKgel UltraSW Aggregate, 3 µm
TSKgel G3000SWXL, 4 µm, (all 7.8 mm ID × 30 cm)
Sample: 1. Thyroglobulin (MW 640,000), 2. γ-Globulin (MW 155,000), 3. Ovalbumin (MW 47,000), 4. Ribonuclease A (MW 13,700), 5. p-Aminobenzoic acid (MW 137)
Mobile phase: 0.2 mol/L phosphate buffer, pH 6.7, 0.05% NaN_3
Flow rate: 1.0 mL/min, 0.35 mL/min (SuperSW mAb HTP)
Temp.: 25°C
Detection: UV @ 280 nm
Inj. vol.: 10 µL, 5 µL (SuperSW mAb HTP)



Columns: TSKgel SW_{XL}, 5 or 8 µm, 7.8 mm ID x 30 cm L
Sample: 1. thyroglobulin (660,000 Da); 2. IgG (160,000 Da) 3. BSA (67,000 Da); 4. ovalbumin (43,000 Da); 5. peroxidase (40,200 Da); 6. β-lactoglobulin (18,400 Da); 7. myoglobin (16,900 Da) 8. ribonuclease A (12,600 Da); 9. cytochrome C (12,400 Da); 10. glycine tetramer (246 Da)
Elution: 0.3 mol/L NaCl in 0.1 mol/L sodium phosphate buffer, pH 7.0
Detection: UV @ 220 nm



Columns: TSKgel SWXL, 5µm, 7.8 mm ID x 30 cm, TSKgel SuperSW, 4 µm, 4.6 mm ID x 30 cm
Sample: proteins: 1. thyroglobulin (660,000 Da); 2. γ-globulin (150,000 Da); 3. BSA (67,000 Da); 4. β-lactoglobulin (18,400 Da); 5. lysozyme (14,500 Da); 6. cytochrome C (12,400 Da); 7. triglycine (189 Da)
Elution: 0.15 mol/L phosphate buffer (pH 6.8)
Flow rate: 0.35 mL/min for SuperSW; 1.0 mL/min for SW_{XL}
Temperature: 25°C

SEC

TSKgel SW-TYPE GEL FILTRATION COLUMNS

Proteins (general)
Choose one of the TSKgel SWXL columns using the calibration curves on PAGE 12 to select the appropriate pore size based on knowledge or estimate of protein size.

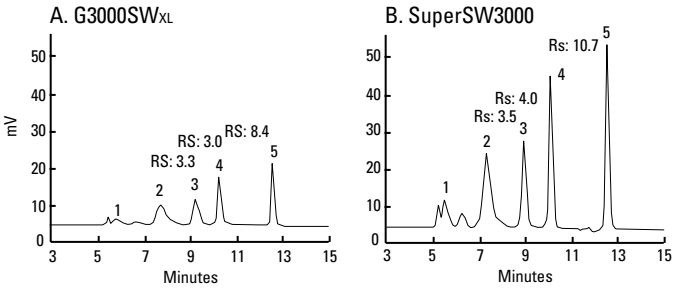
Monoclonal antibodies
TSKgel SuperSW mAb columns have been developed for the analysis of monoclonal antibodies. They provide higher resolution (HR) or faster analysis (HTP) than the TSKgel G3000SWXL which is traditionally used for quality control in many QC labs. TSKgel SuperSW3000 is utilized when sample is limited or at very low concentration.

Peptides
TSKgel G2000SWXL is the first selection for the analysis of peptides. TSKgel SuperSW2000 is utilized when sample is limited or at very low concentration.

Other
The use of TSKgel SuperSW columns requires optimization of the HPLC system with respect to extra-column band broadening. Capillary tubing ID, injection volume, detector cell volume, and detector time constant all need to be reduced to fully benefit from the high column efficiency and small peak volumes of the SuperSW columns. Use SW columns when not sample limited or when larger amounts of sample need to be isolated.

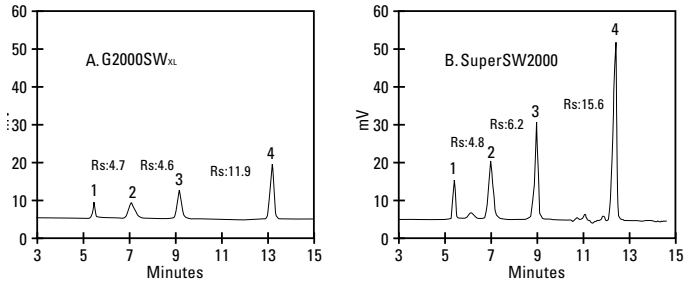
COMPARING TSKgel SW, SWXL AND SuperSW GEL FILTRATION COLUMNS
FIGURE 1 & FIGURE 2 show the increased resolution and sensitivity of the TSKgel SuperSW columns compared to TSKgel SWXL columns. This is due to the smaller particle size (4 vs. 5 µm) and the narrow column diameter (4.6 mm ID).

FIGURE 1
Comparison of TSKgel Super SW3000 and TSKgel G3000SWXL for the separation of proteins



Column: A. TSKgel G3000SWXL, 7.8 mm ID x 30 cm L;
B. TSKgel SuperSW3000, 4.6 mm ID x 30 cm L;
Sample: 5 µL of a mixture of 1. thyroglobulin, 0.5 mg/mL (660,000 Da); 2. γ-globulin, 1.0 mg/mL (150,000 Da); 3. ovalbumin, 1.0 mg/mL (43,000 Da); 4. ribonuclease A, 1.5 mg/mL (12,600 Da); 5. p-aminobenzoic acid, 0.01 mg/mL (137 Da);
Elution: 0.1 mol/L $NaSO_4$ in 0.1 mol/L in phosphate buffer with 0.05 % NaN_3, pH 6.7; Flow rate: 1.0 mL/min for G3000SWXL; 0.35 mL/min for SuperSW3000;
Temp: 25°C; Detection: UV @ 220 nm

FIGURE 2
Comparison of TSKgel Super SW2000 and TSKgel G3000SW for the separation of Proteins



Column: A. TSKgel G2000SWXL, 7.8 mm ID x 30 cm L;
B. TSKgel SuperSW2000, 4.6 mm ID x 30cm L;
Sample: 1. thyroglobulin (0.2 mg/mL); 2. albumin (1.0 mg/mL); 3. ribonuclease A (1.0 mg/mL); 4. p-aminobenzoic acid (0.01 mg/mL);
Inj. Volume: 5 µL; Elution: 0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4 + 0.05 % NaN_3 (pH 6.7);
Flow rate: 0.35 mL/min for SuperSW2000; 1.0 mL/min for G2000SWXL;
Temp: 25°C; Detection: UV @ 280 nm

APPLICATIONS OF TSKgel SW-TYPE GEL FILTRATION COLUMNS

ANALYSIS OF MONOCLONAL ANTIBODIES:

The TSKgel SuperSW mAb size exclusion series consists of three specialized columns designed for the separation and analysis of monoclonal antibodies (mAb). Compared to competitive columns, these new stainless steel, silica-based TSKgel columns offer reduced lot-to-lot variation, long column life, reduction of unspecified adsorption, and superior recovery of aggregates. TSKgel mAb columns are compatible with both HPLC and UHPLC systems.

These columns are available within the TSKgel SW mAb column line:

- TSKgel SuperSW mAb HR
- TSKgel SuperSW mAb HTP
- TSKgel UltraSW Aggregate

TSKgel SuperSW mAb HR and SuperSW mAb HTP both contain 4 µm particles. The HR designation represents the high resolution analysis of mAb monomer, dimer, and fragments, while the HTP stands for "high throughput" due to the smaller dimensions (4.6 mm ID × 15 cm). The TSKgel UltraSW Aggregate column is packed with particles featuring a smaller particle size, 3 µm, and slightly larger pore size. It offers high resolution separation of mAb multimers.

These columns utilize a unique technology, which produces a shallow calibration curve in the molar mass region of a typical antibody. The calibration curve for the TSKgel SuperSW mAb HR column is similar to that of TSKgel G3000SW$_{XL}$. It has a shallower slope than the TSKgel UltraSW Aggregate column around the molar mass range of γ-globulin resulting in a higher resolution for that mass range.

HIGH SPEED ANALYSIS OF THERAPEUTIC mAb

A shorter column length allows the TSKgel SuperSW mAb HTP column to provide fast and efficient run times in the high resolution separation of a mAb monomer and dimer. FIGURE 3 shows no loss in resolution in the analysis of a therapeutic mAb at a 0.50 mL/min flow rate and an increased pressure of 5.0 MPa.

HIGH RESOLUTION SEPARATION OF MONOMER & DIMER

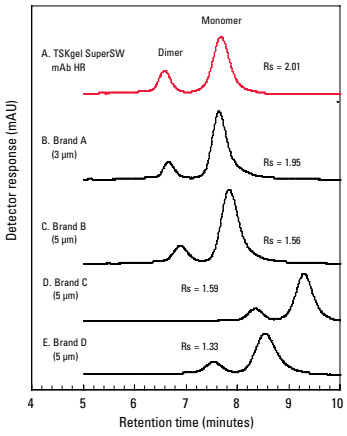
FIGURE 4 demonstrates the superior resolution of the TSKgel SuperSW mAb HR column compared to four competitive columns in the analysis of a mAb monomer and dimer. TSKgel SuperSW mAb HR shows excellent resolution of gamma-globulin dimer and monomer.

DURABILITY OF SuperSW mAb COLUMNS

FIGURE 5 demonstrates the good durability of the TSKgel SuperSW mAb HR column through the reproducibility of resolution for a γ-globulin sample injection.

FIGURE 4

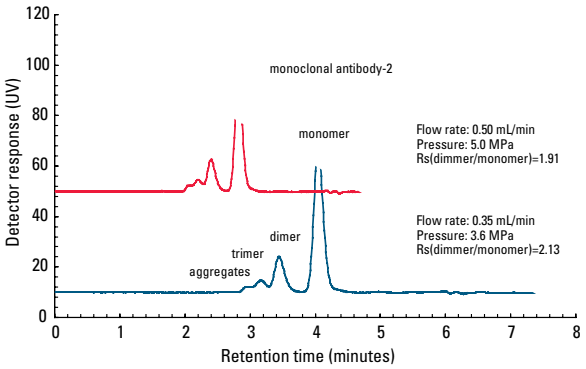
Comparison of resolution of mAb monomer and dimer



Columns: A. TSKgel SuperSW mAb HR, 4 µm, 7.8 mm ID × 30 cm;
B. Brand A, 3 µm, 7.8 mm ID × 30 cm; C. Brand B, 5 µm, 7.8 mm ID × 30 cm;
D. Brand C, 5 µm, 8.0 mm ID × 30 cm; E. Brand D, 5 µm, 8.0 mm ID × 30 cm
Mobile phase: 200 mmol/L phosphate buffer, pH 6.7 + 0.05% NaN_3
Flow rate: 1.0 mL/min; Detection: UV @ 280 nm
Temperature: 25 °C; Injection vol.: 10 µL
Sample: IgG (human polyclonal), 1.0 g/L

FIGURE 3

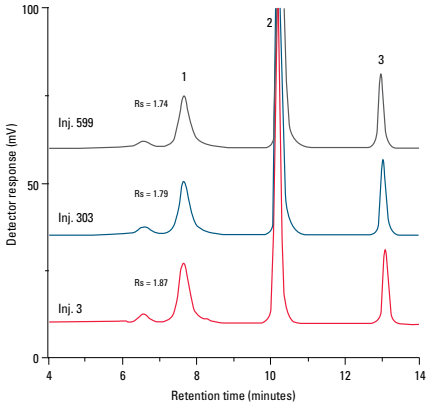
High speed separation of therapeutic mAb



Column: TSKgel SuperSW mAb HTP, 4 µm, 4.6 mm ID × 15 cm
Mobile phase: 0.2 mol/L phosphate buffer, pH 6.7 + 0.05% NaN_3
Flow rate: 0.50 mL/min, 0.35 mL/min; Detection: UV @ 280 nm
Temperature: 25 °C; Sample: monoclonal antibody-2
(mouse-human chimeric IgG, Erbitux®), 5 µL

FIGURE 5

High durability of TSKgel SuperSW mAb HR column



Column: TSKgel SuperSW mAb HR, 4 µm, 7.8 mm ID × 30 cm
Mobile phase: 0.2 mol/L phosphate buffer, pH 6.7 + 0.05% NaN_3
Flow rate: 0.8 mL/min; Detection: UV @ 280 nm
Injection vol.: 10 µL
Samples: 1. γ-Globulin; 2. Cytochrome C; 3. DNP-L-Alanine

SEC

APPLICATIONS OF TSKgel SW-TYPE GEL FILTRATION COLUMNS

SEPARATION OF HIGHER AGGREGATES

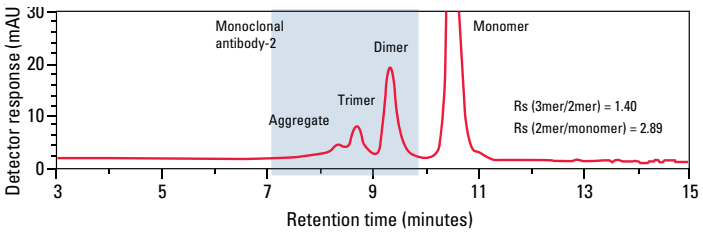
TSKgel UltraSW Aggregate has a smaller particle size than the SuperSW material, and offers high resolution separation of mAb multimers. FIGURE 6 shows the analysis of a mouse-human chimeric IgG using the TSKgel UltraSW Aggregate column. Superior resolution of the mAb trimer and dimer is obtained. The smaller particle size (3 µm) and higher molecular weight exclusion limit (2,500 kDa, globular proteins) of the TSKgel UltraSW Aggregate column, compared to the TSKgel SuperSW mAb HR and HTP columns, allows for high resolution separation of mAb multimers and aggregates.

SEPARATION OF LARGE PROTEINS

TSKgel UltraSW Aggregate provides a larger pore size than TSKgel SuperSW3000. It is therefore not only suited for the analysis of mAb aggregates but can also be used for the analysis of other large proteins and their aggregates. The analysis of a heat denatured, large hydrophobic metalloprotein, apoferritin, is shown in FIGURE 7. A set of six, 0.3 mL HPLC vials each containing 100 µL stock solution of apoferritin was used for protein thermal denaturation. Thermal denaturation was carried out at 60°C using an electric heating block. Individual sample vials were tightly capped and exposed to the heat for 5, 20, 30, 45, and 60 minutes. Samples were analyzed using a TSKgel UltraSW Aggregate column at the end of each incubation time period. The TSKgel Ultra SW Aggregate column yielded high resolution between the monomer and dimer. The trimer, tetramer and higher order aggregates of apoferritin were well separated.

FIGURE 6

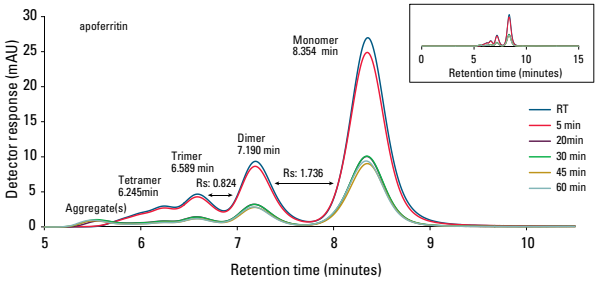
Separation of mAb trimer and dimer



Column: TSKgel UltraSW Aggregate, 3 µm, 7.8 mm ID × 30 cm
Mobile phase: 0.2 mol/L phosphate buffer, pH 6.7 + 0.05% NaN_3
Flow rate: 0.8 mL/min; Detection: UV @ 280 nm
Temperature: 25 ˚C; Sample: monoclonal antibody-2 (mouse-human chimeric IgG, Erbitux), 10 µL

FIGURE 7

Analysis of heat induced forced denatured, large hydrophobic metalloprotein, apoferritin



Protein	Molecular weight (kDa)			
	Monomer	Dimer	Trimer	Tetramer
ferritin and apoferritin	450	900	1350	1800

Column: TSKgel UltraSW Aggregate, 3 µm, 7.8 mm ID × 30 cm
Mobile phase: 50 mmol/L potassium phosphate (monobasic), 50 mmol/L sodium phosphate (dibasic), 100 mmol/L sodium sulfate, 0.05% NaN_3, pH 6.7
Flow rate: 1.0 mL/min; Detection: UV @ 280 nm
Temperature: 30 °C; Injection vol.: 10 µL
Samples: ferritin – Sigma, 4.7 g/L, in saline (0.9% NaCl in water) solution, stored at 2-8 °C apoferritin – Sigma, 5.0 g/L, in 50% glycerol and 0.075 mol/L sodium chloride, stored at -20 °C

APPLICATIONS OF TSKgel SW-TYPE GEL FILTRATION COLUMNS

MEMBRANE PROTEINS

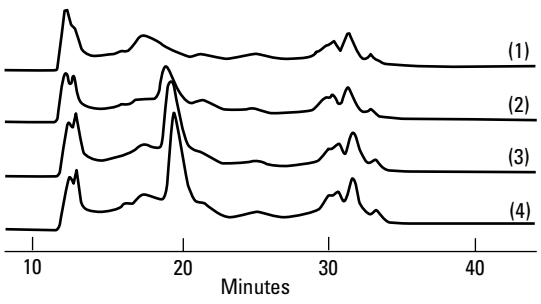
A TSKgel G3000SW column was used to study the effect of different concentrations of the non-ionic surfactant octaethyleneglycol dodecylether on the analysis of membrane proteins from a crude extract from rat liver microsome. The effect of different concentrations of surfactant on the separation of membrane proteins is seen in FIGURE 8. As the concentration of octaethyleneglycol dodecylether increases to 0.05%, the main peak becomes sharper and recovery increases. Caution: we recommend that columns that have been used with a surfactant-containing mobile phase are dedicated for that particular use.

NUCLEIC ACIDS

Separation of four E. coli RNAs, shown in FIGURE 9, confirms the high performance of TSKgel G4000SW columns for samples with a wide high molar mass range. The sample consists of 4S tRNA (2.5×10^4 Da), 5S rRNA (3.9×10^4 Da), 16S rRNA (5.6×10^5 Da), and 23S rRNA (1.1×10^6 Da). All four polynucleotides are within the molar mass range recommended for this TSKgel SW column.

FIGURE 8

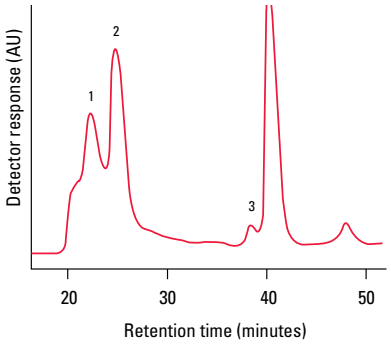
Analysis of membrane protein with differing surfactant concentrations in the mobile phase



Column: TSKgel G3000SW, 10 µm, 7.5 mm ID × 60 cm
Mobile phase: (0.2 mol/L sodium chloride + 20% glycerol + octaethylene glycol dodecylether) in 50 mmol/L phosphate buffer, pH 7.0;
Note: concentration of surfactant: 1.) 0.005% 2.) 0.01% 3.) 0.025% 4.) 0.05%
Flow rate: 1.0 mL/min; Detection: UV @ 280 nm
Sample: membrane protein from a crude extract from rat liver microsome

FIGURE 9

Separation of total E. coli RNA



Columns: TSKgel G4000SW, 13 µm, 7.5 mm ID × 30 cm × 2
Mobile phase: 0.13 mol/L NaCl in 0.1 mol/L phosphate buffer, pH 7.0, + 1 mmol/L EDTA
Flow rate: 1.0 mL/min; Detection: UV @ 260 nm; Injection vol.: 5 µg
Sample: 0.1 mL of 1:10 diluted solution of total E. coli RNA:
1. 23s rRNA (1.1 × 106 Da); 2. 16s rRNA (5.6 × 105 Da)
3. 5s rRNA (3.9 × 104 Da); 4. 4s rRNA (2.5 × 104 Da)

SEC

ENZYMES

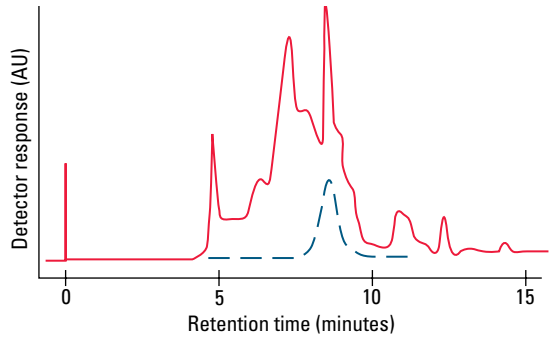
Mobile phase conditions in gel filtration are optimized to ensure little or no interaction of the sample with the packing material. This gentle technique allows for high recovery of enzymatic activity. A crude sample of glutathione S-transferase was separated in only 15 minutes on a TSKgel G3000SWXL column and activity recovery was 98% and 89%, respectively. The elution profile of the separation in FIGURE 10 shows that all of the activity eluted in a narrow band of about 1.5 mL..

SEC-MALS ANALYSIS OF PROTEIN AGGREGATION

TSKgel G3000SWXL is the industry standard for aggregation analysis in quality control of monoclonal antibodies. FIGURE 11 depicts the analysis of mAb Aggregates with UV, refractive index (RI) and multi angle light scattering (MALS) detection.

FIGURE 10

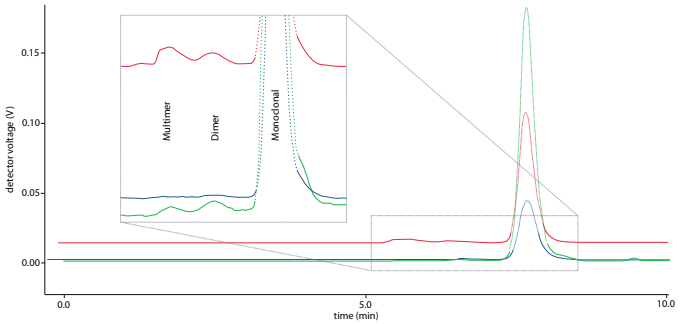
Separation of crude protein sample on TSKgel G3000SWXL



Column: TSKgel G3000SWXL 5 µm, (7.8 mm ID x 30 cm L); Sample: crude glutathione S-transferase from guinea pig liver extract, 0.7 mg in 0.1 mL;
Elution: 0.3 mol/L NaCl in 0.05 mol/L phosphate buffer, pH 7;
Flow rate: 1.0mL/min; Detection: UV@220 nm (solid line) and enzyme assay tests (dashed line); Recovery: enzymatic activity recovered was 89 %

FIGURE 11

SEC-Mals-UV-RI analysis of mAb aggregates



Column: TSKgel G3000SWXL column, 5 µm, 7.8 mm ID x 30 cm L
Sample: monoclonal antibody, Inj.volume: 20 µL;
Mobile phase: phosphate buffered saline (PBS); Flow rate: 1 mL/min;
Detection: MALS (red), refractive index (blue) & UV @ 280 nm (green);
HPLC System: LC-20A prominence, Shimadzu;
MALS detector: miniDAWN™ TREOS, Wyatt Techn. Corp.

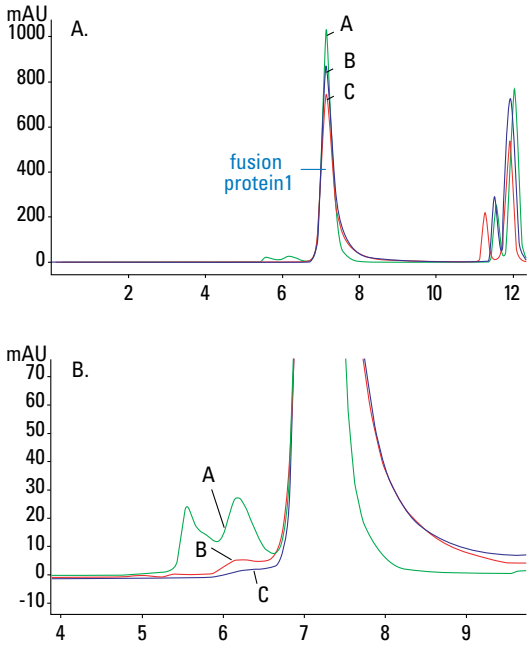
HIGH RESOLUTION ANALYSIS OF FUSION PROTEINS

During method development, many variables are examined to ensure method robustness. Factors such as elution profile, peak shape, and recovery are required to be consistent. During a method re-qualification several variables were investigated to eliminate non-specific binding and increase the robustness of an established QC method using a TSKgel SuperSW3000 column.

As shown in FIGURE 12, excessive peak tailing of "fusion protein 1" is evident with the use of 0.2 mol/L NaCl (chromatogram C). Additionally, the expected protein dimer and trimer aggregates are not visible. By switching from 0.2 mol/L sodium chloride to 0.2 mol/L of the more chaotropic sodium perchlorate salt, together with a two-fold reduction in the buffer concentration, less peak tailing and distinct peaks for the dimer and trimer species of mAb 1 resulted (chromatogram B). Doubling the perchlorate concentration to 0.4 mol/L provided further improvement in the peak shape of fusion protein 1 and associated aggregate species (chromatogram A). FIGURE 12B is an enlargement of the baseline region, showing an improved peak shape of the dimer and trimer aggregates with the use of 0.4 mol/L $NaClO_4$.

FIGURE 12

Overlays of antibody fusion protein analysis



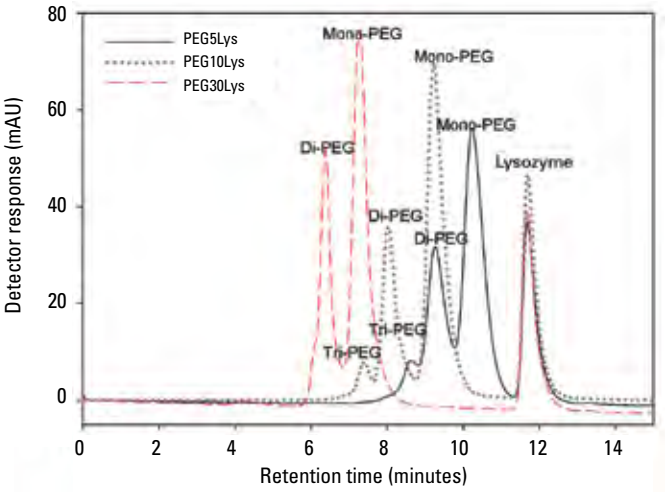
Column: TSKgel SuperSW3000, 4 µm, 4.6 mm ID x 30 cm L;
Mobile phase: c: 0.4 mol/L $NaClO_4$, 0.05 mol/L NaH_2PO_4, b: 0.2 mol/L $NaClO_4$, 0.05 mol/L NaH_2PO_4, a: 0.2 mol/L NaCl, 0.1 mol/L NaH_2PO_4;
Flow rate: 0.35 mL/min; Detection: UV @ 214 nm; Injection vol.: 5 µL;
Samples: antibody fusion protein

PEGYLATED PROTEINS

Chemical modification of therapeutic proteins is of increasing interest. One of the most frequently used protein modification methods, PEGylation, changes the biochemical and physicochemical properties of the protein, which can result in several important benefits, among them more effective target delivery, slower in vivo clearance, and reduced toxicity and immunogenicity of therapeutic proteins. After PEGylation reaction the mixture has to be purified in order to remove non-reacted protein and undesired reaction products. A TSKgel G3000SWXL column was used for the characterization of PEGylated lysozyme, as shown in FIGURE 13. A random PEGylation of lysozyme using methoxy PEG aldehyde of sizes 5 kDa, 10 kDa and 30 kDa was performed. The retention volumes of PEGylated lysozymes were used to assign the peaks based on a standard calibration curve. As a result of PEGylation, a large increase in the size of lysozyme by size exclusion chromatography was observed. The SEC elution position of lysozyme modified with a 30 kDa PEG was equivalent to that of a 450 kDa globular protein. There was a linear correlation between the theoretical molar mass of PEGylated protein and the molar mass calculated from SEC. This result illustrates the strong effect that PEG has on the hydrodynamic radius of the resulting PEGylated protein.

FIGURE 13

SEC analysis of PEGylation reaction mixtures



Column: TSKgel G3000SWXL, 5 µm, 7.8 mm ID × 30 cm
Mobile phase: 0.1 mol/L phosphate buffer, 0.1 mol/L Na_2SO_4, pH 6.7
Flow rate: 1.0 mL/min; Detection: UV @ 280 nm; Injection vol.: 20 µL
Sample: 5, 10, 30 kDa methoxy PEG aldehyde

SEC

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Flow rate (mL/min)	Maximum pressure drop (MPa)
TSKgel Stainless steel columns							
0018674	SuperSW2000	4.6	30	4	≥ 30,000	0.1 -0.35	12.0
0021845	SuperSW3000	1.0	30	4	≥ 18,000	0.016	12.0
0021485	SuperSW3000	2.0	30	4	≥ 25,000	0.065	12.0
0018675	SuperSW3000	4.6	30	4	≥ 30,000	0.1 - 0.35	12.0
0022854	SuperSW mAb HR - NEW -	7.8	30	4	≥ 30,000	0.5 -1.0	12.0
0022855	SuperSW mAb HTP - NEW -	4.6	15	4	≥ 15,000	0.1 -0.35	8.0
0022856	UltraSW Aggregate - NEW -	7.8	30	3	≥ 35,000	0.5 -1.0	12.0
0008540	G2000SWXL	7.8	30	5	≥ 20,000	0.5 -1.0	7.0
0008541	G3000SWXL	7.8	30	5	≥ 20,000	0.5 - 1.0	7.0
0008542	G4000SWXL	7.8	30	8	≥ 16,000	0.5 - 1.0	3.5
0016215	QC-PAK GFC 200	7.8	15	5	≥ 10,000	0.5 -1.0	4.0
0016049	QC-PAK GFC 300	7.8	15	5	≥ 10,000	0.5 -1.0	4.0
0005788	G2000SW	7.5	30	10	≥ 10,000	0.5 -1.0	2.0
0005789	G3000SW	7.5	30	10	≥ 10,000	0.5 -1.0	2.5
0005790	G4000SW	7.5	30	13	≥ 8,000	0.5 -1.0	1.5
0005102	G2000SW	7.5	60	10	≥ 20,000	0.5 -1.0	4.0
0005103	G3000SW	7.5	60	10	≥ 20,000	0.5 -1.0	5.0
0005104	G4000SW	7.5	60	13	≥ 16,000	0.5 -1.0	3.0
0006727	G2000SW	21.5	30	13	≥ 10,000	3.0 -6.0	1.0
0006728	G3000SW	21.5	30	13	≥ 10,000	3.0 -6.0	1.5
0006729	G4000SW	21.5	30	17	≥ 8,000	3.0 - 6.0	1.0
0005146	G2000SW	21.5	60	13	≥ 20,000	3.0 -6.0	2.0
0005147	G3000SW	21.5	60	13	≥ 20,000	3.0 -6.0	3.0
0005148	G4000SW	21.5	60	17	≥ 16,000	3.0 -6.0	2.0
TSKgel PEEK Columns							
0020027	BioAssist G2SWXL	7.8	30	5	≥ 20,000	0.5 - 1.0	7.0
0020026	BioAssist G3SWXL	7.8	30	5	≥ 20,000	0.5 - 1.0	7.0
0020025	BioAssist G4SWXL	7.8	30	8	≥ 16,000	0.5 - 1.0	3.5
TSKgel Glass Columns							
0008800	G3000SW, Glass	8.0	30	10	≥ 10,000	0.4 - 0.8	2.0
0008801	G4000SW, Glass	8.0	30	13	≥ 8,000	0.4 - 0.8	2.0

Suitable SEC guard columns are listed on page 20.

ORDERING INFORMATION

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle size (µm)*	
Guard column products					
0008805	SW Guard column, Glass	8.0	4.0	10	For all 8 mm ID SW glass columns
0018762	SuperSW Guard column	4.6	3.5	4	For 4.6 mm ID SuperSW columns
0002857	SuperSW mAb Guard column - NEW -	6.0	4.0	4	For all 8 mm ID SW glass columns
0002858	SuperSW mAb Guard column - NEW -	3.0	4.0	4	For all 8 mm ID SW glass columns
0002859	UltraSW Guard column - NEW -	6.0	4.0	3	For all 8 mm ID SW glass columns (contains SuperSW3000 packing)
0008543	SWXL Guard column	6.0	4.0	7	For all SWXL columns and P/Ns 0016215 and 0016049 (contains 3000SWXL packing)
0018008	BioAssist SWXL Guard column	6.0	4.0	7	For all BioAssist SWXL, PEEK columns
0005371	SW Guard column	7.5	7.5	10	For all 7.5 mm ID SW columns (contains 3000SW packing)
0005758	SW Guard column	21.5	7.5	13	For all 21.5 mm ID SW columns
Bulk packing					
0008544	SWXL Top-Off, 1g wet gel			5	For SWXL and QC-PAK columns

SEC

TSKgel PW and TSKgel PWXL columns - Gel Filtration Chromatography of water soluble polymers

HIGHLIGHTS

- Hydrophilic, rigid, spherical, porous methacrylate beads pH range of 2 to 12, with up to 50% organic solvent
- Temperatures up to 80°C (50°C for TSKgel G-DNA-PW)
- Wide separation range up to 2 x 10^7 Da for linear polymers
- Linear SEC column line incorporating proprietary multi-pore technology
- Specialty columns for low salt separation of cationic polymers

Polymeric TSKgel PW and high resolution TSKgel PWXL columns are designed for SEC of water soluble organic polymers, polysaccharides, DNA, and RNA. They are based on a hydrophilic polymethacrylate matrix. The range of pore sizes in which TSKgel PW and TSKgel PWXL columns are available permits a wide spectrum of water soluble substances to be analyzed. The properties and molar mass separation ranges for all TSKgel PW series columns are summarized in TABLE II.

Stable from pH 2 to 12, TSKgel PW series columns can be used in mobile phases of water or buffer (up to 20% methanol/80% aqueous) and can tolerate temperatures up to 80 °C (50 °C for TSKgel G-DNA-PW column). For analytical purposes the TSKgel PWXL columns are preferred because of their higher resolution whereas for preparative work the 60 cm TSKgel PW columns are recommended because higher sample amounts can be applied. For the analysis of proteins and peptides we recommend to use silica based SW type columns.

A number of specialty columns include columns for oligosaccharides, nucleic acids, and samples with a broad molecular weight range. A large pore G6000PW phase is available in PEEK column hardware (TSKgel BioAssist G6PW) for ultra-low sample adsorption during virus analysis. TSKgel PWXL-CP columns are especially suited for the separation of cationic polymers.

The latest additions to the TSKgel PW family are high resolution semi-micro SEC columns: TSKgel SuperoligoPW for oligomer analysis and TSKgel SuperMultiporePW columns for MW distribution analysis by linear SEC. The packing material in the TSKgel SuperMultiporePW columns is more hydrophilic than that of TSKgel PWXL columns, which further reduces the risk of adsorption of hydrophilic polymers.

TABLE II

Properties and separation ranges for TSKgel PW-type packings

TSKgel Column	Particle size (µm)	Pore size (nm)	MW range (PEG/PEO)	MW range Dextrans*	MW range Globular Proteins
G2000PW	12	12.5	< 2 x 10^3	-	< 5 x 10^3
G2500PW	12, 17	< 20	< 3 x 10^3	< 3 x 10^3	< 8 x 10^3
G3000PW	12, 17	20	< 5 x 10^4	< 6 x 10^4	5 x 10^2 - 8 x 10^5
G4000PW	17	50	< 3 x 10^5	1 x 10^3 - 7 x 10^5	1 x 10^4 - 1.5 x 10^6
G5000PW	17	100	< 1 x 10^6	5 x 10^4 - 2.5 x 10^6	< 1 x 10^8
G6000PW/ BioAssist G6PW	17	> 100	< 8 x 10^6	5 x 10^5 - 5 x 10^7	< 2 x 10^8
GMPW	17	< 10 - 100	5 x 10^2 - 8 x 10^6	< 5 x 10^7	< 2 x 10^8
G2500PWXL	7	< 20		< 3 x 10^3	< 8 x 10^3
G3000PWXL	7	20	< 5 x 10^4	< 6 x 10^4	5 x 10^2 - 8 x 10^5
G4000PWXL	10	< 50	< 3 x 10^5	1 x 10^3 - 7 x 10^5	1 x 10^4 - 1.5 x 10^6
G5000PWXL	10	100	< 1 x 10^6	5 x 10^4 - 2.5 x 10^6	< 1 x 10^8
G6000PWXL	13	> 100	< 8 x 10^6	5 x 10^5 - 5 x 10^7	< 2 x 10^8
G-DNA-PW	10	> 100	< 8 x 10^6	< 5 x 10^7	
GMPWXL	13	10 - 100	5 x 10^2 - 8 x 10^6	< 5 x 10^7	< 2 x 10^8
G-Oligo-PW	7	12.5	0< 3 x 10^3		< 5 x 10^3
SuperMultiporePW-N	4	n/a	3 x 10^2 - 5 x 10^4		
SuperMultiporePW-M	5	n/a	5 x 10^2 - 1 x 10^6		
SuperMultiporePW-H	8 (6-10)	n/a	1 x 10^3 - 1 x 10^7		
SuperOligoPW	3	n/a	1 x 10^2 - 3 x 10^3		
G3000PWXL-CP	7	20	< 9 x 10^4		
G5000PWXL-CP	10	100	< 1 x 10^6		
G6000PWXL-CP	13	> 100	< 2 x 10^7		

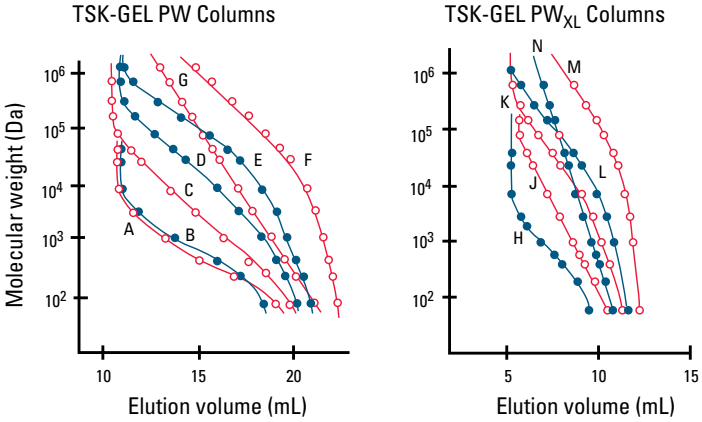
Column: TSKgel PW columns, 7.5 mm ID x 60 cm L; TSKgel PWXL, TSKgel PWXL-CP, G-Oligo-PW & G-DNA-PW, 7.8 mm ID x 30 cm L
Elution: Polyethylene glycols and oxides: distilled water; dextrans: 0.2 mol/L phosphate buffer, pH 6.8
Flow rate: 1.0 mL/min, except for TSKgel SuperMultiporePW and TSKgel SuperOligoPW columns: 0.6 mL/min
*Note: *Maximum separation range determined from estimated exclusion limits*

CALIBRATION CURVES FOR TSKgel PW / SuperMultiporePW GEL FILTRATION COLUMNS

The best results are obtained when selecting a column with the sample's molecular weight in the linear portion of the calibration curve.

FIGURE 14

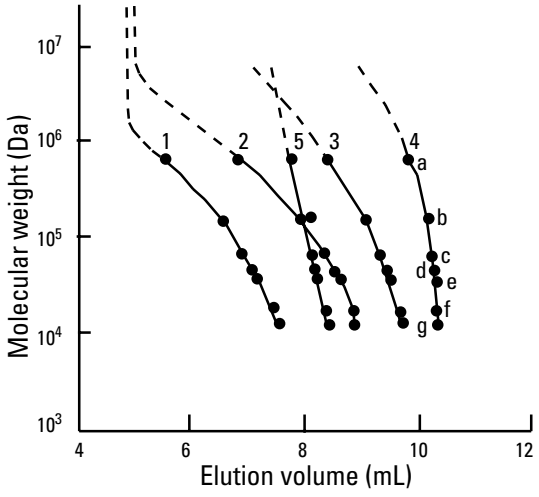
Polyethylene glycol and oxide calibration curves on TSKgel PW and TSKgel PW$_{XL}$ columns



Column: TSKgel PW columns: A. G2000PW, B. G2500PW, C. G3000PW, D. G4000PW, E. G5000PW, F. G6000PW, G. GMPW, all 7.5mm ID x 60 cm L TSKgel PW$_{XL}$ columns: H. G2500PW$_{XL}$, J. G3000PW$_{XL}$, K. G4000PW$_{XL}$, L. G5000PW$_{XL}$, M. G6000PW$_{XL}$, N. GMPW$_{XL}$, all 7.8 mm ID x 30 cm L; Elution: distilled water; Flow rate: 1.0 m L/min; Detection: RI

FIGURE 15

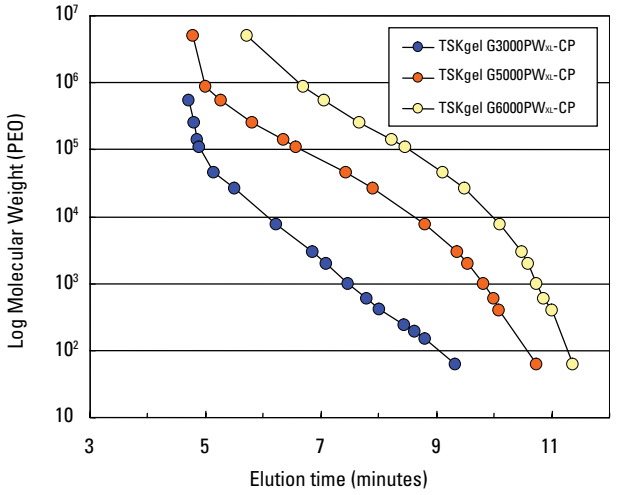
Protein calibration curves on TSKgel PW$_{XL}$ columns



Column: 1. TSKgel G3000PW$_{XL}$, 2. G4000PW$_{XL}$, 3. G5000PW$_{XL}$, 4. G6000PW$_{XL}$, 5. GMPW$_{XL}$; Sample: a. thyroglobulin (660,000 Da), b. γ-globulin (150,000 Da), c. albumin (67,000 Da), d. ovalbumin (43,000 Da), e. β-lactoglobulin (36,000 Da), f. myoglobin (16,900 Da), g. cytochrome C (12,400 Da); Elution: 0.2 mol/L phosphate buffer (pH 6.8); Flow rate: 1.0 mL/min; Detection: UV @ 280 nm

FIGURE 16

Polyethylene glycol and oxide calibration curves for TSKgel PW$_{XL}$-CP columns

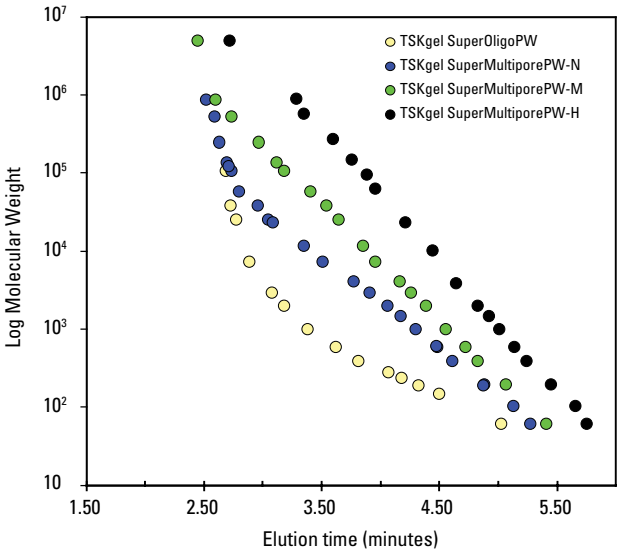


Columns: TSKgel G3000PW$_{XL}$-CP, 7 µm, 7.8 mm ID x 30 cm L, TSKgel G5000PW$_{XL}$-CP, 10 µm, 7.8 mm ID x 30 cm L, TSKgel G6000PW$_{XL}$-CP, 13 µm, 7.8 mm ID x 30 cm L

Mobile phase: 0.1 mol/L $NaNO_3$; Flow rate: 1 mL/min; Detection: RI; Temperature: 25°C; Samples: polyethylene oxides (PEO) standards, polyethylene glycols (PEG) standards

FIGURE 17

Polyethylene glycol, oxide and ethylene glycol calibration curves for TSKgel SuperMultiporePW and SuperOligoPW



Columns: TSKgel SuperOligoPW, SuperMultiporePW-N, SuperMultiporePW-M, SuperMultiporePW-H (each 6.0 mm ID x 15 cm L);

Mobile phase: H_2O; Flow rate: 0.60 mL/min; Detection: RI; Temperature: 25°C; Samples: polyethylene oxides (PEO) standards, polyethylene glycols (PEG) standards, ethylene glycol (EG) standards

SEC

COLUMNS FOR SPECIFIC APPLICATIONS

TSKgel PW$_{XL}$-CP

The new TSKgel PW$_{XL}$-CP columns are designed to facilitate the separation of cationic polymers by SEC at low salt conditions. They are based on the well known PW-type of polymeric resins for aqueous SEC. Cationic surface modification enables low salt elution of cationic polymers with high recoveries. The columns show high theoretical plate numbers, linear calibration curves and high durability. They are produced with three pore sizes for diffrent ranges (G3000-, G5000- and G6000PW$_{XL}$-CP). FIGURE 16 shows the analysis of various cationic polymers on a series of TSKgel PW$_{XL}$-CP columns.

TSKgel SuperOligoPW & G-Oligo-PW

The new TSKgel SuperOligoPW column was developed for the fast determination of molecular mass of aqueous oligomers, particularly oligosaccharides, and low molecular weight aqueous polymers. This is a semi-micro column (6.0 mm ID x 15 cm L) packed with spherical monodisperse polymethacrylate 3 µm particles. The combination of the decreased particle size and small dimensions of the TSKgel SuperOligoPW column enables high speed separation with high resolution - half of the separation time with the same resolution compared to conventional size exclusion columns. An added benefit of the semi-micro and small particle size is lower solvent consumption compared to conventional columns.

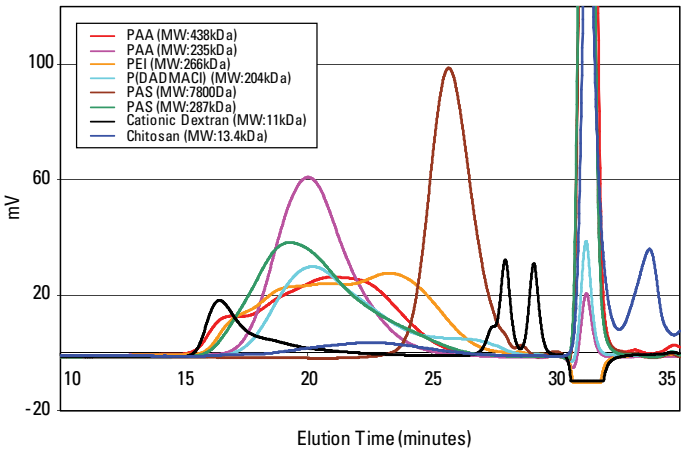
TSKgel G-Oligo-PW was designed for high resolution separations of nonionic and cationic oligomers and oligosaccharides such as hydrolyzed cyclodextrins. Because of the presence of residual cationic groups, this column is not recommended for separating anionic materials. The polyethylene glycol and polythylene oxide calibration curves for TSKgel G-Oligo-PW (not shown) are identical to the calibration curve for TSKgel G2500PW$_{XL}$ (shown on the previous page. FIGURE 18 shows the calibration curve for double stranded DNA for the TSKgel G-DNA-PW column.

TSKgel G-DNA-PW

The TSKgel G-DNA-PW column is dedicated to the separation of large polynucleotides, such as DNA and RNA fragments of 500 to 5,000 base pairs. The exclusion limits for double-stranded DNA fragments are lower than those for rRNAs. The packing of the TSKgel G-DNA-PW column has very large pores (>100 nm) and a small particle size (10 µm).

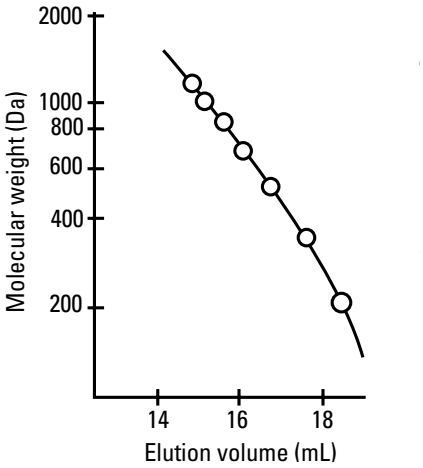
For the separation of large DNA fragments greater than 1,000 base pairs, a four-column system is typically required. Baseline resolution of DNA fragments up to 7,000 base pairs can be achieved, provided there is a two-fold difference in the chain length of the fragments.

FIGURE 18
Double stranded DNA calibration curve for TSKgel G-DNA-PW column



Columns: TSKgel G3000PW$_{XL}$-CP, 7 µm (7.8 mm ID x 30 cm L), TSKgel G5000PW$_{XL}$-CP, 10 µm (7.8 mm ID x 30 cm L), TSKgel G6000PW$_{XL}$-CP, 13 µm (7.8 mm ID x 30 cm L); Eluent: 0.1 mol/L $NaNO_3$; Flow rate: 1 mL/min; Detection: RI; Temperature: 25°C; Sample Load: 3 g/L, 100 µL

FIGURE 19
Oigosaccharides calibration curve for TSKgel G-Oligo-PW column



Column: TSKgel G-Oligo-PW, two 6 µm, 7.8 mm ID x 30 cm L columns in series; Mobile phase: distilled H_2O; Flow rate: 1.0 mL/min; Detection: UV @ 260 nm; Sample: hydrolyzed -cyclodextrin

COLUMNS FOR SPECIFIC APPLICATIONS

TSKgel GMPW AND TSKgel GMPWXL

When the molecular weight range of the sample is broad or unknown, Tosoh Bioscience offers mixed-bed and multipore columns for analysis. The mixed bed column TSKgel GMPW and its high resolution counterpart, TSKgel GMPWXL, are packed with the G2500, G3000 and G6000 PW or corresponding PWXL resins. They offer a broad molecular weight separation range. As shown on page 42, the calibration curve for polyethylene glycols and oxides on these columns is fairly shallow and is linear over the range of 100-1,000,000 Da. The introduction of mixed-bed columns has facilitated the analysis of polydisperse samples. Previously, two-column systems such as TSKgel G3000PW and TSKgel G6000PW, were required to achieve good resolution with wide MW-range samples. The substitution of a TSKgel GMPW series column can save both time and money compared with multi-column systems.

TSKgel SuperMultiporePW

TSKgel SuperMultiporePW columns incorporate the multi-pore particle synthesis technology developed by Tosoh scientists in which monodisperse particles exhibit a broad range of pore sizes. See page 54 for additional information on multipore technology. Each particle, by design, has an extended linear calibration curve, thereby greatly diminishing the appearance of chromatograms with inflection points. This allows better reproducibility when determining molecular mass and molecular mass distribution of polymers.

Three semi-micro (6.0 mm ID x 15 cm L) columns are available within the TSKgel SuperMultiporePW series containing 4, 5 or 8 µm particles. This enables high speed separation for aqueous polymers and low solvent consumption compared to the conventional SEC columns. In addition, a wide separation range can be analyzed with the three different columns, from high molecular mass aqueous polymers to oligomers.

Multi-pore, semi-micro SEC columns provide high resolution and smooth peak shapes without shoulders of inflection points. This leads to better accuracy and reproducibility when determining the molecular mass distribution of water soluble polymers

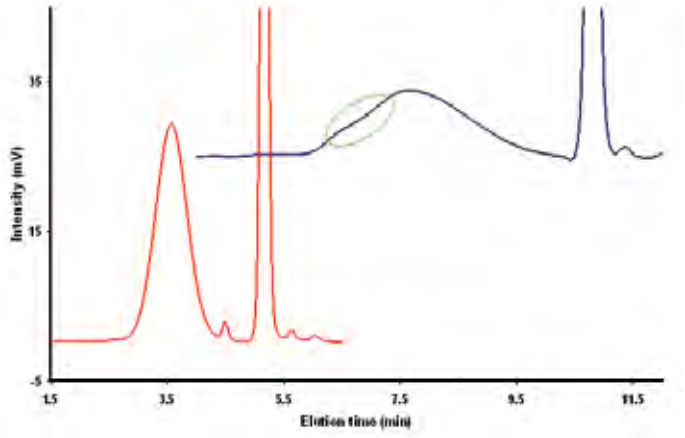
COMPARISON WITH CONVENTIONAL GPC COLUMNS

FIGURE 20 shows the SEC analysis of a real sample Polyvinyl-pyrrolidone (PVP) K-30- on a series of conventional TSKgel G3000PWXL and G5000PWXL columns compared to the one obtained with a single TSKgel SuperMultiporePW-M linear SEC column (MW range 600,000 – 1,500,000). On a series of conventional SEC columns the Polyvinylpyrrolidone peak shows an inflection point, which does not appear on the SuperMultiporePW-M column. Analysis is much faster and more sensitive when applying the new multi-pore packing.

A mixture of polyethylene oxide (PEO) and polyethylene glycol (PEG) was analyzed on a semi-micro TSKgel SuperMultiporePW-M column and on conventional-sized TSKgel G3000PWXL and TSKgel G5000PWXL columns in series. As shown in FIGURE 21, the analysis using the TSKgel SuperMultiporePW-M column was completed in half the time and with higher resolution than the analysis performed using the TSKgel G3000PWXL and TSKgel G5000PWXL columns. This is due to the semi-micro dimensions (6.0 mm ID x 15 cm L) and the smaller particle size (5 µm) of the TSKgel SuperMultiporePW-M column compared to the 7.8 mm ID x 30 cm L size and 7 and 10 µm particle size of the TSKgel G3000PWXL and TSKgel G5000PWXL columns respectively.

FIGURE 20

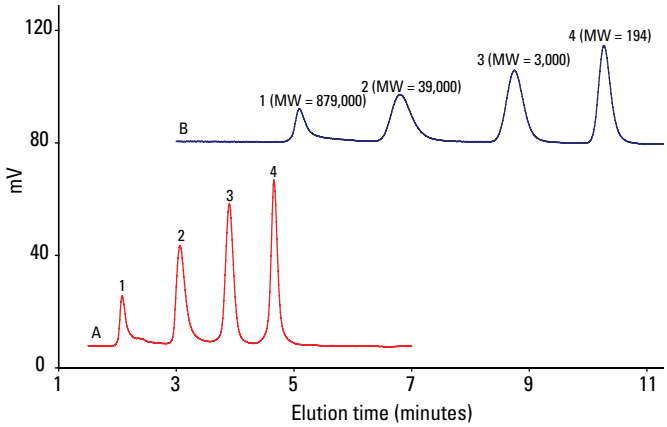
Analysis of polyvenylpyrrolidone



Columns: TSKgel SuperMultiporePW-M, 6 mm ID x 15 cm L x 1 (red) TSKgel G3000PWXL & G5000PWXL, each 7.8 mm ID x 30 cm L in line (blue); Sample: Polyvinylpyrrolidone (K-30); Mobile phase: 0.1 mol/L $NaNO_3$; Flow rate: 0.6 mL/min; Detection: RI

FIGURE 21

Comparison of analysis of a mixture of PEO and PEG



Column: TSKgel SuperMultiporePW-M, 6.0 mm ID x 15 cm L; TSKgel G5000PWXL + G3000PWXL, each 6.0 mm ID x 15 cm L; Mobile phase: H_2O; Flow rate: 0.6 mL/min; Detection: RI; Temperature: 25°C; Injection vol.: A: 20 µL, B: 100 µL; Samples: mixture of PEO and PEG

SEC

OPTIMIZING GEL FILTRATION WITH TSKgel PW AND TSKgel PWXL COLUMNS

SELECTING MOBILE PHASE BUFFERS

SEC separation is based on the difference of apparent molecular size with no additional interaction between the column matrix and the sample molecules. In practice, however, a small number of weakly charged groups on the surface of PW-type packings can cause changes in elution order from that of an ideal system. The eluent composition can vary greatly with TSKgel PW columns to be compatible with a wide range of neutral, polar, anionic, and cationic samples. The table below lists appropriate eluents for GFC of major polymer types.

For some nonionic, nonpolar polymers, such as polyethylene glycols, ideal size exclusion behavior can be obtained by using distilled water. More polar ionic polymers may exhibit abnormal peak shapes or minor peaks near the void volume when eluted with distilled water, due to ionic interactions between the sample and residual charged groups on the resin surface. To eliminate ionic interactions, a neutral salt such as sodium nitrate or sodium sulfate should be added. Generally, a salt concentration of 0.1 to 0.5 mol/L is sufficient to overcome undesirable ionic interactions.

HYDROPHOBIC SAMPLES

TSKgel PW-type resins are more hydrophobic than polysaccharide gels such as cross-linked dextran. Depending on the sample, this can lead to hydrophobic interaction as a secondary retention mechanism. The extent of hydrophobic interaction increases as the salt concentration of the eluent increases, but it can be reduced by the addition of an organic modifier such as acetonitrile. Water soluble organic solvents are frequently used as modifiers to suppress hydrophobic interactions between the sample and the resin surface.

Modifiers are also used for optimizing the elution of both charged and neutral hydrophobic polymers. Typical examples for a variety of sample types are given in the table below. All TSKgel PW-type column packings are compatible with 20 % aqueous solutions of methanol, ethanol, propanol, acetonitrile, dimethyl formamide, dimethyl sulfoxide, formic acid, and acetic acid. In addition, these columns can be operated in 50 % aqueous acetone.

TABLE III

Recommended eluents for GFC of water soluble polymer on TSKgel PW-type columns

Type of polymer	Typical sample	Suitable eluent
Nonionic hydrophilic	polyethylene glycol soluble starch, methyl cellulose, pullulan dextran, hydroxyethyl cellulose, polyvinyl alcohol, polyacrylamide	distilled water 0.01N NaOH 20% DMSO Buffer or salt solution (e.g., 0.1–0.5 mol/L $NaNO_3$)
Nonionic hydrophobic	polyvinylpyrrolidone	Buffer or salt solution with organic solvent (e.g., 20% ACN in 0.1mol/L $NaNO_3$)
Anionic hydrophilic	sodium chondroitin sulfate, sodium alginate, carboxymethyl cellulose, sodium polyacrylate, sodium hyaluronate	Buffer or salt solution (e.g., 0.1 mol/L $NaNO_3$)
Anionic hydrophobic	sulfonated lignin sodium salt, sodium polystyrenesulfonate	Buffer or salt solution with organic solvent (e.g., 20% ACN in 0.1 mol/L $NaNO_3$)
Cationic hydrophilic	glycol chitosan, DEAE-dextran, poly(ethyleneimine), poly(trimethylaminoethyl methacrylate) iodide salt	0.5 mol/L acetic acid with 0.3 mol/L Na_2SO_4, or 0.8 mol/L $NaNO_3$ (0.1 mol/L $NaNO_3$ for PWXL-CP type)
Cationic hydrophobic	poly(4-vinylbenzyltrimethylammonium chloride), poly(N-methyl-2-vinylpyridinium) iodide salt	0.5 mol/L acetic acid with 0.3 mol/L Na_2SO_4
Amphoteric hydrophilic	peptides, proteins, poly-and oligosaccharides, DNA, RNA	Buffer or salt solution (e.g., 0.1 mol/L $NaNO_3$)
Amphoteric hydrophobic	blue dextran, collagen, gelatin, hydrophobic proteins hydrophobic peptides	Buffer or salt solution with organic solvent (e.g., 20% ACN in 0.1 mol/L $NaNO_3$ or 35 - 45% ACN in 0.1% TFA)

APPLICATIONS OF TSKgel PW-TYPE GEL FILTRATION COLUMNS

POLYSACCHARIDES

TSKgel PW columns are recommended for polysaccharide analysis due to their ability to separate a wide molecular weight distribution. Nonionic polysaccharides are the least complicated molecules to analyze by SEC because they seldom exhibit secondary interactions with the solid support. TSKgel G5000PW and TSKgel G3000PW in series are effective for the characterization of clinical dextran.

Cationic samples can be adsorbed on the resin by electrostatic interaction. If the polymer is strongly cationic, a fairly high salt concentration is required to prevent ionic interactions with conventional SEC packings. A mobile phase of 0.5 mol/L acetic acid with 0.3 mol/L Na_2SO_4 can also be used.

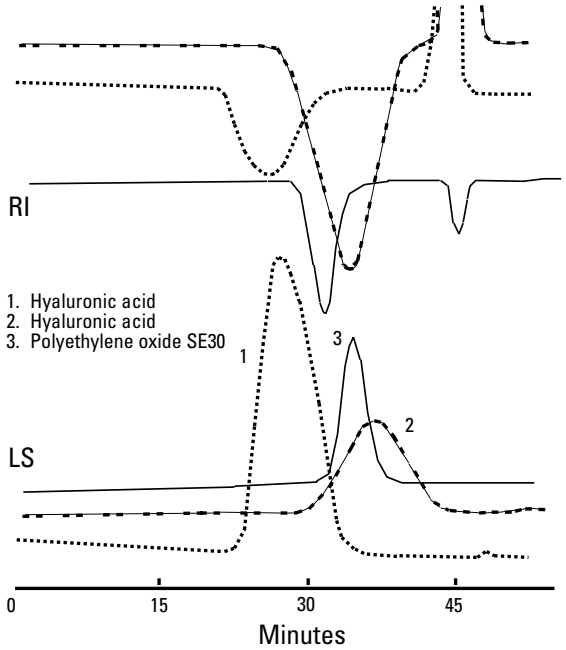
The new TSKgel PW$_{XL}$-CP series enables elution of water soluble, cationic polymers under low salt conditions (e.g. 0.1 mol/L $NaNO_3$). An effective separation of the anionic hydrophilic gluco-saminoglycan, hydraluronic acid, is shown in FIGURE 22 on a TSKgel G6000PW and TSKgel G4000PW column in series with a 0.2 mol/L sodium chloride mobile phase.

OLIGOSACCHARIDES

FIGURE 23 shows the rapid analysis of maltose oligomers using a TSKgel SuperOligoPW column compared to a TSKgel G-Oligo-PW column. The faster analysis time is due to the semi-micro dimensions (6.0 mm ID x 15 cm L) and the small particle size (3 µm) of the TSKgel SuperOligoPW column compared to the 7.8 mm ID x 30 cm L size and 7 µm particle size of the TSKgel G-Oligo-PW column.

FIGURE 22

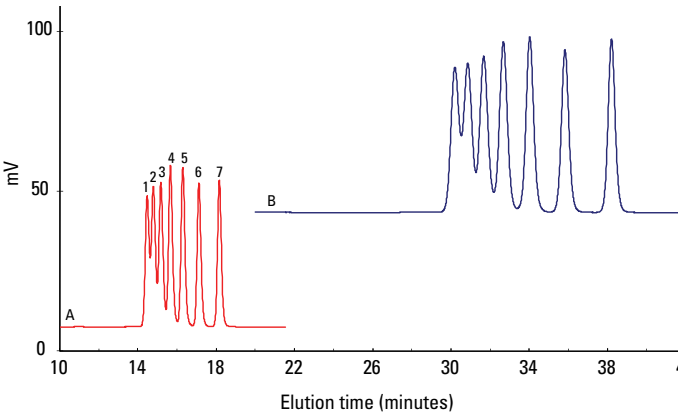
Analysis of oligosaccharides



Column: TSKgel G6000PW + G4000PW, two 7.5 mm ID x 60 cm L columns in series; Mobile phase: 0.2 mol/L NaCl; Flow rate: 0.9 mL/min
Temperature: 40°C; Samples: hyaluronic acid

FIGURE 23

Analysis of maltose oligomers



Column: A: TSKgel SuperOligoPW, 3 µm, 6.0 mm ID x 15 cm L x 4
B: TSKgel G-Oligo-PW, 7 µm, 7.8 mm ID x 30 cm L x 4; Mobile phase: H_2O
Flow rate: A: 0.6 mL/min B: 1.0 mL/min; Detection: RI; Temperature: 40°C
Injection vol.: A: 10 µL B: 50 µL; Samples: 1.maltoheptose, 2. maltohexose, 3. maltopentose, 4. maltotetraose, 5. maltotriose, 6. maltose, 7. glucose

SEC

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Flow rate (mL/min) range	Maximum pressure drop (MPa)
TSKgel Stainless Steel Columns							
0022789	SuperMultiporePW-N	6.0	15	4	>16,000	0.3 - 0.6	4.5
0022790	SuperMultiporePW-M	6.0	15	5	>12,000	0.3 - 0.6	2.7
0022791	SuperMultiporePW-H	6.0	15	8	>7,000	0.3 - 0.6	0.9
0022792	SuperOligoPW	6.0	15	3	>16,000	0.3 - 0.6	5.0
0008031	G-Oligo-PW	7.8	30	7	≥ 16,000	0.5 - 0.8	4.0
0008032	G-DNA-PW	7.8	30	10	≥ 10,000	0.2 - 0.5	2.0
0008020	G2500PW$_{XL}$	7.8	30	7	≥ 16,000	0.5 - 0.8	4.0
0008021	G3000PW$_{XL}$	7.8	30	7	≥ 16,000	0.5 - 0.8	4.0
0008022	G4000PW$_{XL}$	7.8	30	10	≥ 10,000	0.3 - 0.6	2.0
0008023	G5000PW$_{XL}$	7.8	30	10	≥ 10,000	0.3 - 0.6	2.0
0008024	G6000PW$_{XL}$	7.8	30	13	≥ 7,000	0.3 - 0.6	2.0
0008025	GMPW$_{XL}$	7.8	30	13	≥ 7,000	0.3 - 0.6	2.0
0021873	G3000PW$_{XL}$-CP	7.8	30	7	≥ 16,000	1.0	
0021874	G5000PW$_{XL}$-CP	7.8	30	10	≥ 10,000	1.0	
0021875	G6000PW$_{XL}$-CP	7.8	30	13	≥ 7,000	1.0	
0005761	G2000PW	7.5	30	12	≥ 5,000	0.5 - 1.0	2.0
0008028	G2500PW	7.5	30	12	≥ 5,000	0.5 - 1.0	2.0
0005762	G3000PW	7.5	30	12	≥ 5,000	0.5 - 1.0	2.0
0005763	G4000PW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.0
0005764	G5000PW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.0
0005765	G6000PW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.0
0008026	GMPW	7.5	30	17	≥ 3,000	0.5 - 1.0	1.0
0005105	G2000PW	7.5	60	12	≥ 10,000	0.5 - 1.0	4.0
0008029	G2500PW	7.5	60	12	≥ 10,000	0.5 - 1.0	4.0
0005106	G3000PW	7.5	60	12	≥ 10,000	0.5 - 1.0	4.0
0005107	G4000PW	7.5	60	17	≥ 6,000	0.5 - 1.0	2.0
0005108	G5000PW	7.5	60	17	≥ 6,000	0.5 - 1.0	2.0
0005109	G6000PW	7.5	60	17	≥ 6,000	0.5 - 1.0	2.0
0008027	GMPW	7.5	60	17	≥ 6,000	0.5 - 1.0	2.0
0008030	G2500PW	21.5	60	17	≥ 10,000	1.6 - 6.0	2.0
PEEK							
0020024	BioAssist G6PW	7.8	30	17	≥ 3,000	0.5 - 1.0	10
Guard columns							
0022793	SuperMP (PW)-N Guard column	4.6	3.5	4			
0022794	SuperMP (PW)-M Guard column	4.6	3.5	5			
0022795	SuperMP (PW)-H Guard column	4.6	3.5	8			
0022796	SuperOligoPW Guard column	4.6	3.5	3			
0008034	Oligo Guard column	6.0	4.0	13	For 7.8 mm ID G-Oligo-PW columns		
0008033	PW$_{XL}$ Guard column	6.0	4.0	12	For 7.8 mm ID PW$_{XL}$& G-DNA-PW (TSKgel G3000PW packing)		
0021876	PW$_{XL}$-CP Guard column	6.0	4.0	13	For 7.8 mm ID PW$_{XL}$-CP columns		
0006763	PW-L Guard column	7.5	7.5	13	For 7.5 mm ID G2000PW (TSKgel G2000PW packing)		
0006762	PW-H Guard column	7.5	7.5	13	For 7.5 mm ID G2500PW through GMPW columns		
0006758	PW-H Guard column	21.5	7.5	17	For 21.5 mm ID G2500PW through G5000PW columns		
Bulk packing							
0008035	PW$_{XL}$ Top-Off, 1 g wet resin			10	For all PW$_{XL}$ and G-DNA-PW columns		

TSKgel ALPHA AND SuperAW GEL FILTRATION COLUMNS

Gel Filtration and Gel Permeation Chromatography of water soluble and polar organic-soluble polymers

HIGHLIGHTS

- A unique hydrophilic, polymer resin is available in conventional column dimensions (Alpha) and high throughput column format (SuperAW).
- Exhibits strong mechanical stability and minimal swelling characteristics
- A wide range of solvent compatibility, from 100% water to 100% non-polar organic solvents
- The reduced particle size and shorter column length of TSKgel SuperAW columns provide equivalent resolution in one half the time for high throughput applications.
- Unlike polystyrene-divinylbenzene (PS-DVB) resins that may adsorb polymers due to hydrophobic interaction, both the TSKgel Alpha and
- SuperAW columns allow for the separation of polymers soluble in methanol.
- Provide accurate molecular weight determination of samples in dimethyl formamide and exhibit normal retention of polystyrene polymers
- System peaks from salts in the eluent elute away from the oligomer of interest, providing accurate MW determinations.

COLUMN SELECTION

The TSKgel Alpha Series consists of six columns with three particle sizes: 7, 10, and 13 µm. These columns span a wide MW separation range from 10^2 to more than 1 x 10^6 Da when using polyethylene oxide (PEO) as a MW standard. Exclusion limits for the TSKgel Alpha columns for polyethylene oxide (PEO), polyethylene glycols (PEG) and polystyrenes (PS) are shown in the table below. Calibration curves for the TSKgel Alpha Series columns are shown on the next page for polyethylene oxide, polyethylene glycol and polystyrene standards.

The TSKgel SuperAW series contains a similar chemistry as the TSKgel Alpha series but offers the benefit of smaller particle sizes (4 µm to 9 µm) and smaller column dimensions. Reductions in analysis time and mobile phase consumption make SuperAW columns ideal for high throughput applications. TSKgel Alpha and SuperAW columns are offered in 5 discrete exclusion ranges and 1 mixed bed. Both column types can accommodate polymer standards up to several million Dalton molecular weight (see calibration curves on the next page

TABLE IV

Exclusion limits for TSKgel Alpha Series and SuperAW Series columns

TSKgel Column	Particle size (µm)	Exclusion limit (Da) for various standards and eluents		
		PEO[a]/H_2O	PS[b]/10 mmol/L LiBr in DMF	PEG[c]/10 mmol/L LiBr in MeOH
Alpha-2500	7	5 x 10^3	1 x 10^4	1 x 10^4
Alpha-3000	7	9 x 10^4	1 x 10^5	6 x 10^4
Alpha-4000	10	4 x 10^5	1 x 10^6	3 x 10^6
Alpha-5000	10	1 x 10^6	7 x 10^6	N.D.
Alpha-6000	13	> 1 x 10^7	> 1 x 10^7	N.D.
Alpha-M	13	> 1 x 10^7	> 1 x 10^7	N.D.
SuperAW2500	4	5 x 10^3	8 x 10^3	1 x 10^4
SuperAW3000	4	9 x 10^4	8 x 10^4	1 x 10^5
SuperAW4000	6	1 x 10^6	6 x 10^5	6 x 10^5
SuperAW5000	7	1 x 10^{6*}	N.D.	N.D.
SuperAW6000	9	1 x 10^{7*}	N.D.	N.D.
SuperAWM-H	9	1 x 10^{7*}	N.D.	N.D.

N.D. = not determined a Polyethylene oxide b Polystyrene divinyl benzene c Polyethylene glycol

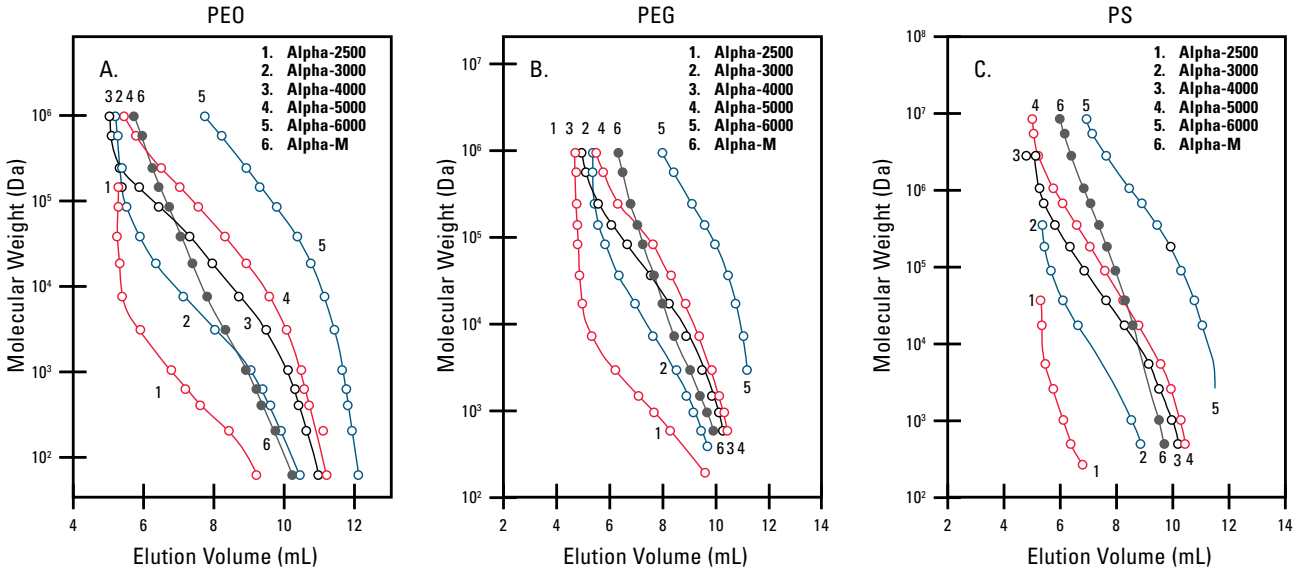
** Exclusion limit for SuperAW5000, SuperAW6000, and SuperAWM-H are estimated, respectively*

SEC

CALIBRATION CURVES FOR TSKgel ALPHA AND SuperAW GEL FILTRATION COLUMNS

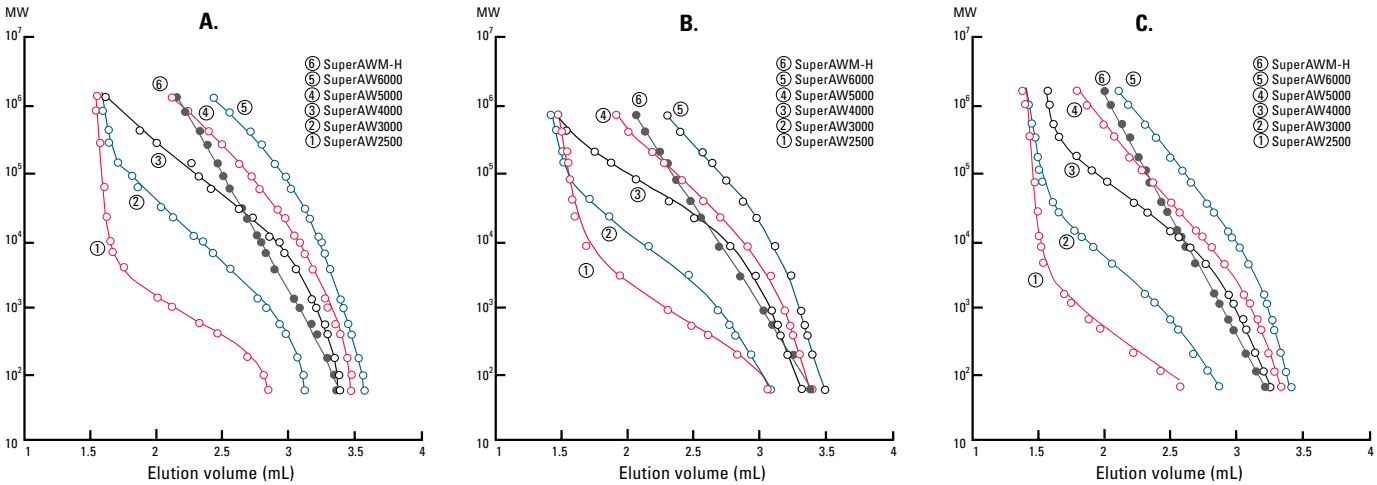
The best results are obtained when selecting a column with the sample's molecular weight in the linear portion of the calibration curve.

Polyethylene oxide (PEO), polyethylene glycol (PEG) and polystyrene (PS) calibration curves for TSKgel Alpha columns



Column: TSKgel Alpha Series, 7.8 mm ID x 30 cm L; Eluent: A. H_2O; B. 10 mmol/L LiBr in Methanol; C. 10 mmol/L LiBr in DMF; Flow rate: 1.0 mL/min; Temperature: A. 25°C; B. 25°C; C. 40°C; Detection: RI

Calibration curves for TSKgel SuperAW series in different solvents with different polarity



Column: TSKgel SuperAW Series (6.0 mm ID x 15 cm L)
Eluent: A. Water; B. MeOH containing 10 mmol/L LiBr; C. DMF containing 10 mmol/L LiBr
Flow rate: 0.6 mL/min; Temperature: 25°C; Detection: Refractive index detector
Samples: Standard polyethylene oxide, polyethylene glycol, ethylene glycol

APPLICATIONS OF TSKgel ALPHA AND SuperAW GEL FILTRATION COLUMNS

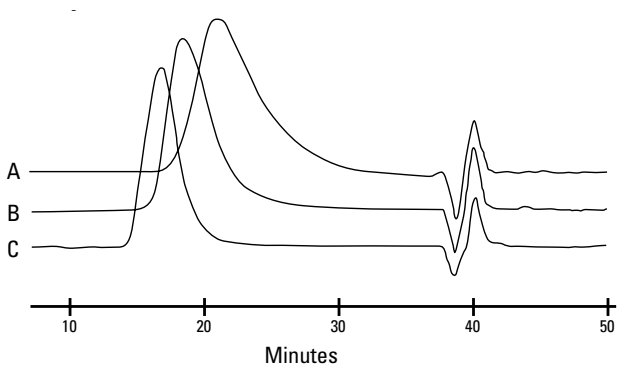
The versatility of using TSKgel Alpha columns with various polar solvents is illustrated in FIGURE 24 for the analysis of cellulose derivatives. A TSKgel Alpha-M column was used to separate ethylcellulose with the polar solvent DMF and ethylhydroxyethyl cellulose with methanol.

The separation of polyvinylalcohol with different degrees of saponification is shown in FIGURE 25. This separation was performed with a TSKgel Alpha-5000 and a TSKgel Alpha-3000 column in series using a hexafluoroisopropanol mobile phase.

FIGURE 26 shows that the column efficiency of TSKgel SuperAW series columns is maintained in a wide variety of polar organic solvents.

FIGURE 25

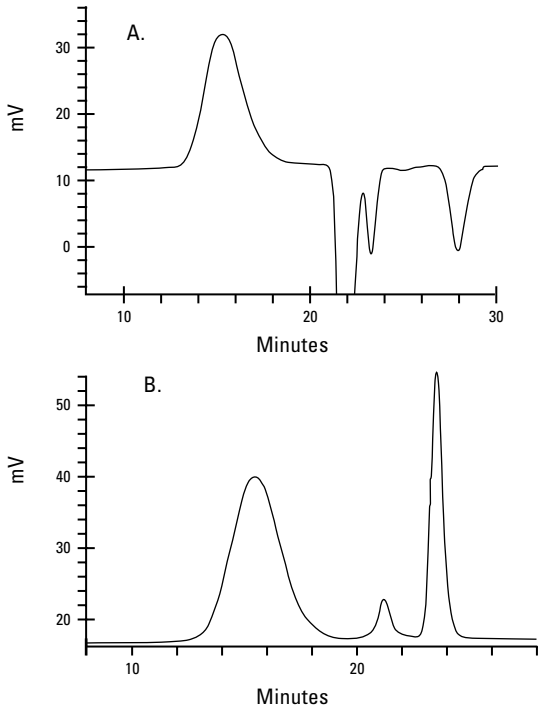
Polyvinylalcohol characterization using TSKgel Alpha-5000 and Alpha-3000 columns in series



Column: TSKgel Alpha-5000 and Alpha-3000, 7.8 mm ID x 30 cm L in series
Sample: degree of saponification of polyvinyl alcohol: A. 75%, B. 88%, C. 100%; Eluent: hexafluoroisopropanol (HFIP); Flow rate: 0.5 mL/min; Temperature: 40°C; Detection: RI

FIGURE 24

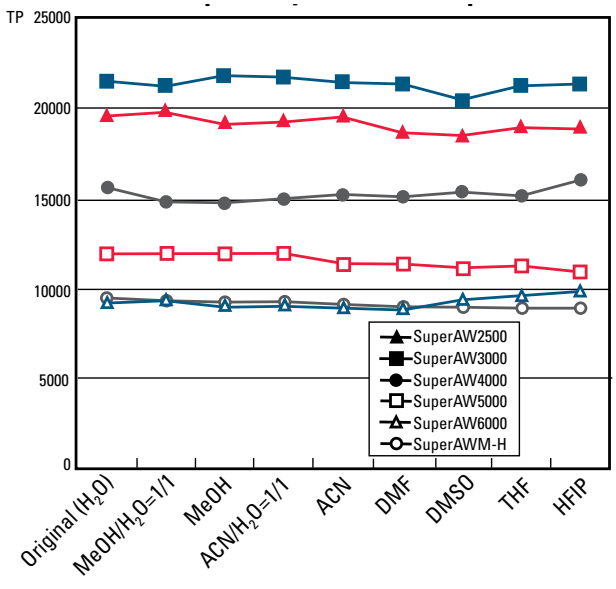
TSKgel Alpha-M separation of cellulose derivatives



Column: TSKgel Alpha-M, 7.8 mm ID x 30 cm L;
Sample: A. 50 µL ethylcellulose, 0.1%; B. 50 µL ethylhydroxyethylcellulose, 0.1%; Elution: A. 10 mmol/L LiBr in DMF; B. 10 mmol/L LiBr in methanol; Flow rate: 0.5 mL/min; Temperature: 40°C; Detection: RI

FIGURE 26

Solvent compatibility of TSKgel SuperAW series



Column: TSKgel SuperAW Series (6.0 mm ID x 15 cm L); Eluent: Water
Flow rate: 0.6 mL/min; Temperature: 25°C; Detection: Refractive index detector
Sample: Ethylene glycol; Inj. volume: 5 µL (2.5 g/L)

SEC

ORDERING INFORMATION

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle size (µm)*	*Number theoretical plates*	*Flow rate (mL/min)*	*Maximum pressure drop (MPa)*	
TSKgel Stainless Steel Columns								
0018339	Alpha-2500	7.8	30	7	≥ 16,000	0.5 - 0.8	1.0	4.0
0018340	Alpha-3000	7.8	30	7	≥ 16,000	0.5 - 0.8	1.0	4.0
0018341	Alpha-4000	7.8	30	10	≥ 10,000	0.3 - 0.6	1.0	3.0
0018342	Alpha-5000	7.8	30	10	≥ 10,000	0.3 - 0.6	1.0	3.0
0018343	Alpha-6000	7.8	30	13	≥ 7,000	0.3 - 0.6	1.0	2.0
0018344	Alpha-M (mixed bed)	7.8	30	13	≥ 7,000	0.3 - 0.6	1.0	2.0
Guard columns								
0018345	Alpha Guard column	6	4	13	For all Alpha columns			
TSKgel VMpak columns*								
0020011	VMpak-25	2.0	5	7	≥ 1,000	0.1 - 0.2	0.25	2.0
0020012	VMpak-25	2.0	15	7	≥ 3,000	0.1 - 0.2	0.25	6.0
TSKgel Stainless Steel Columns								
0019315	SuperAW2500	6.0	15	4	≥ 16,000	0.3 - 0.6	0.6	6.0
0019316	SuperAW3000	6.0	15	4	≥ 16,000	0.3 - 0.6	0.6	6.0
0019317	SuperAW4000	6.0	15	6	≥ 10,000	0.3 - 0.6	0.6	4.0
0019318	SuperAW5000	6.0	15	7	> 10,000	0.3 - 0.6	0.6	3.0
0019319	SuperAW6000	6.0	15	9	> 7,000	0.3 - 0.6	0.6	2.0
0019320	SuperAWM-H	6.0	15	9	> 7,000	0.3 - 0.6	0.6	2.0
Guard columns								
0019321	SuperAW-L Guard Column	4.6	3.5	7	For SuperAW2500-4000 columns.			
0019322	SuperAW-H Guard Column	4.6	3.5	13	For SuperAW5000-AWM-H columns			

**TSKgel VMpak-25 series contains a similar packing as TSKgel Alpha-2500. It can be used for multimodal LC/LC-MS separations.*

TSKgel HXL, HHR, SuperH AND SuperHZ GEL PERMEATION COLUMNS

Polymer-based columns for Gel Permeation Chromatography of organic-soluble polymers

HIGHLIGHTS

- Porous, highly cross-linked, spherical polystyrene divinylbenzene (PS-DVB) resin.
- Five different TSKgel H-type columns are available
- Expanded molecular weight ranges with exclusion limits from 1,000 g/mol to an estimated 4 x 10^8 g/mol
- Minimal shrinking and swelling of the column bed
- Chemically and thermally stable
- Semi-micro SuperMultiporeHZ, SuperHZ and Super H columns for reduced solvent consumption in high throughput analysis
- Multipore columns provide linear calibration curves over a wider MW range for conventional GPC (Multipore HXL) and semi-micro GPC (SuperMultiporeHZ)
- Mixed bed GPC columns for ultra-high temperature GPC up to 220°C

TSKgel H Series columns are recommended for the analysis of organic-soluble polymers and are packed with spherical particles composed of polystyrene cross-linked with divinylbenzene (PS-DVB). Each line of columns within this series differs in degree of inertness and operating temperature range. The packings are available in eight pore sizes and span four different column chemistries. For polymer samples with a broad molecular range, packing of several pore sizes are provided in the mixed bed columns: TSKgel SuperHZM series, TSKgel SuperHM series, TSKgel GMHXL, TSKgel GMHHR, and selected high temperature versions provide linear calibration curves up to several million Daltons (see page 53).

COLUMN SELECTION

Best results are obtained when selecting a column with the sample's molar mass in the linear portion of the calibration curve. The Super prefix refers to the efficiency of the column. The Super series columns contain ultra efficient particles as small as 3 µm, housed in 15 cm length columns. The smaller particle allows for equivalent resolution to conventional HXL columns, with 50% less run time due to the shorter column length. The Super series columns are an excellent choice for high throughput polymer analysis.

TABLE V

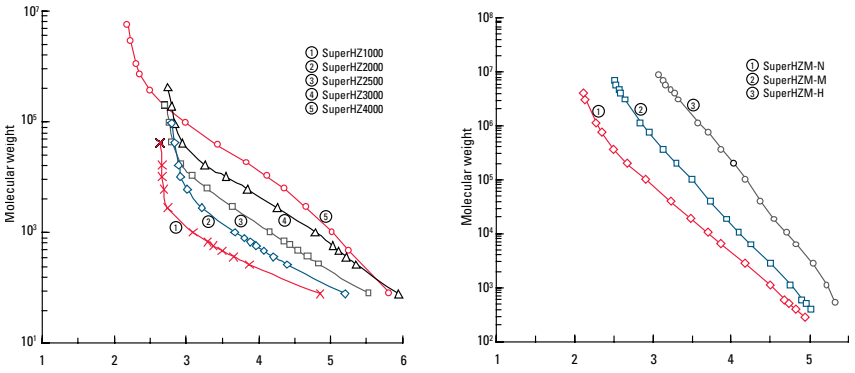
Series Type	SuperMultiporeHZ	SuperHZ	HXL	SuperH	HHR
Application focus	Ultra-high performance with a low dead volume and a wide pore distribution in each particle for superior linearity	High-throughput polymer analysis with ultra low polymer adsorption. Limited solvent compatibility range.	Conventional polymer analysis with ultra low polymer adsorption. Ltd solvent compatibility range.	High-throughput polymer analysis with expanded solvent compatibility.	Conventional polymer analysis with expanded solvent compatibility range.
Particle size	3, 4 and 6 µm, depending on pore size	3, 5 and 10 µm, depending on pore size	5, 9 and 13 µm, depending on pore size	3 and 5 µm, depending on pore size	5 µm
Theoretical plates[1]	20,000/15 cm column	16,000/15 cm column	16,000/30 cm column	16,000/15 cm column	16,000/30 cm column
Maximum temperature	60°C	G1000 - G4000 60°C G5000 - mixed 80°C	G1000 - G4000 60°C G5000 - mixed 80°C	140°C	140°C 220°C for HHR HT2
Standard shipping solvent	THF	THF	THF[2]	THF[2]	THF[2]
THF can be switched to	none	benzene, chloroform, toluene, xylene, dichloromethane[3] and dicholoroethane[3]		see our website for detailed information	
Other shipping solvents available?	yes[4]	yes[4]		no	
Number of solvent substitutions	-	One time only	One time only	Several[5]	Several[5]
Solvent exchange instructions		Linear gradient with a 2 %/min rate of change at a flow rate <0.25 mL/min	Linear gradient with a 2 %/min rate of change at a flow rate <0.5 mL/min	Linear gradient with a 2%/min rate of change according to flow rates listed on our website	

1) Theoretical plates listed are based on smallest particle size listed
2) High-temperature columns (HT) are shipped with OCDB (Orthochlorodivinylbenzene) as standard shipping solvent.
3) Switching from THF to dichloromethane and dichloroethane is not recommended for G1000 pore size columns.
4) See our website for available shipping solvents
5) After switching to a very polar solvent such as acetone, switching back to a nonpolar solvent is not recommended.

SEC

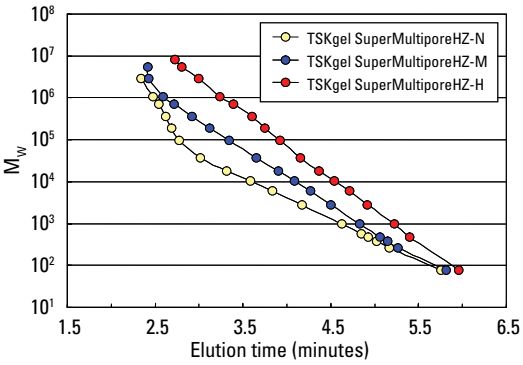
CALIBRATION CURVES FOR TSKgel H-TYPE GEL PERMEATION COLUMNS

Calibration curves for TSKgel SuperHZ columns with polystyrene standards



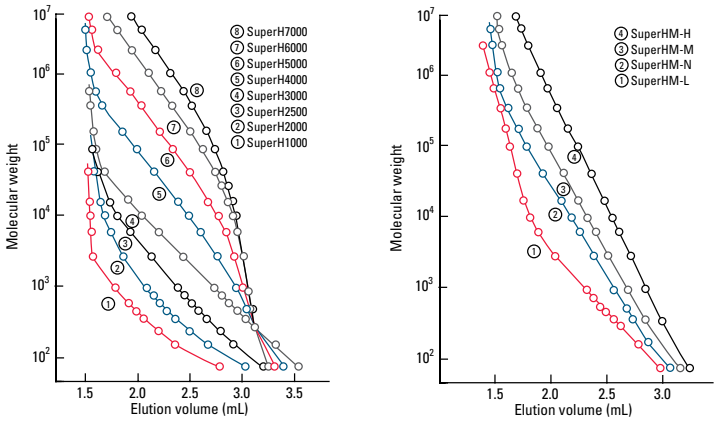
Column: TSKgel SuperHZ series (4.6 mm ID x 15 cm L); Eluent: THF; Flow rate: 0.35 mL/min; Temp.: 25°C; Sample: polystyrene standards; Inj. volume: 2 µL

Calibration curves for TSKgel SuperMultiporeHZ-M, H and N columns



Columns: TSKgel SuperMultiporeHZ-N, 3 µm, 4.6 mm ID x 15 cm L, TSKgel SuperMultiporeHZ-M, 4 µm, 4.6 mm ID x 15 cm L, TSKgel SuperMultiporeHZ-H, 6 µm, 4.6 mm ID x 15 cm L; Mobile phase: THF; Flow rate: 0.35 mL/min; Detection: UV @ 254 nm; Temp.: 25°C; Samples: polystyrene standards

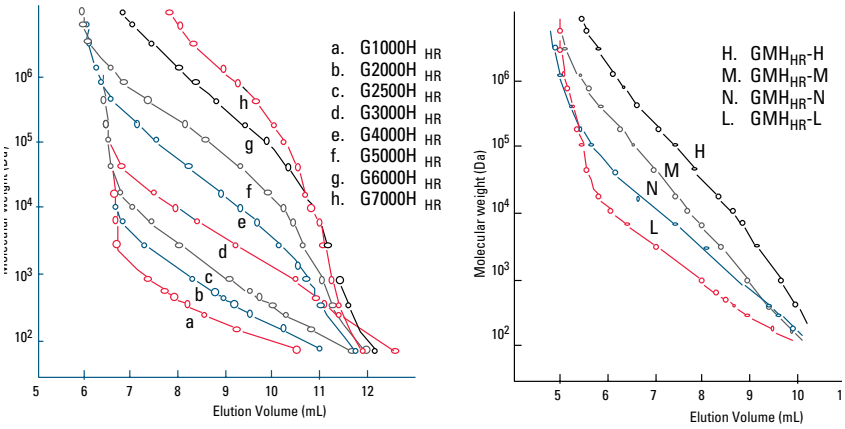
Calibration curves for TSKgel SuperH columns with polystyrene standards



Column: TSKgel SuperH series (6.0 mm ID x 15 cm L); Eluent: THF; Flow rate: 0.6 mL/min; Temp.: 25°C; Detection: UV @ 254 nm; Sample: polystyrene standards

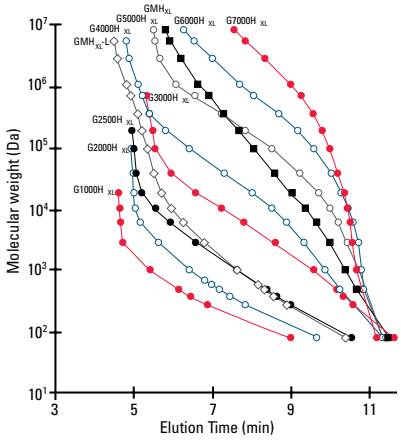
The best results are obtained when selecting a column with the sample's molecular weight in the linear portion of the calibration curve.

Calibration curves for TSKgel H_{HR} columns with polystyrene standards



Column: TSKgel H_{HR} series (7.8 mm ID x 30 cm L); Sample: polystyrene standards; Elution: THF Flow rate: 1.0 mL/min; Temp.: 25°C; Detection: UV @ 254 nm

Calibration curves for TSKgel H_{XL} columns with polystyrene standards



Column size: 7.8 mm ID x 30 cm L; Sample: polystyrene standards; Eluent: THF; Flow rate: 1.0 mL/min; Temp.: 25°C; Detection: UV @ 254 nm

MULTI-PORE SIZE DISTRIBUTION IN A POLYSTERENE PACKING MATERIAL

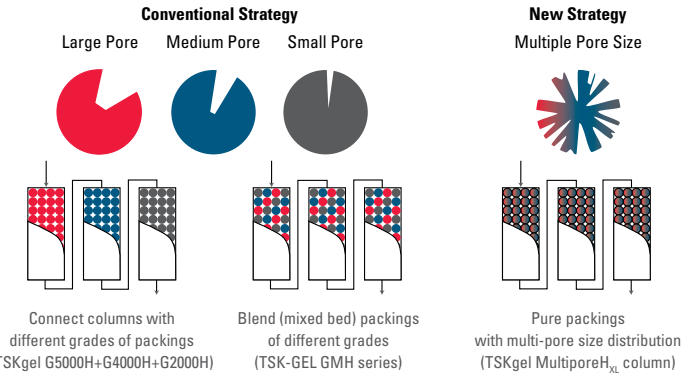
Novel approach to GPC of samples with a wide range of molecular weights

Prior to the introduction of TSKgel MultiporeHXL and SuperMultiporeHZ columns, scientists separating polymers with a wide range of molecular weights were left with two options. One option is to use multiple columns of different pore sizes linked together in series. A second is to use a column packed with a mixed bed resin of different pore sizes at an optimized mix ratio. However, problems can occur with both of these methods, which include distortion of the chromatogram or deviations between the actual calibration curve and the calibration curve approximated from data obtained from the molecular weight standards.

As is shown in FIGURE 27, a novel approach to solve this problem was developed by Tosoh scientists and is incorporated in TSKgel MultiporeHXL and SuperMultiporeHZ Series columns.

FIGURE 27

Strategies for wide range separation using SEC



These columns are packed with particles of uniform size synthesized with a broad distribution of pore sizes. This novel approach creates a linear calibration curve within each particle. Therefore, columns with an extended linear calibration curve can now be prepared without mixing particles of different pore sizes. This results in sharper peaks without inflection points that may be observed using mixed-bed columns.

The pore size distributions of the TSKgel MultiporeHXL-M column and a mixed-bed column are shown in FIGURE 28. The mixed-bed column shows a sharp maximum for pores with a diameter of 0.08 µm, though the overall pore size distribution ranges from 0.006 to 0.6 µm in diameter. In the case of the TSKgel MultiporeHXL-M column, the pore size distribution exhibits a wider maximum range from 0.02 to 0.1 µm in diameter. This difference in pore size distribution may explain the reason for the inflection phenomenon.

The small ID (4.6 mm) and length (15 cm) of the SuperMultiporeHZ columns reduces solvent consumption and results in quick run times, and offers high throughput capabilities. FIGURE 29 demonstrates that inflection points are no longer observed with semi-micro columns packed from particles prepared by multi-pore technology.

TSKgel H series columns can be applied to analyse the molecular mass distribution of a broad variety of organic-soluble polymers. TABLE V lists the recommended solvents by application for TSKgel H series columns. Super H columns are ideally suited to save analysis time and solvent by semi-micro GPC. For optimum performance they should be used in combination with a low dead volume GPC instrument such as the all-in-one EcoSEC system. TABLE VI suggests optimum flow rates to be applied for TSKgel SuperH and TSKgel HHR columns for various solvents.

FIGURE 28

Pore size distribution of TSKgel MultiporeHXL-M column and a mixed-bed column

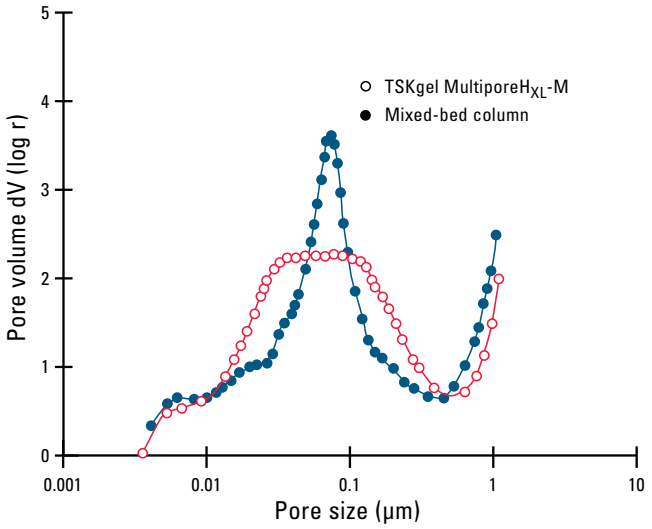
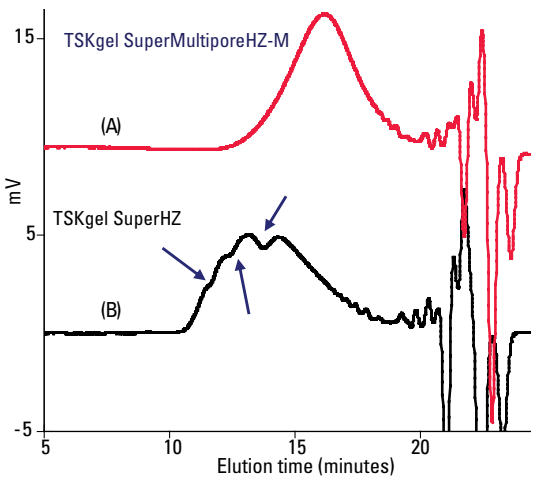


FIGURE 29

Comparison of TSKgel SuperMultiporeHZ-M and TSKgel SuperHZ for separation of Acrylic resin



Column: (A) TSKgel SuperMultiporeHZ-M,4.6 mm ID x 15 cm L, x 4;
(B) TSKgel SuperHZ4000+3000+2500+2000, 4.6 mm ID x 15 cm L x 4
Mobile phase: THF; Detection: RI; Temperature: 40°C; Injection vol.: 10 µL
Samples: acrylic resin

SEC

APPLICATIONS OF TSKgel H-TYPE GEL PERMEATION COLUMNS

SOLVENTS AND FLOW RATES

TSKgel H series columns can be applied to analyse the molecular mass distribution of a broad variety of organic-soluble polymers. Table 5 lists the recommended solvents by application for TSKgel H series columns. Super H columns are ideally suited to save analysis time and solvent by semi-micro GPC. For optimum performance they should be used in combination with a low dead volume GPC instrument such as the all-in-one EcoSEC system. Table 6 suggests optimum flow rates to be applied for TSKgel SuperH and TSKgel HHR columns for various solvents.

TABLE V

Recommended flow rates (mL/min) for TSKgel SuperH and HHR columns

Solvent	TSKgel SuperH 6.0 mm ID × 15 cm	TSKgel HHR 7.8 mm ID × 30 cm
n-Hexane	0.5	0.9
methyl ethyl ketone	0.4	0.7
dichloromethane, ethyl acetate	0.35	0.6
toluene, chloroform	0.3	0.5
dimethylformamide	0.2	0.4
carbon tetrachloride, pyridine	0.15	0.3
dimethyl sulfoxide, dioxane, ethanol, N-methylpyrrolidone, o-dichlorobenzene	0.1	0.2
quinoline, hexafluoroisopropanol, 1-chloronaphthalene	0.05	0.1

TABLE VI

Recommended solvents by application for TSKgel H series columns

Solvent	Application
THF	polystyrene, epoxy resin, phenoxy resin, polycarbonate, polyisoprene, polyvinyl acetate, polyvinyl chloride, monoglycerides, fatty acids, polybutadiene, poly(methyl methacrylate), poly(styrene-butadiene), poly(styrene-acrylonitrile)
n,n-Dimethylformamide (DMF) + 5 mmol/L LiBr	polyvinyl chloride, polyvinyl fluoride, urea resins, polyurethane, polystyrene, polyester, polyimido ether, polyimido ester, polyphenol (aqueous solution), polyacrylonitrile
o-dichlorobenzene (ODCB)	polyethylene, polypropylene
chloroform	polycarboxylic ether, acrylic resin, epoxy resin, polystyrene
m-cresol/chloroform	nylon, polyester, polyamide, poly (ethylene terephthalate)
toluene	polybutadiene, polysiloxane

APPLICATIONS OF TSKgel H-TYPE GEL PERMEATION COLUMNS

PHTHALATE ESTERS

FIGURE 30 demonstrates the high efficiency separation on a TSKgel G1000HXL column for low molecular weight phthalate esters. Resolution was close to baseline, even though the molecular weights of the esters differed by less than 50 Da.

PHENOL RESIN

The TSKgel GMHXL-L column has been designed to provide a complete profile for high molecular weight samples that contain low molecular weight additives. The calibration curve for this mixed-bed column is shallow in the low molecular weight range of oligomers. Sample adsorption is not observed.

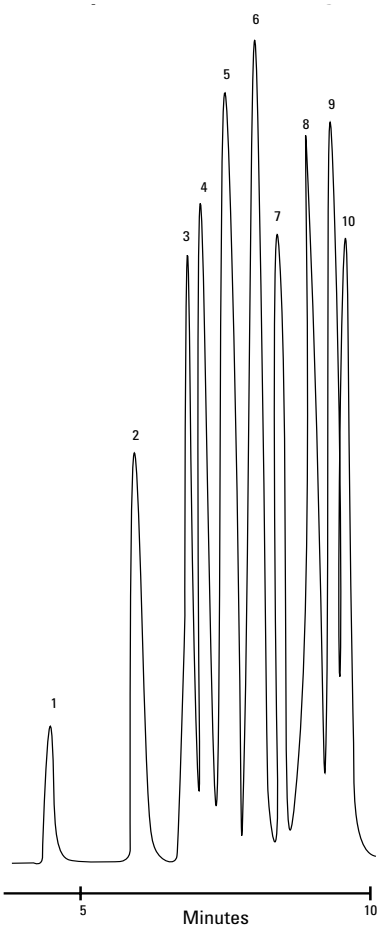
For example, the complete profile of a phenol resin, with high resolution of the low molecular weight components, is shown in FIGURE 31. Other applications for the TSKgel GMHXL-L column include analyses of paint materials, bond and adhesive components and synthetic polymer additives.

FATTY ACIDS

In FIGURE 32, two TSKgel G2000HXL columns in series separate a mixture of fatty acids ranging from C4 to C30.

FIGURE 30

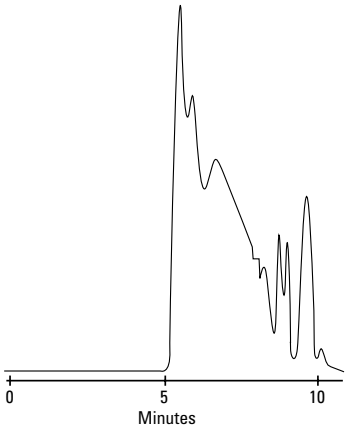
High resolution of phtalate ester on TSKgel G1000HXL



Column: TSKgel G1000HXL, 7.8 mm ID x 30 cm L;
Sample: 1. polystyrene (10,200Da), 2. dioctylphthalate (391Da), 3. dibutylphthalate (278Da), 4.dipropylphthalate (250Da), 5. diethylphthalate (222Da), 6. dimethylphthalate (194Da), 7. n-propylbenzene (120Da), 8. ethylbenzene (116Da), 9. toluene (92Da), 10. benzene (78Da); Elution: THF; Flow rate: 1.0 mL/min; Detection: UV @ 254 nm

FIGURE 31

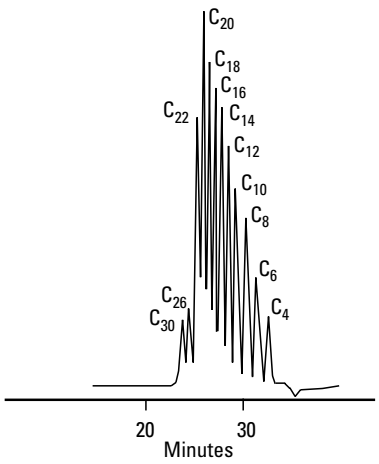
Separation of phenol resin on TSKgel GMHXL-L



Column: TSKgel GMHXL-L, 7.8 mm ID x 30 cm L;
Sample: phenol resin; Elution: THF; Flow rate: 1.0 mL/min;
Detection: UV @ 254 nm

FIGURE 32

Separation of fatty acid



Column: TSKgel G2000HXL, two 7.8 mm ID x 30 cm L in series;
Sample: fatty acids; Elution: THF; Flow rate: 1.0 mL/min; Detection: RI

SEC

APPLICATIONS OF TSKgel H-TYPE GEL PERMEATION COLUMNS

SHEAR DEGRADATION

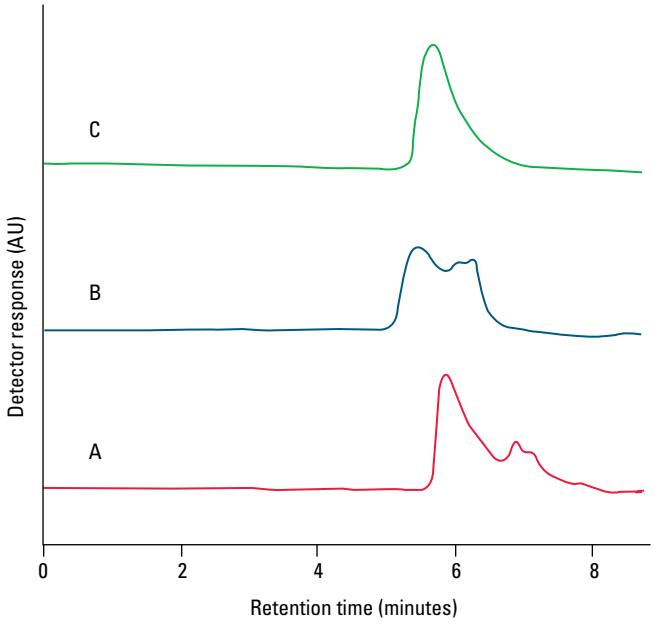
Shear degradation is observed especially when ultra-high molar mass compounds are analyzed. It tends to occur when analysis is carried out at high flow rates using a micro-particle size packing material. FIGURE 33 demonstrates the relationship between shear degradation and particle size of the packing material, when TSKgel GMH columns were used. When the flow rate is 1.0 mL/min, normal elution of an ultra-high molar mass sample (2.06 × 107 Da) is only possible with the TSKgel GMH$_{HR}$-H(S) column, which has a large particle size. However, with the TSKgel GMH$_{XL}$ and GMH$_{HR}$-H columns, shear degradation does take place and new peaks appear in the chromatogram on the smaller molar mass side.

ACRYLIC POLYMER

FIGURE 34 shows the separation of an acrylic polymer on the TSKgel MultiporeH$_{XL}$-M column compared with two commercially available mixed-bed columns. The arrows illustrate the inflections seen in the chromatograms from mixed-bed columns and the improvement achieved when using the TSKgel MultiporeH$_{XL}$-M column.

FIGURE 33

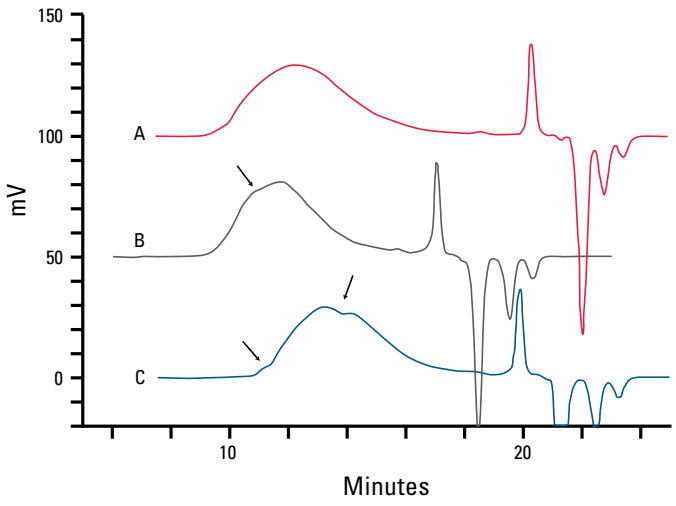
Shear degradation comparison



TSKgel GMH$_{HR}$-H, 5 µm, 7.8 mm ID × 30 cm L; B: TSKgel GMH$_{XL}$, 9 µm, 7.8 mm ID × 30 cm L; C: TSKgel GMH$_{HR}$-H(S), 13 µm, 7.8 mm ID × 30 cm L
Mobile phase: THF; Flow rate: 1.0 mL/min
Detection: UV @ 254 nm; Temperature: 25 °C
Sample: polystyrene standard F2000 (2.06 x 10^7 Da) 20 µL (0.025%)

FIGURE 34

Separation of acrylic resin by SEC on TSKgel MultiporeH$_{XL}$-M and mixed-bed type columns



Column: A. TSKgel MultiporeH$_{XL}$-M, two 7.8 mm ID x 30 cm L in series,
B. Competitor P, two 7.5 mm ID x 30 cm L columns in series, mixed-bed type;
C. Competitor S, two 8.0 mm ID x 30 cm L columns in series, mixed-bed type;
Sample: acrylic polymer (0.1%, 50 µL); Elution: THF; Flow rate: 1.0 mL/min;
Temperature: 40°C; Detection: RI

APPLICATIONS OF TSKgel H-TYPE GEL PERMEATION COLUMNS

POLYMETHYLMETHACRYLATE

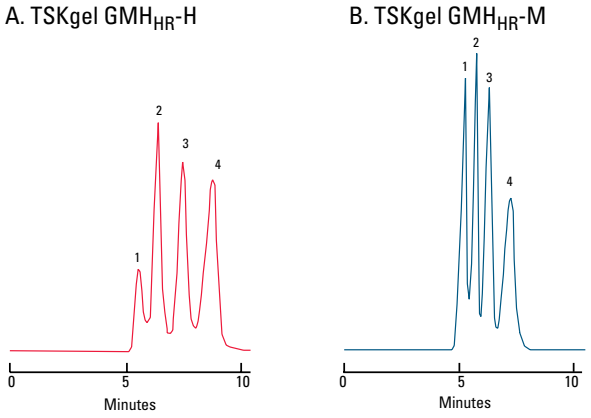
The effect of different pore size distributions in the mixed beds of TSKgel GMH$_{HR}$-H and TSKgel GMH$_{HR}$-M is illustrated in FIGURE 35. The TSKgel GMH$_{HR}$-M produces better resolution in the 8 x 10^5 to 1 x 10^4 Da range.

SEMI-MICRO GPC

Semi-micro columns are referred to as such since their dimensions are smaller than conventional columns in terms of internal diameter as well as in length: 4.6 mm or 6 mm ID x 15 cm vs. 7.8 mm ID x 30 cm of conventional GPC columns. As shown in FIGURE 36, a TSKgel SuperMultiporeHZ-N column provides the same or higher resolution at a much shorter analysis time than multiple conventional sized columns linked together.

FIGURE 35

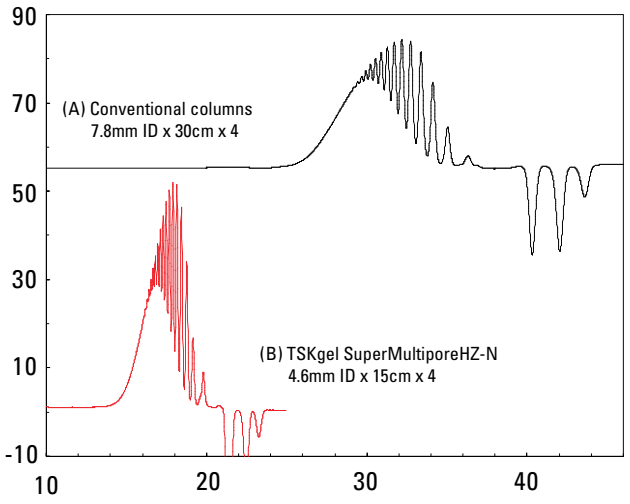
Comparison of TSKgel GMH$_{HR}$-H and -M columns with polymethylmethacrylate standards



Columns: A. TSKgel GMH$_{HR}$-H, 7.8 mm ID x 30 cm L;
B. TSKgel GMH$_{HR}$-M, 7.8 mm ID x 30 cm L;
Sample: polymethylmethacrylate: 1. 820,000 Da, 2. 67,000 Da, 3. 10,200 Da, 4. 1,950 Da; Solvent: 5 mmol/L sodium trifluoroacetate in hexafluoroisopropanol; Flow rate: 1.0 mL/min; Detection: UV @ 220 nm; Temperature: 40°C

FIGURE 36

PTMEG Analysis on conventional and semi-micro TSKgel Columns



Columns: A. Conventional columns, 7.8 mm ID x 30 cm L x 4, B. TSKgel SuperMultiporeHZ-N, 4.6 mm ID x 15 cm L x 4;
Mobile phase: THF; Flow rate: (A) 1.0 mL/min (B) 0.35 mL/min; Temperature: 40°C; Injection vol.: (A) 60 µL (B) 10 µL; Sample: poly(teramethylene ether glycol), (PTMEG 650), 10 µg/µL

SEC

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Flow rate (mL/min) range	Maximum pressure drop (MPa)
TSKgel Stainless Steel Columns							
0017352	G1000H$_{HR}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0017353	G2000H$_{HR}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0017354	G2500H$_{HR}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0017355	G3000H$_{HR}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0017356	G4000H$_{HR}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0017357	G5000H$_{HR}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0017358	G6000H$_{HR}$	7.8	30	5	≥ 10,000	0.5 - 1.0	5.0
0017359	G7000H$_{HR}$	7.8	30	5	≥ 10,000	0.5 - 1.0	5.0
0017362	GMH$_{HR}$-L mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0018055	GMH$_{HR}$-N mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0017392	GMH$_{HR}$-M mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0017360	GMH$_{HR}$-H mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0018393	GMH$_{HR}$-H(S)HT mixed-bed	7.8	30	13	≥ 8,000	5.0 - 1.0	2.0
0018391	GMH$_{HR}$-H(30)HT mixed-bed	7.8	30	30	≥ 4,000		
0018392	GMH$_{HR}$-H(20)HT mixed-bed	7.8	30	20	≥ 6,000		
0016131	G1000H$_{XL}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0016134	G2000H$_{XL}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0016135	G2500H$_{XL}$	7.8	30	5	≥ 16,000	0.5 - 1.0	5.0
0016136	G3000H$_{XL}$	7.8	30	5	≥ 16,000	0.5 - 1.0	3.5
0016137	G4000H$_{XL}$	7.8	30	5	≥ 16,000	0.5 - 1.0	3.5
0016138	G5000H$_{XL}$	7.8	30	9	≥ 14,000	0.5 - 1.0	1.5
0016139	G6000H$_{XL}$	7.8	30	9	≥ 14,000	0.5 - 1.0	1.5
0016140	G7000H$_{XL}$	7.8	30	9	≥ 14,000	0.5 - 1.0	1.5
0016141	GMH$_{XL}$ mixed-bed	7.8	30	9	≥ 16,000	0.5 - 1.0	1.5
0016652	GMH$_{XL}$-L mixed-bed	7.8	30	5	≥ 16,000	0.5 - 1.0	3.5
0018403	Multipore H$_{XL}$-M	7.8	30	5	≥ 16,000	0.5 - 1.0	3.5
0017990	SuperH1000	6.0	15	3	≥ 16,000	0.3 - 0.6	6.0
0017991	SuperH2000	6.0	15	3	≥ 16,000	0.3 - 0.6	6.0
0017992	SuperH2500	6.0	15	3	≥ 16,000	0.3 - 0.6	6.0
0017993	SuperH3000	6.0	15	3	≥ 16,000	0.3 - 0.6	4.0
0017994	SuperH4000	6.0	15	3	≥ 16,000	0.3 - 0.6	4.0
0017995	SuperH5000	6.0	15	3	≥ 16,000	0.3 - 0.6	4.0
0017996	SuperH6000	6.0	15	5	≥ 16,000	0.3 - 0.6	4.0
0017997	SuperH7000	6.0	15	5	≥ 16,000	0.3 - 0.6	4.0
0017998	SuperHM-L	6.0	15	3	≥ 16,000	0.3 - 0.6	4.0
0017999	SuperHM-N	6.0	15	3	≥ 16,000	0.3 - 0.6	4.0
0018000	SuperHM-M	6.0	15	3	≥ 16,000	0.3 - 0.6	4.0
0018001	SuperHM-H	6.0	15	3	≥ 16,000	0.3 - 0.6	4.0

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Flow rate (mL/min) range	Maximum pressure drop (MPa)
TSKgel Stainless Steel Columns							
0019309	TSKgel SuperHZ1000	4.6	15	3	≥ 16,000	0.15 - 0.35	5.6
0019302	TSKgel SuperHZ1000	6.0	15	3	≥ 16,000	0.25 - 0.60	5.6
0019310	TSKgel SuperHZ2000	4.6	15	3	≥ 16,000	0.15 - 0.35	5.0
0019303	TSKgel SuperHZ2000	6.0	15	3	≥ 16,000	0.25 - 0.60	5.0
0019311	TSKgel SuperHZ2500	4.6	15	3	≥ 16,000	0.15 - 0.35	4.0
0019304	TSKgel SuperHZ2500	6.0	15	3	≥ 16,000	0.25 - 0.60	4.0
0019312	TSKgel SuperHZ3000	4.6	15	3	≥ 16,000	0.15 - 0.35	3.0
0019305	TSKgel SuperHZ3000	6.0	15	3	≥ 16,000	0.25 - 0.60	3.0
0019313	TSKgel SuperHZ4000	4.6	15	3	≥ 16,000	0.15 - 0.35	3.5
0019306	TSKgel SuperHZ4000	6.0	15	3	≥ 16,000	0.25 - 0.60	3.5
0019660	TSKgel SuperHZM-N	4.6	15	3	≥ 16,000	0.15 - 0.35	3.5
0019661	TSKgel SuperHZM-N	6.0	15	3	≥ 16,000	0.25 - 0.60	3.5
0019662	TSKgel SuperHZM-M	4.6	15	3 and 5	≥ 16,000	0.15 - 0.35	2.0
0019663	TSKgel SuperHZM-M	6.0	15	3 and 5	≥ 16,000	0.25 - 0.60	2.0
0019664	TSKgel SuperHZM-H	4.6	15	10	≥ 9,000	0.15 - 0.35	1.0
0019665	TSKgel SuperHZM-H	6.0	15	10	≥ 9,000	0.25 - 0.60	1.0
0021488	SuperMultiporeHZ-M	4.6	15	4	≥ 16,000	0.15 - 0.35	2.4
0021815	SuperMultiporeHZ-N	4.6	15	3	≥ 20,000	0.15 - 0.35	4.0
0021885	SuperMultiporeHZ-H	4.6	15	6	≥ 11,000	0.15 - 0.35	1.0
Guard columns							
0018404	MultiporeHXL-M Guard	6.0	4.0	5	For P/N 0018403		
0007113	HXL-L Guard Column	6.0	4.0	7	For G1000HXL through G4000HXL columns		
0013727	HXL-H Guard Column	6.0	4.0	13	For G5000HXL through GMHXL-L mixed-bed columns		
0017368	HHR-L Guard Column	6.0	4.0	13	For G1000-4000HHR and GMHHR-L columns		
0017369	HHR-H Guard Column	6.0	4.0	5	For G5000-7000HHR and and GMHHR-M; -N; -H columns		
0018002	SuperH-L Guard Column	4.6	3.5	3	For SuperH1000-4000		
0018003	SuperH-H Guard Column	4.6	3.5	3	For SuperH5000-7000 and HM-L;-N;-M;-H columns		
0018004	SuperH-RC Ref. Column	6.0	15	4	For EcoSEC		
0019314	SuperHZ-L Guard Column	4.6	2.0	4	For 4.6 mm ID SuperHZ1000-4000 and HZM-N &-M		
0019668	SuperHZ-H Guard Column	4.6	2.0	10	For 4.6 mm ID SuperHZM-H columns		
0019666	SuperHZ-L Guard Column	4.6	3.5	4	For 6.0 mm ID SuperHZ1000-4000 and HZM-N &-M columns		
0019667	SuperHZ-H Guard Column	4.6	3.5	10	For 6.0 mm ID SuperHZM-H columns		
0021489	SuperMP-M Guard	4.6	2.0	4	For SuperMultipore HZ-M P/N 0021488		
0021816	SuperMP-N Guard	4.6	2.0	3	For SuperMultipore HZ-N P/N 0021815		
0021886	SuperMP-H Guard	4.6	2.0	6	For SuperMultipore HZ-H P/N 0021887		
TSKgel GPC columns for high temperature GPC							
0022887	GMHHR-H (30) HT2**	7,8	30		For HT-GPC up to 220°C		
0022888	GMHHR-H (20) HT2**	7.8	30		For HT-GPC up to 220°C		
0022889	GMHHR-H (S) HT2**	7,8	30		For HT-GPC up to 220°C		
0022890	G2000HHR (20) HT2**	7,8	30		For HT-GPC up to 220°C		
0018391	GMHHR-H (30)HT*	7,8	30		For HT-GPC		
0018392	GMHHR-H (20)HT*	7,8	30		For HT-GPC		
0018393	GMHHR-H (S)HT*	7,8	30		For HT-GPC		
Guard columns for high temperature GPC							
0022891	HHR (30) HT2** guardcolumn	7,5	7,5		For HT-GPC up to 220°C		
0022892	HHR (S) HT2** guardcolumn	7,5	7,5		For HT-GPC up to 220°C		
0018397	GMHHR-H (S)HT* guardcolumn	7,5	7,5		For HT-GPC		
0022893	HHR HT-RC Ref. Column	7,5	7,5		For EcoSEC HT		

HHR-HT/HT2 and HXL-HT/HT2 columns are packed in ODCB, HT* Temp. max 170 °C; HT2** Temp. max 220°C

SEC

AMBIENT AND HIGH TEMPERATURE EcoSEC GPC SYSTEM - BASED ON 40 YEARS EXPERIENCE

EcoSEC is a compact, all-in-one GPC system for fast, high resolution, semi-micro GPC. Comprising a precision solvent delivery system, automatic injector, column oven and a high performance refractive index detector, the design of the system components, their configuration and the optimized flow line provides outstanding performance with minimized dead volume. This makes EcoSEC the ideal instrument to be used in combination with the well respected TSKgel semi-micro GPC/SEC columns.

The EcoSEC High Temperature GPC System was issued to meet the demands for reliable results and reproducibility all combined in an easy to use and save instrument specifically for high temperature analyses. The EcoSEC High Temperature GPC System incorporates the proven design and technology used in the ambient EcoSEC GPC system.

For a detailed description of the ambient and high temperature EcoSEC instruments plese refer to our brochures ***EcoSEC GPC/SEC System*** and ***EcoSEC High Temperature GPC System.*** Request a printed copy at sales-marketing.tbg@tosoh.com or visit us at www.ecosec.eu.

ORDERING INFORMATION

TSKgel polymer standards: typical properties

Polystyrene

To calibrate TSKgel SuperMultiporeHZ columns

Part #	Description	Nominal MW (Da)	Amount
0021912	PStQuick MP-N	5.3×10^2 - 4.4×10^4	60 vials
0021913	PStQuick MP-M	5.3×10^2 - 8.0×10^5	60 vials
0021914	PStQuick MP-H	9.5×10^2 - 5.5×10^6	60 vials

To calibrate TSKgel H-type mixed-bed columns

Part #	Description	Nominal MW (Da)	Amount
0021915	PStQuick Kit-L	5.3×10^2 - 4.2×10^5	40 vials
0021916	PStQuick Kit-M	5.3×10^2 - 2.9×10^6	40 vials
0021917	PStQuick Kit-H	5.3×10^2 - 8.4×10^6	60 vials

To calibrate standard TSKgel GPC columns

Part #	Description	Amount
0021911	PStQuick A (A-2500, F-2, F-20, F-128, F-850)	20 vials
0021910	PStQuick B (A-1000, F-1, F-10, F-80, F-550)	20 vials
0021909	PStQuick C (A-500, A-5000, F-4, F-40, F-288)	20 vials
0021908	PStQuick D (A-2500, F-2, F-20, F-128)	20 vials
0021907	PStQuick E (A-1000, A-5000, F-4, F-40)	20 vials
0021906	PStQuick F (A-500, A-2500, F-2, F-20)	20 vials

Part #	Description	Nominal MW (Da)	Amount
0005202	A-300		10 g
0005203	A-500	530 MW	10 g
0005204	A-1000	950 MW	10 g
0005205	A-2500	2.800 MW	5 g
0005206	A-5000	6.200 MW	5 g
0005207	F-1	10.300 MW	5 g
0005208	F-2	16.700 MW	5 g
0005209	F-4	43.900 MW	5 g
0005210	F-10	102.000 MW	5 g
0005211	F-20	186.000 MW	5 g
0005212	F-40	422.000 MW	5 g
0005213	F-80	775.000 MW	5 g
0005214	F-128	1260.000 MW	1 g
0005215	F-288	2.890.000 MW	1 g
0005216	F-380	3.840.000 MW	1 g
0005217	F-450	4.480.000 MW	1 g
0005218	F-550	5.480.000 MW	1 g
0005219	F-700	6.770.000 MW	1 g
0005220	F-850	8.420.000 MW	1 g
0005221	F-2000	20.600.000 MW	1 g

0006476	Oligomer Kit, A-500 thru F-12812 x 1 g
0006477	High MW Kit, F-10 thru F-200012 x 1 g

Polyethylene oxide

Part #	Description	Nominal MW (Da)	Amount
0006211	SE-2	18.000 MW	0.5 g
0006212	SE-5	39.000 MW	0.5 g
0006213	SE-8	86.000 MW	0.5 g
0006214	SE-15	145.000 MW	0.5 g
0006215	SE-30	252.000 MW	0.5 g
0006216	SE-70	594.000 MW	0.5 g
0006217	SE-150	996.000 MW	0.5 g

0005773	Polyethylene Oxide Kit, SE-2 thru SE-150	7 x 0.2 g

The above molecular weights are determined by light scattering except for A-300, A-500, and A-1000, which are based on size exclusion chromatography. Results may vary among individual batches.

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

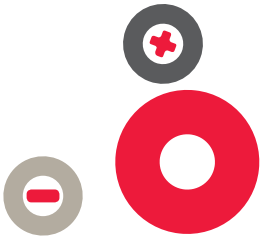
TSKgel SP-STAT
TSKgel CM-STAT
TSKgel BioAssist S
TSKgel SP-5PW
TSKgel CM-5PW
TSKgel SP-2SW
TSKgel SP-NPR
TSKgel CM-2SW
TSKgel CM-3SW
TSKgel SCX

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.

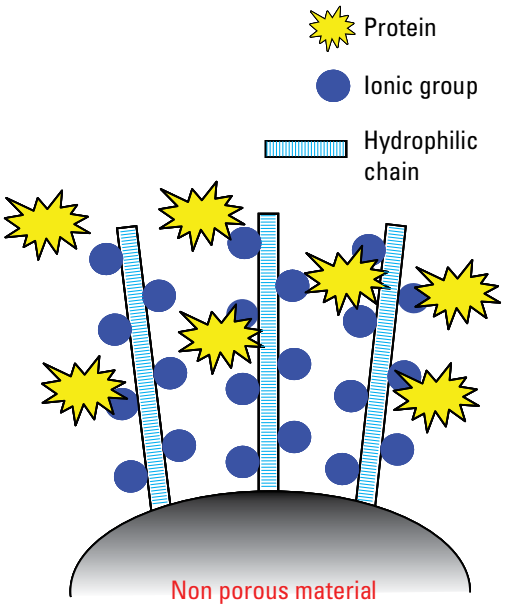
These relatively large particle sizes, combined with an innovative bonding chemistry, result in columns that enable fast equilibration and analysis of complex biomolecular samples, attributes not found in traditional mono-disperse, non-porous stationary phases.

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*

IEC

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

IEC

OLIGONUCLEOTIDES

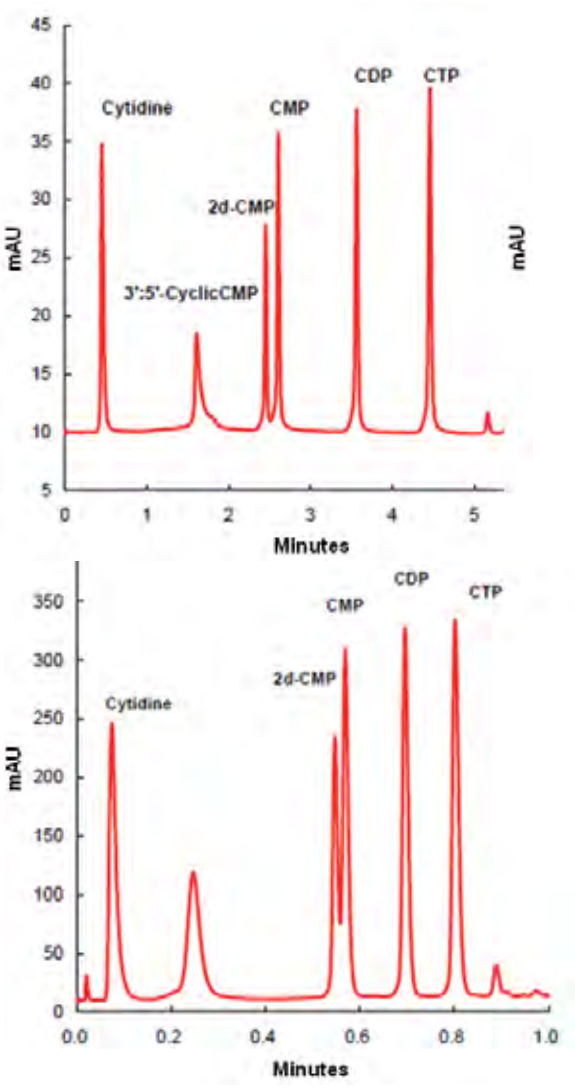
FIGURE 2 compares the high resolution separation of nucleotides on a 10 cm length column to the high throughput separation on a 3.5 cm length column.

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:

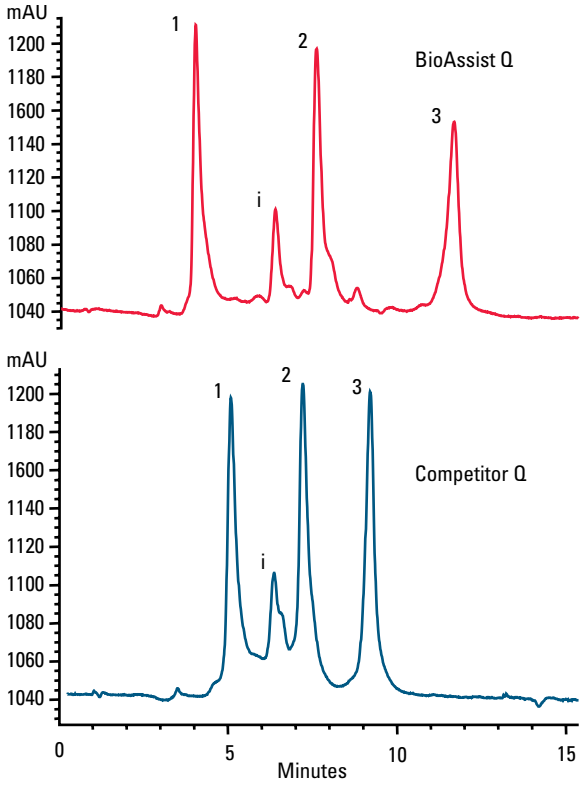
Column: TSKgel Q-STAT, 4.6 mm ID x 10 cm L (7 µm);
Eluent: A) 20 mmol/L Tris-HCl (pH8.5) B) 0.5 mol/L NaCl in A (pH8.5)
Gradient: 0 to 100% B (10 min.); Flow rate: 1.5 mL/min.
Detection: UV @ 260 nm

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.

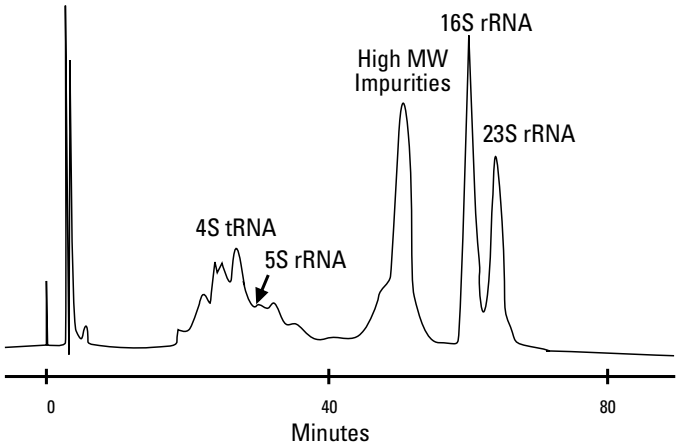
FIGURE 5 shows the fractionation of high molecular weight E. coli RNA on TSKgel DEAE-5PW, effectively utilizing the large 100 nm pores of this base resin.

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

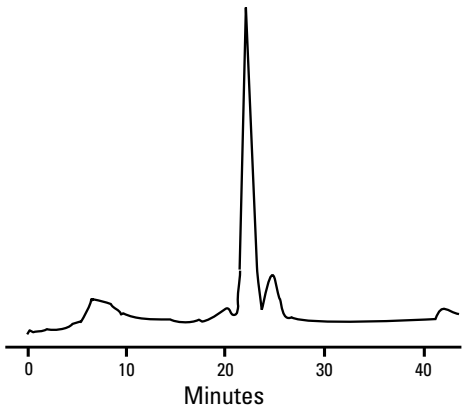
Large pore TSKgel DEAE-5PW resolves high MW RNA



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

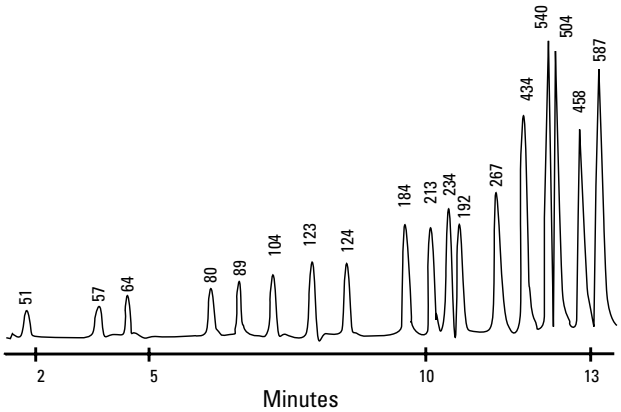
Analysis of synthetic oligonucleotide on TSKgel SuperQ-5PW



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

IEC

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.

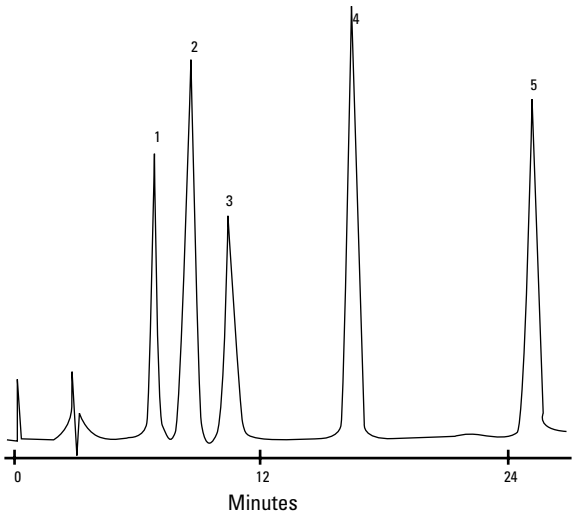
SPECIALTY COLUMNS

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

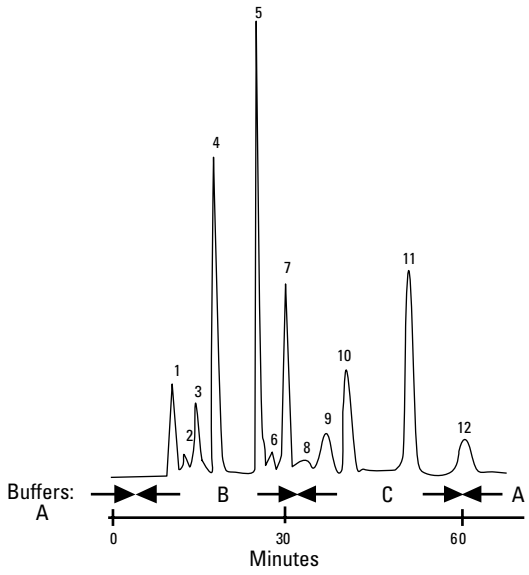
Separation of nucleotides on TSKgel DEAE-2SW



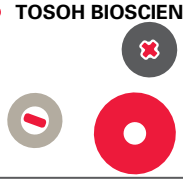
Column: TSKgel DEAE-2SW, 4.6 mm ID x 25 cm L; Sample: 1. AMP, 2. IMP, 3. GMP, 4.ADP, 5. ATP; Buffer A: ACN in 0.1 mol/L phosphate, pH 3.0, 20/80; Buffer B: ACN in 0.5 mol/L phosphate, pH 3.0, 20/80; Elution: 30 min linear gradient from buffer A to B; Flow rate: 1.0 mL/min; Detection: UV @ 260 nm

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	2.5	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		

IEC

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.

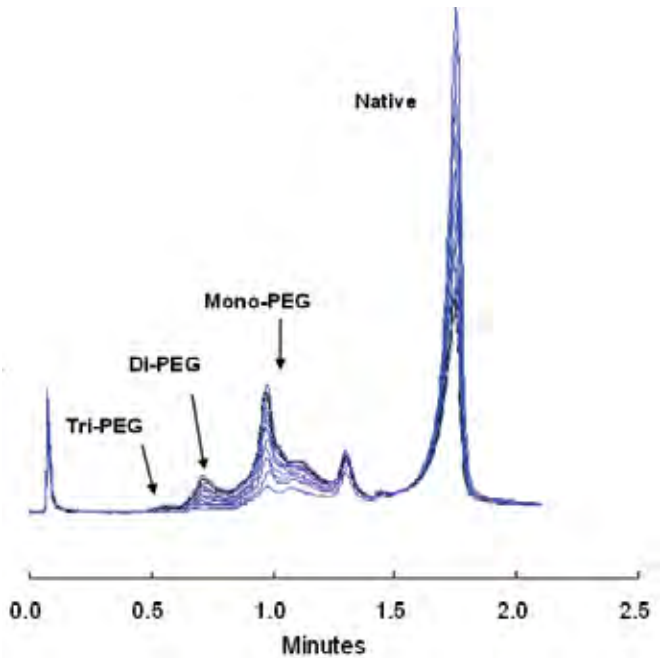
TABLE I I

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

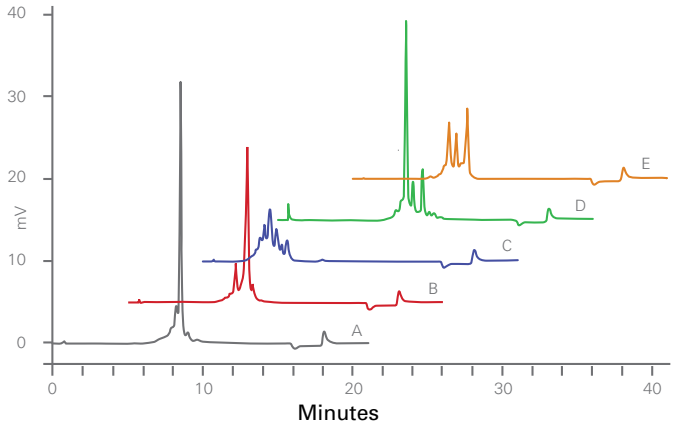
Monotoring of PEGylation of -lactoglobulin



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

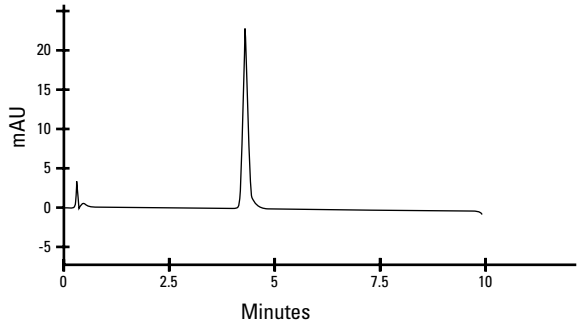
APPLICATIONS - TSKgel CATION EXCHANGE COLUMNS

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

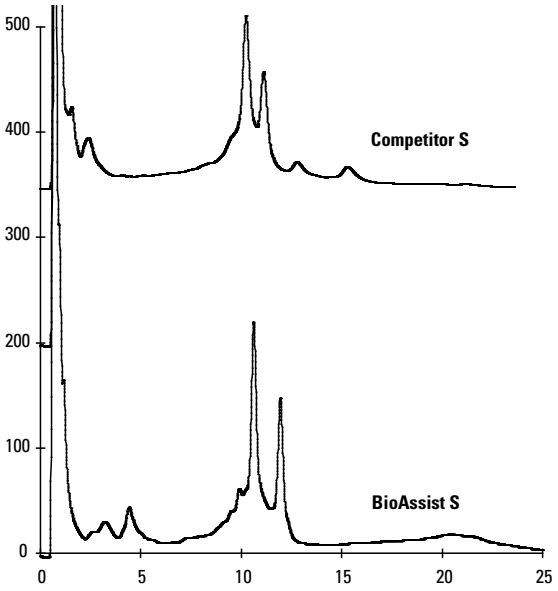
TSKgel Bioassist S

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

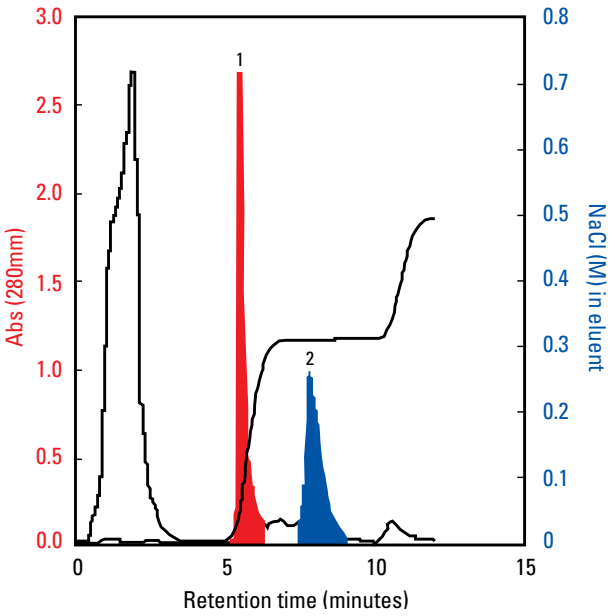
Bromelain Analysis on TSKgel Bioassist S and competitor S Columns



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

IEC

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.

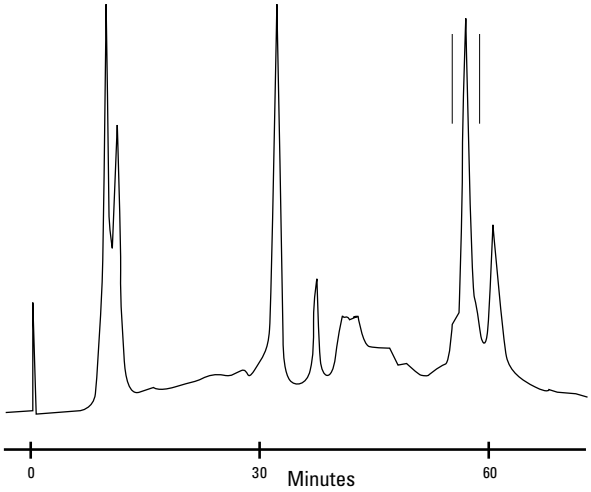
The purification of 200 mg of crude lipoxidase on a 21.5 mm ID TSKgel SP-5PW column is illustrated in FIGURE 15. Scale-up is simplified as only the particle size changes from 10 µm (7.5 mm ID) to 13 µm (21.5 mm ID) or 20 µm (55 mm ID) columns.

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

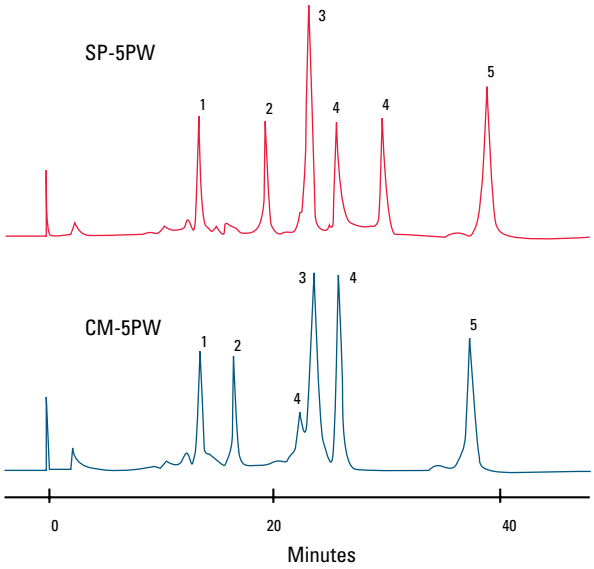
Semi-preparative purification of lipoxidase



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

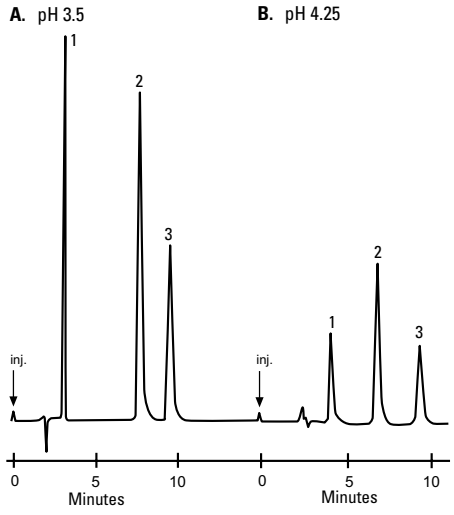
Selectivity on TSKgel strong and weak cation exchangers



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

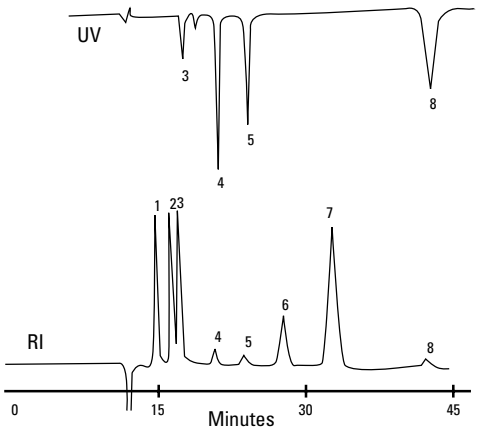
APPLICATIONS - TSKgel CATION EXCHANGE COLUMNS

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



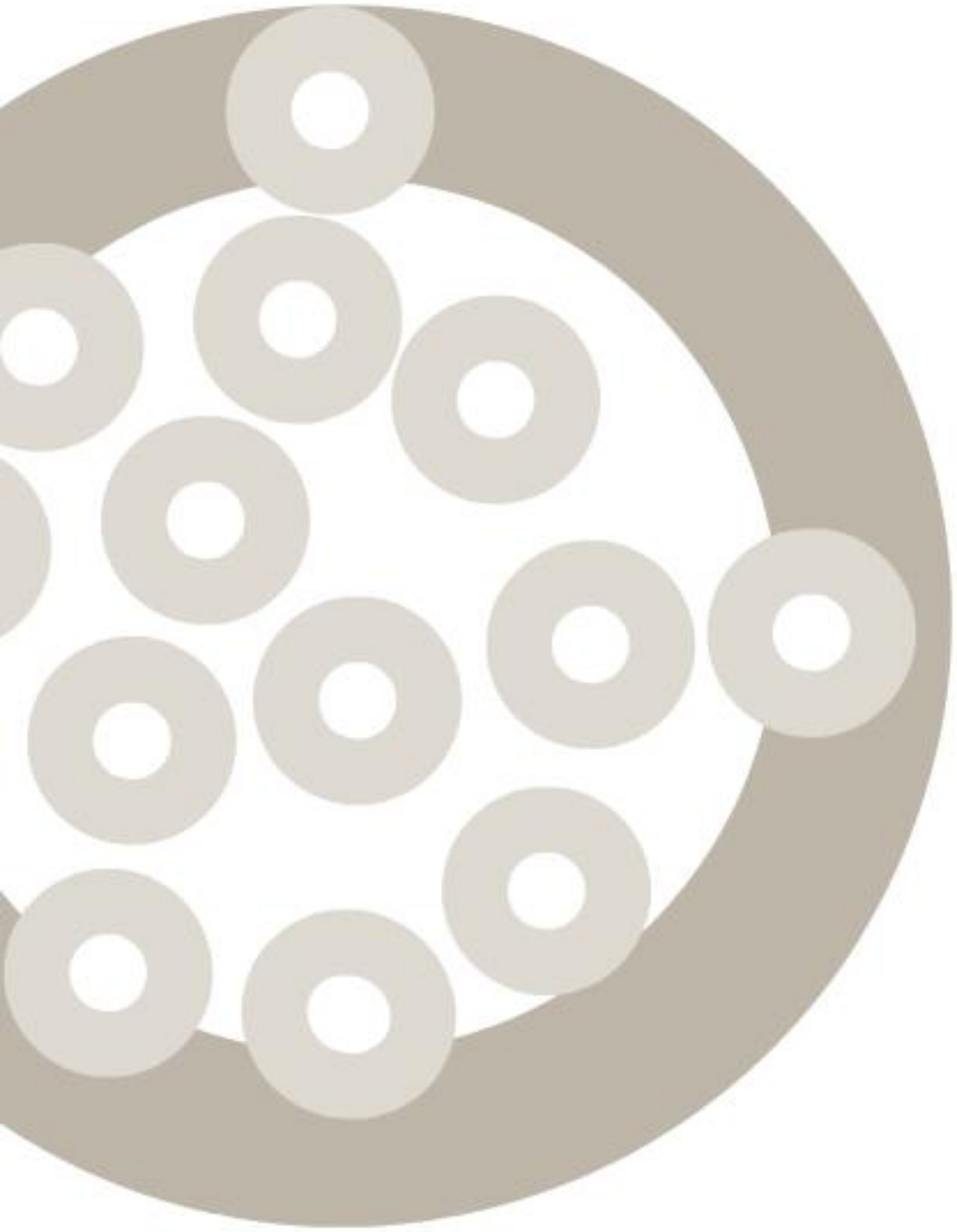
Column: TSKgel SCX (H^+), two 7.8 mm ID x 30 cm L (in series);
Sample: 1. maltose, 2. glucose, 3. fructose, 4. lactic acid, 5. acetic acid, 6. methanol, 7. ethanol, 8. butyric acid; Elution: 0.05 mol/L $HClO_4$; Flow rate: 0.8 mL/min; Detection: UV @ 210 nm, Refractive Index

IEC

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



HILIC
HYDROPHILIC INTERACTION CHROMATOGRAPHY

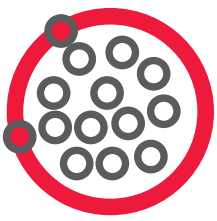
HILIC PRODUCTS

SILICA BASED HILIC COLUMNS

TSKgel Amide-80
TSKgel NH_2-100

TOSOH FACT

The first columns used in chromatography were glass, both for liquid-solid chromatography by Tswett in his separation of plant pigments and by James and Martin in their first gas chromatograph. However, as the technique developed and particle size was reduced, the length of the columns in liquid chromatography was decreased. This resulted in the columns having to be operated at higher pressures. To accommodate these higher pressures, stainless steel columns were introduced. Tosoh introduced its first HPLC (GPC) columns in 1971, which were composed of stainless steel. Recently, columns packed in PEEK, a biocompatable fluorocarbon polymer, became available. PEEK can withstand the pressures commonly encountered in HPLC.



INTRODUCTION TO TSKgel HILIC COLUMNS

HIGHLIGHTS

- Stable bonding chemistries
- Unique polar phases
- Handle a wide spectrum of sample polarities
- Stable in 100% organic
- Separate many different types of polar molecules
- 3 µm particle size for LC/MS analysis

Hydrophilic interaction chromatography (HILIC) is used primarily for the separation of polar and hydrophilic compounds. HILIC has similarities with traditional normal phase chromatography, but the mobile phases for HILIC are similar to those known from reversed phase chromatography (RPC). They include polar organic solvents like acetonitrile. Based on hydrogen bonds the aqueous content of the mobile phase creates a water-rich layer on the particle surface. This allows for partitioning of polar compounds between the more organic mobile phase and the aqueous layer (FIGURE 1). The number of polar groups, as well as the conformation and solubility of the sample in the mobile phase determines the elution order.

Typical mobile phases consist of acetonitrile buffer mixtures. Samples are eluted from the column by increasing the percentage of the aqueous component. Compared to RPC the elution order in HILIC mode is inversed for most substances.

HILIC is often used to separate hydrophilic compounds such as peptides, carbohydrates and small polar drug candidates or metabolites. Hydrophilic compounds are retained on the polar bonded phase column while non-polar sample impurities elute unretained in the void volume. In addition it is ideally suited for sensitive LC-MS analysis of water soluble polar compounds because the high organic content in the mobile phase provides rapid evaporation of solvent during electrospray ionization.

TSKgel HILIC columns are available in various dimensions and particle sizes, functionalized with carbamoyl-groups (TSKgel Amide-80) or amino-groups (TSKgel NH_2-100).This enables the user to perfectly match HILIC selectivity to specific application needs.

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

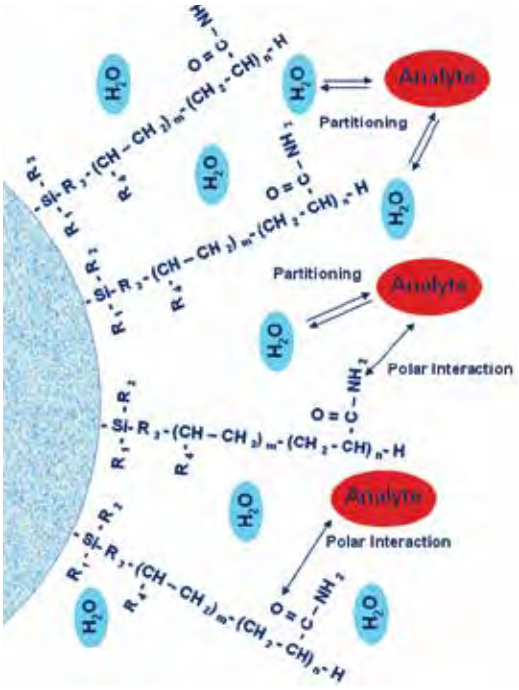
The TSKgel Amide-80 column offers an excellent alternative to amino-bonded stationary phases and consists of 3, 5 or 10 µm silica particles in a stainless steel format. Spherical silica particles are covalently bonded with carbamoyl groups. For years TSKgel Amide-80 columns have been the standard for the analysis of glycans. TSKgel Amide-80 columns packed with 3 µm particles are the newest addition to the TSKgel Amide-80 series. The 3 µm HILIC columns reduce analysis time and improve peak capacity and sensitivity for HPLC and LC-MS analysis.

TSKgel NH_2-100 3 µm columns are the latest addition to the TSKgel HILIC family. They expand the selectivity range of TSKgel HILIC solutions by a new, robust amino-phase. In contrast to conventional silica-based amino phases the new column offers expanded stability under HILIC conditions. It is well suited for the analysis of all types of hydrophilic compounds like carbohydrates, peptides, vitamins, polar drugs or metabolites.

The NH_2-100 phase is based on a silica particle with 3 µm particle and 10 nm pore size, treated with a special endcapping procedure. Amino groups are introduced step wisely after endcapping. These columns are unique in that the bonded phase ligand not only, as expected, has a terminal primary amino group, but that the spacer also incorporates secondary as well as tertiary amino groups. The amino groups act as HILIC functional groups without any peak splits. Due to their high ligand density and large surface area TSKgel NH_2-100 3 µm columns show high retention for very polar compounds. Anionic compounds are retained on the column by ionic interaction. This allows for the use of salt gradients, in addition to gradient elutions with acetonitrile. Since the TSKgel NH_2-100 has cationic sites, it can be used as mixed mode phase under some conditions.

FIGURE 1

HILIC principles



HILIC

COLUMN OPERATION AND SPECIFICATIONS

TSKgel HILIC columns can be operated over a broad range of mobile phase conditions. Factors to consider when employing these columns include:

Sample loading capacity is dependent upon the polarity of the mobile phase. It increases with decreasing mobile phase polarity. For example, on a TSKgel Amide-80 column the highest loading capacity for mannitol (200 µg) occurs with a mobile phase of 75:25 acetonitrile/water. However, <100 µg of mannitol can be loaded in a mobile phase of 65:35 acetonitrile/water. The maximum sample volume for a 4.6 mm ID x 25 cm L Amide-80 analytical column is 50 µL.

Temperature range: TSKgel Amide-80 columns can be operated over a temperature range of 10-80°C (10-50°C for Amide-80 3 µm), TSKgel NH_2-100 columns in the range of 10-50 °C. In general, retention times for carbohydrates decrease with increasing temperature, thereby shortening analysis time. Below certain temperatures some carbohydrates may elute as split peaks. In this case, column heating or addition of triethylamine to the mobile phase is required.

Choice of mobile phase: the pH range of TSKgel Amide-80 and NH_2-100 columns is 2.0-7.5 with a maximum salt concentration of 100 mmol/L. The columns are stable in 100% organic for normal phase separations; however, in HILIC mode a combination of aqueous and organic solvents is necessary in order to create the water-rich surface layer. As the mobile phase polarity decreases (higher organic content) the sample is retained longer on the column.

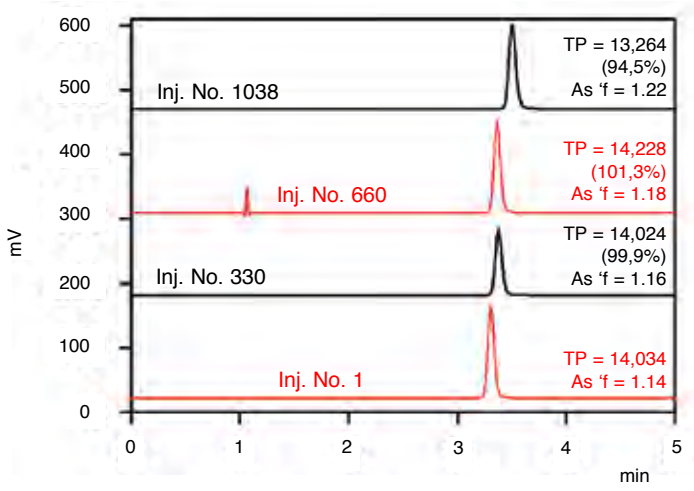
LONG TERM STABILITY

The high stability of TSKgel Amide-80 columns is demonstrated in FIGURE 2 showing the same analysis after 330, 660 and more than 1000 runs compared to the first injection. Only 5% reduction of column performance (theoretical plates) is observed after more than 1000 injections.

FIGURE 3 shows the high stability of TSKgel NH_2-100 columns. Compared to the first injection only a slight reduction of retention time of inositol is observed with the TSKgel NH_2-100 column after more than 400 hours of flushing with mobile phase.

FIGURE 2

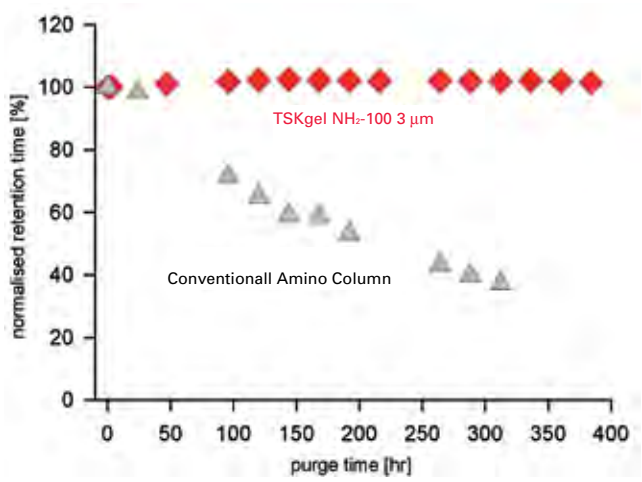
Durability of TSKgel Amide-80 3 µm



Column: TSKgel Amide-80 3 µm (2.0 mm ID x 15 cm L)
Eluent : H_2O/ACN = 15/85; Flow rate: 0.2 mL/min; Inj. volume: 2 µL
Detection : UV @ 254 nm; Temp. : 25 °C; Samples: Uracil (37 mg/L)

FIGURE 3

Long term stability of TSKgel NH_2-100 columns



Column: TSKgel NH_2-100 3 µm, 4.6 mm ID x 15 cm L
Conventional Amino Column, 4.6 mm ID x 25 cm L;
Eluent: H_2O/ACN (25/75); Flow rate: 1.0 mL/min; Detect: RI;
Temp.: 40 °C; Injection: 10 µL; Sample: Inositol

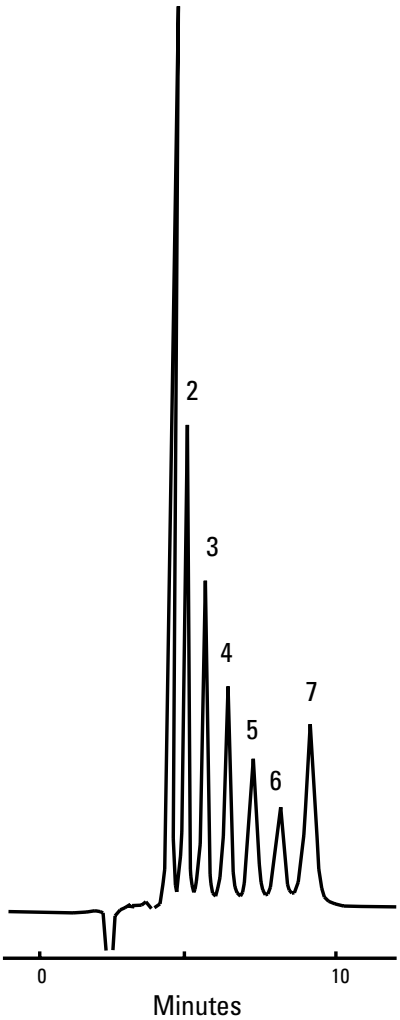
APPLICATIONS OF TSKgel AMIDE-80 COLUMNS

OLIGOSACCHARIDES

The TSKgel Amide-80 can separate oligosaccharides very rapidly and efficiently. FIGURE 4 shows a separation of a ß-cyclodextrin hydrolysate in less than 10 minutes. The labels indicate the number of base sugars such as glucose in each oligomer.

FIGURE 4

Separation of ß-cyclodextrin hydrolysate on TSKgel Amide-80 column



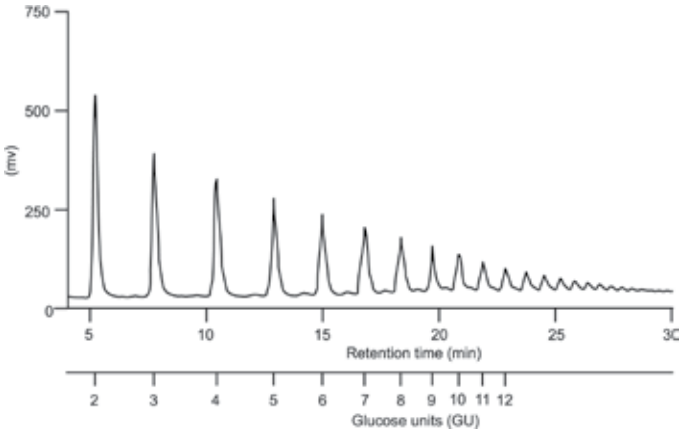
Column: TSKgel Amide-80 (4.6 mm ID x 25 cm L); Sample: 2 µL, β-cyclodextrin hydrolysate, 1 - 7 degrees of polymerization (4.6 mg/mL); Elution: ACN/water (55/45); Flow rate: 1.0 mL/min; Detection: RI; Temperature: 25 ºC

GLYCANS

Glycosylation is one of the most common post-translational modifications in eukaryotic cells. Complex N- and O-linked structures composed of repeating sugar moieties form the so called glycans. HILIC with fluorescence detection is the method of choice to effectively separate, identify and quantify glycans after exoglycosidase cleavage and fluorescent labeling. In order to normalize retention times of complex glycan structures a dextran ladder consisting of glucose oligomers is used as calibration reference. The calculated numbers of glucose units (GU) can be used in subsequent database queries (Glycobase, autoGU) to predict the glycan structure. For years TSKgel Amide-80 columns have been used successfully in glycan analysis. Amide-80 chemistry is ideally suited for the separation of carbohydrate structures. FIGURE 5 shows the high-resolution separation of a 2-aminobenzamide (2AB) labeled dextran ladder within 30 minutes on a TSKgel Amide-80 3 µm column. This ladder can be used as a calibration standard for HPLC and MS analysis of glycans. The ladder contains glucose homopolymer species from degree of polymerization (dp) 1 to dp 22 (i.e. the glucose monomer GU1-2AB to GU22-2AB).

FIGURE 5

Separation of a 2-AB-labeled dextran ladder on TSKgel Amide-80



Column: TSKgel Amide-80 (3 µm, 2.0 mm ID × 15 cm L)
Eluent: A) 50 mM Ammonium formate (pH 4.3), B) Acetonitrile;
Gradient: 0 - 35 min (75 - 35 % B); Flow rate: 0.22 mL/min;
Detection: Fluorescence Ex @ 360 nm, Em @ 425 nm; Temperature: 50 °C;
Injection vol: 3 µl; Sample: CAB-GHP dextran ladder (Ludger; ~300 fmol for GU2)

* Courtesy of K. Darsow & H. Lange, Institute of Bioprocessing, University of Nürnberg/Erlangen

HILIC

High-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) has become a powerful tool when detection sensitivity is an issue. HILIC offers unique advantages for MS detection of very polar compounds when compared to reversed phase mode. The higher organic content of the eluent in HILIC mode supports efficient evaporation of the solvent thus enhancing sensitivity and altering ion suppression.

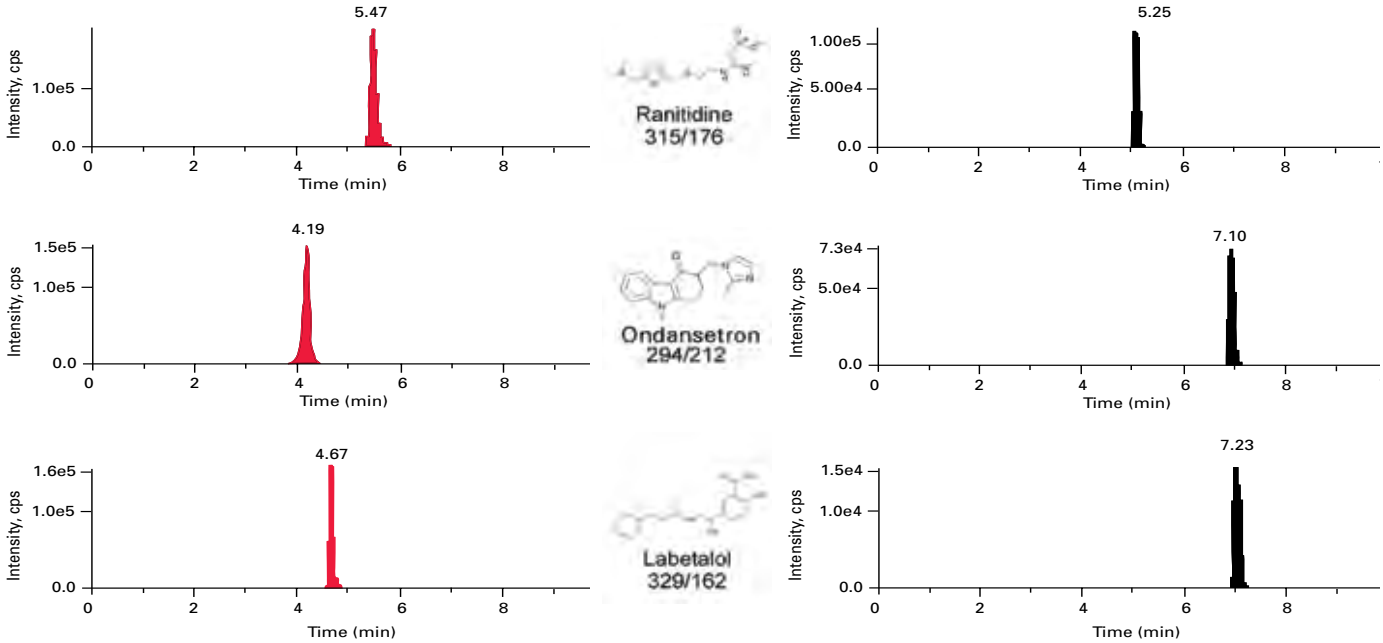
HILIC separations are performed with gradients starting with high percentage of organic solvent and ending with a high portion of aqueous solvent - opposite to typical reversed phase gradients. The elution order of compounds is usually inversed as well. As a result polar compounds are very well separated according to increased polarity in HILIC mode. At the same time the portion of organic solvent in the mobile phase is relatively high.

FIGURE 6 shows the analysis of basic drug substances using a TSKgel Amide-80 3 µm column compared to a reversed phase TSKgel ODS-100V 3 µm column. Ranitidine, a histamine H2 receptor antagonist, Ondansetron, an antiemetic serotonin receptor antagonist, and Labetalol, an alpha-1 and beta adrenergic blocker were selected to demonstrate the differences in selectivity and MS-signal response when applying different chromatographic modes.

Ranitidine has the highest number of polar groups among these molecules and as a result shows the highest retention in HILIC and the lowest retention in RPC mode. Signal intensity is almost doubled for Ranitidine in HILIC mode. For Labetalol a tenfold increase in signal height can be achieved by using HILIC instead of RPC.

FIGURE 6

LC-MS/MS Analysis of basic drugs in HILIC and RPC mode



Column: TSKgel Amide-80 3 µm (2.0 mm ID x 15 cm L)
Eluent : A: 10 mM Ammoniumformiate (pH 3.75); B: ACN
Gradient: 0 min (B 90%) -> 10 min (B 40%) ->13 min (B 40%)
Flow rate: 0.2 mL/min; Inj. volume : 5 µL (50 µg/L)
Detection : QTrap® LC-MS/MS (Applied Biosystems), ESI+

Column: TSKgel ODS-100V 3 µm (2.0 mm ID x 15 cm L)
Eluent: A: 10 mM Ammoniumformiate (pH 3.75); B: ACN
Gradient: 0 min (B 0%) -> 10 min (B 80%) ->13 min (B 80%)
Flow rate: 0.2 mL/min; Inj. volume: 5 µL (50 µg/L)
Detection: QTrap® LC-MS/MS (Applied Biosystems), ESI+

APPLICATIONS OF TSKgel NH_2-100 COLUMNS

SEPARATION OF WATER SOLUBLE VITAMINS

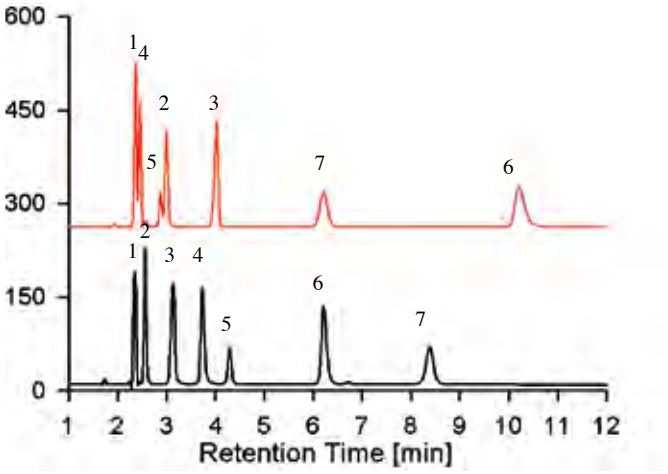
FIGURE 7 shows the separation of a standard solution of water soluble vitamins on a TSKgel NH_2-100 column compared to a TSKgel Amide-80 column. Dimension (4.6 mm ID x 15 cm L), particle size (3 µm), flow rate and mobile phase were identical for both columns. The elution order of the compounds changes when applying the same mobile phase to both columns: The TSKgel NH_2-100 column shows stronger retention for nicotinic acid, vitamin C, and vitamin B12, while retention of vitamin B1, B2, and pyridoxine is reduced.

SEPARATION OF METHOTREXATE AND DERIVATIVES

FIGURE 8 compares the separation of methotrexate and its derivatives (MTXPG2~7) on TSKgel NH_2-100, 3µm HILIC and TSKgel ODS-100V, 3µm reversed phase narrow bore columns. Methotrexate, abbreviated MTX and formerly known as amethopterin is an inhibitor of the folic acid metabolism. It is used in cancer chemotherapy and as a treatment of autoimmune diseases. The MTX and polyglutamate derivatives were eluted in the order of the number of glutamate groups in their molecules on the TSKgel NH_2-100 HILIC column, but eluted in reverse order on the TSKgel ODS-100V column. Despite the early elution of MTX and MTXPG2 on the TSKgel NH_2-100 HILIC column, the overall separation is better than what can be accomplished on the C18 column.

FIGURE 7

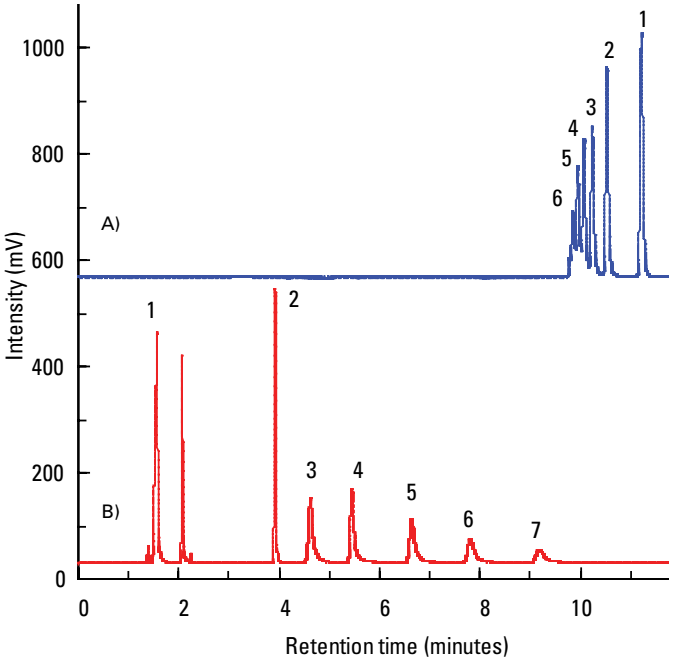
Separation of water soluble vitamins



Columns: TSKgel Amide-80 3 µm, 4.6 mm ID x 15 cm L;
TSKgel NH_2-100 3 µm, 4.6 mm ID x 15 cm L;
Eluent: 25 mM phosphate buffer (pH 2.5)/ACN=30/70
Flow: 1 mL/min; Temp.: 40°C; Detection: UV @ 254 nm
Sample: Vitamin standard mixture: 1 = Nicotinamide, 2 = Vitamin B2, 3 = Pyridoxine, 4 = Nicotinic acid, 5 = Vitamin C, 6 = Vitamin B1, 7 = Vitamin B12
Injection: 5 µL

FIGURE 8

Separation of MTX and derivatives



Column: A) TSKgel ODS-100V, 3 µm, 2.0 mm ID x 15 cm L;
Mobile phase: a) H_2O/ACN (90/10) + 0.1% TFA, b) ACN + 0.1% TFA;
B) TSKgel NH_2-100, 3 µm, 2.0 mm ID x 15 cm L;
Mobile phase: a) H_2O/ACN (10/90) + 0.1% TFA, b) H_2O + 0.1% TFA;
Gradient: 0 % B (0 min), 40 % B (15 min), 0 % B (17 min);
Flow rate: 0.20 mL/min; Detection: UV @ 313 nm; Temperature: 40°C;
Injection vol.: 10 µL; Sample: 1. MTX (MTXPG) 2. MTXPG2 3. MTXPG3 4. MTXPG4 5. MTXPG5 6. MTXPG6 7. MTXPG7

HILIC

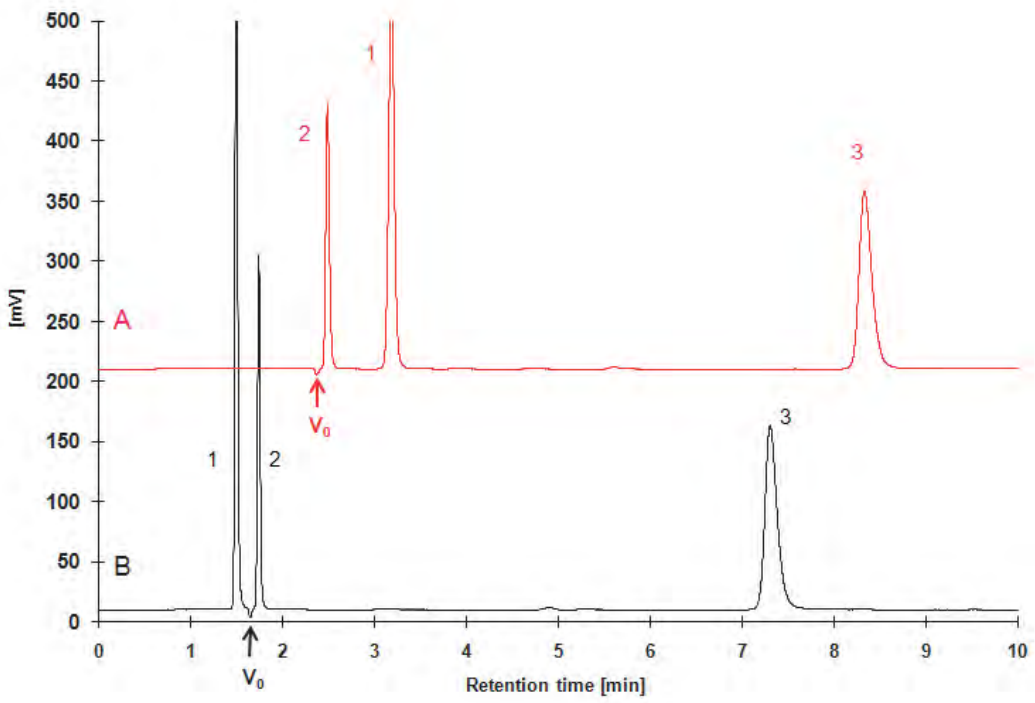
DIRECT CONNECTION HILIC COLUMN FOR DEDICATED APPLICATIONS

The TSKgel NH_2-100 DC column connects directly to other TSKgel HPLC columns. This can be used to combine separations based on polar interactions and non-polar interactions e.g. HILIC/ion exchange and reversed phase without the need of connectors or capillaries. The DC in the name 'TSKgel NH_2-100 DC' emphasizes this 'direct connect' aspect. A male-type outlet end fitting enables the direct connection to the normal end fitting of a TSKgel reversed phase column. This allows for the simultaneous gradient separation of hydrophobic and hydrophilic/acidic compounds - e.g. an active pharmaceutical ingredient (API) and its counter ion - without the loss of column efficiency normally experienced when connecting two columns with capillary tubing. Hydrophilic compounds and anions are retained strongly on the amino-alkyl bonded 3 µm silica phase of the TSKgel NH_2-100 DC 3 µm column. When coupled to a reversed phase column the overall retention of these compounds is thereby shifted from other unretained peaks.

FIGURE 9 demonstrates the use of the TSKgel NH_2-100 DC column in the separation of drug and counter ion. Maleic acid and p-toluene sulfonic acid are commonly used as counter ions in pharmaceutical preparations. Both of these organic acids are hydrophilic and are not retained on a TSKgel ODS-100V reversed phase column at pH 7.0 in 70 % methanol eluent (Chromatogram B). With the connection of a TSKgel NH_2-100 DC column prior to the TSKgel ODS-100V column, the simultaneous determination of maleic acid and the active pharmaceutical ingredient (API) desipramine becomes possible (Chromatogram A). Maleic acid is slightly retained on the TSKgel NH_2-100 DC column by an anion exchange interaction. Desipramine, on the other hand, does not interact with the protonated amino groups as it is positively charged.

FIGURE 9

Simultaneous analysis of maleic acid and desipramine



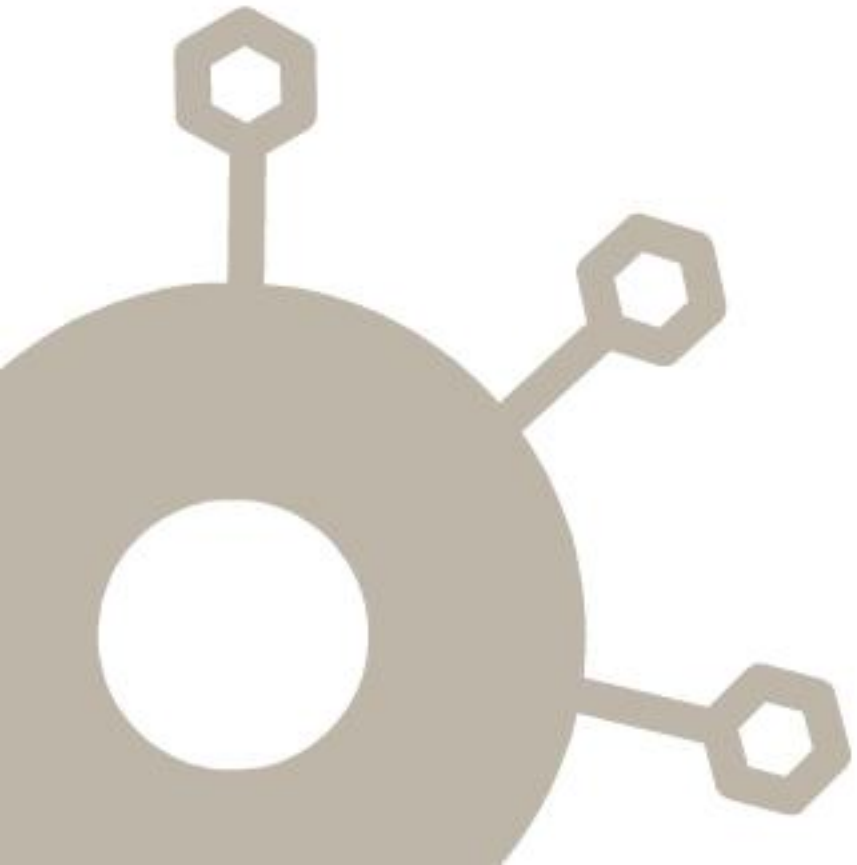
Columns: A: TSKgel NH_2-100 DC 3 µm + TSKgel ODS-100V 3 µm
B: TSKgel ODS-100V 3 µm
Mobile phase: 50 mmol/L phosphate buffer, pH 7.0/MeOH (30/70); Flow rate: 1.0 mL/min; Detection: UV @ 210 nm; Temperature: 40°C; Injection vol.: 5 µL;
Samples: 1. maleic acid (50 mg/L); 2. p-toluene sulfonic acid (50 mg/L); 3. desipramine (50 mg/L)

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Flow rate (mL/min) range	Maximum pressure drop (MPa)
TSKgel Stainless Steel Columns							
0021864	Amide-80	2.0	5.0	3	≥ 3,500	0.2	20.0
0021865	Amide-80	2.0	15.0	3	≥13,000	0.2	20.0
0021866	Amide-80	4.6	5.0	3	≥ 6,000	1.0	20.0
0021867	Amide-80	4.6	15.0	3	≥18,500	1.0	20.0
0020009	Amide-80	1.0	5.0	5	≥ 300	0.03 - 0.05	3.0
0020010	Amide-80	1.0	10.0	5	≥ 600	0.03 - 0.05	6.0
0021486	Amide-80	1.0	15.0	5	≥ 4,000	0.03 - 0.05	9.0
0021487	Amide-80	1.0	25.0	5	≥ 6,000	0.03 - 0.05	12.0
0019694	Amide-80	2.0	5.0	5	≥ 1,000	0.15 - 0.20	4.0
0019695	Amide-80	2.0	10.0	5	≥ 2,000	0.15 - 0.20	8.0
0019696	Amide-80	2.0	15.0	5	≥ 4,000	0.15 - 0.20	10.0
0019697	Amide-80	2.0	25.0	5	≥ 6,000	0.15 - 0.20	15.0
0019532	Amide-80	4.6	5.0	5	≥ 2,500	0.8 - 1.0	5.0
0019533	Amide-80	4.6	10.0	5	≥ 4,000	0.8 - 1.0	5.0
0013071	Amide-80	4.6	25.0	5	≥ 8,000	0.8 - 1.0	15.0
0021982	Amide-80 HR	4.6	25.0	5	≥18,000		15.0
0014459	Amide-80	7.8	30.0	10	≥ 5,000	1.0 - 2.0	7.0
0014460	Amide-80	21.5	30.0	10	≥ 8,000	4.0 - 6.0	3.0
0021967	NH_2-100	2.0	5.0	3	≥ 4,000	0.2	15.0
0021968	NH_2-100	2.0	15.0	3	≥15,000	0.2	20.0
0021969	NH_2-100	4.6	5.0	3	≥ 6,000	1.0	5.0
0021970	NH_2-100	4.6	15.0	3	≥18,000	1.0	15.0
0021999	NH_2-100 DC	4.6	5.0	3	≥ 6,000	1.0	5.0
Guard column products							
0021862	Amide-80 Guard cartridge, pk 3	2.0	1.0	3	For 2.0 mm ID columns		
0021863	Amide-80 Guard cartridge, pk 3	3.2	1.5	3	For 4.6 mm ID columns		
0021941	Amide-80 Guard cartridge, pk 3	2.0	1.0	5	For all 2 mm ID columns		
0019021	Amide-80 Guard column	4.6	1.0	5	For all 4.6 mm ID columns		
0019010	Amide-80 Guard cartridge, pk 3	3.2	1.5	5	For all 4.6 mm ID columns		
0014461	Amide-80 Guard column	21.5	7.5	10	For 21.5 mm ID column		
0021971	NH_2-100 Guard cartridge, pk 3	2.0	1.0	3	For all 2 mm ID columns		
0021972	NH_2-100 Guard cartridge, pk 3	3.2	1.5	3	For all 4.6 mm ID columns		
0019308	Guard cartridge holder				For 2 mm ID x 1 cm L guard cartridges		
0019018	Guard cartridge holder				For 3.2 mm ID x 1.5 cm L guard cartridges		

NOTE: Tosoh Bioscience offers guard columns and guard cartridges to protect your analytical column. Guard cartridges are usually delivered in packages of three and require the appropriate cartridge holder.
In general cartridges for 4.6 mm ID columns are produced in 3.2 mm ID and 1.5 cm length. They require the cartridge holder 19018. Guard cartridges for 2 mm ID columns are 2 mm ID x 1 cm L and require holder 0019308.

HILIC



HIC
HYDROPHOBIC INTERACTION CHROMATOGRAPHY

HIC PRODUCTS

- TSKgel Ether-5PW
- TSKgel Phenyl-5PW
- TSKgel Butyl-NPR

TOSOH FACT

Tosoh Bioscience provides solutions for today's biological purification needs. In fact, some of the first commercial HIC products were manufactured by Tosoh. We take pride in our ability to design new products based on existing chemistries to solve specific customer applications.

We encourage you to have a confidential discussion with us about your specific needs. Whether it is a surface modification of an existing product or the creation of a new one, we encourage you to call on us to meet your needs for a customized solution.

INTRODUCTION TO TSKgel HIC COLUMNS

Hydrophobic Interaction Chromatography (HIC) is based on the interaction between hydrophobic groups on a protein and a hydrophobic ligand on the solid support. HIC offers a distinct advantage for easily denatured proteins; it can be run using moderate concentrations of ammonium sulfate, which favors the stability of many proteins.

The binding of proteins to a hydrophobic matrix is affected by a number of factors including (1) the type of ligand, (2) the ligand density on the solid support, (3) the backbone material of the matrix, (4) the hydrophobic nature of the protein, and (5) the type of salt used. All of these factors help to make HIC a powerful technique for the separation of biomolecules.

Tosoh Bioscience offers three different HIC column types in analytical format: TSKgel Phenyl-5PW, Ether-5PW and Butyl NPR. TSKgel Phenyl-5PW is also available in preparative column formats. See FIGURE 1 for the structure of the HIC resins.

COLUMN SELECTION

TSKgel HIC stationary phases are polymethacrylate-based with a choice of three ligands with varied hydrophobicities from low to high. TSKgel Ether-5PW and Phenyl-5PW are based on a porous base matrix with 100 nm pores and available with various particle sizes depending on column dimensions, while TSKgel Butyl-NPR is based on a 2.5 µm nonporous base particle. Nonporous resins (NPR) re typically used for high-speed analytical applications.

TSKgel ETHER-5PW is less hydrophobic than TSKgel Phenyl-5PW. It displays weaker interaction and thus shorter retention times compared to Phenyl-5PW.

TSKgel Ether-5PW is the best choice for the separation of very hydrophobic proteins such as membrane proteins or monoclonal antibodies.

The TSKgel PHENYL-5PW columns were the first commercially available, polymer-based columns for high performance HIC. These columns have been instrumental to the increase in popularity of this technique for analytical, preparative, and process scale separations of biopolymers.

TSKgel BUTYL-NPR is the least hydrophobic among the three TSKgel HIC columns and requires a higher salt concentration for binding. TSKgel Butyl-NPR columns provide fast and quantitative HIC, because smaller particles provide higher efficiency. By packing the 2.5 µm nonporous resin particles into shorter columns, typical analysis times are reduced to less than 10 minutes. Pore diffusion is often the rate-limiting step in the overall mass transport of large biomolecules through a porous column. Eliminating the pores provides higher resolution at higher flow rates. Another benefit of NPR resins is excellent mass recovery, allowing quantitation down to nanogram levels. These properties make TSKgel Butyl-NPR the preferred choice for process monitoring and quality control. TSKgel Butyl-NPR is getting increasingly popular for the analysis of antibody drug conjugates (ADCs) and is available in two dimensions: 3.5 cm length for high throughput and 10 cm length for high resolution.

TSKgel HIC columns are compatible with water soluble organic solvents at concentration below 50 % (20 % for Butyl-NPR).

FIGURE 1

Structure of TSKgel HIC resins

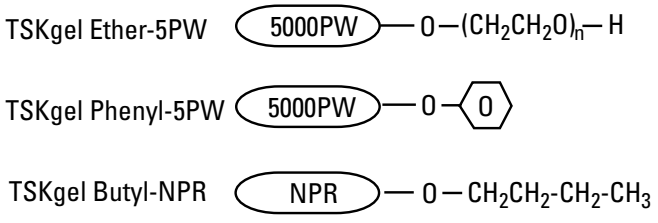


TABLE I

Column selection for the TSKgel HIC columns

Sample	MW range (Da)	TSKgel Column
Peptides	< 10,000	Butyl-NPR
Medium to large proteins	> 10,000	Phenyl-5PW Ether-5PW Butyl-NPR
DNA, RNA, and PCR products	> 500,000	Phenyl-5PW Butyl-NPR
Oligonucleotides	> 10,000	Phenyl-5PW Butyl-NPR

FEATURES

- Choice of three hydrophobic ligands (ether, phenyl or butyl)
- Rigid polymeric base resin
- Similar chemistry to TOYOPEARL resins
- TSKgel Phenyl-5PW offered in PEEK hardware
- Ether and Phenyl available in 2 mm ID format

BENEFITS

- Added flexibility during method development
- Wide pH range (2-12) enabling robust cleaning options
- Seamless scalability from analytical to preparative scaley
- Eliminates undesirable interactions with column hardware
- LC-MS applications

HIC

COMPARISON OF SELECTIVITY

FIGURE 2 compares the separation of standard proteins on the Ether, Phenyl, and Butyl supports under similar operating conditions.

SAMPLE CAPACITY

One definition of sample capacity is the amount of pure compound injected onto the column at which the peak width is 10% larger than the peak width under low loading conditions. Using this definition, the capacity of a 7.5 mm ID x 7.5 cm L TSKgel Phenyl-5PW column varies from 0.1 to 1 mg of protein. Resolution and peak width are dependent on sample loading, as shown in FIGURE 3. Therefore, sample loading should be kept within 0.1 - 0.5 mg in order to obtain the highest resolution.

Separations on TSKgel Ether-5PW columns usually take 30 - 60 minutes. 0.5 mg of pure protein can be purified from a 5 - 10 mg crude protein mixture using a 7.5 mm ID x 7.5 cm L column.

Since almost all of the surface area of a porous particle is inside the pores, the capacity of the 4.6 mm ID x 3.5 cm L TSKgel Butyl-NPR column is significantly less than that for the 7.5 mm ID x 7.5 cm L Phenyl-5PW column. Capacities for the Butyl-NPR column are 100 µg for crude sample and 2 µg for pure sample.

CHEMICAL STABILITY

TSKgel 5PW-type HIC columns are physically and chemically stable in water soluble organic solvents (at < 50% methanol, ethanol, ACN, DMF, DMSO or < 30 % chloroform). Change the solvent gradually by reducing the flow rate (preferably with a gradient) because rapid change may cause degradation of column efficiency. Note: When changing to an organic solvent, reduce the salt concentration to prevent precipitation of the salt on the column. Also, chaotropic agents (urea, SDS, etc.) will reduce the adsorption of biomolecules; therefore, use low levels of these agents (<2 mol/L).

Polymer-based columns are stable when cleaning at alkaline pH. All TSKgel HIC columns can be routinely operated from pH 2-12. TABLE II shows that the phenyl groups on the TSKgel Phenyl-5PW are stable for more than 10 days upon exposure to 0.5 mol/L NaOH or 0.5 mol/L acetic acid.

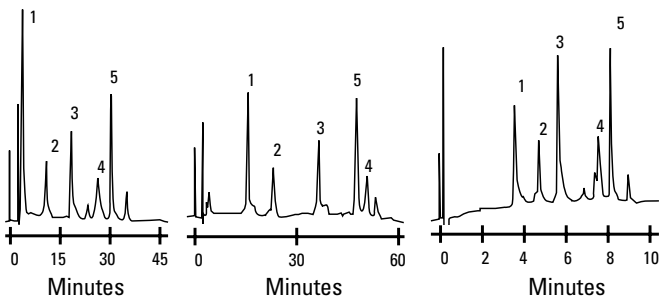
TABLE II

Long-term exposure of TSKgel Phenyl-5PW to acid and base

Acid/base	Phenyl content (mmol/mL - resin)	
	Before exposure	*After 10 days exposure*
0.5 mol/L CH_3COOH	0.105	0.106
0.5 mol/L NaOH	0.105	0.104

FIGURE 2

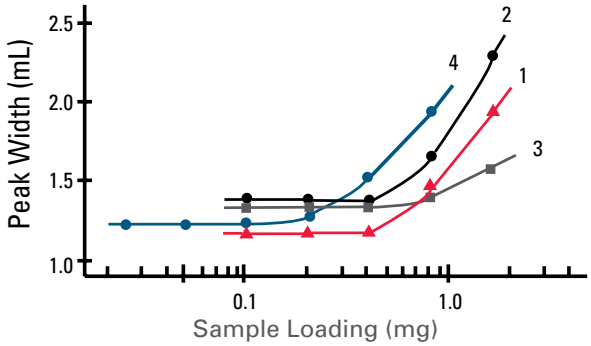
Comparing HIC columns



Column: TSKgel Ether-5PW & TSKgel Phenyl-5PW, 7.5 mm ID x 7.5 cm L TSKgel Butyl-NPR, 4.6 mm ID x 3.5 cm L; Sample: 1. myoglobin, 2. ribonuclease A, 3. lysozyme, 4. α-chymotrypsin, 5. α-chymotrypsinogen; Injection: 5PW-type columns: 100 µL (50-100 µg), NPR-type column: 20 µL (1.5-40 µg); Elution: 60 min linear gradient from 1.8 mol/L to 0 mol/L $(NH_4)_2SO_4$ in 0.1 mol/L phosphate buffer, pH 7.0, for 5PW-type columns; 12 min linear gradient from 2.3mol/L to 0 mol/L $(NH_4)_2SO_4$ in 0.1 mol/L phosphate buffer, pH 7.0 for TSKgel Butyl-NPR; Flow rate: 1.0mL/min; Detection: UV @ 280 nm

FIGURE 3

Dependence of peak width on sample loading in the separation of proteins



Column: TSKgel Phenyl-5PW, 7.5 mm ID x 7.5 cm L; Sample: 1. myoglobin; 2. ribonuclease A; 3. ovalbumin; 4. α-chymotrypsin; concentration: 0.025 % to 1.6 %; Elution: 60 min linear gradient of $(NH_4)_2SO_4$ from 1.5 mol/L to 0 mol/L in 0.1 mol/L phosphate buffer (pH 7.0); Flow rate: 0.5 mL/min; Temperature: 25 °C; Detection: UV @ 280 nm

APPLICATIONS - TSKgel HIC COLUMNS

ANTIBODY FRAGMENTS

FIGURE 4 shows the separation of Fab and Fc fragments of an antibody on TSKgel Butyl-NPR. The appearance of additional Fc fragments is due to the oxidation of methionine residues by 0.10% t-butylhydroperoxide (tBHP). The numbers above the Fc peaks correspond to the number of oxidized residues in each fragment.

ANTIBODY AGGREGATES

The use of a short TSKgel Butyl-NPR column for the separation of a monoclonal antibody and its high molecular weight aggregates is shown in FIGURE 5. The total aggregate content of this sample is about 11 %, which was also confirmed by SEC on TSKgel G3000SW$_{XL}$ (5 micron, 7.8 x 300 mm) the current industrial standard for mAb aggregate analysis. Because of the high efficiency of the nonporous particles of TSKgel Butyl-NPR only low sample amounts are needed for aggregate analysis.

ANTIBODY DRUG CONJUGATES (ADCs)

ADCs are becoming an increasingly important class of therapeutic agents for treatment of cancer. One of the most important quality attributes of an ADC is the drug to antibody ratio (DAR), the average number of drugs that are conjugated. This determines the amount of "payload" that can be delivered to the tumor cell.* Aditya Wakankar and others described the analysis of an ADC on TSKgel Butyl NPR that yielded five predominant peaks that corresponding to mAb containing zero, two, four, six and eight drugs.

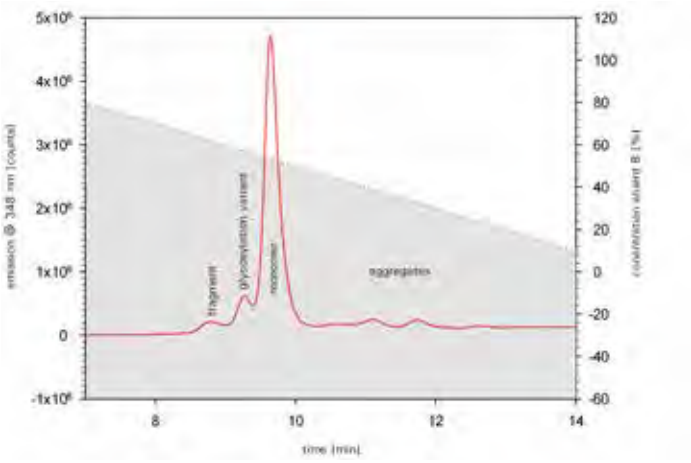
TSKgel ETHER-5PW

ANTIBIOTICS

The TSKgel Ether-5PW column was used to determine the relative purity of the antibiotic components C-1027 and C-1027-AG (FIGURE 6). Antibiotic C-1027 is composed of a protein consisting of many hydrophobic and hydroxyamino acids with a non-protein chromophore. Antibiotic C-1027-AG is composed of the hydrophobic and hydroxyamino acids without the chromophore.

FIGURE 5

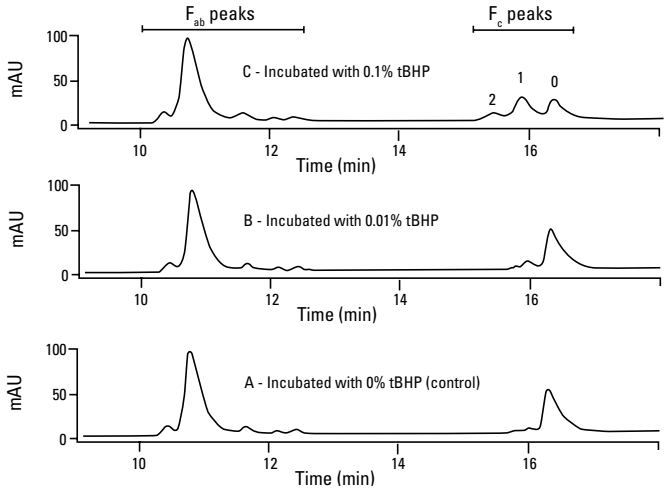
Analysis of monoclonal antibody and aggregates using a TSKgel Butyl-NPR column



Column: TSKgel Butyl-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm L,
Mobile phase: A: 3 mol/L NaCl, B: H_2O, Gradient: 0-100%B in 10 min
Flow rate: 1.0 mL/min, Detection: flourescence, Ex: 280 nm, Em: 348 nm
Injection vol.: 5 µg, Sample: IgG_1

FIGURE 4

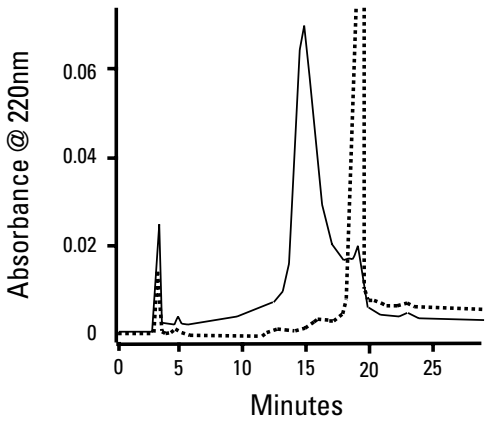
Separation of Fab and Fc fragments on TSKgel Butyl-NPR



Column: TSKgel Butyl-NPR, 4.6 mm ID x 3.5 cm L; Elution: Buffer A: 2 mol/L $(NH_4)_2SO_4$, 20 mmol/L Tris, pH 7, Buffer B: 20 mmol/L Tris, pH 7; Gradient: linear from 10 % B to 100 % B in 34 minutes; Flow rate:1 mL/min; Temperature: 30°C

FIGURE 6

Purification of anti-tumor antibiotic



Column: TSKgel Ether-5PW, 7.5 mm ID x 7.5 cm L; Sample: C-1027, C-1027-AG concentration: 1 mg/mL; Injection: 20 µL; Elution: linear gradient from 1.5 mol/L to 0 mol/L $(NH_4)_2SO_4$ in 0.1 mol/L phosphate buffer, pH 7.0; Flow rate: 0.8 mL/min; Detection: UV @ 220 nm

* *Aditya Wakankar et. al. 'Analytical methods for physicochemical characterization of antibody drug conjugates', mAbs 3:2, pages 161-172; March/April 2011.*

HIC

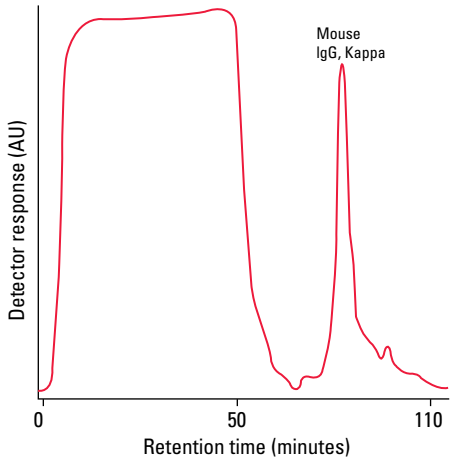
APPLICATIONS - TSKgel HIC COLUMNS

MONOCLONAL ANTIBODIES

Monoclonal antibodies (mAbs) play a part in many research, diagnostic, and therapeutic applications. Monoclonal antibodies are generally the most hydrophobic proteins in ascites fluid and cell culture supernatant.

FIGURE 7

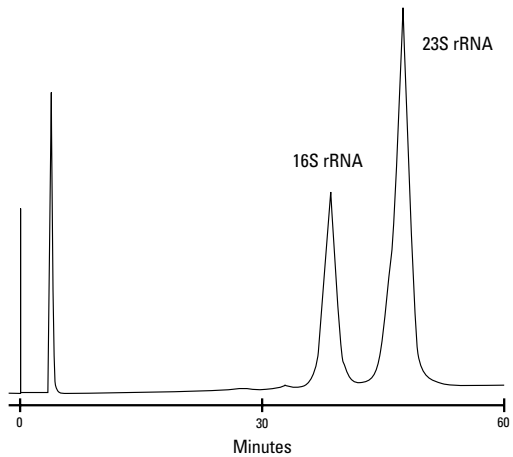
Monoclonal antibody purification



Column: TSKgel Ether-5PW, 10 µm, 8.0 mm ID × 7.5 cm, glass
Mobile phase: 67.5 min isocratic load and wash with 1 mol/L $(NH_4)_2SO_4$ in 1 mol/L glycine, 0.5 mol/L phosphate buffer, pH 7.0, followed by a 37.5 min linear gradient from 1.0 mol/L to 0 mol/L $(NH_4)_2SO_4$ in 1.0 mol/L glycine, 0.05 mol/L phosphate, pH 7.0; Flow rate: 1.0 mL/min; Detection: UV @ 280 nm, 3.0 AUFS; Injection vol.: 50 mL; Sample: 25 mL raw cell culture supernatant, 200 mg total protein, 15 mg total antibody diluted to 50 mL with initial elution buffe

FIGURE 8

Retain large RNAs on TSKgel Phenyl-5PW



Column: TSKgel Phenyl-5PW, 7.5 mm ID x 7.5 cmL;
Sample:16S and 23S rRNA from E. coli, 0.05 mg in 0.1 mL; Elution: 0 min linear gradient from 2 mol/L to 0 mol/L $(NH_4)_2SO_4$ in 0.1mol/L phosphate buffer, pH 7.0; Flow rate: 60.5 mL/min; Detection:UV @ 280 nm

FIGURE 7 shows typical results from the screening of two mAbs using a TSKgel Ether-5PW column.

TSKgel PHENYL-5PW

RNAs

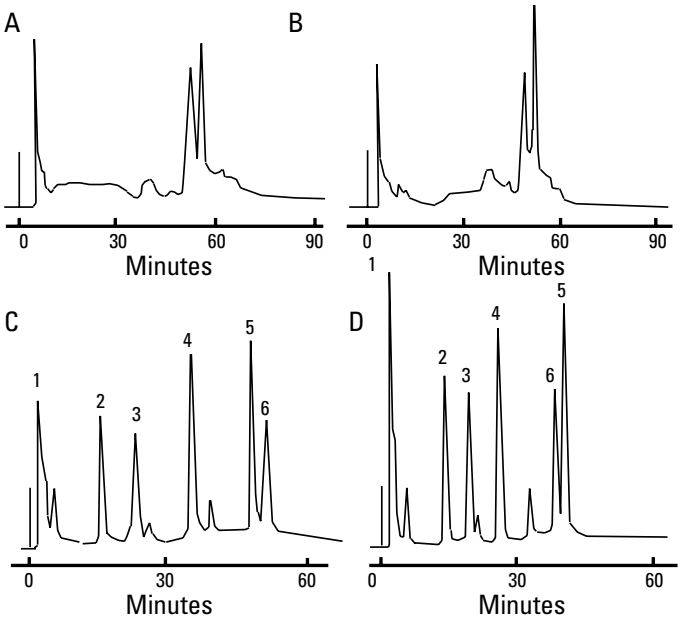
FIGURE 8 illustrates the separation of 16S and 23S ribosomal RNA on a TSKgel Phenyl-5PW column. The approximate molecular weights of these RNAs are 560,000 and 1,100,000 Da, respectively.

MODULATION OF SELECTIVITY

The addition of organic solvents or chaotropic agents in the final buffer can improve separations. However, relative elution positions may change. Therefore, add chaotropic agent and organic solvent in small quantities. See FIGURE 9 for the effect of chaotropic agents and organic solvents on the HIC separation of two different samples.

FIGURE 9

Effect of urea and isopropanol on the separation of commercial lipoxidase and a standard protein mixture



Column: TSKgel Phenyl-5PW, 7.5 mm ID x 7.5 cm L;
Sample: A & B: commercial lipoxidase, C & D: protein mixture: 1. cytochrome C, 2. myoglobin, 3. ribonuclease A, 4. lysozyme, 5. α-chymotrypsinogen, 6. α-chymotrypsin; Elution: A: 60 min linear gradient from 0.1 mol/L phosphate buffer containing 1.5 mol/L $(NH_4)_2SO_4$ (pH 7.0) to 0.1 mol/L phosphate buffer (pH 7.0), B: 60 min linear gradient from 0.1 mol/L phosphate buffer containing 1.5mol/L $(NH_4)_2SO_4$ (pH 7.0) to 0.1 mol/L phosphate buffer containing 2 mol/L urea (pH 7.0), C: 60 min linear gradient from 0.1 mol/L phosphate buffer containing 1.8 mol/L $(NH_4)_2SO_4$ (pH 7.0) to 0.1 mol/L phosphate buffer (pH 7.0), D: 60min linear gradient from 0.1 mol/L phosphate buffer containing 1.8 mol/L $(NH_4)_2SO_4$ (pH 7.0) to 0.1 mol/L phosphate buffer (pH 7.0) containing 7% isopropanol; Flow rate : A & B: 0.5 mL/min; C & D: 1.0 mL/min;
Temp.: 25°C; Detection: UV @ 280 nm

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							
0014013	Ether-5PW Glass, 100 nm	5.0	5.0	10.0	≥ 600	0.5 - 0.8	2.0
0014014	Ether-5PW Glass, 100 nm	8.0	7.5	10.0	≥ 1,000	0.5 - 1.0	2.0
0013063	Phenyl-5PW Glass, 100 nm	5.0	5.0	10.0	≥ 600	0.5 - 0.8	2.0
0008804	Phenyl-5PW Glass, 100 nm	8.0	7.5	10.0	≥ 1,000	0.5 - 1.0	2.0
TSKgel Stainless Steel Columns							
0018760	Ether-5PW, 100 nm	2.0	7.5	10.0	≥ 1,000	0.05 - 0.1	0.6
0008641	Ether-5PW, 100 nm	7.5	7.5	10.0	≥ 1,000	0.5 - 1.0	2.0
0018759	Phenyl-5PW, 100 nm	2.0	7.5	10.0	≥ 1,000	0.05 - 0.1	0.8
0007573	Phenyl-5PW, 100 nm	7.5	7.5	10.0	≥ 1,000	0.5 - 1.0	2.0
0007656	Phenyl-5PW, 100 nm	21.5	15.0	13.0	≥ 3,000	4.0 - 6.0	2.0
0014947	Butyl-NPR, nonporous	4.6	3.5	2.5		0.5 - 1.0	20.0
0042168	Butyl-NPR, nonporous	4.6	10.0	2.5	> 4,000	0.5 - 1.0	20.0
TSKgel PEEK columns							
0020023	BioAssist Phenyl, 100 nm	7.8	5	10.0	≥ 1,000	0.5 - 1.0	2.0

Guard column products		**ID (mm)**	**Length (cm)**	**Particle size (µm)**	
0014025	Ether-5PW Guardgel Kit, Glass			20.0	For P/Ns 0014013 and 0014014
0008643	Ether-5PW Guardgel Kit			20.0	For P/N 0008641
0007652	Phenyl-5PW Guardgel Kit			20.0	For P/N 0007573
0016095	Phenyl-5PW Prep Guardgel Kit			20.0	For P/N 0007656

HIC

RPC
REVERSED PHASE CHROMATOGRAPHY

RPC PRODUCTS

RP COLUMNS FOR BIOMOLECULES

TSKgel Protein C4-300
TSKgel OligoDNA RP
TSKgel TMS-250

UNIVERSAL RP COLUMNS

TSKgel ODS-100V
TSKgel ODS-100Z

FAST RP COLUMNS

TSKgel ODS-140HTP
TSKgel Super-ODS
TSKgel Super-Octyl
TSKgel Super-Phenyl

TRADITIONAL RP COLUMNS

TSKgel ODS-80Ts
TSKgel ODS-80Tm
TSKgel Octyl-80Ts
TSKgel CN-80Ts
TSKgel ODS-120A
TSKgel ODS-120T

POLYMER BASED RP COLUMNS

TSKgel Octadecyl-NPR
TSKgel Octadecyl-2PW
TSKgel Octadecyl-4PW

TOSOH FACT

Tosoh Bioscience, part of the Specialty Group Division of Tosoh Corporation, is a leading supplier of chromatographic columns, media and sophisticated clinical diagnostic systems.

TSKgel, TOYOPEARL and our other branded chromatography products have evolved over more than three decades from the measurement and analysis of polymers and organic compounds to development in the bioscience age with the analysis, separation and purification of proteins.

Experts and knowledgeable industry observers in areas from academia, government and scientific institutions praise the achievements of Tosoh Corporation in the fields of bioanalysis and purification.

TSKgel REVERSRED PHASE CHROMATOGRAPHY COLUMNS

Reversed phase chromatography (RPC) columns can be applied to the analysis of a wide variety of compounds, ranging from neutral polar and non-polar solutes to acidic, basic and amphoteric compounds. RPC is also and efficient technique for the analysis of derivatized amino acids, peptides and proteins, although protein structure is not always maintained due to the high concentration of organic solvent required for elution. Tosoh Bioscience offers 18 distinct RPC column types which are based on either silica or polymer particles (TABLE I).

The silica-based TSKgel RPC product line consists of ten stationary phases designed for the analysis of low molar mass compounds, including active pharmaceutical ingredients (API), forensic compounds, derivatized amino acids, carbohydrates, steroids, lipids, and fatty acids, as well as two stationary phases with larger pore size designed for protein analysis.

TSKgel silica packings consist of spherical particles with uniform pore sizes of 8, 10, 12, 14, 25, or 30 nm bonded with a monomeric or polymeric layer of octadecyl, octyl, cyano, trimethylsilyl, or phenyl groups. Several of the stationary phases are subsequently endcapped by derivatization with trimethylsilyl groups by a proprietary method that deactivates residual silanol groups.

Polymethacrylate-based reversed phase columns are available in a range of pore and particle sizes. Although often not as efficient as and less robust than silica-based RPC columns, key advantages of polymer-based columns are their pH stability from pH 2 to 12, which allows many basic compounds to be analyzed in their uncharged form, thus reducing secondary adsorption and improving peak shape and improving recovery for peptides and proteins due to reduced secondary interactions.

TABLE I

Silica and polymer based TSKgel RPC columns

Silica-based columns	Polymer-based columns
High purity type B silica	Hydrophilic backbone to improve recovery and reduce secondary interactions
High efficiencies	pH stable from 1 to 12
Excellent recoveries	Compatability with organic solvents eliminates swelling
Low bleed for MS	
An excellent choice for analysis of small molecules and peptides	An excellent choice for large MW biomolecules and for analyzing small MM compounds at high pH
TSKgel Protein C_4-300	TSKgel Octadecyl-2PW
High efficiency & throughput TSKgel ODS-100V and 100Z	TSKgel Octadecyl-4PW
Monomeric bonded silica	TSKgel Phenyl-5PW RP
Specialty silica columns	TSKgel Octadecyl-NPR (nonporous)

RPC

RP COLUMNS FOR BIOMOLECULES TSKgel PROTEIN C4-300

HIGHLIGHTS

- Ideal for the separation of proteins
- Endcapping ensures low peak tailing
- Small particle size for high theoretical plate numbers
- Short column for fast separations available

TSKgel Protein C4-300 columns are designed for the optimal recovery and resolution of proteins such as recombinant proteins, antibody fragments or PEGylated proteins. The 30 nm (300 A) pore size of the TSKgel Protein C4-300 columns are ideal for the separation of proteins. A particle size of 3 µm and optimized ligand density and alkyl length result in better protein and peptide resolution compared to other leading RP-C4 HPLC phases.

The C4 short alkyl chain ligand and its controlled bonding density provide moderate hydrophobicity to the stationary phase, which results in protein separations with high recovery and less peak tailing.

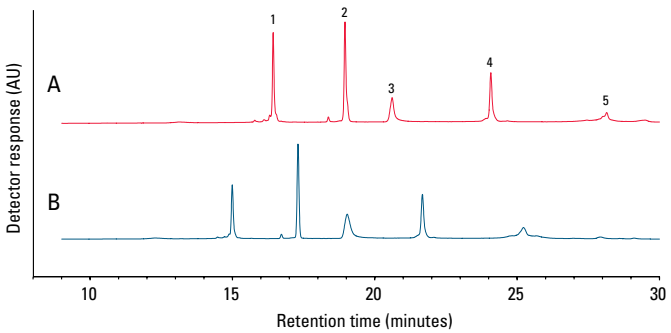
APPLICATIONS

FIGURE 1 shows the separation of a mixture of standard proteins on the TSKgel Protein C4-300 column compared to a competitor column with 3.5 µm particle size. The resolution between cytochrome c and lysozymes reaches 24.8 on the TSKgel Protein C4-300 column compared to 18.6 on the competitor C4 column. Furthermore, the TSKgel column shows higher theoretical plates and less peak tailing, especially for BSA (Peak 3), and also a better resolution of minor peaks.

For high speed separations, the analysis time can be reduced by more than eighty percent when using the short 5 cm TSKgel Protein C4-300 column and increasing the flow rate to 3 mL/min (FIGURE 2). The backpressure remains below 15 MPa, allowing the use of standard HPLC systems. The long term stability of the new C4 phase in acidic solution was tested by flushing the column with 30% acetonitrile, 0.2% TFA (4 times the standard TFA concentration) at 40 °C. There was no change in theoretical plates even after 1,000 hours of run time under this chromatographic condition. Also retention times of standard proteins didn't have significant loss when compared to the initial values.

FIGURE 1

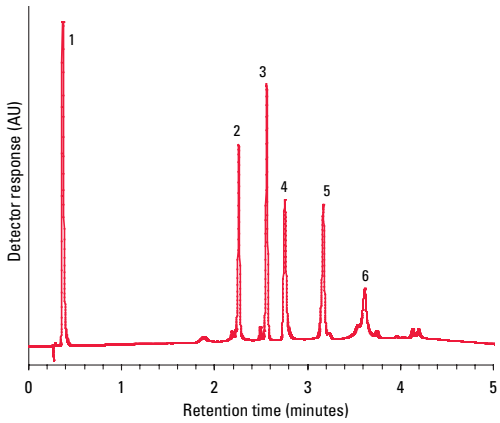
Comparison of standard protein separation



Columns: A. TSKgel Protein C4-300, 3 µm, 4.6 mm ID × 15 cm,
B. Competitor A, 3.5 µm, 4.6 mm ID × 15 cm
Mobile phase: A: $H_2O/CH_3CN/TFA$ = 90/10/0.05 (v/v/v),
B: $H_2O/CH_3CN/TFA$ = 20/80/0.05 (v/v/v)
Gradient: 0 min (0%B) 45 min (100%B), Flow rate: 1.0 mL/min;
Detection: UV @ 210 nm, Temperature: 40 °C; Injection vol.: 10 µL
Samples: 1. cytochrome C, 2. lysozyme, 3. BSA, 4. α-chymotrypsinogen A, 5. ovalbumin (each 2 µg/10 µL)

FIGURE 2

High speed separation of proteins



Column: TSKgel Protein C4-300, 3 µm, 4.6 mm ID × 5 cm
Mobile phase A: $H_2O/CH_3CN/TFA$ = 90/10/0.05 (v/v/v)
Mobile phase B: $H_2O/CH_3CN/TFA$ = 20/80/0.05 (v/v/v)
Gradient: 0 min (0%B) 5 min (100%B)
Flow rate: 3.0 mL/min, Detection: UV @ 210 nm
Temperature: 40 °C, Injection vol.: 10 µL
Samples: 1. phenylalanine, 2. cytochrome C, 3. lysozyme, 4. BSA, 5. α-chymotrypsinogen A, 6. ovalbumin (each 0.2 g/µL)

ORDERING INFORMATION

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle size (µm)*	*Number of theoretical plates*	*Maximum pressure drop (MPa)*
TSKgel Stainless Steel Columns						
0022827	TSKgel Protein C_4-300	4.6	5.0	3 µm	> 6,000	10
0022828	TSKgel Protein C_4-300	4.6	10.0	3 µm	> 11,500	17.5
0022829	TSKgel Protein C_4-300	4.6	15.0	3 µm	> 17,000	25
0022830	TSKgel Protein C_4-300	2.0	5.0	3 µm	> 4,500	15
0022831	TSKgel Protein C_4-300	2.0	10.0	3 µm	> 10,000	22.5
0022832	TSKgel Protein C_4-300	2.0	15.0	3 µm	> 15,500	30
0022833	Protein C_4-300 Guard Cartridge, 3 p	3.2	1.5		For all 4.6 mm ID Protein C_4-300 columns	
0022834	Protein C_4-300 Guard Cartridge, 3 p	2.0	1.0		For all 2 mm ID Protein C_4-300 columns	
0019018	Cartridge holder				For 3.2 mm ID cartridges	
0019308	Cartridge holder				For all 2 mm ID Guard columns	

RPC

RP COLUMNS FOR BIOMOLECULES TSKgel OligoDNA RP / TMS-250

HIGHLIGHTS

- Porous silica with 25 nm (250 A) pore size
- C18 bonded phase in TSKgel OligoDNA RP suited for Oligonucleotides
- C1 bonded phase in TSKgel TMS-250 suited for proteins

TSKgel TMS-250 is exhaustively and repeatedly reacted with trimethyl silyl groups. Standard nomenclature designates the bonded phase as C1. This wide-pore column is recommended for the analysis of proteins.

TSKgel OligoDNA RP contains a monomeric C18 bonded phase that is not endcapped and has a relatively low carbon content of 10%. It is ideal for the purification and analysis of oligonucleotides (up to 500-mer), RNAs, and DNA fragments. It possesses high-resolving power for octamers of similar sequence.

APPLICATIONS

- TSKgel TMS-250

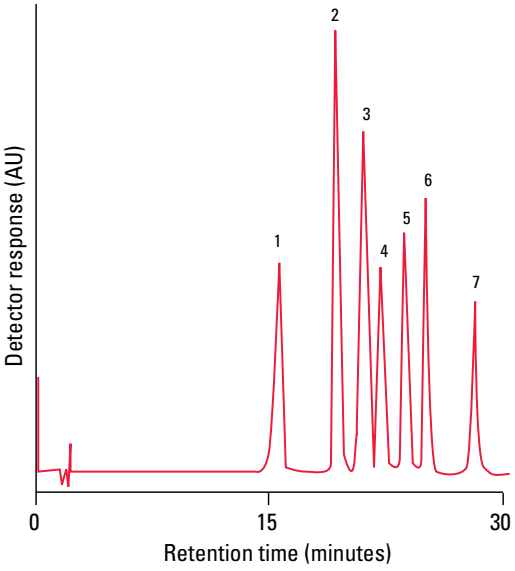
TSKgel TMS-250 is ideal for the separation of proteins which exhibit sharp peaks relative to wide-pore C8 or C18 columns. It can accommodate even large proteins, such as aldolase (158 kDa). The resolution of proteins on TSKgel TMS-250 columns is shown in Figure 3.

- TSKgel OligoDNA RP

The semi-preparative isolation of a 49-mer oligonucleotide from the crude synthetic reaction mixture using a 7.8 mm ID TSKgel OligoDNA-RP column is shown in Figure 4. The purity of the isolated oligonucleotide was subsequently verified on an analytical 4.6 mm ID TSKgel OligoDNA-RP column.

FIGURE 3

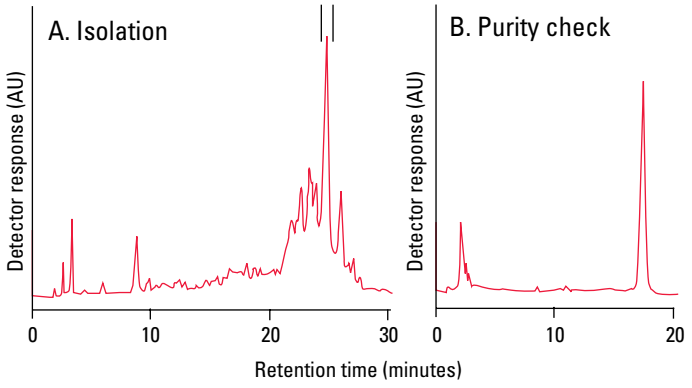
High resolution protein separation on TSKgel TMS-250



Column: TSKgel TMS-250, 4.6 mm ID x 7.5 cm L;
Sample: 5 µg each of: 1. ribonuclease A, 2. cytochrome C, 3. lysozyme, 4. bovine serum albumin, 5. aldolase, 6. carbonic anhydrase, 7. ovalbumin;
Elution: 60 min (TMS-250) linear gradient from 20% to 95% CH_3CN in 0.05% TFA, pH 2.2; Flow rate: 0.61 mL/min; Detection: UV @ 220 nm

FIGURE 4

High resolution protein separation on TSKgel TMS-250



Columns: A. TSKgel OligoDNA-RP, 5 µm, 7.8 mm ID × 15 cm, B. TSKgel OligoDNA-RP, 5 µm, 4.6 mm ID × 15 cm
Mobile phase: A. 120 min linear gradient from 6.25% to 25% CH_3CN (7.8 mm ID) column; B. 90 min linear gradient from 7.5% to 25% CH_3CN (4.6 mm ID) column, both in 0.1 mol/L ammonium acetate, pH 7.0,
Flow rate: A. 2.8 mL/min (7.8 mm ID) B. 1.0 mL/min (4.6 mm ID),
Detection: UV @ 260 m, Sample: synthetic 49-mer oligonucleotide, d(AGCTTGGGCTGCAGGTCGTCTCTAGAGGATCCCCGGGCGAGCTCGAATT)

ORDERING INFORMATION

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle size (µm)*	*Number theoretical plates*	*Flow rate (mL/min) range*	*Maximum pressure drop (MPa)*
TSKgel stainless steel columns							
0013352	OligoDNA RP, 25 nm	4.6	15.0	5	7,000	0.6 - 1.0	12.0
0013353	OligoDNA RP, 25 nm	7.8	15.0	5	7,000	2.0 - 3.0	12.0
0007190	TMS-250, 25 nm	4.6	7.5	10	1,500	0.5 - 0.8	2.0

UNIVERSAL RP COLUMNS TSKgel ODS-100V / ODS-100Z

HIGHLIGHTS

- Ultra-pure silica minimizes sample adsorption
- High surface area (450 m^2/g) silica
- Spherical 3 and 5 µm particles with 10 nm (100 Å) pores
- Very high column efficiency
- Moderate column back pressure
- Two levels of hydrophobicity:
  15% carbon (100V)
  20% carbon (100Z)
- Monomeric bonding chemistry
- Low residual silanol content

TSKgel ODS-100V & TSKgel ODS-100Z columns incorporate the best-in-class surface properties to limit secondary interactions of basic, acidic and chelating compounds. The ultra high purity Type B base silica contains negligible amounts of metal ion impurities.

TSKgel ODS-100V provides strong retention for polar compounds due to its lower C18 ligand density (15% carbon content). Proprietary monomeric bonded phase chemistry provides complete wetting and retention stability in 100% aqueous mobile phases.

The TSKgel ODS-100V and TSKgel ODS-100Z column lines were expanded to include 3 µm packed columns. These columns are well suited for high throughput LC/MS applications, providing fast and efficient separations.

TSKgel ODS-100Z contains a high density (20% carbon content) monomeric C18 bonded phase for maximum retention and selectivity of small molecular weight compounds. Exhaustive endcapping prevents secondary interaction with residual silanol groups.

TABLE II

	TSKgel ODS-100V	TSKgel ODS-100Z
Carbon content	15%	20%
Particle size (µm)	3 and 5	3 and 5
Endcapped	Yes (1)	Yes (2)
Pore size (nm)	10	10
Preferred sample type	Polar, basic, acidic	Hydrophobic
Bonded phase structure	Monolayer	Monolayer
Specific surface area (m^2/g)	450	450
*Asymmetry factor (10%)	0,90 - 1,15	0,90 - 1,15
*Theoretical plates	>14.000	>14.000

* Specifications for 4.6 mm ID x 15 cm L columns packed with 5 µm particles. Conditions: 70% methanol, 30% water; flow rate: 1 mL/min; Temp.: 40°C, N and AF are based on naphthalene peak. Typical pressure: 6 MPa

(1) Prepared by an incomplete first reaction with a difunctional octadecylsilane reagent, which is followed by endcapping with a mixture of two difunctional dialkylsilane reagents.

(2) Prepared by bonding the surface with a difunctional octadecylsilane reagent, followed by repeated endcapping with monofunctional trimethylsilane reagent.

RPC

APPLICATIONS OF TSKgel ODS-100V / ODS-100Z

SRM 870

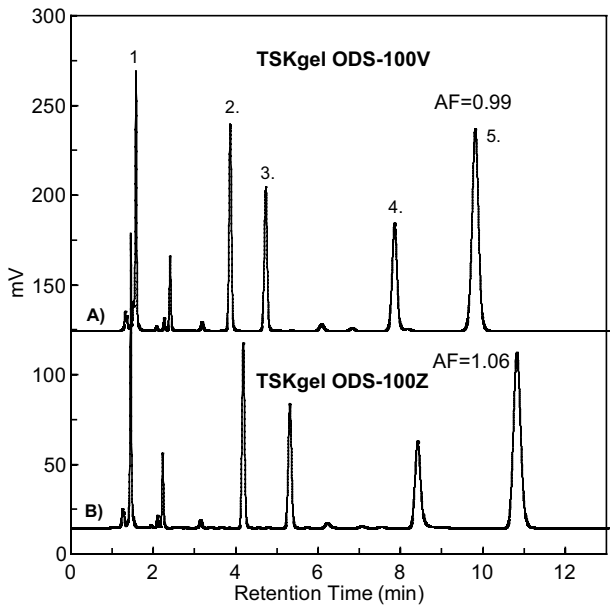
Standard Reference Material SRM 870 was developed by NIST (National Institute of Standards and Technology) as a means to classify the many commercially available reversed phase columns into closely-related groups. Amitriptyline, a tertiary amine, and quinizarin, a strong chelating compound, are included in the SRM 870 mixture, together with more traditional compounds. As shown in FIGURE 5, symmetrical peaks are obtained on TSKgel ODS-100V and TSKgel ODS-100Z for the compounds in this test mixture, clearly demonstrating the superior performance of these columns for the analysis of basic and chelating compounds.

VITAMINS

Simple and fast analysis of water- and lipid-soluble vitamins is possible on the TSKgel ODS-100V and TSKgel ODS-100Z columns, as shown in FIGURE 6. Clearly the TSKgel ODS-100Z column provides better overall resolution for the polar compounds in the mixture, while much shorter analysis time was obtained on TSKgel ODS-100V for the late eluting non-polar compounds.

FIGURE 5

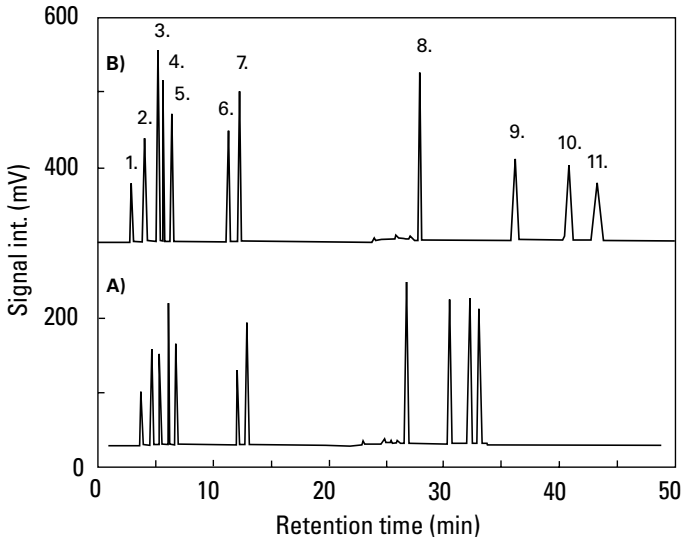
Standard reference material SRM 879



Columns: (A) TSKgel ODS-100V 3 µm (4.6 mm ID x 15 cm L)
(B) TSKgel ODS-100Z 3 µm (4.6 mm ID x 15 cm L);
Eluent: 20 mmol/L Phosphate buffer (pH 7.0)/MeOH (20/80);
Flow rate: 1.0 mL/min; Detection: UV@254nm; Tep.: 40°C; Inj. volume: 10 µL;
Sample: 1. Uracil, 2. Toluene, 3. Ethyl benzene, 4. Quinizarin, 5. Amitriptyline

FIGURE 6

Analysis of vitamins



Columns: (A) TSKgel ODS-100V (4.6 mm ID x 15 cm L)
(B) TSKgel ODS-100Z (4.6 mm ID x 15 cm L);
Eluent: (A) 0.1% TFA in H_2O; (B) 0.1 % TFA in ACN,
Gradient: 0 min (B: 0%) - 20 min (B: 40%) - 22 min (B: 100%) - 50 min (B: 100%);
Flow rate: 1.0 mL/min.; Temp.: 40°C; Detection: UV@280nm;
Inj. volume: 5 µL; Samples: 1. L-Ascorbic acid, 2. Nicotinic acid, 3. Thiamine, 4. Pyridoxal, 5. Pyridoxine, 6. Caffeine, 7. Riboflavin, 8. Retinol, 9. δ-Tocopherol, 10. α-Tocopherol, 11. α-Tocopherol acetate)

APPLICATIONS OF TSKgel ODS-100V /ODS-100Z

ORGANIC ACIDS

Organic acids play an important role in many metabolic processes, fermentation and food products. FIGURE 7 shows a baseline separation of 15 organic acids in less than 25 minutes using a simple 0.1% phosphoric acid mobile phase.

POLYMER ADDITIVES

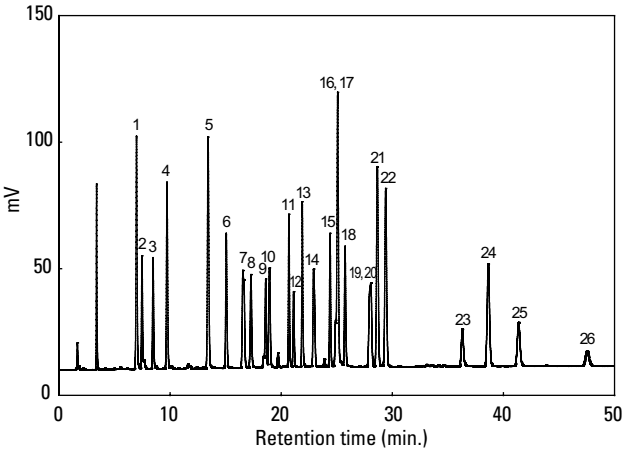
A baseline separation of 26 well known polymer additives is shown in FIGURE 8. Note that while a simple linear acetonitrile gradient was used, the column temperature was increased to 50°C to achieve the required baseline separation on a TSKgel ODS-100V column.

NUCLEOTIDES

The analysis of mono-, di-, and tri-phosphorylated nucleotides on a TSKgel ODS-100V column is shown below (FIGURE 9). The separation is accomplished by adding a short chain ion pairing agent, *t*-butylamine, and adjusting the mobile phase pH to 6.8.

FIGURE 8

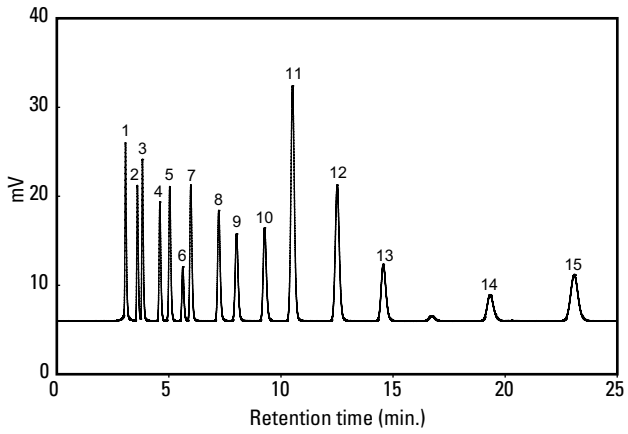
Analysis of polymer additives with TSKgel ODS-100V



Column: TSKgel ODS-100V (4.6mm ID × 15 cm L);
Mobile phases: (A) H_2O (B) ACN; Gradient: 0 min (B: 60%) - 20 min (B: 100%);
Flow rate: 1.0 mL/min; Temp: 50 °C; Detection: UV@225nm;
Inj. Volume: 10 µL; Concentration: 10 mg/L each; Samples: 1. Cyasorb UV-24, 2. BHA, 3. Ionox 100, 4. Seesorb 101, 5. Tinuvin P, 6. Yoshinox SR, 7. Seesorb 202, 8. BHT, 9. Noclizer M-17, 10. Yoshinox 2246R, 11. Topanol CA, 12. Yoshinox 425, 13. Cyanox 1790, 14. Cyasorb UV-531, 15. Ionox 220, 16. Nonflex CBP, 17. Tinuvin 326, 18. Tinuvin 120, 19. Irganox 3114, 20. Uvtex OB, 21. Tinuvin 327, 22. Tinuvin 328, 23. Irganox 1010, 24. Irganox 1330, 25. Irganox 1076, 26. Irgafos 168

FIGURE 7

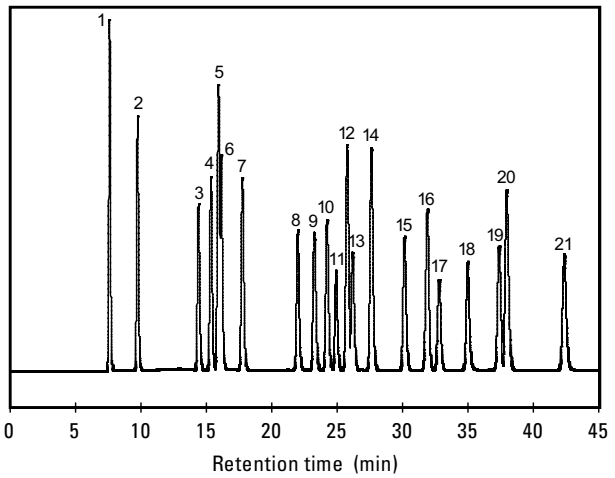
Analysis of organic acids with TSKgel ODS-100V



Column: TSKgel ODS-100V (4.6 mm ID × 25 cm L)
Mobile phase: 0.1 % H_3PO_4, (pH 2.3); Flow rate: 1.0 mL/min;
Temp: 40 °C; Inj. Volume: 10 µL; Samples: 1. Oxalic acid (0.1 mg/mL) 2. L-Tartaric acid (0.5 mg/mL) 3. Formic acid (1.0 mg/mL) 4. L-Malic acid (1.0 mg/mL) 5. L-Ascorbic acid (0.1 mg/mL) 6. Lactic acid (1.0 mg/mL) 7. Acetic acid (1.0 mg/mL) 8. Maleic acid (0.01 mg/mL) 9. Citric acid (1.0 mg/mL) 10. Succinic acid (1.0 mg/mL) 11. Fumaric acid (0.025 mg/mL) 12. Acrylic acid (0.1 mg/mL) 13. Propionic acid (2.0 mg/mL) 14. Glutaric acid (1.0 mg/mL) 15. Itaconic acid (0.025 mg/mL)

FIGURE 9

Analysis of nucleotices with TSKgel ODS-100V



Column: TSKgel ODS-100V (4.6 mm ID × 25 cm L)
Mobile phases: (A) 20 mmol/L t-butylamine + H_3PO_4 (pH 6.8) (B) A/MeOH (90/10); Gradient: 0 min (B: 0%) - 35 min (B: 100%); Flow rate: 1.0 mL/min; Temp: 25 °C; Detection: UV@260nm; Inj. Volume: 2µL; Concentration: 0.3 g/L each; Samples: 1. CMP, 2. UMP, 3. CDP, 4. dUMP, 5. GMP, 6. IMP, 7. UDP, 8. CTP, 9. TMP, 10. GDP, 11. IDP, 12. AMP, 13. UTP, 14. dGMP, 15. TDP, 16. GTP, 17. ITP, 18. ADP, 19. TTP, 20. dAMP, 21. ATP

RPC

ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Maximum pressure drop (MPa)
TSKgel Stainless steel columns						
0021838	ODS-100V, 10 nm	1.0	3.5	3	≥ 2,900	15.0
0021839	ODS-100V, 10 nm	1.0	5.0	3	≥ 4,500	15.0
0021814	ODS-100V, 10 nm, pk 3*	2.0	1.0	3	≥ 500	30.0
0022700	ODS-100V, 10 nm	2.0	2.0	3	≥ 1,500	12.0
0021813	ODS-100V, 10 nm	2.0	3.5	3	≥ 4,000	15.0
0021812	ODS-100V, 10 nm	2.0	5.0	3	≥ 5,700	15.0
0021811	ODS-100V, 10 nm	2.0	7.5	3	≥ 8,600	21.0
0021938	ODS-100V, 10 nm	2.0	10.0	3	≥ 11,500	24.0
0021810	ODS-100V, 10 nm	2.0	15.0	3	≥ 17,500	24.0
0022701	ODS-100V, 10 nm	2.0	25.0	3	≥ 28,000	30.0
0022702	ODS-100V, 10 nm	3.0	2.0	3	≥ 2,000	12.0
0022703	ODS-100V, 10 nm	3.0	3.5	3	≥ 4,000	12.0
0021842	ODS-100V, 10 nm	3.0	5.0	3	≥ 6,000	15.0
0021843	ODS-100V, 10 nm	3.0	7.5	3	≥ 9,000	21.0
0021939	ODS-100V, 10 nm	3.0	10.0	3	≥ 12,000	24.0
0021844	ODS-100V, 10 nm	3.0	15.0	3	≥ 18,000	24.0
0022704	ODS-100V, 10 nm	3.0	25.0	3	≥ 29,000	30.0
0022705	ODS-100V, 10 nm	4.6	2.0	3	≥ 2,500	12.0
0022706	ODS-100V, 10 nm	4.6	3.5	3	≥ 4,500	12.0
0021831	ODS-100V, 10 nm	4.6	5.0	3	≥ 6,500	15.0
0021830	ODS-100V, 10 nm	4.6	7.5	3	≥ 9,750	21.0
0021940	ODS-100V, 10 nm	4.6	10.0	3	≥ 13,500	24.0
0021829	ODS-100V, 10 nm	4.6	15.0	3	≥ 19,500	24.0
0022707	ODS-100V, 10 nm	4.6	25.0	3	≥ 30,000	30.0
0021457	ODS-100V, 10 nm	2.0	5.0	5	≥ 3,000	18.0
0022708	ODS-100V, 10 nm, pk 3*	2.0	1.0	5	≥ 300	28.0
0022709	ODS-100V, 10 nm	2.0	2.0	5	≥ 1,000	9.0
0022710	ODS-100V, 10 nm	2.0	3.5	5	≥ 2,500	9.0
0022711	ODS-100V, 10 nm	2.0	7.5	5	≥ 5,500	18.0
0022712	ODS-100V, 10 nm	2.0	10.0	5	≥ 7,000	18.0
0021458	ODS-100V, 10 nm	2.0	15.0	5	≥ 11,000	18.0
0022713	ODS-100V, 10 nm	2.0	25.0	5	≥ 18,000	18.0
0022714	ODS-100V, 10 nm	3.0	2.0	5	≥ 1,000	9.0
0022715	ODS-100V, 10 nm	3.0	3.5	5	≥ 3,000	9.0
0022716	ODS-100V, 10 nm	3.0	5.0	5	≥ 4,000	12.0
0022717	ODS-100V, 10 nm	3.0	7.5	5	≥ 6,000	18.0
0022718	ODS-100V, 10 nm	3.0	10.0	5	≥ 8,500	18.0
0022719	ODS-100V, 10 nm	3.0	15.0	5	≥ 13,000	18.0
0022720	ODS-100V, 10 nm	3.0	25.0	5	≥ 21,000	18.0
0022721	ODS-100V, 10 nm	4.6	2.0	5	≥ 1,500	9.0
0022722	ODS-100V, 10 nm	4.6	3.5	5	≥ 3,000	9.0
0022723	ODS-100V, 10 nm	4.6	5.0	5	≥ 4,500	12.0
0022724	ODS-100V, 10 nm	4.6	7.5	5	≥ 7,000	18.0
0022725	ODS-100V, 10 nm	4.6	10.0	5	≥ 9,000	18.0
0021455	ODS-100V, 10 nm	4.6	15.0	5	≥ 14,000	18.0
0021456	ODS-100V, 10 nm	4.6	25.0	5	≥ 23,000	21.0
0022726	ODS-100Z, 10 nm, pk 3*	2.0	1.0	3	≥ 500	30.0
0022727	ODS-100Z, 10 nm	2.0	2.0	3	≥ 1,500	12.0

*needs cartridge holder

Part #	Description	ID (mm)	Length (cm)	Particle size (µm)	Number theoretical plates	Maximum pressure drop (MPa)
0022728	ODS-100Z, 10 nm	2.0	3.5	3	≥ 4,000	15.0
0022729	ODS-100Z, 10 nm	2.0	5.0	3	≥ 5,700	15.0
0022730	ODS-100Z, 10 nm	2.0	7.5	3	≥ 8,600	21.0
0022731	ODS-100Z, 10 nm	2.0	10.0	3	≥ 11,500	24.0
0022732	ODS-100Z, 10 nm	2.0	15.0	3	≥ 17,500	24.0
0022733	ODS-100Z, 10 nm	2.0	25.0	3	≥ 28,000	30.0
0022734	ODS-100Z, 10 nm	3.0	2.0	3	≥ 2,000	12.0
0022735	ODS-100Z, 10 nm	3.0	3.5	3	≥ 4,000	12.0
0022736	ODS-100Z, 10 nm	3.0	5.0	3	≥ 6,000	15.0
0022737	ODS-100Z, 10 nm	3.0	7.5	3	≥ 9,000	21.0
0022738	ODS-100Z, 10 nm	3.0	10.0	3	≥ 12,000	24.0
0022739	ODS-100Z, 10 nm	3.0	15.0	3	≥ 18,000	24.0
0022740	ODS-100Z, 10 nm	3.0	25.0	3	≥ 29,000	30.0
0022741	ODS-100Z, 10 nm	4.6	2.0	3	≥ 2,500	12.0
0022742	ODS-100Z, 10 nm	4.6	3.5	3	≥ 4,500	12.0
0022743	ODS-100Z, 10 nm	4.6	5.0	3	≥ 6,500	15.0
0022744	ODS-100Z, 10 nm	4.6	7.5	3	≥ 9,750	21.0
0022745	ODS-100Z, 10 nm	4.6	10.0	3	≥ 13,500	24.0
0022746	ODS-100Z, 10 nm	4.6	15.0	3	≥ 19,500	24.0
0022747	ODS-100Z, 10 nm	4.6	25.0	3	≥ 30,000	30.0
0022748	ODS-100Z, 10 nm, pk 3*	2.0	1.0	5	≥ 300	28.0
0022749	ODS-100Z, 10 nm	2.0	2.0	5	≥ 1,000	9.0
0022750	ODS-100Z, 10 nm	2.0	3.5	5	≥ 2,500	9.0
0021460	ODS-100Z, 10 nm	2.0	5.0	5	≥ 3,000	18.0
0022751	ODS-100Z, 10 nm	2.0	7.5	5	≥ 5,500	18.0
0022752	ODS-100Z, 10 nm	2.0	10.0	5	≥ 7,000	18.0
0021459	ODS-100Z, 10 nm	2.0	15.0	5	≥ 11,000	18.0
0022753	ODS-100Z, 10 nm	2.0	25.0	5	≥ 18,000	18.0
0022754	ODS-100Z, 10 nm	3.0	2.0	5	≥ 1,200	9.0
0022755	ODS-100Z, 10 nm	3.0	3.5	5	≥ 3,000	9.0
0022756	ODS-100Z, 10 nm	3.0	5.0	5	≥ 4,000	12.0
0022757	ODS-100Z, 10 nm	3.0	7.5	5	≥ 6,000	18.0
0022758	ODS-100Z, 10 nm	3.0	10.0	5	≥ 8,500	18.0
0022759	ODS-100Z, 10 nm	3.0	15.0	5	≥ 13,000	18.0
0022760	ODS-100Z, 10 nm	3.0	25.0	5	≥ 21,000	18.0
0022761	ODS-100Z, 10 nm	4.6	2.0	5	≥ 1,500	9.0
0022762	ODS-100Z, 10 nm	4.6	3.5	5	≥ 3,000	9.0
0022763	ODS-100Z, 10 nm	4.6	5.0	5	≥ 4,500	12.0
0022764	ODS-100Z, 10 nm	4.6	7.5	5	≥ 7,000	18.0
0022765	ODS-100Z, 10 nm	4.6	10.0	5	≥ 9,000	18.0
0021461	ODS-100Z, 10 nm	4.6	15.0	5	≥ 14,000	18.0
0021462	ODS-100Z, 10 nm	4.6	25.0	5	≥ 23,000	21.0

TSKgel Guard column products

0021997	ODS-100V Guardgel Cartridge, pk 3*	2.0	1.0	3	For all 3 µm ODS-100V 2 & 3 mm ID columns
0021453	ODS-100V Guard Cartridge, pk 3*	3.2	1.5	5	For all ODS-100V 4.6 mm ID columns
0021841	ODS-100V Guard Cartridge, pk 3*	2.0	1.0	5	For all 5 µm ODS-100V 2 & 3 mm ID columns
0021454	ODS-100Z Guard Cartridge, pk 3*	3.2	1.5	5	For all ODS-100Z 4.6 mm ID columns
0021996	ODS-100Z Guardgel Cartridge, pk 3*	2.0	1.0	3	For all 3 µm ODS-100Z 2 & 3 mm ID columns
0021995	ODS-100Z Guardgel Cartridge, pk 3*	2.0	1.0	5	For all 5 µm ODS-100Z 2 & 3 mm ID columns

*needs cartridge holder

NOTE: Tosoh Bioscience offers guard columns and guard cartridges to protect your analytical column. Guard cartridges are usually delivered in packages of three and require the appropriate cartridge holder. In general cartridges for 4.6 mm ID columns are produced in 3.2 mm ID and 1.5 cm length. They require the cartridge holder 19018. Guard cartridges for 2 mm ID columns are 2 mm ID x 1 cm L and require holder 19308.

RPC

FAST RP COLUMNS TSKgel ODS-140HTP

HIGHLIGHTS

- Moderate pressure at high flow rates
- High resolution and high efficiency
- High throughput applications
- Compatible with HPLC and UPLC systems
- Moderate carbon content
- Polylayer bonding chemistry

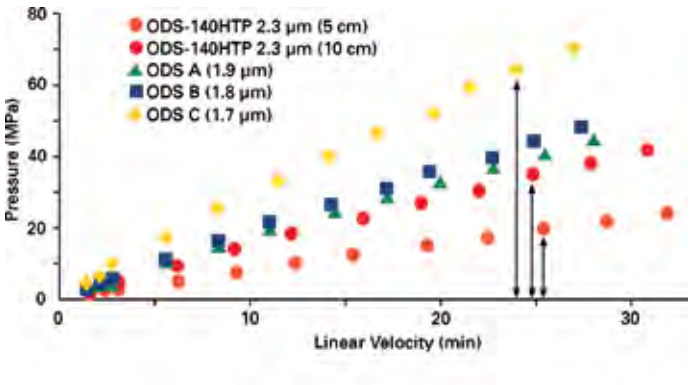
TSKgel ODS-140HTP columns were developed for use in high throughput applications, including drug discovery, pharmacokinetics and peptide digest separations. They are packed with 2.3 µm particles, providing high resolution and short analysis times at moderate pressure. The lower pressure drop reduces the burden on the hardware, allowing TSKgel ODS-140 HTP columns to be used with either UHPLC or conventional HPLC systems. The backpressure of this columns is less than half of the pressure of a sub-2 µm column of the same dimensions (FIGURE 10).

APPLICATIONS

In Vietnamese and Chinese traditional medicine, hot aqueous extract of Crinum latifolium is used because of its antitumor activity. Crinum latifolium is thought to possess antiviral and immunostimulative properties and shows immunomodulatory properties in human peripheral blood mononuclear cells. The analysis of products derived from plant extracts is a challenging chromatographic task. Due to the high number ob components the column needs to provide a high peak capacity, as shown in FIGURE 11.

FIGURE 10

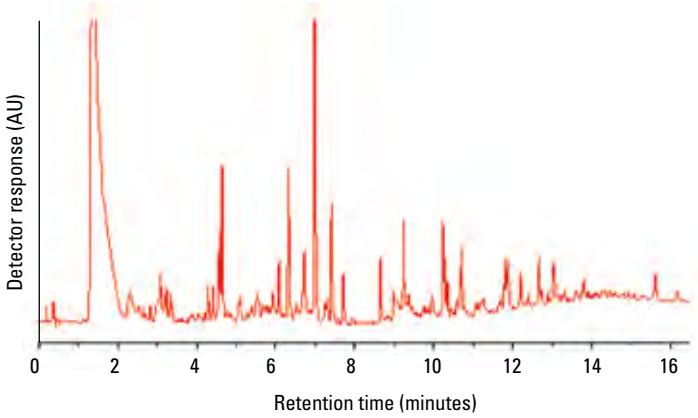
Column backpressure versus particle size



Column: TSKgel ODS-140HTP 2.3 µm (2.0 mm ID x 5.0 cm, 10 cm L)
Sub-2 µm ODS columns (2.1 mm ID x 5.0 cm L); Eluent: H_2O/CH_2CN - 50/50

FIGURE 11

Analysis of crinum latifolium



Column: TSKgel ODS-140HTP 2.3 µm, 2.1 mm ID x 10 cm L;
Sample: Crinum latifolium L extract, 2 µl; Eluent: A: water, B: acetonitrile;
Gradient: 0 min (5% B), 1.2 min (5 % B), 4 min (30 % B), 15 min (68 % B), 15.1 min (100 % B), 20min (100% B); Flow rate: 0.4 mL/min; Temp.: 40°C;
Detection: UV@220 nm; Sampling rate: 80 Hz

ORDERING INFORMATION

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle size (µm)*	*Pore size (nm)*	*Number theoretical plates*	*Maximum pressure drop (MPa)*
TSKgel stainless steel columns							
21927	TSKgel ODS-140HTP	2.1	5.0	2.3	14	≥ 7,000	60.0
21928	TSKgel ODS-140HTP	2.1	10.0	2.3	14	≥ 14,000	60.0

FAST RP COLUMNS TSKgel Super-ODS / Super-Octyl / Super-Phenyl

HIGHLIGHTS

- The silica particles used in Super series columns are monodisperse spherical 2.3 µm beads with 11 nm (110 Å) pores
- TSKgel Super-ODS, Super-Octyl and Super-Phenyl packings are bonded with, respectively, C18, C8 and phenyl functional groups. The bonded phases have a polymeric structure. An exhaustive endcapping reaction minimizes the presence of residual silanol groups
- 2 µm particles provide superior resolution and speed, as well as improved sensitivity
- Pressure drop is not excessive due to the monodisperse particle size distribution

APPLICATIONS

TSKgel SUPER-ODS, SUPER-OCTYL, SUPER-PHENYL
Recommended for small molecular weight compounds (<10,000 Da) such as peptides, amino acids, tryptic digests, nucleotides, pharmaceutical molecules, and food and beverage samples.

OPTIMIZING RESULTS WITH FAST RP COLUMNS
Super series columns can be used on a regular HPLC system if the dead volume is minimized, although optimal results are obtained with an UHPLC system.

The following recommendations are for 4.6 mm ID columns. Use proportionately lower values for 2 mm ID columns.

1. A guard filter is highly recommended to reduce particulate contamination from the sample or system components.
2. Keep sample volume less than 10 µL.
3. To ensure minimal extra-column volume, keep tubing as short as possible (extra-column volume less than 5 µL between column and detector).
4. Conventional 0.1 mm ID connecting tubing may be used (0.005).
5. The smallest detector time constant should be selected (if possible, less than 50 ms).
6. The detector flow cell should be 2 µL or less for best results. A standard HPLC flow cell (10 µL) can be used as an alternative, however, it is recommended that the heating coil is removed.

ORDERING INFORMATION

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle size (µm)*	*Number theoretical plates*	*Flow rate (mL/min) range*	*Maximum pressure drop (MPa)*
TSKgel Stainless Steel Columns							
0020015	Super-ODS, 11 nm	1.0	5.0	2.3	≥ 15,000	0.03 - 0.05	15.0
0019541	Super-ODS, 11 nm	2.0	5.0	2.3	≥ 6,000	0.15 - 0.2	25.0
0019542	Super-ODS, 11 nm	2.0	10.0	2.3	≥ 12,000	0.15 - 0.2	25.0
0018154	Super-ODS, 11 nm	4.6	5.0	2.3	≥ 8,000	1.0 - 2.5	30.0
0018197	Super-ODS, 11 nm	4.6	10.0	2.3	≥ 16,000	1.0 - 2.5	30.0
0020013	Super-Octyl, 11 nm	2.0	5.0	2.3	≥ 15,000	0.15 - 0.20	15.0
0020014	Super-Octyl, 11 nm	2.0	10.0	2.3	≥ 1,500	0.15 - 0.20	30.0
0018275	Super-Octyl, 11 nm	4.6	5.0	2.3	≥ 8,000	1.0 - 2.5	30.0
0018276	Super-Octyl, 11 nm	4.6	10.0	2.3	≥ 16,000	1.0 - 2.5	30.0
0020017	Super-Phenyl, 11 nm	2.0	5.0	2.3	≥ 3,000	0.15 - 0.20	8.0
0020018	Super-Phenyl, 11 nm	2.0	10.0	2.3	≥ 6,000	0.15 - 0.20	15.0
0018277	Super-Phenyl, 11 nm	4.6	5.0	2.3	≥ 8,000	1.0 - 2.5	30.0
0018278	Super-Phenyl, 11 nm	4.6	10.0	2.3	≥ 16,000	1.0 - 2.5	30.0
Guard column products							
0019672	Guard cartridge, pk 3*	2.0	1.0	2.3	For 2 mm ID Super-ODS columns		
0019308	Cartridge holder				For P/N 0019672		
0018207	Guard filter, pk 3*	4.0	0.4		For 4.6 mm ID columns (Super-ODS, -Octyl, -Phenyl)		
0018206	Guard filter holder	4.0	0.4		For P/N 0018207		

*needs cartridge holder

RPC

TRADITIONAL RP COLUMNS TSKgel ODS-80Ts / ODS-80TM / Octyl-80Ts / CN-80Ts

HIGHLIGHTS

- ODS-80 is prepared from spherical silica with 8 nm pores
- Monomeric-bonded phase chemistry for optimal lot-to-lot reproducibility
- High (80TM) or complete (80Ts) endcapping shields the silica surface from participating in solute retention through ionic interaction
- Particles contain 8 nm pores for fast mass transfer of solutes in the 100 to 6,000 Da MW range
- Available in particle sizes of 5 µm, 10 µm, and 20 µm
- Large surface area and high sample capacity

APPLICATIONS

TSKgel ODS-80TM

- Hydrophobic and hydrophilic peptides, synthetic peptides, purity check, peptide mapping
- General purpose column for low MW pharmaceuticals, basic compounds, nucleosides, nucleotides, purines and pyrimidines

TSKgel ODS-80Ts

- Complete endcapping makes the TSKgel ODS-80TS a good choice for strongly basic compounds and for applications that require operation at pH 7.5

TSKgel Octyl-80Ts

- Faster kinetics than ODS, but lower hydrophobic selectivity
- Lower hydrophobic selectivity of Octyl versus ODS

TSKgel CN-80Ts

- Alternative to ODS and Octyl columns for analysis of polar compounds
- Solvent strength should be reduced to obtain similar retention to Octyl and ODS columns when separating non-polar compounds

ORDERING INFORMATION

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle size (µm)*	*Number theoretical plates*	*Flow rate (mL/min) range*	*Maximum pressure drop (MPa)*
TSKgel Stainless Steel Columns							
0018150	ODS-80Ts, 8 nm	2.0	15.0	5	≥ 11,000	0.15 - 0.18	20.0
0018151	ODS-80Ts, 8 nm	2.0	25.0	5	≥ 18,000	0.15 - 0.18	30.0
0017200	ODS-80Ts, 8 nm	4.6	7.5	5	≥ 4,500	0.8 - 1.0	10.0
0017201	ODS-80Ts, 8 nm	4.6	15.0	5	≥ 11,000	0.8 - 1.0	20.0
0017202	ODS-80Ts, 8 nm	4.6	25.0	5	≥ 18,000	0.8 - 1.0	30.0
0017380	ODS-80Ts, 8 nm	21.5	30.0	10	≥ 6,000	4.0 - 6.0	6.0
0016651	ODS-80TM, 8 nm	4.6	7.5	5	≥ 4,500	0.8 - 1.0	10.0
0008148	ODS-80TM, 8 nm	4.6	15.0	5	≥ 11,000	0.8 - 1.0	20.0
0008149	ODS-80TM, 8 nm	4.6	25.0	5	≥ 18,000	0.8 - 1.0	30.0
0014002	ODS-80TM, 8 nm	21.5	30.0	10	≥ 6,000	4.0 - 6.0	6.0
0017344	Octyl-80Ts, 8 nm	4.6	15.0	5	≥ 11,000	0.8 - 1.0	20.0
0017345	Octyl-80Ts, 8 nm	4.6	25.0	5	≥ 18,000	0.8 - 1.0	30.0
0017348	CN-80Ts, 8 nm	4.6	15.0	5	≥ 11,000	0.8 - 1.0	20.0
0017349	CN-80Ts, 8 nm	4.6	25.0	5	≥ 18,000	0.8 - 1.0	30.0
Guard column products							
0019325	ODS-80Ts Guard cartridge, pk 3 *	2.0	1.0	5	For all 2 mm ID ODS-80Ts / ODS-120T columns		
0019011	ODS-80Ts Guard cartridge, pk 3 *	3.2	1.5	5	For all 4.6 mm ID ODS-80Ts columns		
0019012	Octyl-80Ts Guard cartridge, pk 3 *	3.2	1.5	5	For all 4.6 mm ID ODS-80Ts columns		
0017385	ODS-80Ts Guard column	21.5	7.5	10	For P/N 0017380		
0014098	ODS-80TM Guard column	21.5	7.5	10	For P/N 0014002		
0019004	ODS-80TM Guard cartridge, pk 3 *	3.2	1.5	5	For 4.6 mm ID ODS-80TM columns		
0019013	CN-80Ts Guard cartridge, pk 3 *	3.2	1.5	5	For 4.6 mm ID CN-80Ts columns		

TRADITIONAL RP COLUMNS TSKgel ODS-120A - TSKgel ODS-120T

HIGHLIGHTS

- TSKgel ODS-120 contains polymeric-bonded octadecyl groups on 12 nm pore size silica
- TSKgel ODS-120A is not endcapped; TSKgel ODS-120T is endcapped with trimethylsilyl groups
- TSKgel 120T columns are available in 2 mm ID format
- Available in 5 µm and 10 µm particle sizes in analytical and semi-preparative columns respectively. Larger particle sizes are available in preparative columns
- Hardware: stainless steel columns for analytical, semi-preparative, and preparative separations

APPLICATIONS

TSKgel ODS-120A

- Polymeric bonded ODS exhibits improved peak shape for the separation of complex geometric isomers, such as polynuclear aromatic hydrocarbons (PAH)
- TSKgel ODS-120A and 120T provide a similar separation at low pH for a mixture of catecholamines, while at pH 6 the basic solutes interact with negatively charged silanol groups on 120A, but not on 120T

TSKgel ODS-120T

- Endcapped ODS-120T is an alternative to ODS-80TM for peptide and protein separations

ORDERING INFORMATION

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle size (µm)*	*Number theoretical plates*	*Flow rate min) range*	*Maximum pressure drop (MPa)*
TSKgel stainless steel columns							
0007636	ODS-120A, 12 nm	4.6	15.0	5	≥ 7,000	0.8 - 1.0	15.0
0007124	ODS-120A, 12 nm	4.6	25.0	5	≥ 10,000	0.8 - 1.0	20.0
0007129	ODS-120A, 12 nm	7.8	30.0	10	≥ 6,000	1.0 - 2.0	7.5
0006172	ODS-120A, 12 nm	21.5	30.0	10	≥ 6,000	4.0 - 6.0	6.0
0018152	ODS-120T, 12 nm	2.0	15.0	5	≥ 6,500	0.15 - 0.18	15.0
0018153	ODS-120T, 12 nm	2.0	25.0	5	≥ 10,000	0.15 - 0.18	20.0
0007637	ODS-120T, 12 nm	4.6	15.0	5	≥ 7,000	0.8 - 1.0	15.0
0007125	ODS-120T, 12 nm	4.6	25.0	5	≥ 10,000	0.8 - 1.0	20.0
0007130	ODS-120T, 12 nm	7.8	30.0	10	≥ 6,000	1.0 - 2.0	7.5
0007134	ODS-120T, 12 nm	21.5	30.0	10	≥ 6,000	3.0 - 6.0	6.0
Guard column products							
0019006	ODS-120T Guard cartridge, pk 3 *	3.2	1.5	5	For all 2 mm ID ODS-120T columns		
0019005	ODS-120A Guard cartridge, pk 3*	3.2	1.5	5	For 4.6 mm ID ODS-120T columns		
0019018	Guard cartridge holder	3.2	1.5		For 3.2 mm ID cartridges		
0019308	Guard cartridge holder	2.0	1.5		For all 2 mm ID Guard columns		

RPC

POLYMER BASED RP COLUMNS TSKgel Octadecyl-NPR / -2PW / -4PW/ -Phenyl-5PW RP

HIGHLIGHTS

- Polymer-based RPC columns are chemically stable at pH 2-12, allowing operation at basic pH where silica-based columns have limited chemical stability.
- Polymer-based columns can be cleaned and impurities removed by using either strong acid or base.
- Non-porous resins (NPR) or porous resins of various pore sizes available. Column selection is based on sample MW or application.
- 2.5 µm particle size TSKgel Octadecyl-NPR resin features fast kinetics resulting in high column efficiency and quantitative protein recovery at sub-microgram loads.
- TSKgel Octadecyl-2PW with 5 µm particle size and 12.5 nm pores size.
- TSKgel Octadecyl-4PW with 7 µm particle size and 50 nm pores size.
- TSKgel Phenyl-5PW with 10 µm particle size and an average pore size of 100 nm. In comparison with the Phenyl-5PW packing material used in HIC, the greater level of hydrophobicity in TSKgel Phenyl-5PW RP makes this material more suitable for use in RPC.

APPLICATIONS

TSKgel OCTADECYL-NPR

- High efficiency purification of proteins and peptides at sub-microgram loads
- Stable to higher pressures than porous particles
- Improved recovery at low sample concentration over traditional porous resins

TSKgel OCTADECYL-2PW

- For analyzing small MW pharmaceutical compounds at basic pH
- Faster analysis than competitive polymeric RPC columns

TSKgel OCTADECYL-4PW

- Recommended for peptides and small proteins

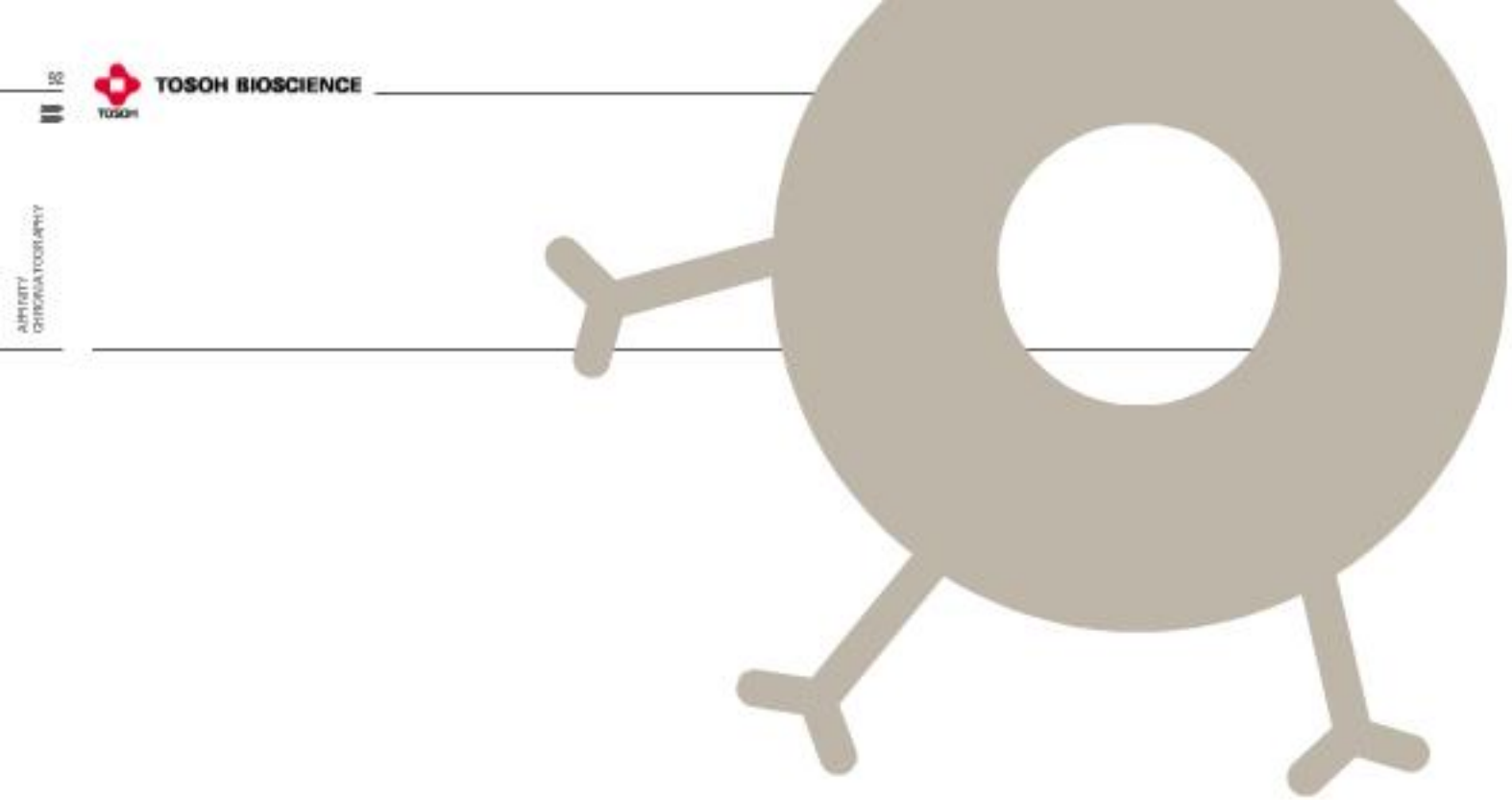
TSKgel PHENYL-5PW RP

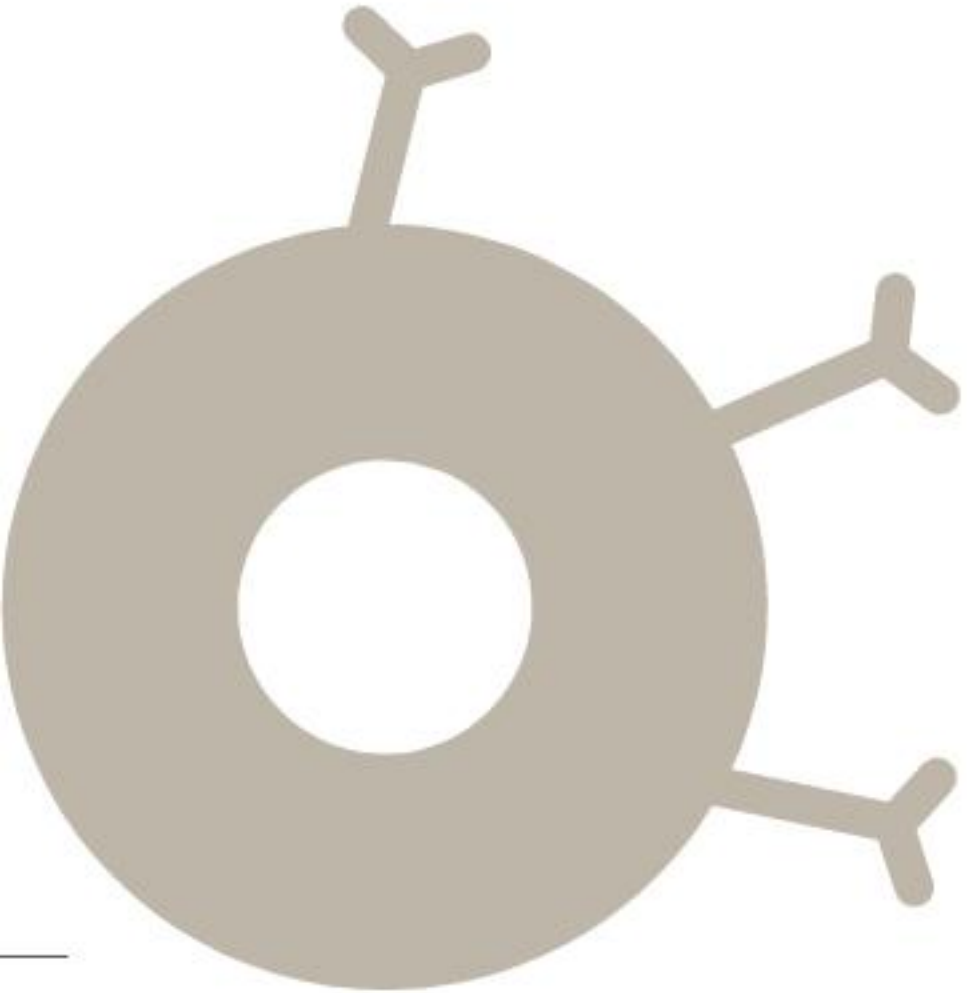
- Ideal for the separation of proteins, including high MW
- Able to handle high loads (high capacity)

ORDERING INFORMATION

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle size (µm)*	*Number theoretical plates*	*Flow rate (mL/min) range*	*Maximum pressure drop (MPa)*
TSKgel stainless steel columns							
0014005	Octadecyl-NPR nonporous	4.6	3.5	2.5	≥ 1,000	1.0 - 1.5	20.0
0018754	Octadecyl-2PW, (10 - 12.5 nm)	2.0	15.0	5	≥ 5,000	0.07 - 0.11	7.0
0017500	Octadecyl-2PW, (10 - 12.5 nm)	4.6	15.0	5	≥ 6,000	0.4 - 0.6	10.0
0017501	Octadecyl-2PW, (10 - 20 nm)	6.0	15.0	5	≥ 6,000	0.5 - 1.0	10.0
0018755	Octadecyl-4PW, 50 nm	2.0	15.0	7	≥ 2,000	0.08 - 0.17	10.0
0013351	Octadecyl-4PW, 50 nm	4.6	15.0	7	≥ 2,000	0.5 - 1.0	12.0
0016257	Octadecyl-4PW, 50 nm	21.5	15.0	13	≥ 2,000	3.0 - 6.0	2.5
0018756	Phenyl-5PW RP, 100 nm	2.0	7.5	10	≥ 400	0.05 - 0.1	1.0
0008043	Phenyl-5PW RP, 100 nm	4.6	7.5	10	≥ 500	0.5 - 1.0	3.0
0016260	Phenyl-5PW RP, 100 nm	21.5	15.0	13	≥ 1,000	6.0 - 8.0	3.0
Glass columns							
0014007	Phenyl-5PW RP Glass, 100 nm	8.0	7.5	10	≥ 700	1.0 - 2.0	2.0
Guard column products							
0019007	Phenyl-5PW RP Cartridge, pk 3 *	3.2	1.5	10	For P/N 0008043		
0017502	Octadecyl-2PW Guard column	4.6	1.0	5	For P/N 0017500		
0017503	Octadecyl-2PW Guard column	6.0	1.0	5	For P/N 0017501		
0019008	Octadecyl-4PW Cartridge, pk 3 *	3.2	1.5	7	For P/N 0013351		
0019308	Guard cartridge holder	2.0	1.0		For all 2 mm ID cartridges		
0019018	Guard cartridge holder	3.2	1.5		For 4.6 mm ID Octadecyl 4-PW and Phenyl-5PW RP columns		

*needs cartridge holder





AFC
AFFINITY CHROMATOGRAPHY

AFC PRODUCTS

- TSKgel BORONATE-5PW
- TSKgel CHELATE-5PW
- TSKgel TRESYL-5PW

TOSOH FACT

The Tosoh logo symbolizes the corporate philosophy of Tosoh's vision of the ideal .

The curved lines represent the realization of happiness, reflecting Tosoh's management philosophy of putting people first. The square in the center expresses the advanced nature of Tosoh's technology and also represents the outstanding quality of Tosoh's products. The right-angle cut at the top portrays an image of contributing to society, Tosoh's stance towards the outside world. The red corporate color symbolizes the Tosoh spirit, which guides the ceaseless efforts to realize the ideal.

INTRODUCTION TO TSKgel AFFINITY CHROMATOGRAPHY COLUMNS

The Tosoh Bioscience TSKgel Affinity Chromatography (AFC) column line consists of two group-specific stationary phases: TSKgel BORONATE-5PW and TSKgel CHELATE-5PW as well as one activated packing material called TSKgel TRESYL-5PW. Affinity chromatography offers the highest level of specificity and selectivity in biomolecular separations and purifications. Tosoh Bioscience supplies a full range of products for analytical, preparative and process scale affinity chromatography.

TSKgel affinity chromatography columns are based on the well-known G5000PW porous resin, which is the basis for high performance size exclusion chromatography columns. The TSKgel 5PW-type resin is a hydrophilic media with 100 nm pores and an estimated protein exclusion limit of 5 x 10^6 Da. Tosoh Bioscience's process scale affinity media are based on the 65 µm particle size, semi-rigid TOYOPEARL HW-65 resin. Since analytical and semi-preparative columns are made from the same polymer chemistry as the process scale media, seamless scale-up from lab to process scale is achievable. Consult the chapter on bulk media for more information about resins for packing columns to purify medium to large volume samples.

COLUMN SELECTION

TABLE I lists the ligand concentration, adsorption capacity and the test analyte used to determine the capacity of each column type.

The structures of the functional ligands available from Tosoh Bioscience are shown in FIGURE 1. The choice of a specific ligand is dictated by the expected interaction between the sample and column bonded phase. For example, the TSKgel Chelate-5PW column will bind high concentrations of Zn^{2+} ions. If a given protein is known to bind to Zn^{2+} ions, the Chelate-5PW would be a candidate column for the isolation of that target compound.

Tosoh Bioscience offers AFC columns in both glass and stainless steel formats. Glass columns are available in 5 mm ID x 5 cm L and 8 mm ID x 7.5 cm L. Stainless steel columns are available as 7.5 mm ID x 7.5 cm L and 6 mm ID x 4 cm L (Tresyl-5PW only). TSKgel BioAssist Chelate is packed in 7.8 mm ID x 5 cm L PEEK hardware. The shipping solvent is distilled water for Boronate-5PW. The Chelate-5PW is shipped in 10 mmol/L acetate buffer, pH 4.5, and the Tresyl-5PW column shipping solvent is acetone.

Stainless steel or Pyrex frits are employed in the body of the column end-fittings for the metal and glass columns, respectively. The nominal frit size for stainless steel columns is engraved in the end-fittings and all Pyrex® frits are 10 µm nominal pore size.

TABLE I

Characteristics of TSKgel AFC columns

Column packing	Ligand type	Ligand concentration	Adsorption capacity	Sample
Boronate-5PW	*m*-aminophenyl-boronate	not available	40 µmol/mL resin	sorbitol
Chelate-5PW	iminodiacetic acid	20 µmol/mL resin	not available	not available
Tresyl-5PW	tresyl	ca. 20 µmol/mL resin	>60 mg/g dry resin (coupling capacity)	soybean trypsin inhibitor

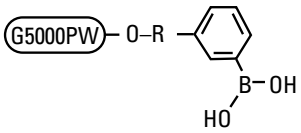
FEATURES	BENEFITS
High size exclusion limit (> 5 x 10^6 Da)	Enhanced access of large proteins to affinity ligands
Small particle size	High efficiency for analytical (10 µm) and semi-preparative (13 µm) affinity applications.
Rigid polymeric base resin	Wide pH range (2-12) of the base resin, enabling robust cleaning options
Stable affinity ligands	Long lifetime, solvent compatibility, autoclavable
Choice of four affinity ligands	Application flexibility, scalability from lab to commercial production.
TSKgel BioAssist Chelate offered in PEEK hardware	Eliminates undesirable interactions with column hardware.

AFC

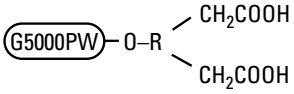
APPLICATIONS OF TSKgel AFFINITY CHROMATOGRAPHY COLUMNS

FIGURE 1
TSKgel affinity chromatography column packings

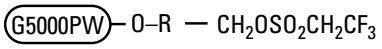
TSKgel Boronate-5PW



TSKgel Chelate-5PW



TSKgel Tresyl-5PW



Separation columns should be protected with a guard column. Tosoh Bioscience offers a unique Guardgel kit consisting of guard column hardware and gel packing, allowing the user to repack the guard column as required. Guardgel kits are available for most affinity columns, both glass and stainless steel.

TSKgel BORONATE-5PW

Coupling of m-aminophenyl boronate to the TSKgel 5PW-type polymeric support results in a ligand capable of forming a tetrahedral boronate anion under alkaline pH conditions. This anionic structure can bind with 1,2 cis-diol groups such as those found in carbohydrates, carbohydrate-containing compounds, and catecholamines. Interaction between the boronate anion and the 1,2 cis-diol groups is enhanced in the presence of Mg^{2+} ions and is inhibited by amine-containing buffers. Adsorption onto the TSKgel Boronate-5PW takes place in basic buffers such as HEPES and morpholine, while desorption takes place in carbohydrate or amine-containing mobile phases like sorbitol or Tris.

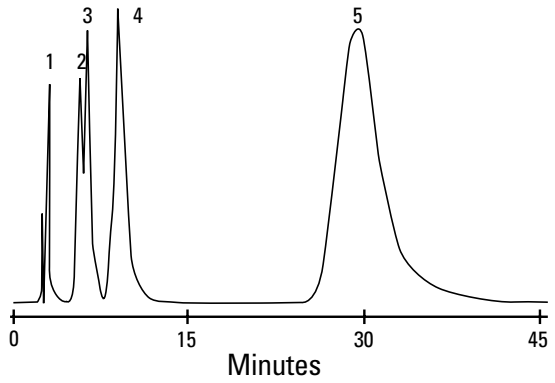
Applications for TSKgel Boronate-5PW include: nucleic acids, nucleotides and nucleosides. This affinity column has also been used to isolate catecholamines and other biomolecules containing the 1,2 cis-diol functionality (FIGURE 2).

TSKgel CHELATE-5PW

TSKgel Chelate-5PW utilizes the ability of iminodiacetic acid (IDA) to chelate ions such as Zn^{2+}, Ni^{2+} and Cu^{2+}. The column is pre-loaded with divalent metal ions by chelation. Peptides and proteins containing histidine residues will normally adsorb to these chelated ions at neutral pH. The retained compounds are then eluted with buffer containing imidazole or glycine.

The key to making successful use of this retention mechanism is the proper selection of metal ions for chelation and the elution buffer to desorb the analytes. In general, Cu^{2+} interacts better with protein; however, resolution is usually enhanced with Zn^{2+} ions. A gradient mobile phase containing increasing imidazole or glycine concentrations is used to elute the retained compounds. A decreasing pH gradient can also be used. Glycine, as well as HEPES buffers, will also elute the metallic ion so column regeneration is necessary. Conversely, imidazole in phosphate buffer will extract the metal ions very slowly, avoiding frequent column regeneration.

FIGURE 2
Separation of catecholamines on TSKgel Boronate-5PW



Column: TSKgel Boronate-5PW, 7.5 mm ID x 7.5 cm L; Sample: 1. tyrosine, 2. normetanephrine, 3. metanephrine, 4. DOPA, 5. epinephrine;
Elution: 0.1 mol/L phosphate buffer, pH 6.5; Flow rate: 1.0 mL/min;
Detection: UV @ 280 nm

APPLICATIONS OF TSKgel AFFINITY CHROMATOGRAPHY COLUMNS

Applications for TSKgel Chelate-5PW include: immunoglobulins, transferrin, lectins, milk proteins, membrane proteins, and peptides.

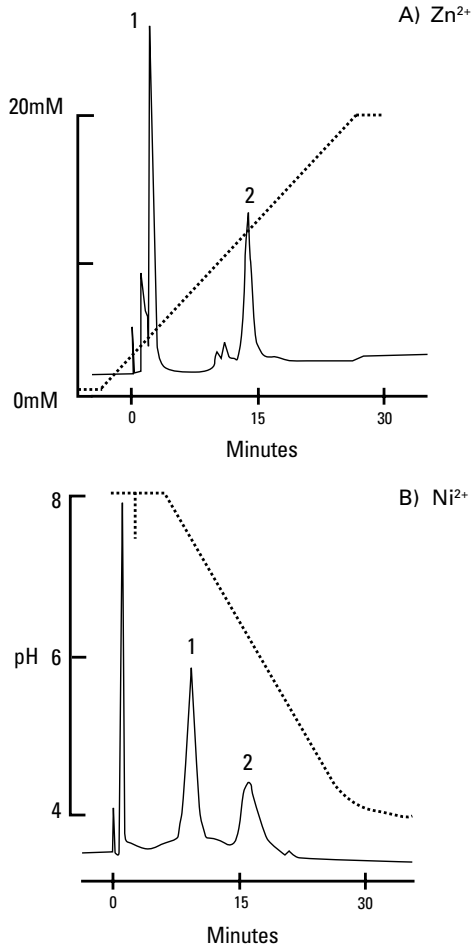
In FIGURE 3, the separation of ribonuclease A (bovine) and transferrin (human) are compared on TSKgel Chelate-5PW columns (glass, 5 mm ID x 5 cm L) containing different metal ions.

TSKgel TRESYL-5PW

Unlike other TSKgel affinity columns, the TSKgel Tresyl-5PW (tresyl; 2,2,2-trifluoroethanesulfonyl) requires activation with a user-selected ligand containing amino, thiol, phenol, or imidazole groups. The resulting structure is literally a custom affinity ligand with excellent pH stability and minimal ligand loss due to leaching. TSKgel Tresyl-5PW readily reacts with amino or thiol groups to form stable covalent alkylamines or thioethers.

FIGURE 3

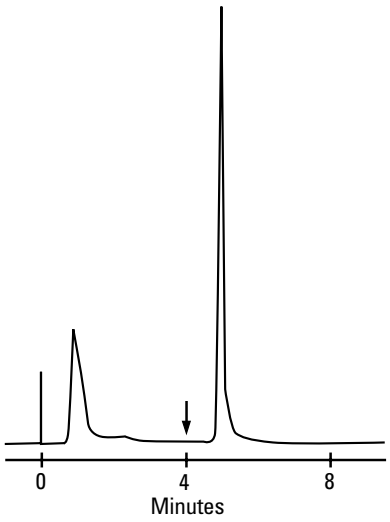
Separation of standard proteins by immobilized metal ion affinity chromatography



Column: TSKgel Chelate-5PW, 5 mm ID x 5 cm L; Metal Ion: A) Zn^{2+} and B) Ni^{2+}
Sample: 1. ribonuclease A (bovine), 2. transferrin (human)
Elution: A): 30 min linear gradient from 1 mmol/L to 20 mmol/L imidazole in 20 mmol/L HEPES-NaOH buffer, pH 8.0, containing 0.5 mol/L NaCl
B) 30 min linear pH gradient from 20 mmol/L HEPES-MES-acetic acid, pH 8.0, to 20 mmol/L HEPES-MES-acetic acid, pH 4.0, both in 0.5 mol/L NaCl; Flow rate: 0.8 mL/min; Detection: UV @ 280 nm

FIGURE 4

Purification of peroxidase on concanavalin A coupled to TSKgel Tresyl-5PW



Washing step: Wash TSKgel Tresyl-5PW, 6 mm ID x 4 cm L, with DI water; Ligand solution: Dissolve 40 mg of concanavalin A in 10 mL of 0.1 mol/L $NaHCO_3$, pH 8.0, containing 0.5 mol/L NaCl; Coupling step: Recycle the ligand solution overnight through the column at 0.2 mL/min at 25°C; Blocking step: Block residual tresyl groups with 0.1 mol/L Tris-HCl, pH 8.0, at 1.0 mL/min for 1 h at 25°C; Column: TSKgel Tresyl-5PW modified with concanavalin A; Sample: Crude peroxidase, 0.5 mg; Binding: 0.05 mol/L acetate buffer, pH 5.0, containing 0.5 mol/L NaCl and 1 mmol/L each of $CaCl_2$, $MnCl_2$, and $MgCl_2$; Elution: Step gradient at 4 min (see arrow on diagram) to 25 mmol/L -methyl-D-glucoside in binding buffer; Flow rate: 1.0mL/min; Detection: UV @ 403 nm

AFC

Principal applications for TSKgel Tresyl-5PW include the selective purification of antigens after coupling the appropriate antibody to the solid support. The antibody coupling yield at pH >7.5 is more than 90 %, with the maximum binding occurring at pH 7.5. Antigen adsorption to the antibody ligand is most effective when the antibody concentration is < 2-3 mg/mL of affinity resin. To increase binding capacity, more antibody should be added to the coupling reaction.

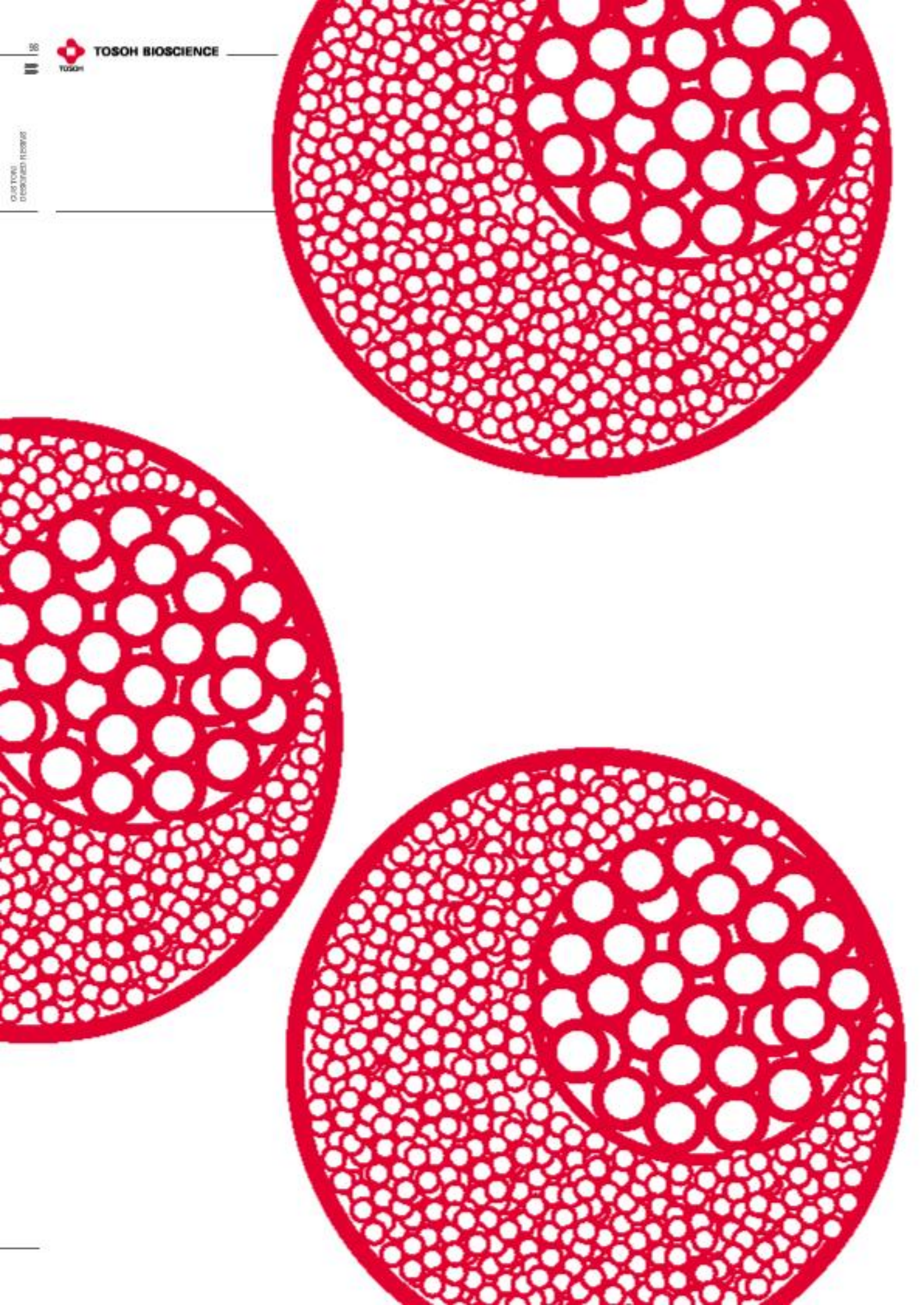
However, higher concentrations of antibody can result in steric hindrance, thus lowering the binding capacity of the column. As a general rule, the time required for antibody attachment to the TSKgel Tresyl-5PW column is directly proportional to the antibody concentration. Small amounts of antibody require about 2 hours to complete the cross-linking reaction, whereas it may take 6-7 hours to fully attach an antibody at the concentration of 10 mg/mL-resin.

Examples of the wide range of applications using TSKgel Tresyl-5PW include the binding of such ligands as concanavalin A (a lipoprotein lectin that binds to glycoproteins), numerous antibodies and enzymes. The chromatogram in FIGURE 4 shows the purification of peroxidase by the concanvalin A ligand coupled to the TSKgel Tresyl-5PW affinity support resin.

ORDERING INFORMATION

Part #	*Description*	*ID (mm)*	*Length (cm)*	*Particle size (µm)*	*Number theoretical plates*	*Flow rate (mL/min) range*	*Maximum pressure drop (MPa)*
Glass columns							
0014449	Boronate-5PW Glass, 100 nm	5.0	5.0	10	≥ 500	0.5 - 1.0	2.0
0014440	Chelate-5PW Glass, 100 nm	5.0	5.0	10	≥ 500	0.5 - 0.8	2.0
TSKgel Stainless Steel Columns							
0013066	Boronate-5PW, 100 nm	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.0
0008645	Chelate-5PW, 100 nm	7.5	7.5	10	≥ 1,300	0.5 - 1.0	1.0
0014455	Tresyl-5PW, 100 nm	6.0	4.0	10		0.2 - 0.5	1.0
0014456	Tresyl-5PW, 100 nm	7.5	7.5	10		0.5 - 1.0	1.0
TSKgel PEEK columns							
0020022	BioAssist Chelate, 100 nm	7.8	5.0	10	≥ 800	0.5 - 1.0	1.0
Guard column products							
0014451	Boronate-5PW Glass Guardgel Kit			20	For P/N 0014449		
0013125	Boronate-5PW Guardgel Kit				For P/N 0013066		
0008647	Chelate-5PW Guardgel Kit				For P/N 0008645		
Bulk packing							
0016208	Tresyl-5PW, 2 g dry gel*			10			

** 1 g is approximately 3.5 mL*



PROCESS DEVELOPMENT PRODUCTS AND BULK RESINS FOR LABORATORY SCALE PURIFICATION

PROCESS DEVELOPMENT & RESINS

- ToyoScreen PROCESS DEVELOPMENT COLUMNS
  RoboColumn PROCESS DEVELOPMENT COLUMNS
  MiniChrom PROCESS DEVELOPMENT COLUMNS
  TOYOPEARL AND TSKgel LabPAK
  TOYOPEARL AND TSKgel BULK RESINS

TOSOH FACT

Tosoh Bioscience offers a range of technical support services to our TSKgel, ToyoScreen, and TOYOPEARL chromatography products.

Whether you need help developing an HPLC assay for the analysis of a new therapeutic target, want to know how to monitor drug metabolites in the human body or need regulatory files to support a submission to the FDA, our technical support specialists will provide assistance in all of these areas and more.

We offer on-site training and application-specific seminars and are committed to providing prompt and courteous service for these and other requests.

ToyoScreen PROCESS DEVELOPMENT COLUMNS

ToyoScreen Process Development columns are easy-to-use, pre-packed columns containing the most popular TOYOPEARL resins. These columns provide a convenient, low-cost method for the evaluation of TOYOPEARL ligand chemistries. ToyoScreen Process Development columns are available in volumes of 1 mL and 5 mL for affinity, ion exchange, mixed-mode and hydrophobic interaction chromatography. See the chapter on bulk resins for detailed information on TOYOPEARL resins.

SCREENING

Historically, resin screening was accomplished by manually packing various bulk resins into small columns requiring a significant investment in time and cost. In order to improve the efficiency of resin screening experiments, pre-packed ToyoScreen Process Development columns were developed for the evaluation of different TOYOPEARL resins.

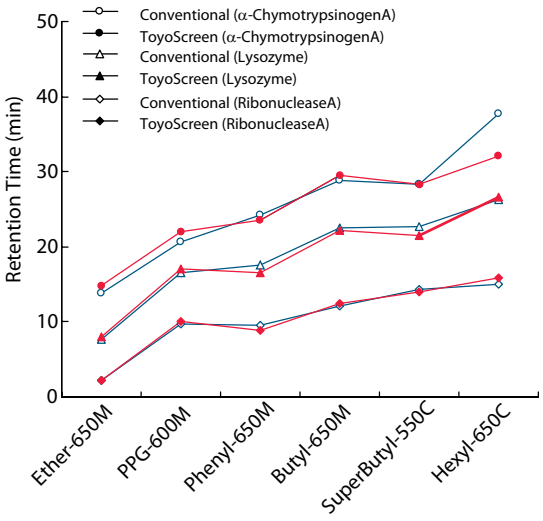
SCALABILITY

Initial results from resin screening and optimization with ToyoScreen columns can accurately predict the separation behavior at larger scales. FIGURE 1 illustrates the similar retention time behavior between 1 mL ToyoScreen columns and con-ventional 7.5 mm ID x 7.5 cm L analytical columns. Additionally, FIGURE 2 depicts a practical antibody scale up in which conditions were set using a 1 mL ToyoScreen column and applied to a 10 mL semi-preparative column with a different inner diameter and length. Similar resolution results are predicted by the following equation:

$$Rs \propto \frac{1}{dp} \frac{z^{1/2}}{u^{1/2}(g(V_t\text{-}V_o))^{1/2}}$$

FIGURE 1

Comparison of selectivity between Toyoscreen and conventional column



Columns: ToyoScreen (6.4 mm ID x 3 cm L), Conventional Column (7.5 mm ID x 7.5 cm L);
Eluent A: 0.1 mol/L phosphate buffer + 1.8 mol/L sodium sulfate (pH 7.0),
Eluent B: 0.1 mol/L phosphate buffer (pH 7.0); Flow rate: 1 mL/min
Gradient: 30 min linear; Inj. Vol.: 50 µL; Samples: Ribonuclease A, Lysozyme, α-Chymotrypsinogen, 1 mg/mL

Retention time of conventional column was plotted after converting following equation: plotted value = actual measurement value - 4.82

METHOD OPTIMIZATION

Besides the determination of what sticks during resin screening experiments, ToyoScreen Process Development columns can be used to quickly establish optimum elution conditions. Varying pH, salt type, salt gradients and flow rate are common experimental parameters explored. The effect of varying salt type and pH are shown in FIGURES 3 & 4 for anti-TSH in cell culture supernatant on ToyoScreen Phenyl-650M.

FEATURES

- Pre-packed columns
- 1 mL and 5 mL bed volume
- Cartridge design
- Ready to connect to ÄKTA, FPLC and HPLC systems
- Six pieces offered in mixed or single chemistry

BENEFITS

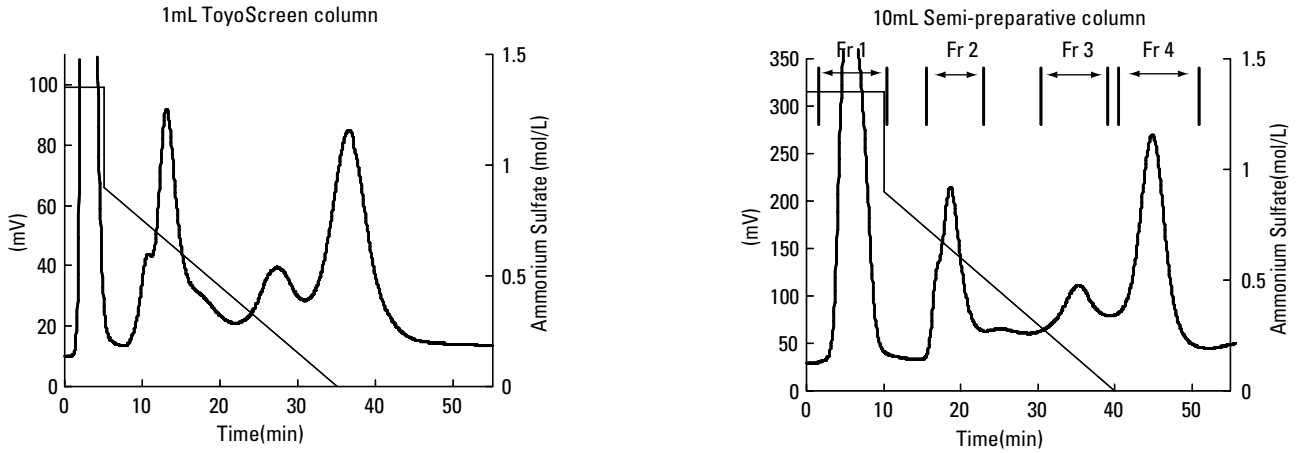
- Easy to set up and screen an entire resin series for a specific chromatographic mode
- For sample limited applications with up to milligram purifications
- Provides low cost, efficient alternative to hand packing with bulk resin
- Seamless integration into any platform
- For cost savings in screening or process experiments

PROCESS DEVELOPMENT

APPLICATIONS - ToyoScreen PROCESS DEVELOPMENT COLUMNS

FIGURE 2

Comparison chromatograms between ToyoScreen and semi-preparative columns



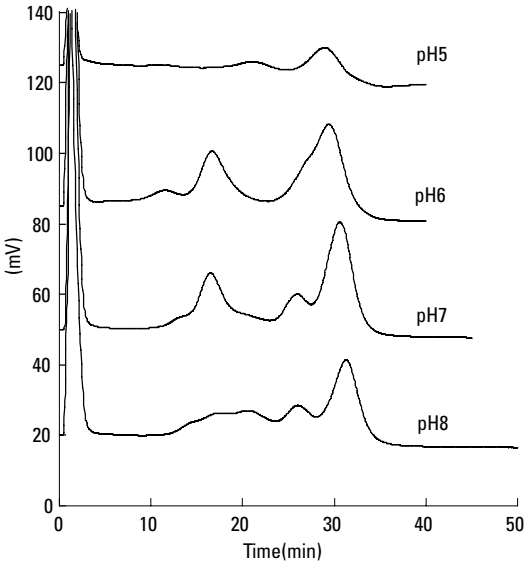
Packing: TOYOPEARL Phenyl-650M; Eluent: (A) 0.1 mol/L phosphate buffer containing 1.8 mol/L $(NH_4)_2SO_4$, pH 7.0 (B) 0.1 mol/L phosphate buffer, pH7.0; Sample: Anti-TSH from cell culture supernatant (x4 diluted)

	1 mL ToyoScreen	10 mL Semi-preparative
Column Dimensions:	6.4 mm ID x 3 cm L	14.6 mm ID x 6 cm L
Injection Volume:	500 µL	5000 µL
Flow rate:	0.5 mL/min; 0.5 CV/min; 93 cm/h	2.5 mL/min; 0.25 CV/min; 90 cm/h
Gradient Profile:	25% B; 0-5 min (isocratic)	25% B; 0-10 min (isocratic)
	50% B: 5 min (step)	50% B: 10 min (step)
	50% to 100% B; 5-35 min (linear)	50% to 100% B; 10-40 min (linear)
Gradient Slope*:	0.06 M/mL	0.012 M/mL

* The gradient slope is the change in ionic strength per unit volume. Gradient volume is the product of flow rate and gradient time.

FIGURE 3

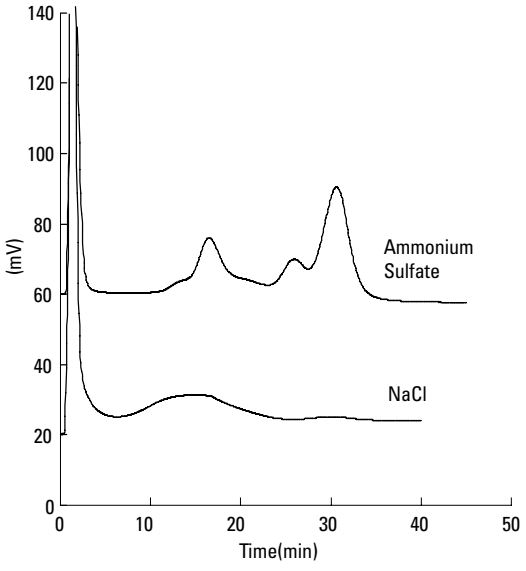
Optimizing eluent pH in HIC



Column: ToyoScreen Phenyl-650M (1 mL); Eluent A: 0.1 mol/L phosphate buffer + 1.8 mol/L ammonium sulfate (pH7.0); Eluent B: 0.1 mol/L phosphate buffer (pH 7.0); Flow rate: 1 mL/min; Gradient: 30 min linear (30 CV); Inj. Vol.: 200 µL; Sample: Cell culture supernatant (x4 diluted) (antibody: Anti-TSH)

FIGURE 4

Optimizing salt conditions in HIC



Column: ToyoScreen Phenyl-650M (1 mL); Eluent A: 0.1 mol/L phosphate buffer containing 1.8 mol/L each salt (pH7.0); Eluent B: 0.1 mol/L phosphate buffer (pH 7.0); Flow rate: 1 mL/min; Gradient: 30 min linear (30 CV); Inj. Vol.: 200 µL; Sample: Cell culture supernatant (x 4 diluted) (antibody: Anti-TSH)

ORDERING INFORMATION

Part #	Description	Package description
Ion Exchange		
0021360	ToyoScreen DEAE-650M, 1 mL	1 mL x 6 ea
0021361	ToyoScreen DEAE-650M, 5 mL	5 mL x 6 ea
0021362	ToyoScreen SuperQ-650M, 1 mL	1 mL x 6 ea
0021363	ToyoScreen SuperQ-650M, 5 mL	5 mL x 6 ea
0021364	ToyoScreen QAE-550C, 1 mL	1 mL x 6 ea
0021365	ToyoScreen QAE-550C, 5 mL	5 mL x 6 ea
0021992	ToyoScreen Q-600C AR, 1 mL	1 mL x 6 ea
0021993	ToyoScreen Q-600C AR, 5 mL	5 mL x 6 ea
0021859	ToyoScreen GigaCap Q-650M, 1 mL	1 mL x 6 ea
0021860	ToyoScreen GigaCap Q-650M, 5 mL	5 mL x 6 ea
0022873	ToyoScreen GigaCap DEAE-650M, 1 mL	1 mL x 6 ea
0022872	ToyoScreen GigaCap DEAE-650M, 5 mL	5 mL x 6 ea
0023443	ToyoScreen NH_2-750F	1 mL x 6 ea
0023444	ToyoScreen NH_2-750F	5 mL x 6 ea
0021870	ToyoScreen MegaCapII SP-550EC, 1 mL	1 mL x 6 ea
0021871	ToyoScreen MegaCapII SP-550EC, 5 mL	5 mL x 6 ea
0021366	ToyoScreen CM-650M, 1mL	1 mL x 6 ea
0021367	ToyoScreen CM-650M, 5mL	5 mL x 6 ea
0021951	ToyoScreen GigaCap CM-650M, 1 mL	1 mL x 6 ea
0021952	ToyoScreen GigaCap CM-650M, 5 mL	5 mL x 6 ea
0021368	ToyoScreen SP-650M, 1mL	1 mL x 6 ea
0021369	ToyoScreen SP-650M, 5mL	5 mL x 6 ea
0021370	ToyoScreen SP-550C, 1mL	1 mL x 6 ea
0021371	ToyoScreen SP-550C, 5mL	5 mL x 6 ea
0021868	ToyoScreen GigaCap S-650M, 1 mL	1 mL x 6 ea
0021869	ToyoScreen GigaCap S-650M, 5 mL	5 mL x 6 ea
0021392	ToyoScreen IEC Anion Mix Pack, 1 mL (DEAE-650M, SuperQ-650M, QAE-550C, GigaCap Q-650M, Q-600C AR)	1mL x 5 Grades
0021393	ToyoScreen IEC Anion Mix Pack, 5 mL (DEAE-650M, SuperQ-650M, QAE-550C, GigaCap Q-650M, Q-600C AR)	5mL x 5 Grades
0021394	ToyoScreen IEC Cation Mix Pack, 1 mL (CM-650M, SP-650M, SP-550C, GigaCap CM-650M /S-650M)	1 mL x 5 Grades
0021395	ToyoScreen IEC Cation Mix Pack, 5 mL (CM-650M, SP-650M, SP-550C, GigaCap CM-650M /S-650M)	5 mL x 5 Grades
0021396	ToyoScreen IEC Mix Pack, 1 mL (GigaCap Q-650M/ CM-650M/S-650M, SuperQ-650M, Q-600C AR)	1mL x 6 Grades x 1 ea
0021397	ToyoScreen IEC Mix Pack, 5 mL (GigaCap Q-650M/ CM-650M/S-650M, SuperQ-650M, Q-600C AR)	5mL x 6 Grades x 1 ea
Mixed-Mode		
0022824	ToyoScreen MX-Trp-650M, 1 mL	1 mL x 6 ea
0022825	ToyoScreen MX-Trp-650M, 5 mL	5 mL x 6 ea
Hydrophobic Interaction		
0021372	ToyoScreen Ether-650M, 1 mL	1 mL x 6 ea
0021373	ToyoScreen Ether-650M, 5 mL	5 mL x 6 ea
0021892	ToyoScreen Phenyl-600M, 1 mL	1 mL x 6 ea
0021893	ToyoScreen Phenyl-600M, 5 mL	5 mL x 6 ea
0021374	ToyoScreen Phenyl-650M, 1 mL	1 mL x 6 ea
0021375	ToyoScreen Phenyl-650M, 5 mL	5 mL x 6 ea
0021494	ToyoScreen Butyl-600M, 1 mL	1 mL x 6 ea
0021495	ToyoScreen Butyl-600M, 5 mL	5 mL x 6 ea
0021376	ToyoScreen Butyl-650M, 1 mL	1 mL x 6 ea
0021377	ToyoScreen Butyl-650M, 5 mL	5 mL x 6 ea
0021378	ToyoScreen Hexyl-650C, 1 mL	1 mL x 6 ea
0021379	ToyoScreen Hexyl-650C, 5 mL	5 mL x 6 ea
0021380	ToyoScreen PPG-600M, 1 mL	1 mL x 6 ea
0021381	ToyoScreen PPG-600M, 5 mL	5 mL x 6 ea
0021382	ToyoScreen SuperButyl-550C, 1 mL	1 mL x 6 ea
0021383	ToyoScreen SuperButyl-550C, 5 mL	5 mL x 6 ea
0021398	ToyoScreen HIC Mix Pack, 1 mL (PPG-600M, Butyl-600M/-650M, Phenyl-600M/-650M, Hexyl-650C)	1 mL x 6 Grades x 1 ea
0021399	ToyoScreen HIC Mix Pack, 5 mL (PPG-600M, Butyl-600M/-650M, Phenyl-600M/-650M, Hexyl-650C)	5 mL x 6 Grades x 1 ea
Affinity		
0023430	ToyoScreen AF-rProtein A HC-650F, 1 mL	1 mL x 5 ea
0023431	ToyoScreen AF-rProtein A HC-650F, 5 mL	5 mL x 1 ea
0023432	ToyoScreen AF-rProtein A HC-650F, 5 mL	5 mL x 5 ea
0022809	ToyoScreen AF-rProtein A-650F, 1 mL	1 mL x 5 ea
0022810	ToyoScreen AF-rProtein A-650F, 5 mL	5 mL x 1 ea
0022811	ToyoScreen AF-rProtein A-650F, 5 mL	5 mL x 5 ea
0021386	ToyoScreen AF-Blue HC-650M, 1 mL	1 mL x 6 ea
0021387	ToyoScreen AF-Blue HC-650M, 5 mL	5 mL x 6 ea
0021384	ToyoScreen AF-Chelate-650M, 1 mL	1 mL x 6 ea
0021385	ToyoScreen AF-Chelate-650M, 5 mL	5 mL x 6 ea
0021390	ToyoScreen AF-Heparin HC-650M, 1 mL	1 mL x 6 ea
0021391	ToyoScreen AF-Heparin HC-650M, 5 mL	5 mL x 6 ea
0021388	ToyoScreen AF-Red-650M, 1 mL	1 mL x 6 ea
0021389	ToyoScreen AF-Red-650M, 5 mL	5 mL x 6 ea
ToyoScreen accessories		
0021400	ToyoScreen column holder	

PROCESS DEVELOPMENT

RoboColumns for TOYOPEARL

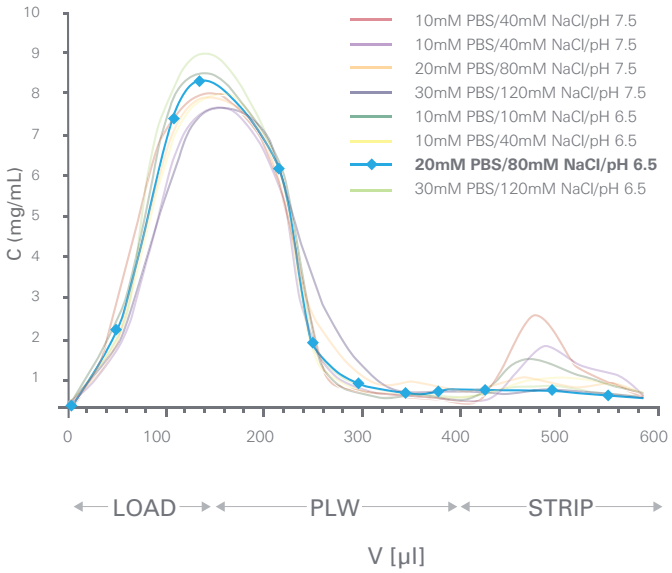
Tosoh Bioscience offers TOYOPEARL media now also in the well-known RoboColumn® format packed by Atoll GmbH. RoboColumns are miniaturized chromatographic columns pre-packed with the most popular TOYOPEARL ion exchange, mixed-mode, hydrophobic interaction or affinity media. The columns are available in different volumes and can be operated with a robotic liquid handling system. This approach allows automated high-throughput, small-scale chromatographic separations of protein samples by running up to eight individual columns simultaneously.

RoboColumns are available in two formats with 200 µL (bed height of 10 mm) and 600 µL (bed height of 30 mm) resin volume, respectively. They are supplied in a row of eight units pre-packed with the same TOYOPEARL resin and sealed with two removable silicon cover seals for proper storage. A 96-well array plate is available to arrange the up to 96 RoboColumn units.

Figure 1 shows a screening experiment to optimize the parameters for the intermediate flow-through anion exchange step in a mAb purification platform. Protein binding of a Protein A capture eluate on RoboColumns packed with TOYOPEARL SuperQ-650M was analyzed by varying salt concentration and pH of loading and washing buffer. Best results were achieved using 20 mmol/L sodium phosphate, 80 mmol/L sodium chloride, pH 6.5.

FIGURE 5

Optimization of anion exchange conditions



Elution profile of a protein A capture eluate on RoboColumns packed with Toyopearl SuperQ-650M at various conditions. Data kindly provided by T. Schröder, Atoll GmbH.

FEATURES

- Pre-packed columns for use with robotic systems
- 200 and 600 µl bed volume
- Miniaturized column format

BENEFITS

- High throughput parallel chromatography
- Fast resin screening and evaluation of design space
- Low consumption of sample and buffers

ORDERING INFORMATION

Part #	Description	Package description
ToyoScreen RoboColumns for fast automated screening of resins		
0045099		Array Plate
Gel Filtration / Desalting		
0045071	RoboColumn HW-40F	0.2 mL*8 cols
0045072	RoboColumn HW-40F	0.6 mL*8 cols
Ion Exchange		
0045023	RoboColumn GigaCap S-650S	0.2 mL*8 cols
0045024	RoboColumn GigaCap S-650S	0.6 mL*8 cols
0045001	RoboColumn GigaCap S-650M	0.2 mL*8 cols
0045002	RoboColumn GigaCap S-650M	0.6 mL*8 cols
0045025	RoboColumn GigaCap Q-650S	0.2 mL*8 cols
0045026	RoboColumn GigaCap Q-650S	0.6 mL*8 cols
0045002	RoboColumn GigaCap S-650M	0.6 mL*8 cols
0045003	RoboColumn GigaCap Q-650M	0.2 mL*8 cols
0045004	RoboColumn GigaCap Q-650M	0.6 mL*8 cols
0045005	RoboColumn GigaCap CM-650M	0.2 mL*8 cols
0045006	RoboColumn GigaCap CM-650M	0.6 mL*8 cols
0045007	RoboColumn GigaCap DEAE-650M	0.2 mL*8 cols
0045008	RoboColumn GigaCap DEAE-650M	0.6 mL*8 cols
0045011	RoboColumn Q-600C AR	0.2 mL*8 cols
0045012	RoboColumn Q-600C AR	0.6 mL*8 cols
0045021	RoboColumn NH_2-750F	0.2 mL*8 cols
0045022	RoboColumn NH_2-750F	0.6 mL*8 cols

Part #	Description	Package description
Mixed-Mode		
0045051	RoboColumn MX-Trp-650M	0.2 mL*8 cols
0045052	RoboColumn MX-Trp-650M	0.6 mL*8 cols
Hydrophobic Interaction		
0045031	RoboColumn Phenyl-600M	0.2 mL*8 cols
0045032	RoboColumn Phenyl-600M	0.6 mL*8 cols
0045033	RoboColumn Butyl-600M	0.2 mL*8 cols
0045034	RoboColumn Butyl-600M	0.6 mL*8 cols
0045035	RoboColumn PPG-600M	0.2 mL*8 cols
0045036	RoboColumn PPG-600M	0.6 mL*8 cols
0045037	RoboColumn Phenyl-650M	0.2 mL*8 cols
0045038	RoboColumn Phenyl-650M	0.6 mL*8 cols
Affinity		
0045061	RoboColumn AF-rProtein A-650F	0.2 mL*8 cols
0045062	RoboColumn AF-rProtein A-650F	0.6 mL*8 cols
0045063	RoboColumn AF-rProtein A HC-650F	0.2 mL*8 cols
0045064	RoboColumn AF-rProtein A HC-650F	0.6 mL*8 cols

PROCESS DEVELOPMENT

MiniChrom for TOYOPEARL and TSKgel

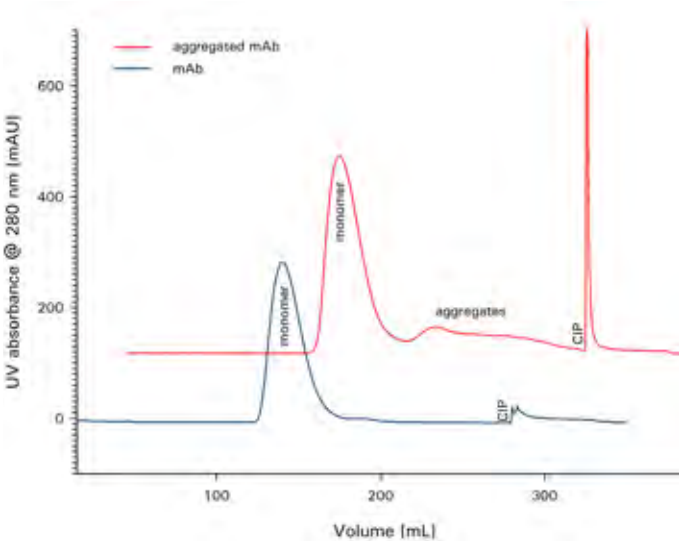
TOYOPEARL and TSKgel media re now available in the well-known 5 mL MiniChom format (8 mm ID x 100 mm) for parameter screening, method optimization and/or small scale purifications. The 5 mL MiniChrom columns are the ideal tools to further optimize the purification method and to confirm the operational window after having selected a resin for a certain purification task by resin screening, e.g. with ToyoScreen cartridges on conventional LC systems or by high throughput screening using RoboColumns on robobotic workstations.

MiniChrom columns are made of biocompatible polyethylene and polypropylene. Each column is individually packed under optimum compression, ensuring consistent experimental results. The columns can be connected directly to any laboratory liquid chromatography system via standard connectors (M10-32 for 1/16'' tubing) and are ready for equilibration in the buffer of choice. Two columns can be connected in series to increase the bed height in order to model real conditions in pilot scale or for scale- down experiments.

MiniChrom columns for TOYOPEARL and TSKgel are packed by Atoll GmbH and are available with a broad range of ion exchange, hydrophobic interaction, mixed-mode, and Protein A affinity resins. Figure 1 shows the mixed-mode separation of a monoclonal antibody and its aggregates on a 5 mL MiniChrom MX-Trp-650M column.

FIGURE 6

Mixed-mode separation on MiniChrom MX-Trp-650M



Column: MiniChrom MX-Trp-650M, 8 mm ID x10 cm L, 5 mL
Mobile phase: A: 100 mmol/L acetate buffer (pH 4.3) + 200 mmol/L NaCl, B: 100 mmol/L acetate buffer (pH 5.6) + 500 mmol/L NaCl;
Flow rate: 150 cm/h; Gradient: 5 CV 100% A, 50 CV linear gradient from 100% A to 100% B
Sample: 5 mL monoclonal antibody 5 mg/mL
5 mL aggregated monoclonal antibody (1 h, pH 2.7 @ RT) 5 mg/L

FEATURES

- Pre-packed with TOYOPEARL or TSKgel media
- Common column format (5 mL, 8 x 100 mm)
- Reliable packing quality

BENEFITS

- Ready to use with any LC system
- Ideal for method optimization and small scale purifications
- Reproducible results

ORDERING INFORMATION

MiniChrom Columns

Ion Exchange

Part #	Description	Package description
0045108	MiniChrom TOYOPEARL NH_2-750F, 5 mL	8 mm ID x 100 mm L
0045101	MiniChrom TOYOPEARL GigaCap S-650M, 5 mL	8 mm ID x 100 mm L
0045102	MiniChrom TOYOPEARL GigaCap S-650S, 5 mL	8 mm ID x 100 mm L
0045103	MiniChrom TOYOPEARL GigaCap CM-650M, 5 mL	8 mm ID x 100 mm L
0045104	MiniChrom TOYOPEARL GigaCap Q-650M, 5 mL	8 mm ID x 100 mm L
0045105	MiniChrom TOYOPEARL GigaCap Q-650S, 5 mL	8 mm ID x 100 mm L
0045106	MiniChrom TOYOPEARL GigaCap DEAE-650M, 5 mL	8 mm ID x 100 mm L
0045107	MiniChrom TSKgel SuperQ-5PW (20), 5 mL	8 mm ID x 100 mm L
0045109	MiniChrom TOYOPEARL Super Q-650M	8 mm ID x 100 mm L
0045110	MiniChrom TOYOPEARL SP-650M	8 mm ID x 100 mm L
0045111	MiniChrom TOYOPEARL SP-650S	8 mm ID x 100 mm L
0045112	MiniChrom TOYOPEARL DEAE-650M	8 mm ID x 100 mm L

Hydrophobic Interaction

Part #	Description	Package description
0045121	MiniChrom TOYOPEARL Phenyl-650M, 5 mL	8 mm ID x 100 mm L
0045122	MiniChrom TOYOPEARL Phenyl-650S, 5 mL	8 mm ID x 100 mm L
0045123	MiniChrom TOYOPEARL Phenyl-600M, 5 mL	8 mm ID x 100 mm L
0045124	MiniChrom TOYOPEARL PPG-600M, 5 mL	8 mm ID x 100 mm L
0045125	MiniChrom TOYOPEARL Butyl-650M, 5 mL	8 mm ID x 100 mm L
0045126	MiniChrom TOYOPEARL Butyl-650S, 5 mL	8 mm ID x 100 mm L
0045127	MiniChrom TOYOPEARL Butyl-600M, 5 mL	8 mm ID x 100 mm L

Mixed Mode

Part #	Description	Package description
0045151	MiniChrom TOYOPEARL MX-Trp-650M 5 mL	8 mm ID x 100 mm L

Affinity

Part #	Description	Package description
0045161	MiniChrom TOYOPEARL AF-rProtein A HC-650M 5 mL	8 mm ID x 100 mm L

PROCESS DEVELOPMENT

TOYOPEARL AND TSKgel LABPAK MEDIA

TOYOPEARL and TSKgel LabPak media products are small package sizes of TOYOPEARL and TSKgel bulk media products. Typically they contain three or four different ligand types offered for a particular chromatography mode.

They are useful for developmental scientists and engineers who wish to familiarize themselves with the physical properties of resins in different buffer systems:

- slurry and reslurry mechanics
- resin handling during column packing
- mechanical strength relative to other resin backbones
- degree of compressibility

The larger resin amounts in LabPak products allow the packing of wider bore and longer columns than available in the ToyoScreen products. This helps the developmental scientist or engineer to more accurately determine the resin's:

- dynamic binding capacity
- selectivity
- column efficiency
- operating conditions

ORDERING INFORMATION

TSKgel LABPAKS

Ion Exchange

Part #	*Description*	*Container size*
0043380	IEXPAK PW, 20 µm (DEAE-5PW, SP-5PW, SuperQ-5PW)	3 x 25 mL
0043280	IEXPAK PW, 30 µm (DEAE-5PW, SP-5PW, SuperQ-5PW)	3 x 25 mL

Hydrophobic Interaction

0043278	HICPAK PW, 20 µm (Ether-5PW, Phenyl-5PW)	2 x 25 mL
0043175	HICPAK PW, 30 µm (Ether-5PW, Phenyl-5PW)	2 x 25 mL

TOYOPEARL LABPAKS

Size Exclusion

0019820	SECPAK HP, 30 µm (HW-40, 50, 55, 65S)	4 x 150 mL
0019821	SECPAK LMW, 45 µm (HW-40, 50, 55F)	3 x 150 mL
0019819	SECPAK HMW, 45 µm (HW-55, 65, 75F)	3 x 150 mL

Ion Exchange

0019817	IEXPAK HP, 35 µm (DEAE-650S, SP-650S,CM-650S, SuperQ-650S)	4 x 25 mL
0043210	AIEXPAK, 75/100 µm (GigaCap Q-650M, SuperQ-650M, Q-600C AR)	3 x 100 mL
0043220	CIEXPAK, 75/100 µm (GigaCap CM-650M/ S-650M, SP-550C)	3 x 100 mL

Hydrophobic Interaction

0043150	HICPAK HP, 35 µm (Ether, Phenyl, Butyl-650S)	3 x 25 mL
0019806	HICPAK, 65 µm (Ether, Phenyl, Butyl-650M)	3 x 25 mL
0043125	HICPAK-C, 100 µm (Phenyl, Butyl, Hexyl-650C)	3 x 25 mL

Affinity

0043400	AFFIPAK ACT, 65 µm (AF-Epoxy, Tresyl-650M)	2 x 5 g*
0043410	AFFIPAK, 65 µm (AF-Amino, Carboxyl, Formyl-650 M)	3 x 10 mL

***1 g is approximately 3.5 mL**

INTRODUCTION TO BULK RESINS FOR LABORATORY PURIFICATION

Tosoh Bioscience offers TOYOPEARL and TSKgel resins (media) in bulk quantities for laboratory-scale applications.

Although the resins can be applied to the purification of small as well as large MW compounds, TOYOPEARL and TSKgel resins are most useful for the separation of peptides, proteins, and oligonucleotides.

The focus of this section is on the use of bulk resins in laboratory applications. Please request the Process Chromatography Catalog for information about the use of TOYOPEARL and TSKgel for larger scale separations or visit our website at: www.tosohbioscience.de.

TOYOPEARL BULK RESIN

TOYOPEARL resins are hydrophilic, macroporous media for medium pressure liquid chromatographic applications.

The polymethacrylate backbone structure of TOYOPEARL packings assure excellent pressure/flow characteristics. TOYOPEARL is mechanically stable up to 0.3 MPa, which simplifies column packing by reducing the setup time and improving reproducibility from column to column.

The media is stable over the range of pH 2-12 for normal operating conditions and pH 1-13 for cleaning conditions. In most modes, TOYOPEARL is available in three grades, S (superfine) for highest performance, F (fine) and M (medium) for economical purification, and C (coarse) and EC (extra coarse) for capture. Consult TABLE I for particle sizes associated with the various chemistries and pore sizes.

FEATURES	BENEFITS
chemistries available in Size Exclusion, Ion Exchange, Mixed-Mode, Hydrophobic Interaction and Affinity chromatography	added flexibility during method development
methacrylate backbone has hydrophilic surface properties	less non-specific adsorption
	high recovery of proteins, enzymes, glycoproteins
TSKgel and TOYOPEARL bulk resin product lines feature the same ligand and backbone chemistries from 20 µm to 150 µm particle sizes	simplified scale up from laboratory separation to process
SEC product line available in 5 pore sizes	suitable for fractionation of large and small biopolymers
IEC, HIC and AFC products are based on 100, 75 and 50 nm pore size particles.	high capacity and efficient chromatography of small protein and large biopolymers due to unrestricted access of available surface area
chemical stability	cleanable resins in strong base or acid (pH 1-13)
	compatible with all water soluble organic solvents
	stable in chaotropic agents such as: guanidine hydrochloride, sodium dodecyl sulfate and urea
thermal stability	autoclavable at 120°C
	wide range of operating temperature (4-60°C)
mechanical stability	linear relationship between flow rate and pressure drop
column bed stability	constant bed volume over a wide range of salt concentrations

PROCESS DEVELOPMENT

PROCESS DEVELOPMENT BULK MEDIA

TOYOPEARL HW-type resins, available in pore sizes ranging from 5 nm to >100 nm, are employed in size exclusion chromatography (SEC). TOYOPEARL HW-65 and HW-55 resins are used as starting materials for the production of all other functionalized TOYOPEARL resins. The large pore size of HW-65 (100 nm) allows unhindered access of large proteins to the stationary phase, resulting in faster separation and shorter recycling times.

For predictable results during scale up, TOYOPEARL resins are based on the same chemistry as the prepacked TSKgel columns. This allows for seamless scale up from the laboratory to manufacturing.

TSKgel BULK RESINS

TSKgel resins are larger particle size versions of the chemically equivalent methacrylic packing of analytical-scale TSKgel columns used for protein analysis and purification. The TSKgel resin product line consists of DEAE-5PW, SuperQ-5PW, SP-5PW, and SP-3PW resins for ion exchange, Tresyl-5PW resins for afffinity chromatography, and Ether-5PW and Phenyl-5PW resins for HIC. TSKgel resins are often employed to simplify scale-up from analytical columns, as only the particle size is different. Their small particle sizes, high degree of cross-linking and high mechanical stability make TSKgel resins the preferred choice for high efficiency purifications.

TABLE I

Characteristics of TOYOPEARL and TSKgel media

Mode	Resin	Grade/particle size (µm)	Pore size (nm)**	MW range Proteins (Da)	Operating pH range	Max. pressure (MPa)
SEC	TOYOPEARL HW-40	S (20-40), F (30-60), C (50-100)	5	1×10^2 - 1×10^4	2–12	0.3
	TOYOPEARL HW-50	S (20-40), F (30-60)	12.5	5×10^2 - 8×10^4	2–12	0.3
	TOYOPEARL HW-55	S (20-40), F (30-60)	50	1×10^3 - 7×10^5	2–12	0.3
	TOYOPEARL HW-65	S (20-40), F (30-60)	100	4×10^4 - 5×10^6	2–12	0.3
	TOYOPEARL HW-75	S (20-40), F (30-60)	> 100	5×10^5 - 5×10^7	2–12	0.3
IEC	TSKgel SuperQ-5PW	20 and 30	100	< 5×10^6	2–12	2.0
	TSKgel DEAE-5PW	20 and 30	100	< 5×10^6	2–12	2.0
	TSKgel SP-5PW	20 and 30	100	< 5×10^6	2–12	2.0
	TSKgel SP-3PW	30	25	< 1×10^4	2–12	2.0
	TOYOPEARL SuperQ-650	S (20-50), M (40-90), C (50-150)	100	< 5×10^6	2–12	0.3
	TOYOPEARL DEAE-650	S (20-50), M (40-90), C (50-150)	100	< 5×10^6	2–12	0.3
	TOYOPEARL GigaCap Q-650	S (20-50), M (50-100)	100	< 5×10^6	2–12	0.3
	TOYOPEARL GigaCap DEAE-650	M (50-100)	100	< 5×10^6	2–12	0.3
	TOYOPEARL SP-650	S (20-50), M (40-90), C (50-150)	100	< 5×10^6	2–12	0.3
	TOYOPEARL CM-650	S (20-50), M (40-90), C (50-150)	100	< 5×10^6	2–12	0.3
	TOYOPEARL GigaCap S-650	S (20-50), M (50-100)	100	< 5×10^6	2–12	0.3
	TOYOPEARL GigaCap CM-650	M (50-100)	100	< 5×10^6	2–12	0.3
	TOYOPEARL QAE-550	C (50-150)	50	< 5×10^5	2–12	0.3
	TOYOPEARL Q-600C AR	C (50-150)	75	< 2.5×10^6	2-12	0.3
	TOYOPEARL NH_2-750	F (30-60)	>1000	< 5×10^7	2–12	0.3
	TOYOPEARL SP-550	C (50-150)	50	< 5×10^5	2–12	0.3
	TOYOPEARL MegaCap II SP-550	EC (100-300)	50	<5×10^5	2–12	0.3
MMC	TOYOPEARL MX-Trp-650M	M (50-100)	100	< 5×10^6	2–12	2.0
HIC	TSKgel Ether-5PW	20 and 30	100	< 5×10^6	2–12	2.0
	TSKgel Phenyl-5PW	20 and 30	100	< 5×10^6	2–12	2.0
	TOYOPEARL Ether-650	S (20-50), M (40-90)	100	< 5×10^6	2–12	0.3
	TOYOPEARL PPG-600	M (40-90)	75	< 5×10^6	2–12	0.3
	TOYOPEARL Phenyl-600	M (40-90)	75	< 5×10^6	2–12	0.3
	TOYOPEARL Butyl-600	M (40-90)	75	< 5×10^6	2–12	0.3
	TOYOPEARL Phenyl-650	S (20-50), M (40-90), C (50-150)	100	< 5×10^6	2–12	0.3
	TOYOPEARL Butyl-650	S (20-50), M (40-90), C (50-150)	100	< 5×10^6	2–12	0.3
	TOYOPEARL Super Butyl-550	C (50-150)	50	< 5×10^5	2–12	0.3
	TOYOPEARL Hexyl-650	C (50-150)	100	< 5×10^6	2–12	0.3
AFC	TSKgel Tresyl-5PW	10	100	< 5×10^6	2–12	1.0
	TOYOPEARL AF-Chelate-650	M (40-90)	100	< 5×10^6	2–12	0.3
	TOYOPEARL AF-rProtein A HC-650	F (30-60)	100	< 5×10^6	N/A	0.3
	TOYOPEARL AF-rProtein A	F (30-60)	100	< 5×10^6	N/A	0.3
	TOYOPEARL AF-Tresyl-650	M (40-90)	100	< 5×10^6	N/A	0.3
	TOYOPEARL AF-Epoxy-650	M (40-90)	100	< 5×10^6	N/A	0.3
	TOYOPEARL AF-Formyl-650	M (40-90)	100	< 5×10^6	6-9	0.3
	TOYOPEARL AF-Amino-650	M (40-90)	100	< 5×10^6	2-12	0.3
	TOYOPEARL AF-Carboxy-650	M (40-90)	100	< 5×10^6	2-12	0.3
	TOYOPEARL AF-Red-650	M (40-90)	100	< 5×10^6	4-9	0.3
	TOYOPEARL AF-Blue HC-650	M (40-90)	100	< 5×10^6	4-9	0.3
	TOYOPEARL AF-Heparin HC-650	M (40-90)	100	< 5×10^6	5-10	0.3

** nominal values; Pore size of base matrix

TOYOPEARL BULK RESINS FOR SEC

HIGHLIGHTS

- Pore sizes ranging from 5 nm to >100 nm
- Three particle sizes (S, F, C)
- HW-40 is ideal for desalting applications
- Easy to pack in semi-preparative and process scale columns

Size exclusion chromatography (SEC) is a common technique for separating molecules based on their apparent molecular size. For nearly twenty-five years, TOYOPEARL SEC bulk resins, with their macroporous packings, have been used for laboratory and production-scale biochromatography.

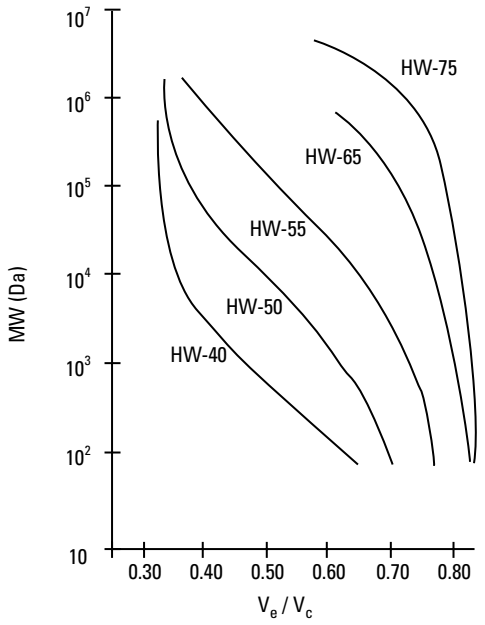
TOYOPEARL SEC resins are semi-rigid, spherical polymethacrylate beads. The resins have hydrophilic surfaces due to the presence of ether and hydroxyl groups. The numerous surface hydroxyl groups provide attachment points for other functional groups and ligands. TABLE II provides an overview of the TOYOPEARL SEC resin product line including corresponding molecular weight ranges of common target samples. Calibration curves of the TOYOPEARL HW-type resins determined with globular proteins are presented in FIGURE 5.

Ordering information for quantities <1 L is provided at the end of this section. For larger quantities, please contact customer service at **+49 (0) 6155 70437-30**. LABPAK kits are also available in popular combinations of TOYOPEARL media. See the page 99 for additional information.

Applications: proteins, peptides, amino acids, nucleic acids, and small molecular weight molecules. Please visit our website: www.tosohbioscience.de for extensive data on applications.

FIGURE 5

Calibration curves for globular proteins on TOYOPEARL HW-type resins



Column: 22 mm ID x 30 cm L; Sample: protein standards; Elution: 0.06 mol/L phosphate buffer, pH 7, in 0.06 mol/L KCl; Legend: Ve=elution volume, Vc=column volume

TABLE II

Properties and molecular weight separation ranges for TOYOPEARL HW-type resins (HW = Hydrophilic, water-compatible polymeric base resins)

			Molecular weight of sample (Da)		
TOYOPEARL resin	**Particle size (µm)**	**Pore size (nm)**	**PEG and PEO**	**Dextrans**	**Globular proteins**
HW-40S HW-40F HW-40C	20 - 40 30 - 60 50 - 100	5 5 5	1×10^2 - 3×10^3	1×10^2 - 7×10^3	1×10^2 - 1×10^4
HW-50S HW-50F	20 - 40 30 - 60	12.5 12.5	1×10^2 - 1.8×10^4	5×10^2 - 2×10^4	5×10^2 - 8×10^4
HW-55S HW-55F	20 - 40 30 - 60	50 50	1×10^2 - 1.5×10^5	1×10^3 - 2×10^5	1×10^3 - 7×10^5
HW-65S HW-65F	20 - 40 30 - 60	100 100	5×10^2 - 1×10^6	1×10^4 - 1×10^6	4×10^4 - 5×10^6
HW-75F	30 - 60	>100	4×10^3 - 5×10^6	1×10^5 - 1×10^7	5×10^5 - 5×10^7

PROCESS DEVELOPMENT

TOYOPEARL AND TSKgel BULK RESINS FOR IEC

HIGHLIGHTS

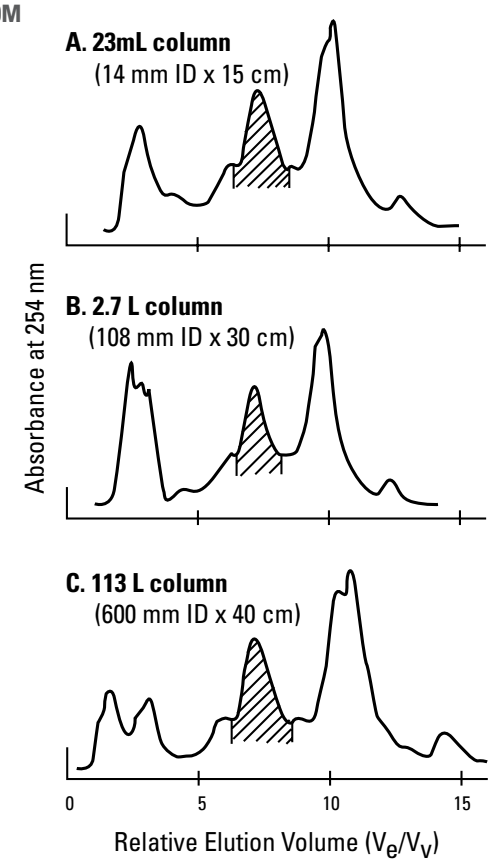
- TOYOPEARL GigaCap®S-650, CM-650M, DEAE-650, and Q-650 resins are high capacity ion exchange resins featuring high dynamic binding capacities for both small molecules like insulin and larger proteins like monoclonal antibodies.
- Salt tolerant TOYOPEARL NH_2-750F anion exchanger with >100 nm pore size.
- Weak and strong anion and cation exchangers are offered in both product lines.
- Standard 100 nm pore size for large biopolymers and 50 nm pore size packing for optimal binding capacity are available.
- High efficiency TSKgel resins scale up directly from TSKgel analytical columns.

For separating mixtures of biomolecules, Ion Exchange Chromatography (IEC) is known for its high resolution and high capacity. It is very effective in the initial capture step of a chromatography process. IEC is also useful for further purification and/or polishing. It can complement other chromatographic techniques in the design of an economical downstream purification process. IEC is often used as a purification step before HIC, SEC, and RPC. IEC will also purify and concentrate the target molecule in one step when the sample is diluted. This also allows it to be used as a concentration step after SEC. A 5000-fold scale-up of a α-galactosidase enzyme purification was accomplished using TOYOPEARL DEAE-650M. The chromatograms in FIGURE 6 demonstrate the excellent scale up characteristics of TOYOPEARL ion exchange media. Gradient slope and particle diameter remained unchanged. Linear velocity was reduced by 15% in the largest scale separation, and resolution actually improved relative to the smallest scale separation. This may be partly attributed to increased bed height and the slower linear velocity. Although the column volume was increased in part by increasing the bed height, the principal change in column volume was a result of the greater column diameter (1.4 cm to 60 cm L). This example illustrates how TOYOPEARL media can be conveniently scaled up from laboratory to production scale applications using the same particle size if desired.

Because the correct choice of an ion exchange resin can have a considerable impact on the economy of a process, Tosoh Bioscience provides many product options in both TOYOPEARL and TSKgel IEC bulk polymeric media. See TABLE III for a complete listing of available particle sizes. Ordering information for quantities < 1 L is provided at the end of this section.

FIGURE 6

Process scale-up purification of β-galactosidase with TOYOPEARL DEAE-650M



Column: TOYOPEARL DEAE-650 M; Sample: 1% β-galactosidase: A. 8 mL; B. 1L; C. 40L Elution: linear gradient from 0.03 to 0.10 mol/L NaCl in 0.014 mol/L Tris-HCl (pH7.7); Flow rate: A. 1.0 mL/min; B. 60 mL/min; C. 1.6 L/min; Linear velocity: A. 39 cm/h; B. 40 cm/h; C. 34 cm/h; Detection: UV@254nm

TABLE III

TOYOPEARL and TSKgel Ion Exchange resins

Description	Type*	Part. size (µm)
Anion Exchange		
TSKgel DEAE-5PW	W	20, 30
TSKgel SuperQ-5PW	S	20, 30
TOYOPEARL DEAE-650	W	35, 65, 100
TOYOPEARL SuperQ-650	S	35, 65, 100
TOYOPEARL QAE-550	S	100
TOYOPEARL Q-600 AR	S	100
TOYOPEARL GigaCap Q-650M	S	35, 75
TOYOPERL GigaCap DEAE-650M	W	75
TOYOPEARL NH_2-750F	S	45
Cation Exchange		
TSKgel SP-5PW	S	20, 30
TSKgel SP-3PW	S	30
TOYOPEARL CM-650	W	35, 65, 100
TOYOPEARL GigaCap CM-650M	W	75
TOYOPEARL SP-550	S	100
TOYOPEARL SP-650	S	35, 65, 100
TOYOPEARL MegaCap II SP-550EC	S	100-300
TOYOPEARL GigaCap S-650M	S	35, 75

**W = Weak; S = Strong*

TOYOPEARL AND TSKgel BULK RESINS FOR MIXED-MODE CHROMATOGRAPHY

HIGHLIGHTS

- TOYOPEARL MX-Trp-650M is a multimodal cation exchange resin
- It provides high binding capacity for IgG and other proteins
- It tolerates high conductivity feedstocks
- Target molecules elute under mild conditions in sharp peaks

Multimodal or mixed-mode chromatography expands the range of chromatographic modes applied in biopurification. Mixed-mode media combine ionic and hydrophobic interactions and offer new selectivities and a higher salt tolerance than traditional ion exchange media. Mixed-mode media can be used for direct processing of clarified feedstocks at physiological salt concentrations as well as for intermediate and polishing applications. The salt tolerance of the recently introduced TOYOPEARL NH_2-750F anion exchange resin is to a certain extent also based on mixed-mode interactions. Nevertheless, this resin is listed in the ion exchange section.

TOYOPEARL MX-Trp-650M is a multimodal cation exchange resin with unique selectivity and high recovery. It provides high protein binding capacities and tolerates high conductivity feedstocks. In addition to ionic groups its ligand also carries hydrophobic regions. Thus, the binding of target molecules is determined by electrostatic and hydrophobic contributions. TOYOPEARL MX-Trp-650M is especially suited for the purification of target molecules that are difficult to purify using common purification platforms.

FIGURE 7

TOYOPEARL MX-Trp-650M STRUCTURE

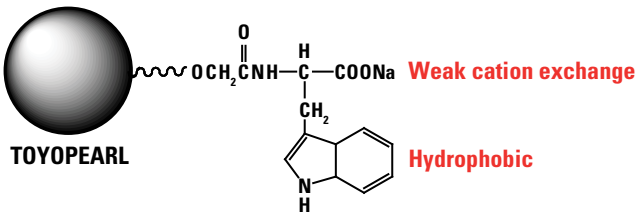
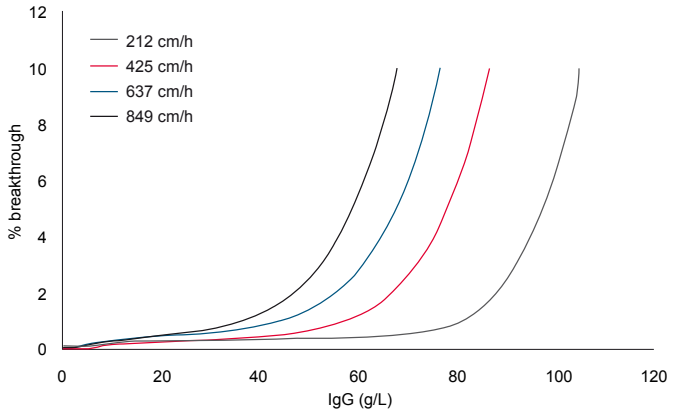


FIGURE 8

IgG binding capacity



Column: TOYOPEARL MX-Trp-650M (6 mm ID x 4 cm);
Sample: polyclonal human IgG (1 mg/mL) in 0.05 mol/L NaAc + 0.1 mol/L sodium chloride (pH 4.7); Linear velocity: 212, 425, 637, 849 cm/h;
Detection: UV @ 280 nm

PROCESS DEVELOPMENT

TOYOPEARL AND TSKgel BULK RESINS FOR HIC

HIGHLIGHTS

- A wide range of hydrophobicities is suitable for most proteins.
- Standard 100 nm pore size is available for large biopolymers, and three Butyl pore sizes (50 nm, 75 nm and 100 nm) are available.
- TOYOPEARL "600M" series of HIC resins with optimized pore size of 75 nm for antibody separation. Phenyl-600M and Butyl-600M with highest DBCs for IgG.
- Seamless scale up from high efficiency TSKgel 5PW-type analytical columns is possible.

Hydrophobic Interaction Chromatography (HIC) has become a popular mode of chromatography for the purification of biopolymers at analytical as well as preparative scale. This is accomplished by the interaction of hydrophobic ligands on the base matrix with the hydrophobic areas located on the surface of proteins. HIC is an excellent complement to size exclusion and ion exchange chromatography in difficult separations, particularly those where the contaminants are of similar pI or molecular weight. It is often preferred over reversed phase chromatography when preservation of biological activity of the protein is of utmost importance.

Tosoh Bioscience offers both the TSKgel and TOYOPEARL resin product lines for HIC. See TABLE IV for a complete listing of functionalities. Each product line has similar backbone chemistry. TSKgel 5PW-type resins possess a higher degree of cross-linking than the corresponding TOYOPEARL resins. Additionally, choices in particle size are offered to match the desired resolution and throughput. A variety of HIC bulk media are offered as LABPAK kits in quantities < 1 L and in a combination of resins with varying functionalities. Additionally, HIC media are available in ToyoScreen process development columns for convenient scouting and methods development.

Ordering information for quantities < 1 L is provided at the end of this section.

TABLE IV
TOYOPEARL and TSKgel HIC resins

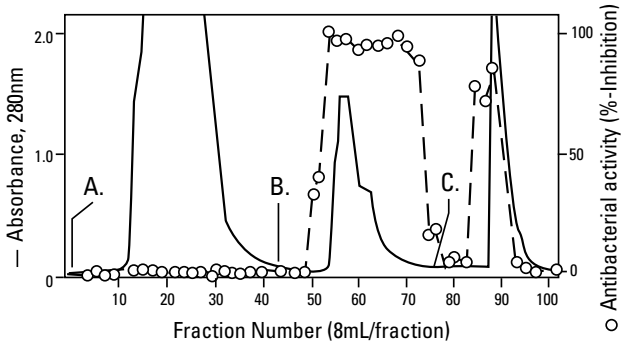
Description	Strength*	Part. size grades (µm)
TSKgel Ether-5PW	1	20, 30
TOYOPEARL Ether-650	1	35, 65
TOYOPEARL PPG-600	2	65, 100
TSKgel Phenyl-5PW	3	20, 30
TOYOPEARL Phenyl-650	3	35, 65, 100
TOYOPEARL Phenyl-600	4	65
TOYOPEARL Butyl-600	4	65
TOYOPEARL Butyl-650	4	35, 65, 100
TOYOPEARL SuperButyl-550	4	100
TOYOPEARL Hexyl-650	5	100

* Relative scale: 1 = least hydrophobic, 5 = most hydrophobic.

APPLICATIONS:

HIC resins can be applied to separate/purifiy proteins with similar chemical or structural properties, plasmids and monoclonal antibodies. See FIGURE 9 for separation of large glycoprotein from crude extract on TOYOPEARL Butyl-650S. Please visit our website: www.tosohbioscience.de for extensive application data.

FIGURE 9
Large glycoprotein purified on TOYOPEARL Butyl-650S



Column: TOYOPEARL Butyl-650S, 22 mm ID x 26 cm L;
Sample: crude protein from sea hare *Aplysia kurodai*;
Elution: multi-step $(NH_4)_2SO_4$ in 50 mmol/L phosphate buffer, pH 7.0 A. load & wash: 40 % saturated $(NH_4)_2SO_4$
B. 20% saturated $(NH_4)_2SO_4$
C. 0% saturated $(NH_4)_2SO_4$

PROCESS DEVELOPMENT

TOYOPEARL RESINS FOR AFC

HIGHLIGHTS

- New AF-rProtein A-HC 650F resin for antibody purification.
- Active, reactive and group specific resins
- Provided in standard 100 nm pore size for high capacity of large biopolymers.
- TOYOPEARL AF-Blue HC-650M is available for albumin and interferon applications with the lowest leaching blue.
- TOYOPEARL AF-Heparin HC-650M high capacity resin exhibits an Antithrombin III dynamic capacity of 4 mg/mL.

TOYOPEARL media for Affinity Chromatography (AFC) are based on TOYOPEARL HW-65 resin and functionalized with either chemically active groups or group-specific ligands. Resins with activated functional groups are ready for direct coupling of a protein or other ligand, while resins with reactive groups employ coupling or reductive amination to achieve covalent bonding. The 100 nm pore size common to all TOYOPEARL affinity resins accommodates proteins up to 5,000,000 Da.

In general, TOYOPEARL AF-Tresyl-650M and AF-Formyl-650M are recommended for coupling proteins, while AF-Epoxy-650M is suited for coupling low molecular weight ligands. TOYOPEARL AF-Amino-650M and TOYOPEARL AF-Carboxy-650M may be used in either application. TOYOPEARL AF-Heparin HC-650M interacts with a wide range of biomolecules including plasma components, lipoprotein lipase, collagenase, and DNA polymerase. The structures of TOYOPEARL activated and reactive ligands are given in FIGURE 10, while the structures of TOYOPEARL group-specific ligands are listed in FIGURE 11.

FIGURE 10

Activated and reactive TOYOPEARL affinity resins

Toyopearl AF-Tresyl-650M (1) HW-65—O-R-O-SO_2-CH_2-CF_3
Ligand Density: 80 mol/g (dry)

Toyopearl AF-Epoxy-650M (1) HW-65—O-R-O-CH_2-CH-CH_2 (O)
Ligand Density: 800 mol/g (dry)

Toyopearl AF-Formyl-650M (2) HW-65—O-R-O-CH_2-CHO
Ligand Density: 60 eq/mL

Toyopearl AF-Amino-650M (3) HW-65—O-R-O-CH_2-CHOH-CH_2NH_2
Ligand Density: 100 eq/mL

Toyopearl AF-Carboxy-650M (3) HW-65—O-R-O-CH_2-COOH
Ligand Density: 100 eq/mL

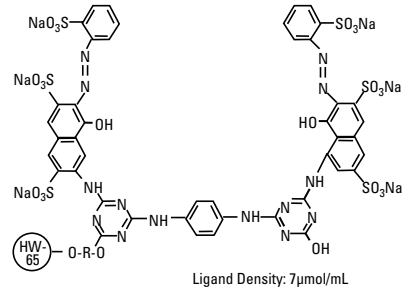
(1) Provided as dry, free-flowing powder.
One gram of dry powder produces about 3.5 mL of hydrated resin.
(2) Provided as aqueous slurry, containing 1% gluteraldehyde.
(3) Provided as aqueous slurry, containing 20% ethanol.

TOYOPEARL AF-rProtein A HC-650F designed for efficient and robust purification of antibodies. The newly developed recombinant protein A ligands are derived from one of the IgG-binding domains of the staphylococcus aureus protein A (FIGURE 12). TOYOPEARL AF-rProtein A HC-650F binds immunoglobulin G with high binding capacity and at high flow rates. This reduces column and buffer volumes and allows fast loading procedures.

FIGURE 11

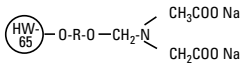
Group-specific TOYOPEARL affinity resins

Toyopearl AF-Red-650M (1)



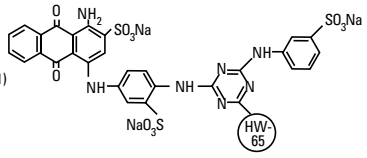
Ligand Density: 7µmol/mL

Toyopearl AF-Chelate-650M (2)

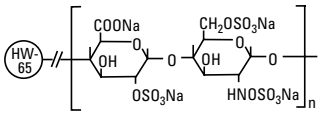


Ligand Density: 20µmol/mL

Toyopearl AF-Blue HC-650M (1)



Toyopearl AF-Heparin HC-650

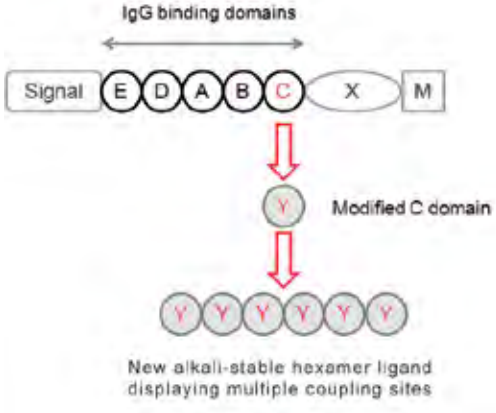


Approximate Ligand Density: 5mg/mL

(1) Provided as an aqueous slurry containing 20% ethanol, v/v in 1mol/L NaCl.
(2) Provided as an aqueous slurry containing 20% ethanol.

FIGURE 12

Recombinant Protein A derived ligand



ORDERING INFORMATION

A. Size Exclusion Chromatography

TOYOPEARL bulk resins

Part #	Description	Container size
0019809	HW-40S, 30 µm	150 mL
0007451	HW-40S, 30 µm	250 mL
0019808	HW-40F, 45 µm	150 mL
0007448	HW-40F, 45 µm	500 mL
0019807	HW-40C, 75 µm	150 mL
0007449	HW-40C, 75 µm	500 mL
0019811	HW-50S, 30µm	150 mL
0007455	HW-50S, 30µm	250 mL
0019810	HW-50F, 45 µm	150 mL
0007453	HW-50F, 45 µm	500 mL
0019813	HW-55S, 30 µm	150 mL
0007459	HW-55S, 30 µm	250 mL
0019812	HW-55F, 45 µm	150 mL
0007457	HW-55F, 45 µm	500 mL
0019815	HW-65S, 30 µm	150 mL
0007467	HW-65S, 30 µm	250 mL
0019814	HW-65F, 45 µm	150 mL
0007465	HW-65F, 45 µm	500 mL
0021481	HW-65C, 75 µm	150mL
0007466	HW-65C, 75 µm	500mL
0019816	HW-75F, 45 µm	150 mL
0007469	HW-75F, 45 µm	500 mL

B. Anion Exchange Chromatography

TSKgel bulk resins

0043381	DEAE-5PW (20)	25 mL
0014710	DEAE-5PW (20)	250 mL
0043281	DEAE-5PW (30)	25 mL
0014712	DEAE-5PW (30)	250 mL
0043383	SuperQ-5PW (20)	25 mL
0018535	SuperQ-5PW (20)	250 mL
0043283	SuperQ-5PW (30)	25 mL
0018536	SuperQ-5PW (30)	250 mL

TOYOPEARL bulk resins

0019823	SuperQ-650S, 35 µm	25 mL
0017223	SuperQ-650S, 35 µm	250 mL
0043205	SuperQ-650M, 65 µm	100 mL
0017227	SuperQ-650M, 65 µm	250 mL
0043275	SuperQ-650C, 100 µm	100 mL
0017231	SuperQ-650C, 100 µm	250 mL
0043271	QAE-550C, 100 µm	100 mL
0014026	QAE-550C, 100 µm	250 mL
0021985	Q-600C AR, 100 µm	100 mL
0021986	Q-600C AR, 100 µm -	250 mL
0019804	DEAE-650S, 35 µm	25 mL
0007472	DEAE-650S, 35 µm	250 mL
0043201	DEAE-650M, 65 µm	100 mL
0007473	DEAE-650M, 65 µm	250 mL
0007988	DEAE-650C, 100 µm	250 mL
0022865	GigaCap DEAE-650M, 75 µm	100 mL
0022866	GigaCap DEAE-650M, 75 µm	250 mL
0022881	GigaCap Q-650S, 35 µm	25 mL
0022882	GigaCap Q-650S, 35 µm	250 mL
0021854	GigaCap Q-650M, 75 µm	100 mL
0021855	GigaCap Q-650M, 75 µm	250 mL
0023438	NH_2-750F, 45 µm	100 mL
0023439	NH_2-750F, 45 µm	250 mL

C. Cation Exchange Chromatography

TSKgel bulk resins

0043382	SP-5PW (20)	25 mL
0014714	SP-5PW (20)	250 mL
0043282	SP-5PW (30)	25 mL
0014716	SP-5PW (30)	250 mL
0021976	SP-3PW (30)	25 mL
0021977	SP-3PW (30)	250 mL

TOYOPEARL bulk resins

0019803	CM-650S, 35 µm	25 mL
0007474	CM-650S, 35 µm	250 mL
0043203	CM-650M, 65 µm	100 mL
0007475	CM-650M, 65 µm	250 mL
0007991	CM-650C, 100 µm	250 mL
0019822	SP-650S, 35 µm	25 mL
0008437	SP-650S, 35 µm	250 mL
0043202	SP-650M, 65 µm	100 mL
0007997	SP-650M, 65 µm	250 mL
0007994	SP-650C, 100 µm	250 mL
0043272	SP-550C, 100 µm	100 mL
0014028	SP-550C, 100 µm	250 mL
0021804	MegaCap II SP-550EC, 160 µm	100 mL
0021805	MegaCap II SP-550EC, 160 µm	250 mL
0022875	GigaCap S-650S, 35 µm	25 mL
0022876	GigaCap S-650S, 35 µm	250 mL
0021833	GigaCap S-650M, 75 µm	100 mL
0021834	GigaCap S-650M, 75 µm	250 mL
0021946	GigaCap CM-650M, 75 µm	100 mL
0021947	GigaCap CM-650M, 75 µm	250 mL

D. Mixed-Mode

TOYOPEARL bulk resins

0022817	MX-Trp-650M, 75 µm	25 mL
0022818	MX-Trp--650M, 75 µm	100 mL

PROCESS DEVELOPMENT BULK RESINS

ORDERING INFORMATION

E. Hydrophobic Interaction Chromatography

Part #	Description	Container size
TSKgel bulk resins		
0043276	Ether-5PW (20)	25 mL
0016052	Ether-5PW (20)	250 mL
0043176	Ether-5PW (30)	25 mL
0016050	Ether-5PW (30)	250 mL
0043277	Phenyl-5PW (20)	25 mL
0014718	Phenyl-5PW (20)	250 mL
0043177	Phenyl-5PW (30)	25 mL
0014720	Phenyl-5PW (30)	250 mL
TOYOPEARL bulk resins		
0019955	SuperButyl-550C, 100 µm	25 mL
0019956	SuperButyl-550C, 100 µm	100 mL
0021448	Butyl-600M, 65 µm	25 mL
0021449	Butyl-600M, 65 µm	100 mL
0043153	Butyl-650S, 35 µm	25 mL
0007476	Butyl-650S, 35 µm	100 mL
0019802	Butyl-650M, 65 µm	25 mL
0007477	Butyl-650M, 65 µm	100 mL
0043127	Butyl-650C, 100 µm	25 mL
0007478	Butyl-650C, 100 µm	100 mL
0043151	Ether-650S, 35 µm	25 mL
0016172	Ether-650S, 35 µm	100 mL
0019805	Ether-650M , 65 µm	25 mL
0016173	Ether-650M , 65 µm	100 mL
0044465	Hexyl-650C, 100 µm	25 mL
0019026	Hexyl-650C, 100 µm	100 mL
0021887	Phenyl-600M, 65 µm	25 mL
0021888	Phenyl-600M, 65 µm	100 mL
0043152	Phenyl-650S, 35 µm	25 mL
0014477	Phenyl-650S, 35 µm	100 mL
0019818	Phenyl-650M, 65 µm	25 mL
0014478	Phenyl-650M, 65 µm	100 mL
0043126	Phenyl-650C, 100 µm	25 mL
0014479	Phenyl-650C, 100 µm	100 mL
0021301	PPG-600M, 65 µm	25 mL
0021302	PPG-600M, 65 µm	100 mL

F. Affinity Chromatography

Part #	Description	Container size
TSKgel bulk resins		
0016208	Tresyl-5PW (10)	2 g*
TOYOPEARL bulk resins		
0023425	AF-rProtein A HC-650F, 45 µm	10 mL
0023426	AF-rProtein A HC-650F, 45 µm	25 mL
0023427	AF-rProtein A HC-650F, 45 µm	100 mL
0022803	AF-rProtein A-650F, 45 µm	10 mL
0022804	AF-rProtein A-650F, 45 µm	25 mL
0022805	AF-rProtein A-650F, 45 µm	100 mL
0043411	AF-Amino-650M, 65 µm	10 mL
0008002	AF-Amino-650M, 65 µm	25 mL
0008039	AF-Amino-650M, 65 µm	100 mL
0019688	AF-Blue HC-650M,65 µm	25 mL
0019689	AF-Blue HC-650M, 65 µm	100 mL
0043412	AF-Carboxy-650M, 65 µm	10 mL
0008006	AF-Carboxy-650M, 65 µm	25 mL
0008041	AF-Carboxy-650M, 65 µm	100 mL
0014475	AF-Chelate-650M, 65 µm	25 mL
0019800	AF-Chelate-650M, 65 µm	100 mL
0043402	AF-Epoxy-650M, 65 µm	5 g*
0008000	AF-Epoxy-650M, 65 µm	10 g*
0008038	AF-Epoxy-650M, 65 µm	100 g*
0043413	AF-Formyl-650M, 65 µm	10 mL
0008004	AF-Formyl-650M, 65 µm	25 mL
0008040	AF-Formyl-650M, 65 µm	100 mL
0020030	AF-Heparin-HC-650M, 65 µm	10 mL
0020031	AF-Heparin-HC-650M, 65 µm	100 mL
0008651	AF-Red-650M, 65 µm	25 mL
0019801	AF-Red-650M, 65 µm	100 mL
0014471	AF-Tresyl-650M, 65 µm	5 g*
0014472	AF-Tresyl-650M, 65 µm	100 g*

*1 g is approximately 3.5 mL


TOYOPEARL®
PPG-600M
TOYOPEARL®
AF-rProtein A HC-650F
TOYOPEARL®
SP-650S
TOYOPEARL®
DEAE-650C
TOYOPEARL®
Butyl-650M
TOYOPEARL®
GigaCap S-650M
TOYOPEARL®
Q-600C AR

APPENDIX

APPENDIX A

ABOUT TSKgel COLUMNS, THEIR MAINTENANCE AND SCALE UP

Tosoh Corporation closely monitors all stages of the manufacturing process for chromatographic media that is used to pack TSKgel columns. Packing materials are produced in large gel batches which must pass stringent quality control specifications for particle size distribution, pore size distribution, pore volume, and surface area. After producing the particles, each lot is then used to prepare multiple batches of bonded phase by attaching the appropriate ligand. Each gel lot is again tested to ensure that it meets the specifications for parameters such as ligand density, retention, selectivity, etc.

TSKgel columns are designed for general purpose HPLC or FPLC applications. They are not guaranteed to work for specific customer applications. Suitability of a column has to be determined by the end user. Good Laboratory Practice (GLP) demands that a rugged method must be developed by testing at least three different gel lots to understand the type of variability in retention and selectivity that may be encountered with future columns.

Tosoh Bioscience recommends that shipments are inspected for the presence of the Inspection Data sheet, Operating Conditions and Specifications (OCS) sheet, and column appearance. After review of the shipping contents, the column should be tested within 30 days according to the conditions listed in the Inspection Data sheet to confirm that the column meets the specifications listed in the OCS sheet.

TROUBLESHOOTING COLUMN PROBLEMS

Listed below are the five most common causes of poor column performance and the precautions that must be taken to prevent these problems:

1. VOID OR DEAD SPACE AT THE COLUMN INLET OR CHANNELING OF THE PACKING

Sudden pressure surges and higher than recommended flow rates can compress the column packing, which can result in a void or a channel, especially with large pore size columns such as TSKgel G4000SW and TSKgel G4000SW$_{XL}$. We recommend using an injector that ensures continuous flow onto the column during injection, i.e., no pressure pulse due to interrupted flow, and installation of a pulse dampener to suppress the sudden pressure surges encountered with quick-return pumps.

Bulk packing material is available to refill voids in some of the analytical and semi-preparative columns. We highly recommend the use of a guard column to protect your analytical column from pressure surges and to prevent irreversibly binding impurities from reaching the analytical column. A guard column also helps to neutralize the pH of the sample solvent if it is different from that of the mobile phase. The pH of the sample will be equilibrated with the mobile phase before it reaches the analytical column. This is particularly important in the silica-based SW-type columns because this silica-type is not stable at a pH higher than 7.5.

2. AIR IN COLUMN

The column should be tightly capped when not in use to prevent air from entering it. Air dissolved in the mobile phase must be removed before it can enter the column. This is particularly important for polymer-based columns. Air can be removed by sparging with helium, mobile phase filtration or other degassing procedures. If air does enter the column, follow the rehydration procedure described on page 107.

3. COLUMN CONTAMINATION OR INCOMPLETE SAMPLE RECOVERY

Cleaning conditions for all column types are provided on the OCS sheets that are shipped with each column. Cleaning solvents are discussed in the cleaning section below.

4. FRIT PLUGGING AND HIGH PRESSURE

Solvents and samples should be filtered through at least a 0.45 µm filter to prevent clogging the column frits. If the frit becomes partially plugged, the result may be split peaks or high pressure. The entire end-fitting can be removed and sonicated in 6 M nitric acid. Rinse the end-fitting thoroughly after cleaning. (Be careful not to disturb the packing.) Alternatively, this end-fitting can be replaced. Installing a membrane filter prior to the injector is recommended to prevent particles created by pump seal wear from reaching the analytical column. Consult the price list for these and other hardware products.

5. PEAK SPLITTING

Column overload, whether in volume or concentration, can cause peak splitting and poor resolution. Consult the sample capacity information for each column type to determine the appropriate concentration and volume of analyte.

CLEANING

Columns should be cleaned at regular intervals. The frequency depends on the purity of the samples. Occasionally, samples are run which adsorb onto the packing material. If one of the performance characteristics (asymmetry factor, retention time, theoretical plates, or resolution) changes by 10% or more, it is prudent to clean the column.

A Data Inspection sheet and an Operating Conditions and Specifications (OCS) sheet accompanies all TSKgel columns. The Data Inspection sheet identifies the testing method that was used to verify the column's performance. The column's specifications are listed on the OCS sheet. However, a well resolved sample component could be used to monitor the column. Establish that the column is performing properly using the standard test probes listed on the Data Inspection sheet. Calculate the asymmetry factor, theoretical plates and resolution of one or more of the sample components. Note the retention time. This becomes the baseline test mix which provides a basis for comparison.

APPENDIX

BASIC RULES FOR CLEANING TSKgel COLUMNS - ALL TYPES

1. Clean the column in the reverse flow direction.
2. During cleaning, do not connect the column to the detector.
3. Run the column at half the maximum flow rate making sure to monitor the pressure.
4. If cleaning with a high or low pH solution, make certain that the rest of the chromatographic system (pump, pump seals, injector, etc.) is compatible.
5. Use at least 5 column volumes (CV) of each cleaning solution and rinse with 5 CV of ultra pure water between each cleaning step.
6. Equilibrate with 5 CV of the mobile phase for the method.

Each type of TSKgel column has a recommended set of cleaning solutions specific to the column, as described below and on the OCS sheet. Choose a cleaning solution based upon the column and sample type. In general low pH salt solution will remove basic proteins, and organics will remove hydrophobic proteins. Chaotropic agents will remove strongly adsorbed materials (e.g. hydrogen bonded). For columns or column types not listed below, please contact Tosoh Bioscience Technical Service Specialists at **+49 (0) 711 13257-57**.

CLEANING SOLUTIONS

SIZE EXCLUSION, TSKgel SW AND SW$_{XL}$ TYPES

1. Concentrated salt (e.g. 0.5 mol/L Na_2SO_4) at low pH (e.g. pH 3.0)
2. Water soluble organic (MeOH, ACN, EtOH, 10 % - 20 %) in aqueous buffer
3. Note: Detergents are difficult to remove. They require rinsing with 20 to 40 CV of 20% ACN. Therefore they should be used only when the previous cleaning solutions are not effective.
   Buffered solutions of SDS (0.1 %), urea (8 mol/L), or guanidin (6 M)

SIZE EXCLUSION, TSKgel PW AND PW$_{XL}$ TYPES

1. High concentration salt (e.g. 0.5 mol/L - 1.0 mol/L Na_2SO_4) in aqueous buffer
2. Buffered solutions at low pH (e.g. 2 - 3) or high pH (e.g. 11 - 12)
3. Water soluble organic (MeOH, ACN, EtOH, 10% - 20%) in aqueous buffer
4. Note: Detergents are difficult to remove. They require rinsing with 20 to 40 CV of 20% ACN. Therefore they should be used only when the previous cleaning solutions are not effective.
   Buffered solutions of SDS (0.1 %), urea (8 mol/L), or guanidine (6 mol/L).

ION EXCHANGE, TSKgel SW-TYPE

1. High concentration salt (e.g. 0.5 mol/L - 1.0 mol/L Na_2SO_4) in aqueous buffer
2. Buffered solutions at low pH (e.g. 2 - 3)
3. Water soluble organic (MeOH, ACN, EtOH, 10% - 20%) in aqueous buffer
4. Note: Chaotropic agents are difficult to remove. They require rinsing with 20 to 40 CV of 20% ACN. Therefore they should be used only when the previous cleaning solutions are not effective.
   Urea (8 mol/L) or non-ionic surfactant in buffer solution.

ION EXCHANGE, TSKgel PW-TYPE

1. Inject up to 1 CV in 250 µL increments of 0.1 mol/L - 0.2 mol/L NaOH on analytical columns. Inject proportionally larger volumes on semi-preparative columns.
2. 20 % - 40 % aqueous acetic acid* (Since acid can precipitate protein it should be used after other cleaning methods.)
3. Water soluble organic (MeOH, ACN, EtOH, 10% - 20%) in aqueous buffer
4. Note: Chaotropic agents are difficult to remove. They require rinsing with 20 to 40 CV of 20% ACN. Therefore they should be used only when the previous cleaning solutions are not effective.
   Urea (8 mol/L) or non-ionic surfactant in buffer solution.

Note: Rinse Ion Exchange columns with 5 CV of the appropriate solution to restore the correct counter-ion before equilibrating with loading buffer.

HYDROPHOBIC INTERACTION, TSKgel PW-TYPE

1. 0.1 mol/L - 0.2 mol/L NaOH*
2. 20 % - 40 % aqueous acetic acid* (Since acid can precipitate protein it should be used after other cleaning methods.)

REVERSED PHASE, SILICA-BASED

1. 100% acetonitrile or methanol
2. Gradient from 10% - 100% acetonitrile in 0.05% trifluoro- acetic acid

REVERSED PHASE, POLYMER-BASED

1. 100 % acetonitrile or methanol
2. 0.1 mol/L - 0.2 mol/L NaOH*
3. 20 % - 40 % aqueous acetic acid* (Since acid can precipitate protein it should be used after other cleaning methods.)

HILIC, TSKgel SW-TYPE

1. Water
2. 45 % acetonitrile or acetone
3. 0.1 % triethylamine in at least 75 % acetonitrile
4. 50 mmol/L phosphate buffer pH 6.0 in 50 % acetonitrile

Affinity Columns, TSKgel PW-type

Consult the OCS sheet of the specific column type for cleaning directions.

**Inject up to 1 CV in 250 µL increments of solutions 2 & 3 on analytical columns. Inject proportionally larger volumes on semi-preparative columns.*

APPENDIX

GUARDING YOUR COLUMN

GLP procedures often specify that the separation column be protected by a guard column. The guard column is installed between the injector and the analytical column. It is designed to protect the analytical column from unwanted materials, such as highly retained or irreversibly adsorbed compounds and particulate matter. Tosoh Bioscience supplies an assortment of packed guard columns, guardgel kits, guard cartridges, and guardfilters.

Guardgel kits contain the hardware and the gel packing material to fill a guard column using an aspirator. In addition, step-by-step instructions are avaible on the Tosoh Bioscience YouTube channel (www.youtube.com/tosohbiosciencellc). Figure 1 is an example of a guardgel kit, in this case for a TSKgel DEAE-5PW column.

FIGURE 1
Guardgel kit

Guard cartridges (Figure 2) are pre-packed, small replaceable columns easily inserted into a hand-tight guard cartridge holder (Figure 3).

FIGURE 2
Guard cartridges

FIGURE 3
Guard cartridge holder

Guardfilters (Figure 4) are pre-packed, small replaceable columns easily inserted into a hand-tight guardfilter holder (Figure 5).

FIGURE 4
Guard filter

FIGURE 5
Guardfilter holder

For those columns where a guard product is not available, Tosoh Bioscience recommends the use of an in-line filter with a 0.5 µm cutoff to avoid frequent plugging of the 1.0 µm pores in the column frit of TSKgel ODS-140HTP, Super-ODS, Super-Octyl, and Super-Phenyl columns. A pre-injector membrane filter is also recommended to prevent particles generated by pump seal wear from reaching the column.

REHYDRATION

Dehydration of TSKgel liquid chromatography columns can occur during long-term storage or from improper use. Dehydration can also occur if the plugs are not tightened or if air inadvertently is pumped into the column during use. It is easier to detect dehydration in glass columns because the dry packing will appear to pull away from the column walls. This condition can be remedied by using the following procedure:

1. Connect the column to your LC system in the reverse flow direction.
2. Do not connect the column to the detector.
3. Pump a filtered mobile phase of 20 % methanol in ultrapure water over the column at half of the recommended maximum flow rate.
   Note: reversed phase columns require 60 % methanol.
4. Continue this procedure until the column has been rehydrated. Rehydration can take several hours, depending on the column size.
5. Connect the column to the LC system in the proper flow direction.
6. Rinse with 3 column volumes (CV) of ultra pure water to remove the organic if it is not part of the normal mobile phase.
7. Equilibrate with loading buffer (usually 3-5 CV).
8. Perform the recommended QC tests to ensure that the column is performing properly. Evaluation methods are available from the Technical Service Department of Tosoh Bioscience.

APPENDIX

COLUMN STORAGE

When the column will be used the next day, allow it to run overnight at a low flow rate in a buffer that does not contain a halide salt. When the column will not be used for more than a day, clean it first, then flush salt from the column and store in 0.05 % sodium azide or 20 % ethanol. Seal tightly to prevent the column from drying out.

SCALING UP

FOR SIZE EXCLUSION CHROMATOGRAPHY

Tosoh Bioscience offers semi-preparative (21.5 mm ID), preparative (55 mm ID), and larger ID stainless steel columns packed with TSKgel SW-type or PW-type resin for seamless scale-up to commercial production of therapeutic proteins and other biopharmaceuticals. These packing materials have a larger particle size that is appropriate for use in process scale equipment. The packing materials, however, have the same pore size and provide the same selectivity as the corresponding TSKgel analytical column. The column volume (CV) of the preparative column that is needed to produce the required amount of product (per injection) is given by the relationship:

$(CV)_{pc} / (CV)_{ac} = (\text{mg product})_{pc} / (\text{mg product})_{ac}$

in which pc and ac refer to the preparative and analytical column respectively. The volume of a column is equal to $^1/4\ \pi\ (ID)^2L$, in which ID is the internal diameter and L the length of the column. In scaling up, column length (L) is usually kept constant. If so, to achieve a 100-fold increase in product per run, the ID of the prep column should be 10 times larger than that of the analytical column. As noted, the particle size in the preparative column is usually larger, and one should select a larger ID column than predicted by the above equation. As a rule of thumb, a 2-fold increase in particle size reduces resolution and thus output by the square root of 2.

Since scale-up from analytical columns is relatively straightforward, preparative TSKgel SW columns may be an economical route for the rapid production of biomolecules for clinical testing. See the SEC section of this catalog for more information and request a copy of the process media catalog. For more detailed analysis of your scale-up requirements, please contact Tosoh Bioscience's Technical Service Specialists .

FOR HYDROPHOBIC INTERACTION AND ION EXCHANGE CHROMATOGRAPHY

Tosoh Bioscience provides various ID preparative columns for hydrophobic interaction (HIC) and ion exchange (IEC) chromatography. As shown above, to calculate the sample capacity of a larger column, multiply the capacity obtained on a 7.5 mm ID column by the ratio of the column volumes. The table below lists the column volumes for TSKgel HIC and IEC columns and their ratios relative to the 7.5 mm ID x 7.5 cm L column.

Dimensions (mm ID x cm L)	Volume (mL)	Volume ratio*
5 x 5	1.0	0.3
7.5 x 7.5	3.3	1.0
8.0 x 7.5	3.8	1.2
20 x 15	47.1	14.3
21.5 x 15	54.4	16.4
55 x 20	474.9	143.6
108 x 20	1831.2	554.8

** Relative to 7.5 mm ID x 7.5 cm L column*

Based on a 1 mg capacity for a 7.5 mm ID x 7.5 cm L column, the capacity for a 55 mm ID x 20 cm L column is expected to be about 150 mg. Much larger amounts of crude sample can be injected as long as impurities do not co-elute from the column with the compound of interest.

APPENDIX

APPENDIX C

United States Pharmacopeia (USP) specifications and corresponding Tosoh Bioscience columns

L1 - Octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 1.5 to 10 µm in diameter, or a monolithic rod.
Recommendations: TSKgel ODS-100V, ODS-100Z, ODS-100S, Super-ODS, ODS-80TM, ODS-80TS, ODS-120A, ODS-120T
See: Reversed Phase section

L7 - Octylsilane chemically bonded to totally porous silica particles, 1.5 to 10 µm in diameter.
Recommendations: TSKgel Super-Octyl, Octyl-80TS
See: Reversed Phase section

L8 - An essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 1.5 to 10 µm in diameter.
Recommendations: TSKgel NH_2-100, TSKgel NH_2-100 DC
See: Hydrophilic Interaction section

L-9 - Irregular or spherical, totally porous silica gel having a chemically bonded, strongly acidic cation-exchange coating, 3 to 10 µm in diameter.
Recommendations: TSKgel SP-2SW
See: Ion Exchange section

L10 - Nitrile groups chemically bonded to porous silica particles, 3 to 10 µm in diameter.
Recommendations: TSKgel CN-80TS
See: Reversed Phase section

L11 - Phenyl groups chemically bonded to porous silica particles, 1.5 to 10 µm in diameter.
Recommendations: TSKgel Super-Phenyl
See: Reversed Phase section

L13 - Trimethylsilane chemically bonded to porous silica particles, 3 to 10 µm in diameter.
Recommendations: TSKgel TMS-250
See: Reversed Phase section

L14 - Silica gel having a chemically bonded, strongly basic quaternary ammonium anion exchange coating, 5 to 10 µm in diameter.
Recommendations: TSKgel QAE-2SW
See: Ion Exchange section

L20 - Dihydroxypropane groups chemically bonded to porous silica particles, 5 to 10 µm in diameter.
Recommendations: TSKgel QC-PAK 200 and 300, SWXL, SW, SuperSW, and SW mAb series
See: Size Exclusion section

L21 - A rigid, spherical styrene-divinylbenzene copolymer, 5 to 10 µm in diameter
Recommendations: TSKgel HXL and HHR, SuperH, SuperHZ, and SuperMultipore HZ series
See: Size Exclusion section

L22 - A cation-exchange resin made of porous polystyrene gel with sulfonic acid groups, about 10 µm in size
Recommendations: TSKgel SCX
See: Ion Exchange section

L23 - An anion-exchange resin made of porous polymethacrylate or polymethacrylate gel with quartenary ammonium groups, about 10 µm in size.
Recommendations: TSKgel SuperQ-5PW, BioAssist Q, Q-STAT, and DNA-STAT
See: Ion Exchange section

L24- A semi-rigid hydrophilic gel consisting of vinyl polymers with numerous hydroxyl groups in the matrix surface, 32 to 63 µm in diameter.
Recommendations: TOYOPEARL HW-type
See: Size Exclusion in the Bulk Resin section

L25- Packing having the capacity to separate compounds with a molecular weight range from 100-5000 (as determined by polyethylene oxide), applied to neutral, anionic, and cationic water soluble polymers.
Recommendations: TSKgel G2500PW, G2500PWXL, Alpha-2500, SuperAW2500
See: Size Exclusion section

L26- Butyl silane chemically bonded to totally porous silica, 1.5 to 10 µm in diameter.
Recommendations: TSKgel Protein C_4-300
See: Reversed Phase section

L33- Packing having the capacity to separate dextrans by molecular size over a range of 4,000 to 500,000 daltons. It is spherical, silica-based, and processed to provide pH stability.
Recommendations: TSKgel SuperSW, SWXL, QC-PAK, SW, and Super mAb series
See: Size Exclusion section

L37- Packing having the capacity to separate proteins by molecular size over a range of 2,000 to 40,000 daltons. It is a polymethacrylate gel.
Recommendations: TSKgel G3000PWXL, G3000PW, G3000PWXL-CP
See: Size Exclusion section

APPENDIX

L38- A methacrylate-based size-exclusion packing for water soluble samples
Recommendations: TSKgel PW$_{XL}$, PW$_{XL}$-CP, PW, Alpha, and SuperAW series
See: Size Exclusion section

L39- A hydrophilic polyhydroxymethacrylate gel of totally porous spherical resin.
Recommendations: TSKgel PW, PW$_{XL}$, PW$_{XL}$-CP, Alpha, and SuperAW series
See: Size Exclusion section

L52- A strong cation exchange resin made of porous silica with sulfopropyl groups, 5 to 10 µm in diameter.
Recommendations: SP-2SW
See: Ion Exchange section

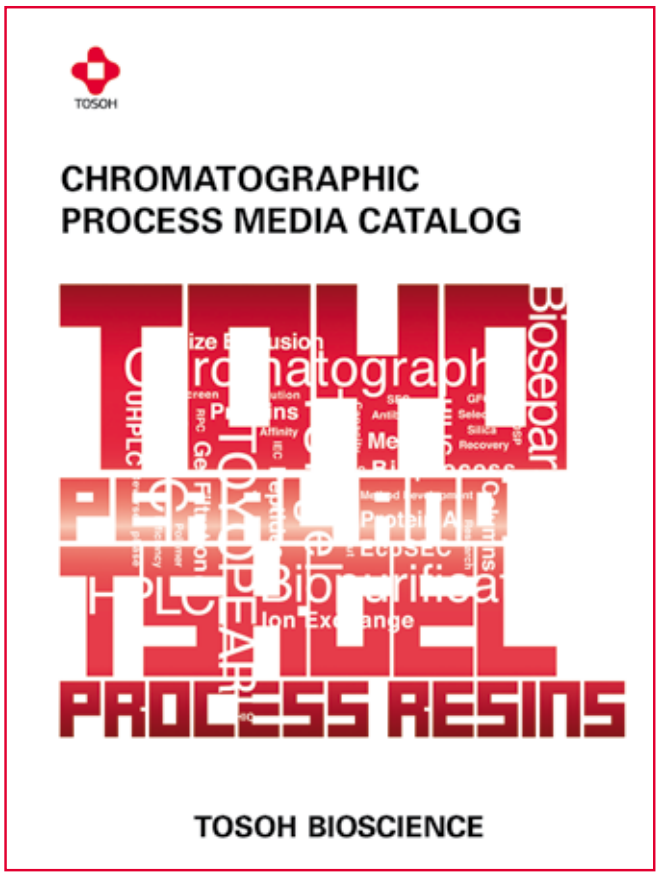
L58- Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the sodium form, about 7 to 11 µm diameter.
Recommendations: TSKgel SCX (Na^+)
See: Ion Exchange section

L59- Packing having the capacity to separate proteins by molecular weight over the range of 10 to 500 kDa. It is spherical (10 µm), silica-based, and processed to provide hydrophilic characteristics and pH stability.
Recommendations: TSKgel G2000SW, G3000SW and G4000SW
See: Size Exclusion section

L68 - Spherical, porous silica gel, 10 µm or less in diameter, the surface of which has been covalently modified with alkyl amide groups and not endcapped.
Recommendations: TSKgel Amide-80
See: HILIC section

FURTHER INFORMATION

To get an overview of the whole range of TOYOPEARL and TSKgel bulk media, ask for our **Process Media Catalog**.



For a deeper insight into applications and all questions related to the practical use of TSKgel and TOYOPEARL, check out the website **www.tosohbioscience.de** and related catalogs or instruction manuals.

Our technical experts are happy to discuss your specific separation needs, call: **+49 (0)711 13257-57** or **techsupport.tbg@tosoh.com**

MZ ANALYSENTECHNIK

AUTHORIZED DISTRIBUTOR
MZ-Analysentechnik GmbH, Barcelona-Allee 17 • D-55129 Mainz
Tel +49 6131 880 96-0, Fax +49 6131 880 96-20
e-mail: info@mz-at.de, www.mz-at.de

TOSOH BIOSCIENCE

Im Leuschnerpark 4, 64347 Griesheim, Germany
Tel: +49 (0)6155 70437 00 Fax: +49 (0)6155 83579 - 00
info.tbg@tosoh.com www.tosohbioscience.de

C15L03A